TOXOCARA PTEROPODIS BAYLIS, 1936

LIFE-CYCLE, EPIZOOTIOLOGY AND ZOONOTIC POTENTIAL

Paul Prociv MB BS (Sydney) FRACP

Department of Parasitology
University of Queensland
St. Lucia, Queensland
Australia, 4067

Submitted for the degree of
Doctor of Philosophy
within the
University of Queensland

November, 1987

ABSTRACT

The aim of this study was to establish whether a human epidemic of hepato-enteritis, the "Palm Island mystery disease", could have been a manifestation of nematode visceral *larva migrans* caused by the flying fox parasite, *Toxocara pteropodis*. An assessment of the risk of human exposure to such an infection, and of its pathogenicity, required collection of data from field and laboratory studies.

The life-cycle was elucidated in *Pteropus poliocephalus*, the predominant flying fox in the Brisbane region. Adult worms develop in the intestines of suckling juveniles, and infections may become patent in bats as young as 35 days. About 50% of juveniles develop patent infections, and each harbours an average of 1 adult male worm and 2 adult females. These worms are voided spontaneously before the bats leave their summer camps. Each female worm produces about 25,000 eggs per day, most of which are shed into the environment of the summer camp. Eggs become infective from 10 days after voiding; few remain viable longer than 6 weeks. As very little fruit remote from the camps was found contaminated with eggs, adult bats probably become infected in the camps by licking foliage and grooming during wet weather. Eggs ingested by bats hatch in the intestine and the released third-stage larvae penetrate the mucosa to reach the liver via the portal vein. These larvae may remain indefinitely in the liver, where they grow over 3 months from a length of about 420 µm to about 600 µm. In male bats, hepatic larvae gradually accumulate, but in females many leave the livers at the time of parturition and pass through the mammary glands to reach the juvenile intestines in the first 3 weeks after birth. Many developing intestinal larvae, predominantly males, are expelled spontaneously in the faeces of suckling bats. This explains the low average worm burdens in patent infections and their unequal sex representation.

Virtually all adult male *P. poliocephalus* harbour larvae in their livers; in adult females the prevalence and intensity of hepatic infection

is lower. In the P. alecto and P. conspicillatus populations of northern Queensland, the prevalence pattern of T. pteropodis appears to be similar whereas in southern P. alecto, and in P. scapulatus, infection is much less common. T. pteropodis was also found in Pteropus specimens from New Guinea, Indonesia and India. Nematodes from Rousettus sp. from the Philippines, and poorly-preserved type specimens of Toxocara cynonycteridis, from a Rousettus sp. in Burma, were very similar to, if not identical with, T. pteropodis.

On detailed examination, T. pteropodis had some features distinct from those of other members of the genus. The relatively thick egg-shell may protect against desiccation. The second moult occurs in the egg. Third-stage larvae grow in the livers of definitive (and some abnormal) hosts. Adult worms have cephalic surface features which may be unique, and do not possess a cervical alar "supporting bar" typical of the genus.

Experimental mammals other than bats exhibit varying degrees of susceptibility to infection with T. pteropodis. In mice, about 10% of an oral egg dose is recoverable as hepatic larvae, some of which may persist longer than 2 years. Suckling rats are less susceptible, and larvae are short-lived. Adult rats are even more resistant, and rabbits and pigs appear to be totally refractory to infection. In guinea-pigs and monkeys, larvae reach the liver but perish within 6 weeks, eliciting focal granulomatous responses and blood eosinophilia. Histological findings in bats, mice and monkeys, and limited experimental studies, indicate that viable larvae in tissues are capable of undergoing considerable migrations following host death.

In view of the failure of infected monkeys to develop clinical illness or significant metabolic disturbances, T. pteropodis is not likely to cause human hepato-enteritis. Alternative explanations are considered and, in an article submitted as an appendix to this thesis, copper sulphate poisoning is proposed as the cause of the Palm Island epidemic.

ACKNOWLEDGEMENTS

I am grateful to Dr H M D Hoyte, Head of the Department of Parasitology at the time of my appointment to its staff, for encouraging my study, and to his successor, Professor C Dobson, for continuing support.

Dr D E Moorhouse, my supervisor and colleague in teaching, not only provided the inspirational basis for this project, but also gave generously of ideas, time and material support.

The interest and assistance of other students and staff-members of the Department, in particular Professor J F A Sprent, were most gratefully appreciated. This study could not have been completed without the generous contributions of numerous individuals; because of space limitations and unintentional oversight, the following list is incomplete.

Of considerable help in both the laboratory and in field collections was my assistant, Catherine Creevey, who also performed most of the blood counts. Histological specimens were processed by Marjorie Barrett. Les Hall, of the Anatomy Department, advised in the location and capture of flying foxes. Collections were greatly facilitated by the interest and generous support of Jim and Kaye Davidson, of Beenleigh; Clarrie Rose, of Eagleby; Bill Allen, of Flinders Peak; Tom and Kit Rodda, of "Kurrajong Park" via Moura; Charlie and Val McCracken, of Mossman; Greg and Anne Richards, formerly of Atherton; and Doug and Barbara Holdsworth, of Portland Roads. Linesmen of the South-East Queensland Electricity Generating Board retrieved bats from powerlines, and many other people, including Des Spittle of the Currumbin Bird Sanctuary, provided both live and dead specimens. Also very helpful were Veterinary Clinic staff, particularly Wendy Blanchard and her surgical skills.

Fruit inspectors at the Rocklea Markets were sympathetic in catering for the dietary preferences of captive bats. Graeme Orr and others at "The Farm" obliged in helping to maintain captive bats and other

experimental animals, as were Denise Rogers and Margaret Owen in the care of mice. Mal Graydon, of the Anatomy Department, Dr Shirley Butler, Dr Paul Brindley and Reg and Pauline Christensen, cared for my bats during unavoidable absences.

Overseas, Dr Chris Tidemann collected and dissected bats for me on Christmas Island; Misi Nicholas sent specimens from Niue; Dr Karl Koopman forwarded specimens from the American Museum of Natural History in New York; at the British Museum (Natural History), Mr David Hill gave me access to the chiropteran collection and Dr David Gibson and his staff were most helpful in my search for worms; Dr Gianna Arbocco located type specimens in the Genoa Natural History Museum; in the Cook Islands, Mr John Campbell was generous in hospitality and the collection of bats, as were Mr Tauira Teuruaa and Mr Atingakau Tangatakino; in the Malaysian Institute for Medical Research, Kuala Lumpur, Dr Mak Joon Wah expertly managed our experimental infections in macaques, and his staff helped to find tissue larvae; and in Sri Lanka, Dr John Roberts searched for *Pteropus giganteus*.

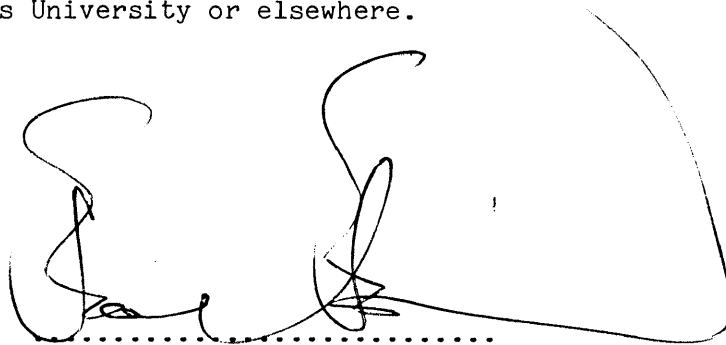
Mr Peter Foxwell forwarded mangoes from Townsville. Dr John Hardy and Mr Bob Grimmer, of the Electron Microscope Department, were most helpful in all aspects of specimen preparation, viewing and photography. Mr Tony Barnes provided useful statistical guidance.

Early manuscripts for publication were typed by the late Mrs Betty Siddell; later manuscripts and thesis drafts were typed by Mrs Sally Roth; the bulk of drafts, and final thesis were expertly prepared by Mrs Wendy Gardiner, who also assisted in field collections.

The success of this project depended very much upon the support and understanding of my long-suffering wife, Judy, and children, Nicholas and Andrew.

DECLARATION

The studies presented herein are entirely my own, unless otherwise acknowledged, and no part of this material has been submitted for another degree, either in this University or elsewhere.



Paul Prociv

November, 1987

TABLE OF CONTENTS

	Page
ABSTRACT	i
ACKNOWLEDGEMENTS	iii
DECLARATION	v
TABLE OF CONTENTS	vi
LIST OF TABLES	xiii
LIST OF FIGURES	xv
LIST OF ABBREVIATIONS	xviii
CHAPTER 1 INTRODUCTION	
1.1 THE PALM ISLAND EPIDEMIC	1
1.1.1 Description	1
1.1.2 Possible aetiology	3
1.1.2.1 Toxocariasis	3
1.1.2.2 Algal intoxication	4
1.2 AIMS OF THIS STUDY	6
1.3 *LARVA MIGRANS* IN HUMANS	6
1.3.1 Historical outline	6
1.3.2 Definition	9
1.3.3 Aetiology	10
1.3.4 Biological significance	12
1.3.5 Pathogenesis and symptomatology	16
1.3.6 Diagnosis	18
1.3.7 Epidemiology	19
1.4 THE GENUS *TOXOCARA*	20
1.4.1 Taxonomy and description	20
1.4.2 *Toxocara pteropodis*	22
1.4.3 Life-cycles	22
1.4.3.1 Life-cycle of *T. canis*	23

1.4.3.2 Life-cycle of *T. cati* 25

1.5 MATERNAL TRANSMISSION OF HELMINTHIC INFECTIONS 27

1.6 BATS AS RESERVOIRS OF ZOONOTIC INFECTIONS 28

1.6.1 Bat taxonomy 28

1.6.2 The genus *Pteropus* 29

1.6.3 Infections in bats 33

CHAPTER 2 MATERIALS AND METHODS

2.1 NATURALLY-INFECTED BATS 36

2.1.1 Collection of bats 36

2.1.2 Maintenance in captivity 38

2.1.3 Blood sampling 40

2.1.4 Tissue larval recovery 41

2.1.5 Faecal examination 42

2.1.6 *T. pteropodis* egg collection 43

2.1.7 Embryonation of eggs 43

2.1.8 Recovery of infective eggs 44

2.1.9 Recovery of worms 45

2.1.10 Bats from overseas 45

2.2 *TOXOCARA CANIS* AND *T. CATI* 46

2.3 EXPERIMENTAL ANIMALS 46

2.3.1 Bats 46

2.3.2 Mice 46

2.3.3 Other experimental animals 47

2.4 HAEMATOLOGICAL STUDIES 47

2.5 HISTOLOGICAL EXAMINATION 48

2.5.1 Tissues 48

2.5.2 Worms 48

2.6 SCANNING ELECTRON MICROSCOPY 48

CHAPTER 3 NATURAL INFECTIONS IN *PTEROPUS POLIOCEPHALUS*

3.1 INTRODUCTION 49

3.2 RESULTS 49

3.2.1 Adult males 49

3.2.2 Adult females 55

3.2.3 Adult females with young 57

3.2.4 Patent infections in suckling juveniles 64

3.3 DISCUSSION 67

CHAPTER 4 EXPERIMENTAL INFECTIONS AND OBSERVATIONS IN *PTEROPUS POLIOCEPHALUS*

4.1 INTRODUCTION 74

4.2 EXPERIMENTAL PROCEDURES AND RESULTS 74

4.2.1 Larval distribution in adult bats 74

4.2.2 Larval migration in juvenile bats 75

4.2.3 Parenteral inoculation of *T. pteropodis* eggs 77

4.2.4 Infection of juvenile *P. poliocephalus* with *T. canis* 79

4.2.5 Longevity and growth of hepatic larvae 81

4.2.6 Maternal transmission of infection 84

4.2.7 Growth of intestinal *T. pteropodis* larvae 87

4.2.8 Infection of suckling young *P. poliocephalus* with hepatic larvae 87

4.2.9 Induction of pseudo-pregnancy 89

4.3 HAEMATOLOGICAL RESPONSES TO *T. PTEROPODIS* INFECTIONS 89

4.4 HISTOLOGICAL RESPONSES TO INFECTION 95

4.4.1 Non-pregnant bats 95

4.4.1.1 Intestine 95

4.4.1.2 Lymph nodes 96

4.4.1.3 Lungs 96

4.4.1.4 Livers 96

4.4.2 Juvenile bats inoculated subcutaneously 101

4.4.2.1 Livers 101

4.4.2.2 Lungs 101

4.4.3 Post-paturient females 103

4.4.3.1 Livers 103

4.4.3.2 Mammary glands 104

4.4.4 Suckling juveniles with intestinal infections .. 108

4.4.5 *T. canis* infections in juvenile bats 108

4.5 DISCUSSION 109

CHAPTER 5 INFECTION PATENCY AND EGG PRODUCTION, MORPHOLOGY, DEVELOPMENT, SURVIVAL AND DISPERSAL

5.1 INTRODUCTION 134

5.2 PRE-PATENT INTERVAL 134

5.3 EGG OUTPUT IN EXPERIMENTAL INFECTION 134

5.4 EGG OUTPUT FROM NATURALLY-INFECTED BATS 136

5.5 INFERTILE EGG OUTPUT 137

5.6 SPERMATOZOA OF *T. PTEROPODIS* 138

5.7 FAECAL EGG CONCENTRATION 140

5.8 SPONTANEOUS LOSS OF ADULT WORMS 141

5.9 MORPHOLOGY OF *T. PTEROPODIS* EGGS 142

5.9.1 Fertile eggs 142

5.9.2 Infertile eggs 144

5.10 DEVELOPMENT OF EGGS 147

5.10.1 Conditions for embryonation 147

5.10.2 Embryonation and infectivity of eggs 149

5.11 ENVIRONMENTAL DISTRIBUTION OF *T. PTEROPODIS* EGGS 153

5.12 DISCUSSION 154

CHAPTER 6 THE DEVELOPMENT OF *T. PTEROPODIS*

6.1 INTRODUCTION 168

6.2 OBSERVATIONS 168

6.2.1 Larval development in the egg 168

6.2.2 Larval development in the liver; comparison with *T. canis* and *T. cati* 173

6.2.3 Larval development in mammary glands 183

6.2.4 Larval development in the intestine 183

6.2.5 The adult *T. pteropodis* 193

6.3 DISCUSSION 207

CHAPTER 7 *T. PTEROPODIS* IN OTHER MEGACHIROPTERA

7.1 INTRODUCTION 220

7.2 THE BLACK FLYING FOX, *PTEROPUS ALECTO* 220

7.2.1 Natural infections 220

7.2.1.1 Adult males 220

7.2.1.2 Adult females 221

7.2.1.3 Autopsied young 221

7.2.1.4 Suckling juveniles 221

7.2.2 Experimental infections 223

7.2.2.1 Recovery and growth of hepatic *T. pteropodis* larvae 223

7.2.2.2 Maternal transmission of infection 223

7.2.2.3 Haematological responses to infection 224

7.2.2.4 Liver histology 224

7.3 THE SPECTACLED FLYING FOX, *PTEROPUS CONSPICILLATUS* 226

7.4 THE SMALL RED FLYING FOX, *PTEROPUS SCAPULATUS* 227

7.4.1 Natural infections 227

7.4.2 Experimental infections 228

7.4.3 Liver histology 231

7.5 THE BARE BACKED FRUIT BAT *DOBSONIA MOLUCCENSIS* 234

7.6 *PTEROPUS TONGANUS TONGANUS* 235

7.7 *PTEROPUS MELANOTUS (NATALIS)* 235

7.8 MEGACHIROPTERA FROM MUSEUM COLLECTIONS 235

7.9 *CYNONYCTERIS AMPLEXICAUDATA* 239

7.10 DISCUSSION 240

CHAPTER 8 *T. PTEROPODIS* INFECTIONS IN NON-CHIROPTERAN HOSTS

8.1 INTRODUCTION 252

8.2 EXPERIMENTAL PROCEDURES AND RESULTS 252

8.2.1 Mice 252

8.2.1.1 Summary of published findings 252

8.2.1.2 Post-mortem larval migration 253

8.2.2 Rats 258

8.2.3 Guinea-pigs 258

8.2.4 Rabbits 259

8.2.5 Dogs 259

8.2.6 Cats 260

8.2.7 Brush-tailed possums (*Trichosurus vulpecula*) 260

8.2.8 Chickens and ducklings 261

8.2.9 Pigs 261

8.2.10 Cynomolgus monkeys (*Macaca fascicularis*) 262

8.2.10.1 Methodology 262

8.2.10.2 Results 263

8.3 DISCUSSION 269

CHAPTER 9 CONCLUSIONS

9.1 HOST RANGE AND GEOGRAPHICAL DISTRIBUTION OF *T. PTEROPODIS* .. 281

9.2 LIFE-CYCLE OF *T. PTEROPODIS* 282

9.3 TAXONOMIC AFFINITIES 284

9.4 T. PTEROPODIS AS A MODEL OF ASCARIDOID INFECTIONS 285

9.5 POST-MORTEM LARVAL MIGRATION 286

9.6 PATHOGENICITY OF T. PTEROPODIS 286

9.6.1 Bats 286

9.6.2 Other experimental animals 287

9.6.3 Humans 287

9.7 THE PALM ISLAND EPIDEMIC 288

CHAPTER 10 REFERENCES 289

APPENDIX I Observations on the transmission and development of T. pteropodis in P. poliocephalus 302

" II Seasonal behaviour of P. scapulatus 307

" III Observations on the prevalence and possible infection source of T. pteropodis in Queensland flying foxes 309

" IV Observations on T. pteropodis infections in mice .. 312

" V Oral, parenteral and paratenic infections of mice with T. pteropodis 317

" VI Susceptibility of rats to infection with T. pteropodis 320

" VII T. pteropodis, T. canis and T. cati infections in guinea pigs 323

" VIII Parasites of Australian flying foxes 329

" IX Faecal synthesis of vitamin B12 in Australian Pteropus sp. 332

" X Toxocariasis - an unlikely cause of Palm Island mystery disease 335

" XI Palm Island reconsidered. Was it copper poisoning? 336

LIST OF TABLES

TABLE

3.1 Numbers and dimensions of *T. pteropodis* larvae from male *P. poliocephalus* using tryptic digestion 50

3.2 Numbers and dimensions of *T. pteropodis* larvae from male *P. poliocephalus* using saline separation 51

3.3 *P. poliocephalus* adult females 56

3.4 *P. poliocephalus* females with young 58

3.5 Number and size of *T. pteropodis* from intestines of young *P. poliocephalus* correlated with age and maternal infection 59

3.6 Patent infections in and recovery of adult *T. pteropodis* from suckling *P. poliocephalus* 65

3.7 Findings in livers of suckling *P. poliocephalus* 66

4.1 Numbers of *T. pteropodis* larvae from tissues of experimentally-infected juvenile *P. poliocephalus* .. 76

4.2 Numbers of *T. pteropodis* larvae from tissues of juvenile *P. poliocephalus* inoculated subcutaneously with embryonated eggs 78

4.3 Recovery of larvae from tissues of juvenile *P. poliocephalus* fed embryonated *T. canis* eggs 78

4.4 Lengths of larvae recovered from bats infected orally and subcutaneously with *T. pteropodis* and orally with *T. canis* eggs 80

4.5 Lengths of *T. pteropodis* larvae recovered from livers of *P. poliocephalus* at varying intervals 82

4.6 Numbers and lengths of *T. pteropodis* recovered post-partum from experimentally-infected female *P. poliocephalus* and their offspring 86

4.7 Haematological indices of *P. poliocephalus* exposed orally to *T. pteropodis* 90

4.8 Haematological indices of experimentally-infected female *P. poliocephalus* and offspring 92

5.1 Development of *T. pteropodis* eggs in sulphuric acid .. 150

6.1 Key dimensions of *Toxocara* larvae from tissues of experimentally-infected animals 178

6.2 Dimensions of male *T. pteropodis* larvae from intestine of juvenile *P. poliocephalus* 185

6.3 Dimensions of female *T. pteropodis* larvae from intestine of juvenile *P. poliocephalus* 186

6.4 Dimensions of adult *T. pteropodis* passed spontaneously by juvenile *P. poliocephalus* with patent infections .. 195

7.1 Numbers and lengths of *P. pteropodis* larvae recovered from livers of adult male *P. alecto* 222

7.2 Lengths of *T. pteropodis* larvae recovered from livers of experimentally-infected *P. alecto* 222

7.3 Numbers and lengths of *T. pteropodis* larvae from livers of adult male *P. scapulatus* 227A

7.4 Numbers of lengths of *T. pteropodis* larvae from livers of experimentally-infected adult *P. scapulatus* 227A

7.5 Key dimensions of nematode larvae recovered from Megachiroptera in the American Museum of Natural History collection 237

7.6 Dimensions of type specimens of *T. cynonycteridis* examined in Genoa Museum of Natural History 241

8.1 Recovery of *T. pteropodis* larvae from mouse livers incubated at 37 C over varying intervals in different media .. 255

8.2 Recovery of *T. pteropodis* and *T. canis* larvae from peritoneal cavities of mice immediately and 4 hours post-mortem 255

8.3 Infection schedules, timing of autopsy and recovery of larvae from tissues of monkeys fed infective *T. pteropodis* eggs 264

8.4 Numbers and lengths of *T. pteropodis* larvae recovered from tissues of experimentally-infected monkeys 270

LIST OF FIGURES

FIGURE		Page
1.1	World distribution of family Pteropodidae	30
1.2	Distribution of flying foxes in Australia	31
2.1	Locations of facilities and *Pteropus* roosts visited in Brisbane environs	37
2.2	Maintenance of captive bats	39
3.1	Intensity of hepatic infection with larval *T. pteropodis* in naturally-infected *P. poliocephalus*	53
3.2	Length distribution of *T. pteropodis* larvae from livers of naturally-infected male *P. poliocephalus*	54
4.1	Mean lengths of *T. pteropodis* larvae recovered from livers of experimentally-infected juvenile and adult *P. poliocephalus*	83
4.2	Length dispersal of *T. pteropodis* from intestines of *P. poliocephalus*	88
4.3	Peripheral blood eosinophil levels in experimentally-infected *P. poliocephalus* mother-young pairs	93
4.4	Peripheral blood total white cell counts and eosinophil levels following oral administration of *T. pteropodis* eggs to juvenile *P. poliocephalus*	94
4.5	Liver histology of *P. poliocephalus* following ingestion of *T. pteropodis* eggs	98
4.6	Tissue changes in *P. poliocephalus* experimentally-infected with *T. pteropodis*	102
4.7	Histological findings in post-parturient adult *P. poliocephalus* infected with *T. pteropodis* and juvenile infected with *T. canis*	105
4.8	Sections of mammary gland of adult female *P. poliocephalus* 9 days post-partum	107
5.1	Twenty-four hour faecal output of *T. pteropodis* eggs from juvenile *P. poliocephalus* born to experimentally-infected mothers	135
5.2	Uterine contents from adult *T. pteropodis* passed by *P. poliocephalus* with patent infections	139
5.3	Fertile eggs of *T. pteropodis* from faeces of naturally-infected juvenile *P. poliocephalus*	143
5.4	SEMs of fertile eggs from uterus of adult *T. pteropodis*	146

5.5 Infertile eggs of T. pteropodis from faeces of juvenile P. poliocephalus 148

5.6 Intraovular development of T. pteropodis 152

6.1 Larvae expressed from T. pteropodis eggs embryonating 6-7 days in sulphuric acid 170

6.2 Larvae expressed from T. pteropodis eggs embryonating 8-11 days in sulphuric acid 171

6.3 Third-stage larvae of T. pteropodis 174

6.4 Typical third-stage T. pteropodis larva 176

6.5 SEMs of larvae of T. canis, T. cati and T. pteropodis from brains of mice, skeletal muscle of mice and livers of bats 179

6.6 SEMs of T. canis, T. cati and T. pteropodis third-stage larvae 181

6.7 Relative position of genital primordium in T. pteropodis larvae from livers of female P. poliocephalus .. 182

6.8 Developing T. pteropodis larvae from mammary gland and juvenile intestine of P. poliocephalus 184

6.9 Developmental changes at anterior end of T. pteropodis larvae 188

6.10 Position of anterior extremity of genital primordium in relation to body length of T. pteropodis from intestines of juvenile P. poliocephalus 191

6.11 Developmental stages of genital primordium in T. pteropodis 192

6.12 Caudal extremity of T. pteropodis larvae from intestine of juvenile P. poliocephalus 194

6.13 SEMs of cephalic extremity of adult T. pteropodis and T. canis 197

6.14 Anterior body structures of adult T. pteropodis 201

(In text) Excretory system of T. pteropodis 202

6.15 Caudal structures of adult male T. pteropodis, male T. canis and female T. pteropodis 205

7.1 Peripheral blood total white cell counts and eosinophil levels following oral administration of T. pteropodis eggs to juvenile P. poliocephalus and P. alecto 225

7.2 Lengths of 149 intact T. pteropodis larvae from intestine of juvenile P. scapulatus born to mother infected 66 days post-partum 230

7.3 Histological changes in livers of adult P. scapulatus experimentally-infected with T. pteropodis 233

7.4 Flying fox camps near Brisbane and T. pteropodis fourth-stage larvae recovered from R. amplexicaudatus from the Philippines 238

(In text) Parona's Ascaris cynonycteridis 239

8.1 Absolute blood eosinophil counts in monkeys given T. pteropodis eggs 266

8.2 Histological sections from livers of monkeys infected with T. pteropodis 268

LIST OF ABBREVIATIONS USED IN TEXT

cm	=	centimetre
d	=	day(s)
epg	=	eggs per gram
g	=	gram(s)
GIT	=	gastrointestinal tract
h	=	hours
H & E	=	haematoxylin and eosin
ip	=	intraperitoneal
l	=	litre(s)
m	=	metre(s)
mg	=	milligram(s)
ml	=	millilitre(s)
mm	=	millimetre(s)
MNGC	=	multinucleated giant cell
µl	=	microlitre(s)
µm	=	micron(s)
n	=	number in sample examined
N saline	=	normal (0.85%) saline
OLM	=	ocular larva migrans
pi	=	post-infection
pm	=	post-mortem
pp	=	post-partum
s	=	second(s)
sc	=	subcutaneous
SD	=	standard deviation of the mean
SEM	=	scanning electron microscopy/micrograph
VLM	=	visceral larva migrans
$\bar{x}$	=	mean
$\pm$	=	standard deviation

CHAPTER 1

INTRODUCTION

1.1 THE PALM ISLAND EPIDEMIC

1.1.1 Description

"In November, 1979, 138 children and 10 adults of Aboriginal/Islander descent [on Palm Island, north Queensland] were afflicted with an illness, the cause of which is still a mystery . . . The ages of the children ranged from two to 16 years . . . No babies were affected. They presented in successive waves over a three-week period" (Byth, 1980). Considerably more people were affected but not severely enough to seek medical attention (Byth, pers. comm.). The population of Palm Island was about 2000.

The illness began with malaise, anorexia, vomiting, constipation and in many patients, headache and abdominal pain. Tender hepatomegaly was found in all patients and 21% had a fever above 38 ^{o}C. Urine analysis showed proteinuria in 89%, glycosuria in 74%, ketonuria in 53%, haematuria in 20% and urobilinogenuria in 8%. Following admission, vomiting ceased and the children maintained high oral fluid intakes and urinary outputs. Within three days of admission, 82% developed hypokalaemia and acidosis, often with serum potassium below 2mmol/l and bicarbonate less than 5mmol/l. In 69%, intravenous fluid and electrolyte replacement was required, and in 12% protein infusion was administered for hypovolaemic shock. Within 2 days of this severe fluid and electrolyte disturbance, 39% of children developed profuse diarrhoea, and in almost all of these frank blood was passed in the stools. This bloody diarrhoea in some cases persisted for three weeks. Other features observed in some children included peripheral oedema, hyperaemic and bleeding mucous membranes, parotitis and acute abdominal tenderness with peritonism.

On faecal microscopy there were no unexpected findings - ova of Trichuris trichiura in 81% and Ascaris lumbricoides in 38%, Entamoeba histolytica cysts in 37% and amoebic trophozoites in 6%. On culture, enteropathogenic Escherichia coli was isolated from 13% and salmonellae from 1%. According to Moorhouse (pers. comm.) there were reports of "atypical Strongyloides larvae" from some cases, but the source of such reports could not be traced. Viral cultures of faeces were negative and viral serology inconclusive. Urinary toxins were not detected. "Full blood counts in most children showed a neutrophilic leucocytosis with eosinophilia" (Byth, 1980) although Byth subsequently felt (pers. comm.) that these findings probably were not outside the usual range for that population. In 32%, hepatic transaminases were elevated, but serum bilirubin levels were normal in all cases (Byth, pers. comm.).

The illness lasted between 4 and 26 days, and no likely cause was found. In retrospect, Byth (pers. comm.) was of the opinion that the illness was not as homogenous as reported, and that perhaps 2 or 3 aetiological agents may have been active at the same time.

Unusual features which may have contributed (Byth, 1980) included:

(i) very late onset of the wet season, so the island was still dry; the dam water level was low and the reticulated water was distasteful and malodorous for 2 months prior to the outbreak, so that many people resorted to drinking water from scattered springs and wells which later demonstrated high coliform counts;

(ii) an algal overgrowth in the island's dam was treated with copper sulphate 5 days before the 1st case presented (Bourke et al., 1983);

(iii) local watermelons, harvested for the 1st time on the island, were sold at the local stall just before the outbreak; these had been watered directly from the dam, and fungicides and pesticides had not been used.

1.1.2 Possible aetiology

By the end of the outbreak, several possibilities had been considered and the 2 most plausible subsequently published.

1.1.2.1 Toxocariasis

Every affected individual had been eating mangoes, and because of the particularly dry season the mango crop had been excellent that year (Moorhouse, 1982). Investigating further, Moorhouse recovered "many hundreds of viable eggs of *Toxocara pteropodis*" from 13 mangoes collected on Palm Island towards the end of the epidemic. He also found adult *T. pteropodis* in the intestines of 3 out of 7 suckling juvenile black fruit bats, *Pteropus alecto*, shot in Townsville. Because some of the clinical features of the epidemic were consistent with toxocariasis, Moorhouse proposed that the affected individuals had developed visceral *larva migrans* (VLM) as a result of ingesting *T. pteropodis* eggs while eating mangoes. Although the life-cycle of this nematode was not known, Moorhouse assumed that young bats were infected transplacentally, as occurs with *T. canis* in dogs (Sprent, 1958). Fruit was then contaminated with faeces containing worm eggs as the young bats were carried around at night on the mothers' feeding flights.

To explain the diarrhoea, which was not a feature of classical toxocariasis, Moorhouse speculated that the continued ingestion of *T. pteropodis* eggs may have precipitated an allergic gastroenteritis. The hepatomegaly could be attributed to toxocariasis but it is difficult to explain the other features of the outbreak on this basis. Electrolyte and acid-base disturbances, notably hypokalaemic acidosis, may complicate severe diarrhoea as seen in cholera (Carpenter, 1983), but in the Palm Island cases these changes *preceded* the diarrhoea. In the absence of blood biochemical data, it is impossible to interpret the significance of the glycosuria and ketonuria Byth reported (1980), but proteinuria and

haematuria imply renal pathology which must have been an early feature of the syndrome. Larvae of T. canis have been found in the kidneys of experimental primates (Tomimura et al., 1976) and humans (Dent et al., 1956), but nephritis has not been reported as a signficant feature of toxocariasis.

Nevertheless, the possibility remained that in humans T. pteropodis larvae may have undergone a different migration pattern from that of T. canis to explain most, if not all, of the features of the Palm Island disease.

1.1.2.2 Algal intoxication

Bourke et al. (1983) chronicled the evolution of the Palm Island epidemic in relation to the build-up of algae in the water supply dam and its subsequent treatment with copper sulphate. Their impression was that the illness "was due to chemical intoxication" and that the epidemic "was of a common-source type". The distribution of cases was unremarkable, except that "no illness had been reported among approximately 50 persons living in an isolated area" not supplied with reticulated water.

Questionnaires which had been "distributed to 100 school children did statistically implicate green mangoes and wells located in the area serviced by reticulated water. However, most of those who had eaten green mangoes had also ingested well water [and] all . . . had drunk the reticulated water . . . The older aboriginal residents [suspected] green mangoes". Bourke et al. considered that hepatomegaly was the only feature of the outbreak suggestive of toxocariasis and reasoned that the epidemiological characteristics were much more consistent with an algal toxin in the reticulated water supply than with the ingestion of contaminated mangoes. Copper sulphate treatment of the dam water could have disrupted the algal cells to release endotoxins.

In an extensive review of algal toxicity, Schwimmer & Schwimmer (1968) analysed the features of many episodes of poisoning in domestic and wild animals, as well as in a smaller number of human cases. In animals, acute toxicity manifested most commonly with vomiting, diarrhoea and thirst. Other features included neuromuscular disturbances, such as spasms, twitching, convulsions or paralysis, and respiratory difficulties. Autopsies had shown hepatosplenomegaly, hepatic necrosis and haemorrhage, gut inflammation and haemorrhage, pulmonary congestion with oedema and pleural effusion, myocardial haemorrhage and pericardial effusions, and renal focal necrosis with tubular degeneration. Human cases, usually people swimming in contaminated lakes, were much less common and poorly documented. Major symptoms included headache, nausea and vomiting occurring within hours of exposure and lasting up to 4 days. Other problems included acute respiratory irritation and allergic and toxic skin eruptions. Hepatitis with electrolyte disturbances, haematuria and delayed diarrhoea have not been reported in humans.

Intraperitoneal inoculation into mice of a toxin from Microcystis aeruginosa, a common bloom from Australian lakes and dams (Falconer et al., 1981) within 1 h produced hepatic necrosis and haemorrhage. Changes in the lungs could be explained on the basis of disseminated intravascular coagulation secondary to the hepatic damage, whereas other organs remained histologically normal. Chronic toxicity studies in mice (Elleman et al., 1978) again showed early hepatic necrosis and haemorrhage leading to progressive fibrosis. There was very little change in other organs. Subsequently, Falconer et al. (1983) produced biochemical evidence of transient liver damage in a rural Australian population exposed to water from a dam in which a M. aeruginosa bloom had been treated with copper sulphate. They did not prove a causal association, and there was no associated clinical illness. Then, in 1985, Hawkins et al. isolated Cylindrospermopsis raciborskii, a ubiquitous tropical blooming

cyanobacterium, from the Palm Island dam, and demonstrated that it produced a toxin which, injected intraperitoneally into mice, resulted in acute haemorrhagic hepatic necrosis and diarrhoea. Despite the route of administration and the acute pathological changes, they concluded that their findings strongly implicated this blue-green algal species in the Palm Island epidemic.

Clearly, a syndrome similar to the Palm Island disease has not been previously documented in humans. On the basis of animal studies, some of its features could be attributed to algal toxicity. However, this did not explain the chronological evolution of the epidemic, which remained a puzzle.

1.2 AIMS OF THIS STUDY

The purpose of this study was, firstly, to assess the risk of human exposure to infection with *T. pteropodis* by determining its life-cycle and distribution in flying fox populations and, secondly, to explore its behaviour in various experimental animals so as to gain an indication of its potential pathogenicity to humans.

1.3 *LARVA MIGRANS* IN HUMANS

1.3.1 Historical outline

The discovery that nematode larvae are capable of invading human tissues must be attributed to James Paget who, as a student in 1835, recognised the cysts of *Trichinella spiralis* (named later by Owen) while dissecting cadavers (Kean et al., 1978). Zenker, in 1860 (Kean et al., 1978), in describing the life-cycle and pathogenesis of this parasite, clearly established that larval infection could directly lead to specific symptomatology, and its association with blood eosinophilia was shown later by Brown (Thayer, 1897). Yet the concept of helminthic *larva migrans* was

not developed until well into the 20th Century. According to Abraham (1896), Robert Lee had introduced the term "creeping eruption" in 1874 for the skin lesions caused by Gasterophilus larvae, for which Radcliffe-Crocker (1907) later designated the term "larva migrans", referring to the aetiological agent rather than the lesions.

Penetration of the skin by Ancylostoma duodenale larvae was first demonstrated by Looss (1901), who later traced their passage through lymphatics, blood vessels and lungs (Looss 1911). Although Bentley (1902) associated the lesions of "ground itch" with hookworm larvae in the soil, according to Beaver (1956) it was Looss in 1911 who first described "creeping eruption caused by a normal parasite of man". In 1926, Fülleborn described in detail the cutaneous lesions resulting from Strongyloides larval migration and experimentally induced cutaneous lesions with larvae of Uncinaria stenocephala, while Kirby-Smith et al. (1926) found nematode larvae, subsequently identified as Ancylostoma braziliense (White & Dove, 1928), in biopsies from people with creeping eruption. Meanwhile, Tamura (1921) had implicated Gnathostoma in a number of human cases of "creeping disease". Numerous case reports about that time from around the world, including Australia (Heydon, 1929) confirmed that the larvae of dog and cat hookworms were more frequently involved in human creeping eruption than Gasterophilus or Gnathostoma spinigerum larvae.

In early studies of ascaridoid life-cycles, speculation concerning the possible accidental infection of humans failed to arouse interest in the absence of convincing evidence. Ransom & Foster (1917), in reporting the earlier work of Stewart (1916) on the migration of A. lumbricoides and A. suum, were the first to demonstrate hepatic-pulmonary-tracheo-oesophageal migration, and concluded that ascarid larvae probably caused "lung troubles" in children, pigs and other animals. In 1921, Fülleborn described the intrauterine infection of pups with Belascaris marginata

(*Toxocara canis*), the granulomatous response to larvae in the tissues of adult dogs fed *T. canis* eggs, and the migration of larvae and resulting lesions in mice and guinea-pigs. He was able to conclude that these experimental hosts were not "accidental" but functioned as a form of intermediate host, and speculated that in view of the ubiquity of *Toxocara* eggs, humans could be infected in a similar fashion.

However, it was not until 1952 that Beaver et al. reported the first confirmed human case of *Toxocara* infection, labelling the syndrome "visceral *larva migrans*". Beaver's suspicions had been raised by earlier case reports, the first being that of a child with chronic marked blood eosinophilia, lung infiltration and hepatic granulomas attributed to ascariasis (Perlingiero & György, 1947). Although no larvae had been found in these hepatic lesions on biopsy, in cases described shortly afterwards by Mercer et al. (1950) and Behrer (1951) intestinal ascariasis was associated with the presence of larvae in the liver. In 1951, Beautyman & Woolf reported the first finding of a nematode larva, tentatively diagnosed as *A. lumbricoides*, from a human brain, that of a 6-year-old girl who had died of poliomyelitis. As the child had played with dogs and cats, they conceded that the larva could have been another ascarid, and it was subsequently shown to be *T. canis* (Beautyman et al., 1966).

In 1953, Smith & Beaver reported the induction of chronic blood eosinophilia in the absence of clinical signs in 2 mentally retarded infants, each of whom had been fed 200 eggs of *T. canis*. From limited mouse experiments, they concluded that the behaviour of this nematode in humans and in mice was similar. In the same year, Dent & Carrera (1953) described in detail the clinical syndrome of VLM based on 13 cases, of which 1 had been confirmed at autopsy, 5 on finding larvae in liver biopsies, 3 on characteristic liver histology in the absence of larvae and the remainder on strong clinical grounds. Detailed study of the morphology of *T. canis* and other larvae in histological sections by Nichols (1956)

facilitated a more confident identification of these larvae from human tissues. Hence, Dent et al. (1956) were able to conclude that "some of the nematodes found in the eye by Wilder" (1950) were, in fact, *T. canis* and not hookworms as had been suspected originally.

Once *T. canis* had been established beyond doubt as a common cause of VLM in children, it became obvious that it was responsible for many typical cases reported earlier as Loeffler's syndrome, tropical eosinophilia, familial eosinophilia, benign eosinophilic leukaemia, or disseminated visceral ascariasis with eosinophilia (Dent et al., 1956).

By 1962 about 150 cases of VLM had been reported (Beaver, 1962), mainly from North America but also from other parts of the world. Most probably were caused by *T. canis*, and in 36 *Toxocara* larvae had been identified in tissues. By 1971, the number of recorded likely cases exceeded 200 (Arean & Crandall, 1971), although by then the majority were no longer being reported (Beaver, 1966). By 1981, over 1900 cases had been recorded from 48 countries throughout the world (Glickman & Schantz, 1981).

1.3.2 Definition

Beaver (1956) defined *"larva migrans"* as the "prolonged migration of a larval parasite in the skin or internal organs of an abnormal host, usually man". The organism is usually a nematode whose "path through the tissues is marked by a progressive linear lesion . . . in the vicinity of the wandering larva . . .". The qualification, "visceral", was proposed by Beaver et al. in 1952 for larval nematode migration in deeper tissues, although some of the agents of cutaneous *larva migrans* were capable of penetrating into deeper organs (Beaver, 1956). Conversely, organisms typically causing visceral lesions may at times migrate into cutaneous structures.

1.3.3 Aetiology

Larvae of a wide range of metazoan parasites, including insects, have been described from lesions in human tissues. According to Beaver (1956), "in most instances the offending larva is a nematode", and mature infections following a normal prepatent period do not eventuate, so that these infections could be referred to as "non-patent nematodiases". He classified the more important causative nematodes in four groups according to their normal life-cycle patterns:

I. Hookworms and Strongyloides, whose larvae develop in the soil and penetrate the skin. Although these are responsible mainly for cutaneous lesions, Strongyloides and human hookworm larvae normally undergo tracheal migration which may be symptomatic. The larvae of dog and cat hookworms also may proceed into deeper tissues, as first reported by Muhleisen (1953), and theoretically cause visceral LM.

II. Ascarids, acquired by ingestion of eggs containing infective larvae. For many years, only T. canis had been implicated as a cause of VLM under natural conditions. However, A. lumbricoides larvae may cause seasonal pneumonia (Gelpi & Mustafa, 1967) and A. suum has caused pneumonia and hepatitis following the ingestion of large numbers of eggs (Phills et al., 1972). Whether this complies with Beaver's definition of VLM would depend on how long these larvae remain in the tissues.

III. Gnathostoma and other spiruroids, eaten as infective-stage larvae within intermediate or transport hosts. Although human gnathostomiasis was originally diagnosed from skin lesions (Tamura, 1921), involvement of deeper tissues is probably more common (Beaver, 1956).

IV. Filarial nematodes, which enter the skin following the "bite" of an arthropod intermediate host. Filarial larvae may migrate through various tissues during development.

In addition to these nematode groups, "certain insects, pentastomes, tapeworms and flukes properly might be included among the larva migrans

producing parasites" (Beaver, 1956).

Subsequent to the confirmation of T. canis as the major cause of VLM in humans, a number of "new" nematodes have been shown to produce classical syndromes which may be considered as "variants" of VLM in humans, including Angiostrongylus cantonensis (Rosen et al., 1962) and Anisakis sp. (van Thiel et al., 1960; van Thiel, 1966). Two major factors appear to explain the increasing discovery of non-patent, zoonotic helminthiasis in humans (Beaver, 1962). Firstly, there has been an increasing awareness of the possibility of such infections coupled with a diminishing inclination to presume that parasites found in human tissues are normal parasites of humans. Secondly, the marked decrease in prevalence of the common human parasites in many communities has enhanced the discovery of zoonotic helminthic infections. These zoonoses "are nevertheless of minor importance when considered in relation to other health problems of either man or the animals which harbour the infections [but] have several features which .. . . . command the attention of health workers" (Beaver, 1966).

In many cases of larva migrans the organism is impossible to detect or identify, and with time more parasitic species will be implicated. "Since any species whose normal life-cycle includes tissue-invading stages in mammalian hosts may be regarded as a potential cause of disease in man, a wide variety of nematode parasites of those mammals which live in close association with man must be considered" (Beaver, 1956). A recent case in point is that of Baylisascaris procyonis, a common parasite of raccoons in North America. Based upon earlier findings in non-primate experimental animals, then in squirrel monkeys, Kazacos et al. (1981) predicted that this ascaridoid nematode was capable of causing neurological disease in humans. Subsequently, a fatal human case of eosinophilic meningoencephalitis was attributed to this worm (Fox et al., 1985).

1.3.4 Biological significance

Fülleborn (1921) speculated on the finding of nematode larvae in the tissues of various animals. He agreed with Leuckart's suggestion that these represented an essential developmental stage of these parasites, i.e. in an intermediate host, and discounted the proposals of Dujardin and von Siebold that these larvae had gone astray in the wrong host. Working with T. canis, Fülleborn produced patent infection in a newborn pup by feeding it infective eggs. He also demonstrated intrauterine infection and tracheal migration of larvae in pups, and concluded that this nematode could develop directly in the right host or could use adult dogs as a form of intermediate host. By feeding T. canis eggs to mice and guinea-pigs, Fülleborn demonstrated the tissue migration of larvae which, when fed to pups, underwent tracheal migration and could have led to patent infection. He concluded that mice and other small animals functioned as intermediate hosts, even though T. canis larvae did not develop in them, and speculated that this could be the role of humans as well. He also pre-empted the discovery of human infections from nematode larvae in herrings.

In a subsequent consideration of larval nematode distribution in host tissues, Fülleborn (1923) rejected the term "wandering" on the grounds that these larvae were in the main passively distributed by the circulation, although at "critical locations" initiated behaviour which determined their ultimate anatomical dispersal. Nematode larvae entering the "wrong" host, either through the skin or gastrointestinal tract, may be capable of reaching the circulation to undergo either pulmonary-tracheal migration or somatic dispersal. Surviving larvae ultimately could be ingested by the natural host in which they developed, and then such a pathway, through reinforcement, could become incorporated into the normal life cycle. Hence, T. canis could infect dogs "directly" or through "larvae gone astray" in "transportation hosts". Evolutionary pressures would select larvae which acquired a "histotropism" for tissues which ensured their

ultimate transfer to the definitive host. Fülleborn (1929) then expanded these ideas to conclude that "the far advanced *Ascaris* larvae of the lung" in man and pig are analogous to related larvae in intermediate hosts, such as those of aquatic bird nematodes which develop in fish liver - "there can be no doubt that . . . man can infect his intestine with *Ascaris* by eating the larvae with the lung of a rabbit [or through cannibalism] . . . but such an infective meal has become dispensable" because man is not only the definitive host "but also one of its many 'intermediate' hosts".

Eventually it became clear that in many parasitic life cycles, larvae could pass through one or more intermediate hosts which were not essential to development. Baer (1952) introduced the term "paratenic host" for "an optional intermediate host . . . which larvae usually enter passively . . . [in which they] remain at the same stage of development . . . [and which] is not at all necessary for the successful completion of the life-cycle". In other words, paratenic hosts facilitate the transport of parasitic larvae through complex food pyramids back to their definitive hosts. Because in animals otherwise considered paratenic hosts, the larvae of some ascarids of carnivores may undergo considerable growth (Tiner, 1953; Sprent, 1954), Beaver (1956) suggested that the larval growth qualification should be removed from the definition and added a further proviso: "the larvae that use paratenic hosts tend to be capable of survival in a wide range of hosts, without developing to the mature adult stage and without loss of infectivity to its final host". With the exception of filarial nematodes, those causing *larva migrans* in man, viz. ascaridoids, hookworms and spiruroids, "all may eventually be shown to have become adapted to natural passage through paratenic intermediate hosts". Hence, "it is incorrect to regard man as an abnormal host except that he is not a normal final host". Because the *larva migrans*-causing organisms included worms which had predilections for various anatomical locations,

which could develop to sexual maturity (e.g. Capillaria hepatica) and in other instances were "normal" parasites of man with behaviour rendered atypical by unusual host immune or tissue responses, Beaver (1956) concluded that classification was impossible without an accurate identification of the worm. "Probably the best designation for the nematode infections . . . known collectively as larva migrans . . . is a non-patent nematodiasis".

Sprent (1963) analysed VLM in a broader biological perspective, defining the concept as "the invasion of, and migration through, any of the tissues of the animal body by nematode larvae . . . which are natural parasites of the affected host, or . . . other hosts". He emphasized that detailed knowledge of the structure, physiology and behaviour of nematode larvae in tissues was essential to the development of a rational clinical approach to human cases. According to Sprent, because in the primitive rhabditoid-strongyloid life-cycle there is only a single host, humans invaded by such larvae will adopt the role of final host or accidental host. On the other hand primitive ascaridoid life-cycles involved a series of hosts, so such species will utilize humans either as a final host harbouring fourth-stage larvae or adults, or as an intermediate host infected with second or third stage larvae. Sprent's wider definition allowed him to include human-specific filarioids such as Onchocerca volvulus, as well as trichuroids such as T. spiralis and C. hepatica, amongst the nematodes causing VLM. He also included "aberrant" larvae, i.e. those which are not potentially seeking paratenic transmission, but have strayed from their usual migratory patterns in the tissues of a host in which they have no biological future, unless developing to maturity. Amongst examples of these in humans, Sprent included A. cantonensis, Dirofilaria immitis and possibly Lagochilascaris minor.

Beaver (1969) took issue with Sprent's more general definition of VLM, advocating a restriction of the definition "to include only the prolonged

migration and long persistence of larvae whose behaviour clearly reflects that which occurs in a normal intermediate or paratenic host", such as seen with *T. canis* or *G. spinigerum*. This would exclude filarioids, *Angiostrongylus*, *Anisakis*, *Capillaria* and others whose development in humans resembles that in the final hosts. Instead of redefining VLM, Beaver classified zoonotic helminthic infections into four groups:

(i) humans normal and adequate, but unnatural, final host, e.g. *C. hepatica* and *T. spiralis*;

(ii) humans normal but inadequate final host, e.g. *Dirofilaria tenuis*, *D. immitis*, *A. cantonensis* and *Anisakis* spp.;

(iii) humans normal but unnatural intermediate host, e.g. larval tapeworms such as cysticerci or hydatids, pentastomes and perhaps some ascaridoids;

(iv) humans normal but unnatural paratenic host, e.g. *T. canis*, *T. cati*, *Gnathostoma*, sparganosis and perhaps animal hookworms.

Beaver concluded that no purpose would be served in precisely defining VLM until the identity and life-cycles of nematodes invading human tissues were understood in greater detail and seen in their biological perspective. Nevertheless, he later defined VLM as "a syndrome caused by the migration of larval stages of *T. canis* and certain other parasitic helminths of dogs, cats and other carnivorous animals in the deeper parts of the body" (Beaver et al., 1984). The implication was that in VLM humans adopted the role of paratenic hosts. Once the larva was diagnosed the condition should then be labelled specifically. The biologically distinct non-paratenic larval invasion of human tissues by helminths was excluded from Beaver's definition of VLM. In this group were parasites naturally developing to maturity in humans, e.g. *Ascaris*, *Necator* and *Strongyloides*, and those for which humans replaced the definitive host, e.g. *Capillaria*, *Angiostrongylus*, *Anisakis* and *Dirofilaria*.

It is possible to re-define "*larva migrans*" taking into account both Sprent's and Beaver's considerations. If a tissue larva is identifiable, the condition should be labelled accordingly. However, until a metazoan larva found in human tissues is specifically identified, then its biological significance and the status of the host must remain unclear. Hence, "*larva migrans*" simply could be termed "a clinical condition caused by the presence of unidentified helminthic larvae in human tissues", and classified according to the organs affected.

1.3.5 Pathogenesis and symptomatology

The host response and hence clinical features of VLM are determined by the numbers, organ distribution and longevity of larvae coupled with host factors such as age, physiological suitability, sensitization, and immune status (Beaver, 1962; Glickman & Schantz, 1981). *T. canis* is the best studied and most prevalent cause of human VLM. Since the original description (Beaver et al., 1952), numerous case reports have added little to the classical picture of clinical toxocariasis. Even by 1962, Beaver claimed that "a review of the 56 best described cases to date introduces no important new features". The classical syndrome develops in children aged less than four years who have a habit of eating dirt. However, adults are also susceptible to toxocariasis canis (Arean & Crandall, 1971). Recurrent fever, anorexia, lassitude, irritability, coughing, wheezing, shortness of breath, allergic manifestations, behavioural changes, seizures and other neurological symptoms are common (in decreasing frequency), although those with light infections may be asymptomatic (Smith & Beaver, 1953). Physical signs include hepatomegaly, respiratory and sometimes neurological abnormalities. Ocular involvement, in the form of endophthalmitis or retinal granuloma, occurs usually in older children without the other features described above. In rare fatal cases there has been extensive involvement of the myocardium or central nervous system

(Glickman & Schantz, 1981).

In virtually all cases, even those free of symptoms, blood leukocytosis with pronounced eosinophilia has been a major feature. In 86% of cases (Beaver, 1962) eosinophils accounted for more than half of the circulating white blood cells, and in more than 50% absolute eosinophil counts exceeded $20,000/mm^3$. Serum globulins were elevated in most cases. About 50% of patients with chest signs demonstrated transient pulmonary infiltrates radiologically. All of these changes can be explained by the larval migration through liver, lungs, and other tissues following ingestion of infective _T. canis_ eggs.

In their extensive review, Glickman & Schantz (1981) distinguished in more detail between the classical syndrome (VLM) and ocular _larva migrans_ (OLM). Rarely were the two associated in one individual, although occasionally VLM preceded OLM by several years. OLM tended to occur in older children who did not habitually ingest soil. Glickman & Schantz argued that in view of the different epidemiological pattern and the lower specific antibody titres and eosinophilia in cases of OLM, the affected children must have been exposed to fewer _T. canis_ eggs, assuming that high numbers of larvae stimulate an aggressive immune response which prevents their reaching the eye. Nevertheless, "the pathogenicity of _T. canis_ at low doses must be relatively low, since many children have serologic evidence of infection, but few develop ocular lesions". Extensive low-dose studies have not been undertaken in experimental animals. Smith & Beaver (1953) induced persistent eosinophilia by feeding 200 eggs of _T. canis_ to each of two mentally defective infants, without causing illness in either except for transient mild hepatomegaly in one. In another study (Chaudhuri & Saha, 1959) an adult volunteer fed 100 eggs developed a persistent cough and blood eosinophilia. It may be concluded that as few as 100 eggs of _T. canis_ can induce VLM in humans, although in neither of these studies could

pre-sensitization with a related infection (e.g. ascariasis) be excluded, or apparently even had been considered. In more severe cases, many thousands of eggs must have been ingested, e.g. at the autopsy of a 19-month-old boy who had died of serum hepatitis complicating blood transfusions for anaemia associated with VLM, 60 T. canis larvae per gram of liver tissue, 5 larvae/g muscle and 3-5 larvae/g brain were recovered (Dent et al., 1956).

The histological responses in tissues of humans and experimental animals, reviewed in detail by Arean & Crandall (1971), consist essentially of acute inflammation followed by granulomatous reactions to migrating and stationary larvae.

1.3.6 Diagnosis

In a typical case, the diagnosis may be suspected on clinical grounds, but the signs of toxocariasis are non-specific, with a wide differential diagnosis. Confirmation ultimately relies on the detection and identification of larvae in tissues. There may be radiological chest abnormalities. Liver function tests are usually normal (Arean & Crandall, 1971). Blood eosinophilia is almost universal, but this occurs with a variety of other syndromes, including numerous other helminthoses, as well as allergies, asthma and the very rare eosinophilic leukaemia (Bunch, 1983). As biopsy cannot be justified in most cases, a wide variety of immunological tests have been employed, generally with unreliable results (Glickman & Schantz, 1981). More recently, the utilization of an ELISA with antigens from cultured T. canis larvae appears to have rendered serological diagnosis more sensitive and specific (Cypess et al., 1977; de Savigny et al., 1979). However, its assessment and refinement has been hampered by the inability to find larvae in most cases studied, in which the initial diagnosis still rests on clinical and other laboratory features. There is a paucity of studies evaluating the genus-specificity

of this test, let alone its species-specificity. Furthermore, non-infected control groups include individuals with asymptomatic toxocariasis.

The major role of serology is in supporting a presumptive clinical diagnosis of VLM, for which purpose the ELISA appears to be adequately sensitive and specific (Glickman & Schantz, 1981). The results of serological population surveys have varied widely according to the methods used.

1.3.7 Epidemiology

Clinical toxocariasis is most prevalent in children, and human infections may occur wherever dogs harbour _T. canis_, particularly where human living conditions are poor. However, most reported cases have been from countries where medical diagnostic facilities are sophisticated and human parasites are not major problems (Arean & Crandall, 1971). Infective _Toxocara_ eggs have been found in the soil of parks and playgrounds in North America and Europe (Beaver et al., 1984). As these eggs take about 3 weeks to embryonate, direct exposure to dogs is not a major risk factor (Borg & Woodruff, 1973), whereas soil ingestion in affected children is very common (Beaver, 1962). Glickman et al. (1981) found a higher prevalence of _Toxocara_ antibodies in children with geophagy than those with paint or plaster pica. In their review of risk factors for human toxocariasis, Glickman & Schantz (1981) cited 1 study which showed an increased prevalence of _Toxocara_ antibodies amongst a group of dog breeders, and 2 studies which failed to show an association between dog-handling and subclinical infection.

Subjecting to ELISA 8,457 serum samples from a wide cross-section of the civilian, non-institutionalised population of the USA, Glickman & Schantz (1981) found 2.8% positive for _Toxocara_ antibodies. The prevalence was highest in the 1-11 year age groups, and higher in blacks than in

whites at all ages. It was generally higher in males except in the 1-5 year age group, and black females in the 56+ age group. This latter finding was explained on the basis of the common practice of clay ingestion by older black women.

It is clear, therefore, that geophagy is the major risk factor for toxocariasis, as by this means the individual is exposed to maximal numbers of infective eggs. Paratenic transmission from the ingestion of raw liver has been suggested (Beaver, 1962) but must be uncommon even though natural *Toxocara* infection in pigs has been demonstrated (Stevenson, 1979).

1.4 THE GENUS *TOXOCARA*

1.4.1 Taxonomy and description

Ascaridoid nematodes are found in the gastrointestinal tracts of all vertebrate groups. Their taxonomy is still far from settled (Sprent, 1983; Gibson, 1983). According to Sprent (1983), the modern classification of ascaridoids is that formulated by Hartwich in 1957, reproduced in the form of a key (Hartwich, 1974) in which the genera *Toxocara*, *Porrocaecum* and *Paradujardinia* comprise the subfamily Toxocarinae of the family Ascarididae in the superfamily Ascaridoidea, order Ascaridida. Because "the superfamily Ascaridoidea cannot be defined by a single characteristic feature", Sprent (1983) reviewed the structures significant in taxonomy, including the excretory system, oesophagus, intestinal caeca, labial structures, male tail and body size, and life history patterns, to propose a new classification based on his revised key to the five component subfamilies. He placed the genus *Toxocara* in the subfamily Ascaridinae, whose features he defined as follows: "Excretory system filamentar, excretory nucleus near commissure, excretory pore usually near nerve ring; ventriculus rectangular, ill-defined or absent; oesophageal gland nuclei enlarged; more than eight precloacal papillae; gubernaculum absent".

Warren (1971a) reviewed in detail the genera Toxocara and Neoascaris, subjugating the latter into the former. After a detailed analysis of the literature and examination of available specimens, he concluded that only 11 species could be assigned with certainty to the genus Toxocara, as follows:

Genotype: Toxocara canis (Werner, 1782) Stiles, 1905

T. alienata (Rudolphi, 1819) new comb.

T. apodemi (Olsen, 1957) new comb.

T. cati (Shrank, 1788) Brumpt, 1927

T. mackerrassae (Sprent, 1957) new comb.

T. paradoxura Kou, 1958

T. pteropodis Baylis, 1936

T. suricattae (Ortlepp, 1940) new comb.

T. tanuki Yamaguti, 1941

T. vincenti Puylaert, 1967

T. vitulorum (Goeze, 1782) new comb.

Warren compiled a key to the adults of this genus, and described the features of its members thus: "Cervical alae sometimes manifest, variable in width; supporting V-shaped bar always present. Lips with pulp forming an unpaired internal lobe and 2 larger external lateral lobes; dentigerous ridges prominent; interlabia absent. Oesophagus divided into anterior muscular portion and posterior glandular ventriculus without appendix. Intestinal caecum absent. Male: Spicules equal or subequal; alate. Tail with terminal pointed appendix bearing up to 4 pairs of papillae. One large double papilla on either side and immediately posterior to cloaca. Preanal papillae variable in number, in 2 distinct rows. No pericloacal roughened areas. Gubernaculum absent. Female: Opisthodelphic; vulva in anterior half of body length. Oviparous; eggs globular or subglobular with thick, pitted shell. Parasites of terrestrial mammals".

Subsequently, Sprent described *T. vajrasthirae* in 1972, Warren described *T. genettae* and *T. sprenti* in 1972 and Maung described *T. hippopotami* in 1975. A new species, *T. indica*, was added in 1981 (Naidu, 1981), bringing to 16 the number of species in the genus.

1.4.2 *Toxocara pteropodis*

Baylis (1936) described *T. pteropodis* from "a number of Ascarids" collected from the alimentary tract of a suckling fruit-bat, *Pteropus geddiei*, in the northern New Hebrides (now Vanuatu), and found it to be distinct from the "three well-authenticated species" of *Toxocara*, viz. *T. canis*, *T. mystax (cati)* and *T. melis*. The major differential features were body length, very narrow cervical alae, short spicules of the male, caudal papillae of the male and egg size and appearance. Warren (1971a) re-examined the paratype specimens and added some measurements, but otherwise did not contribute further to Baylis' description of *T. pteropodis*.

1.4.3 Life-cycles

Sprent (1954) adopted the view, with strong justification, that ascaridoid nematodes originated in marine environments and that the life-cycles utilizing aquatic arthropods and fish as intermediate hosts and marine vertebrates as definitive hosts were the most primitive of this group. These parasites then radiated through semi-aquatic hosts to terrestrial predators, depending on smaller mammalian intermediate hosts (usually rodents), and their life-cycles have attained the highest development in non-carnivorous hosts, for which intermediate hosts are no longer required. In dispensing with intermediate hosts, ascaridoids of non-carnivores adopted various migratory pathways other than the somatic to ensure arrival in the gut of the host, including tracheal (as occurs with *A. lumbricoides*, *A. suum* and *Parascaris equorum*), transplacental (then known for *T. canis* and suspected in *Neoascaris vitulorum*) and abdominal

migration, e.g. Toxascaris leonina.

Later, Sprent (1962) elaborated on these ideas, proposing a relationship between ascaridoid life-cycle evolution and the development of the excretory system. As more ascaridoid life-cycles were elucidated, his proposals for the evolution of ascaridoids became even more specific (Sprent, 1982; 1983). He considered that the aquatic group representing the earliest vertebrate hosts of ascaridoids were the Crocodilia. In the more "primitive" life-cycles invertebrates were the 1st intermediate hosts, with a number of subsequent vertebrate intermediate or paratenic hosts interposed en route to the definitive carnivorous host at the apex of the "life pyramid". The great majority of ascaridoids occur in hosts whose main food source is other animals. Ascaridoids of herbivorous hosts are more likely to have direct life-cycles, e.g. A. lumbricoides and A. suum, and this small group could be considered as the most modern representatives. Sprent also noted that in some species, e.g. T. vitulorum of cattle and T. pteropodis of fruit bats, suckling animals harboured the adult worms, and in the former they were infected via the mother's milk. Nevertheless, he cautioned that "it is not possible to generalize about life history patterns among ascaridoids because relatively few species have been investigated" (Sprent, 1983). In fact, within the genus Toxocara only 2 life-cycles are known in any detail.

1.4.3.1 Life-cycle of T. canis

Fülleborn (1921), Shillinger & Cram (1923) and Augustine (1927) demonstrated pre-natal infection of pups from bitches which had been infected with T. canis eggs (larvae in Fülleborn's case) during gestation. Yutuc (1949) showed that this also occurred in bitches infected before conception, suggesting that larvae accumulated in the tissues and then migrated across the placenta. Fülleborn (1921) also found that intestinal

infection resulted from feeding T. canis eggs to newborn pups, but in adult dogs larvae migrated to the liver, lungs and kidneys. Sprent (1958) re-confirmed the high prevalence of gut infections in young pups but not in older dogs, the establishment of patent infections in dogs after eating tissue larvae in mice, and the susceptibility of pups younger than 3 weeks to develop patent infections after eating eggs whereas older dogs developed non-patent tissue infections. He described in detail the development of T. canis from second-stage larvae through to adults. Webster (1958) fed 2,000 T. canis eggs to dogs of varying ages, and found that age susceptibility to patent infection did not change as abruptly as suggested by Sprent (1958), who had given much higher egg doses to his adult dogs. In 3-week-old dogs, Webster found predominantly tracheal migration leading to patent gut infections, with a few larvae migrating to tissues. In dogs aged 2 and 3 months, larvae underwent both migratory routes, whereas in a 6-month-old dog most larvae followed the somatic route although a few developed in the gut. Dubey (1978), working with ascarid-naive beagles, showed that the establishment of gut infection partly depended on the egg dose. Patent infections developed in all 9-week-old dogs fed 50-1,000 eggs (pre-patent period 30-39 d), but in none fed 10,000 eggs. Three of 6 adults fed 100 eggs each also developed patent infections. Douglas & Baker (1959) reported that in pregnant bitches fed T. canis eggs, larvae required 2 weeks to reach the placenta and foetus, and that foetal infection occurred after 6 weeks of gestation. They also noted that post-parturient bitches developed patent infections, which persisted on average for 60 d (range 9-108 d), at the same time as their litters. It was then shown by Sprent (1961) that in post-parturient bitches, 3rd-stage T. canis larvae, passed in the faeces of the pups and ingested by the dam while grooming, were capable of developing into adult worms. Douglas & Baker (1965) also demonstrated that not all larvae are mobilized in the pregnant bitch to cross the placenta, so that some may persist and infect litters of

subsequent pregnancies.

Stone & Girardeau (1967) reported finding larvae of T. canis in milk expressed from a lacatating greyhound 22 days post-partum. Stoye (1976) confirmed that some T. canis larvae infected the pup after birth via the transmammary route. However, in an elegant experiment, Burke & Roberson (1985a) showed that the transplacental route was by far more important, accounting for 98.5% larvae, with only 1.5% being transmitted lactationally in beagles infected 2-4 months prior to mating.

In summary, most dogs develop patent T. canis infections in the neonatal period. Eggs shed in their faeces contaminate the environment to embryonate and be ingested by other dogs or paratenic hosts. In young pups, larvae from eggs may undergo tracheal migration leading to patent infection, but with increasing age of the dog more larvae undergo somatic migration, although patent infections still may develop in adult dogs which have eaten larvae in paratenic hosts. The tissue reservoirs of larvae in the pregnant bitch are presumably in liver, lungs, kidneys and muscle (Noda, 1958; Greve, 1971), and they are mobilized, probably in response to hormonal changes, around the 42nd day of gestation. They lodge in the foetal liver, from which about the time of parturition they undergo tracheal migration leading to patent infection 23-40 d after birth. Some 3rd-stage larvae passing through the pup's intestine may develop to adults in the intestine of the lactating bitch.

The natural definitive hosts for T. canis include many members of the family Canidae and occasionally some Felidae (Sprent, 1958). Records of adult T. canis from humans are questionable (Beaver et al., 1984).

1.4.3.2 Life-cycle of Toxocara cati

This nematode occurs naturally in many of the Felidae, although there have been well-documented reports of adult worms in humans (Sprent, 1956;

Beaver et al., 1984). The life-cycle was first studied in detail by Sprent (1956), who found 34% of cats in Brisbane infected, with prevalence in kittens (69%) higher than in adult cats (25%). In mice fed infective eggs, larvae accumulated in skeletal musculature. Eggs were also infective to earthworms, cockroaches, chickens, dogs and sheep, but relatively minute numbers of larvae were recovered from the last 3 groups. Only 1 of 22 wild rats and 1 of 4 mice harboured Toxocara larvae.

In kittens fed infective eggs, larvae underwent predominantly tracheal migration although some dispersed throughout skeletal muscle early in the infection. Development from the 3rd to the 4th stage seemed to occur mainly in the stomach wall. In kittens fed infected mice, larvae again developed in the stomach wall to the 4th-stage before re-entering the gastrointestinal lumen, with small numbers undergoing somatic and perhaps also tracheal migration. Sprent found no evidence of transplacental infection. He described in detail developmental stages from 2nd-stage larvae to adults, and concluded that paratenic hosts played an important role in the life-cycle of T. cati. Swerczek et al. (1971) demonstrated convincingly that prenatal infection of kittens does not occur, but that transmammary transmission of T. cati larvae from queen to suckling kittens is a major route, even when eggs are fed as late as 1 d ante-partum. Doses of 2,000 eggs were fed daily, with larvae recovered from milk samples until the experiment was terminated 22 d post-partum. Larvae developed directly to adults in the kittens' alimentary tracts. Hence, in cats, patent T. cati infections develop following the ingestion of infective eggs, or of larvae in paratenic hosts or in milk.

The tissue reservoir of larvae in cats is presumably in skeletal muscle; the hormonal or other influences which stimulate larval migration to the mammary glands remain a mystery.

1.5 MATERNAL TRANSMISSION OF HELMINTHIC INFECTIONS

Anatomically, helminthic larvae can pass from the mother to young via either intrauterine (presumably transplacental) or transmammary routes. Yutuc (1949) claimed that since the finding by Fuijinami & Nakamura of *Schistosoma japonicum* in the foetus of a dog in 1911, "prenatal helminthiasis has been observed a number of times in natural infections". However, the only life-cycle in which signficant transplacental migration has been shown to occur naturally is that of *T. canis*.

A variety of other nematode infections more prevalent in juvenile hosts have recently been shown to undergo lactogenic transmission. According to Stone & Smith (1973), Salzer in 1916 found *Trichinella* larvae in the milk of a woman with trichinosis and, in 1928, Kotake reported *Ancylostoma caninum* and *T. canis* larvae in the mammary glands and milk of experimentally-infected guinea pigs. However, this route of infection in a normal life-cycle was not demonstrated until 1960, for *Uncinaria lucasi* in Arctic fur seals (Olsen & Lyons, 1965). Then, *Strongyloides ransomi* of pigs, previously thought to be transmitted placentally, was shown by Moncol & Batte (1966) to pass through colostrum. The next life-cycle shown to utilize this route was that of a trematode, *Pharyngostomoides procyonis*, of raccoons (Harris et al., 1967).

At about this time, the significance of the transmammary passage of larvae in the life-cycle of *A. caninum* was being established (Stone & Girardeau, 1968), and *T. canis* larvae had been found in dog's milk (Stone & Girardeau, 1967). Subsequently, this route was demonstrated for *Strongyloides westeri* in horses (Lyons et al., 1969), *T. vitulorum* in cattle (Warren, 1969; 1971b), *Strongyloides papillosus* in sheep and cattle (Lyons et al., 1970), *T. cati* in cats (Swerczek et al., 1971), *Strongyloides ratti* in rats (Zamirdin & Wilson, 1974), *Strongyloides fulleborni* in humans (Brown & Girardeau, 1977), *Necator americanus* in humans (Setasuban et al., 1980) and *Mesocestoides corti* in mice (Conn &

Etges, 1983).

In his review, Miller (1981) listed the helminths so far shown either naturally or experimentally to undergo prenatal and transmammary transmission, and cautioned against accepting previous studies on prenatal infections because "not all evidence presented by some authors is convincing". Furthermore, "some neonatal infections of helminths, once considered to be prenatal, are either partially or exclusively milk-borne". Nevertheless, he documented 13 species as being capable of prenatal and 17 of transmammary transmission.

In the past 25 years transmammary infection has been shown to be an important route in many nematode life cycles. Without doubt, the movement of larvae from various maternal reservoir organs to the mammary glands must ultimately be influenced by hormonal changes. However, because of the variety of hosts and parasites involved, the differences in the timing of such migrations and the relative numbers and developmental stages of larvae involved, and the general ignorance of the endocrinology of pregnancy in these host animals, the field at present remains open to speculation.

1.6 BATS AS RESERVOIRS OF ZOONOTIC INFECTIONS

1.6.1 Bat taxonomy

Bats are placental mammals comprising the order Chiroptera. Members of the suborder Microchiroptera are small (weight less than 170 g and wingspan less than 30 cm), use echolocation and feed on insects, although a few are predators of small vertebrates and some are blood feeders (Richards, 1983). Bats of the suborder Megachiroptera generally are large (weight up to 1 kg or more, and wingspan up to 1.6 m), do not use echolocation (although members of the cave-roosting Africo-Asian genus, *Rousettus*, have a simple echolocatory system of oral clicks), are

herbivorous and are restricted mainly to tropical regions.

The Megachiroptera comprise only 1 family, Pteropodidae, which includes about 150 species (Richards, 1983). Eight species occur in Australia: 4 *Pteropus* species (flying foxes), *Dobsonia moluccensis* (bare-backed fruit-bat), *Nyctimene robinsoni* (Queensland tube-nosed bat), *Syconycteris australis* (Queensland blossom bat) and *Macroglossus lagochilus* (northern blossom bat).

1.6.2 The genus *Pteropus*

According to Andersen (1912), 85 species are found in this, the largest genus of Megachiroptera. Its geographical range extends "from the island of Pemba (north of Zanzibar), through the Malagasy, Oriental, and Australian regions to the Samoa Islands" (Fig. 1.1). Their greatest diversity is attained in the Indo-Malayan (14 species) and Austro-Malayan (36 species) regions. Koopman (1970) considered there were only 65 species of *Pteropus*, 39 in the Australian, 24 in the Indo-Malayan and 9 in the Ethiopian regions.

The distribution of the 4 species in Australia is shown in Figure 1.2. *P. poliocephalus* is found from about Rockhampton in central coastal Queensland to islands in the Bass Strait; *P. alecto* from about Grafton in northern New South Wales to Cape York and across to northern Western Australia, *P. conspicillatus* from Townsville to Cape York and *P. scapulatus* from Carnarvon in Western Australia, across the north of the continent and south to Victoria, being sometimes seen on Kangaroo Island off South Australia. Andersen (1912) concluded that this "small number of species, representing as many groups, is evidence that the genus forms no part of the orginal fauna of the continent . . . *P. scapulatus* and *poliocephalus* are decidedly the most peculiarly modified, hence perhaps the earliest immigrants into the continent". In fact, these two species are endemic

FIGURE 1.1

World distribution of family Pteropodidae (bounded by broken line)

Distribution of genus _Pteropus_ within this area is indicated by vertical hatching. _Pteropus_ does not occur on mainland Africa. _Extremities_ of its distribution are: 1 - Pemba Island; 2 - _Volcano Island_; 3 - northern Mariana Islands and Guam; 4 - Fiji; 5 - Samoa; 6 - Rarotonga and Mangaia in the Cook Islands (from Hall, 1987).

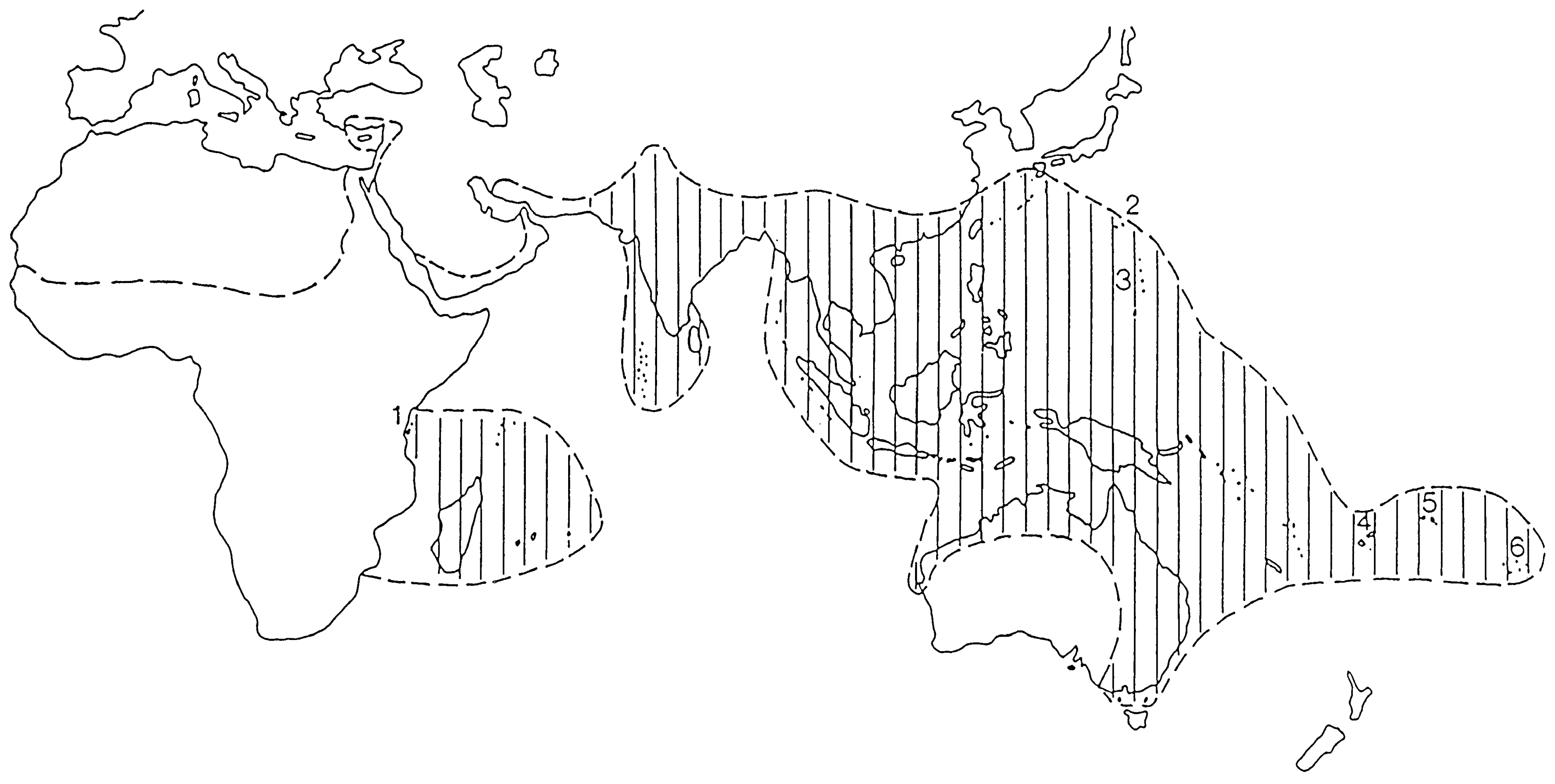


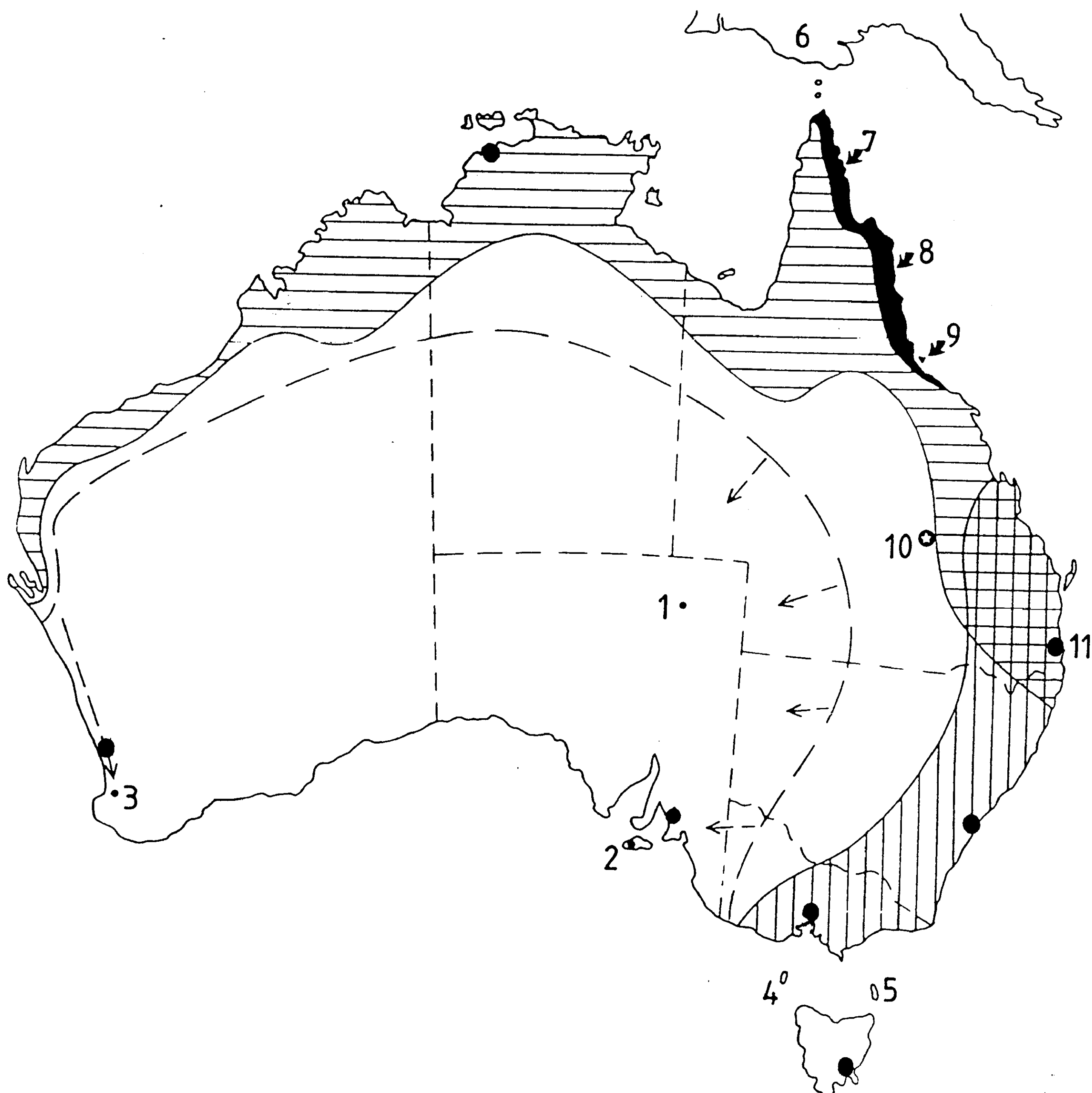
FIGURE 1.2

Distribution of flying foxes in Australia (from Hall, 1987) and location of camps visited in the present study

black = P. conspicillatus; horizontal hatching = P. alecto; vertical hatching = P. poliocephalus; broken line = inland limit of P. scapulatus
● = capital cities

Broken arrows indicate periodic movement of P. scapulatus along inland rivers or following blossoms, resulting in specimens having been collected at Cowarie (1), Kangaroo Island (2) and Donnybrook (3). P. poliocephalus has been recorded from King (4) and Flinders (5) Islands, but not Tasmania. P. alecto and P. conspicillatus occur in New Guinea (6).

In this study, Dobsonia moluccense was collected at the mouth of the Pascoe River (7), P. conspicillatus at the mouth of the Mossman River (8), and P. scapulatus near Bauhinia Downs (10). Palm Island is indicated by arrow at 9. Camps in the Brisbane environs (11) are shown in Figure 2.1



only to Australia, whereas *P. conspicillatus* also occurs in New Guinea and its offshore islands and *P. alecto* (formerly *P. gouldii*) extends through New Guinea into eastern Indonesia (Hall, 1987). The distribution, movement and behaviour of these Australian species have been described by Ratcliffe (1931) and Nelson (1965a,b). *P. poliocephalus*, *P. alecto* and *P. conspicillatus* establish their camps (communal roosts) near or along the coast. In summer these camps are situated in mangroves, eucalypts, melaleucas and other trees usually along estuaries and rivers, and comprise from several hundred to hundreds of thousands of individuals. The young are born in these camps in late spring, and the adult bats copulate there in late autumn, following which most individuals usually disperse to establish smaller winter camps often away from the coast. There is a free and poorly understood movement of individuals between camps, which are located often within 40 km of each other. At dusk each day the adult bats fly out of the camps to forage, feeding on blossom, fruit and young foliage, covering perhaps 100 km or so by the time they return to camp before sunrise. In the first weeks of its life, the young bat is carried on the mother's feeding flights, clinging onto an axillary nipple with its milk teeth and to the mother's fur with its sharp claws. From about 3-5 weeks juveniles are left behind in the camps at night, and relocated by their mothers each morning presumably by olfactory and auditory characteristics. The young actively exercise their wings, both in daylight and at night, and by 2 months of age are capable of flight between trees in and around the camp. Probably by 3 months the young independently accompany their mothers on feeding flights, at which time fruit and blossom begin to make a major contribution to their diets. As the time of mating approaches most juveniles have left their mothers to join "adolescent groupings" on the outskirts of the camp, and shortly thereafter the colony disperses. Sexual maturity is attained at 18 months of age. These bats do not undergo extensive seasonal migrations as Ratcliffe (1931) had

originally supposed (Nelson, 1965b).

P. scapulatus is smaller than the 3 "coastal" species and much more nomadic, venturing up to 600 km inland. Its summer camps are often formed adjacent to those of other species, but usually for short periods of up to several weeks. Copulation occurs in early summer and the young are born in the "winter camps" in autumn (Appendix II).

1.6.3 Infections in bats

Very little systematic work has been published on infections in bats, perhaps reflecting their insignificance as a reservoir of major zoonoses. Most studies have related to the presence of virus infections, particularly rabies, in microchiropterans (Baer, 1975). Nevertheless, reports have appeared of infections in megachiropterans which may be of potential public health concern. Presumed rabies virus infection has been described in a Pteropus species from India (Pal et al., 1980) and there have been reports of cases of human infections with related viruses possibly acquired from fruit bats in Africa (Crick et al., 1982). Virus infections of bats which could be pathogenic to humans were reviewed by Sulkin & Allen (1970). In Australia, antibodies to Ross River Virus (RRV) have been found in P. poliocephalus and P. scapulatus (Gard et al., 1973), and antibodies to RRV and Murray Valley Encephalitis in P. gouldii (= alecto) and P. scapulatus (Doherty et al., 1971).

Hill & Smith (1984) outlined the variety of infective organisms found in bats which were potentially pathogenic to humans, but stressed the rarity of the actual transmission of any of these. Their discussion included various arthropod-borne and rabies viruses; bacteria, such as Salmonella, Shigella, mycobacteria, Leptospira, Borrelia and various rickettsiae; and fungi, such as Histoplasma capsulatum, Cryptococcus neoformans and dermatophytes. They also briefly mentioned various protozoa, helminths and ectoparasites.

Ubelaker (1970) briefly reviewed the ecto- and endo-parasites of bats, noting that nematodes were the most successful helminths in these hosts and commented: "Although more than 100 species of nematodes have been described from bats, . . . no life cycles . . . have been elucidated". Since then Humphery-Smith (1982) has described the life-cycle of the strongyle nematodes, Nycteridostrongylus uncicollis and Molinostrongylus heydoni, in the microchiropteran Miniopterus schreibersii. Of the order Ascaridida, Ubelaker (1970) mentioned only Contracaecum, occurring in bats as larvae. In fact, 2 ascaridoid species have been reported from megachiropterans as definitive hosts, these being Ascaris cynonycteridis, described from juvenile Xantharpyia [Cynonycteris] amplexicaudata from Burma by Parona (1889), and T. pteropodis (Baylis, 1936). The order Strongylida was most strongly represented, with others including spiruroids, filarioids and trichuroids, but Ubelaker (1970) did not indicate which species occurred in fruit-bats, and his discussion related entirely to microchiropterans. Likewise, most cestodes and all the trematodes of bats have been reported from microchiropterans. Of the protozoa found in bats, only species of Leishmania and Trypanosoma seem to be potentially zoonotic, but their taxonomy is far from clear and the species from bats may be innocuous to humans.

This predominance of infections in the Microchiroptera could simply reflect their accessibility to research workers, as the Megachiroptera are restricted to "third-world" countries, with the exception of Australia. Nevertheless, the densely-packed and often large colonies of Microchiroptera, their dark humid roosts and insectivorous diets facilitate the acquisition and transmission of a variety of infections, including helminths, from ingested prey and from larval recycling in the roost environment. Close contact between individuals promotes transmission of agents utilizing respiratory droplets or ectoparasitic vectors.

Although the prevalence of infections in pteropids seems much lower, a

wide variety of organisms has been reported in this group, and are briefly reviewed in Appendix VIII. As already mentioned, some are exposed to arbovirus infections. In Madagascar, wild-caught Pteropus rufus have been found to harbour Salmonella typhi, S. typhimurium, Shigella flexneri and Sh. sonnei, but the source of these infections was not determined (Brygoo et al., 1971). Australian Pteropus species harbour Hepatocystis pteropi and Trypanosoma pteropi (Mackerras, 1959). The vectors for these are not known, but it is likely that Hepatocystis is transmitted by Culicoides (Boulard et al., 1985), and the trypanosome by nycteribiid flies of the genus Cyclopodia (Reid, 1984). A filarial nematode, Makifilaria inderi, has been described from P. hypomelanus in Malaysia (Krishnasamy et al., 1981), and this or a closely-related species has been seen in Australian flying foxes (Moorhouse, unpublished).

The high, open roosts of the fruit-bats, often over water, the spacing between individuals (except with P. scapulatus, which roost densely), their habit of inverting to defaecate and urinate and their herbivorous diet limit opportunities for the transmission of infective organisms. Most of those which have succeeded are dependent upon potential vectors such as the ectoparasitic mites and nycteribiid flies, or bloodsucking freeliving arthropods, such as mosquitoes, tabanids and ceratopogonids, which live and breed in the vicinity of the roost.

Opportunities for transmission of potential zoonoses to humans are afforded usually when people venture into bat habitats and expose themselves to direct droplet-, dust-, soil- or vector-borne infections. In the case of fruit-bats, there is the added possibility of infection transmission via the faecal contamination of fruit (Moorhouse, 1982).

CHAPTER 2

MATERIALS AND METHODS

2.1 NATURALLY-INFECTED BATS

2.1.1 Collection of bats

This study commenced in July, 1981, when flying foxes were protected by law and special permission from the Department of National Parks and Wildlife was required to kill these animals and collect live specimens. At intervals from then until March 1986, *P. poliocephalus* were shot in a winter camp situated in rainforest at Beenleigh, a winter camp in rainforest at Mt. Flinders and a summer camp in mangroves along the Logan River at Eagleby (Fig. 2.1).

Animals were shot with a 0.22 calibre rifle. A shotgun used on one occasion injured many animals without bringing them down, and caused rapid dispersal of the colony. A telescopic sight fitted to the rifle enabled careful observation of roosting animals, accurate selection and more efficient killing. These specimens were supplemented with freshly dead adult bats collected from power lines, the Animal Refuge at Yeerongpilly, the Veterinary Clinic at the University of Queensland and from interested members of the public.

Live *P. poliocephalus* mothers with young were caught by shaking them from mangroves in summer camps at the North Pine River, Indooroopilly Island and Eagleby. Many were also obtained from the other sources mentioned above.

Live and dead *P. alecto* were obtained, in smaller numbers, from the same sources, except the winter camp at Mt. Flinders which comprised only *P. poliocephalus*.

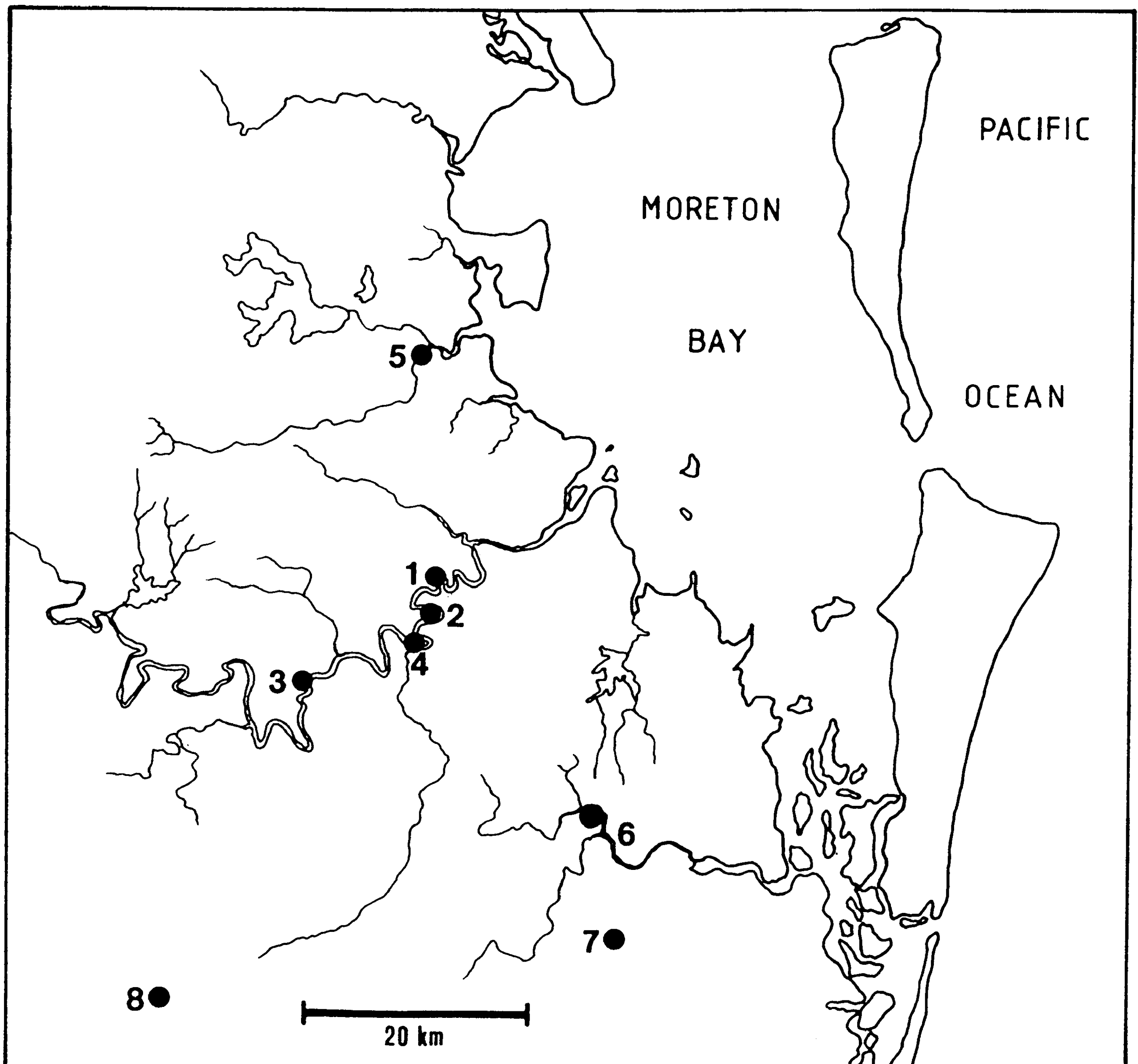
Abandoned *P. conspicillatus* juveniles were caught by shaking out of tall thin mangroves in a camp deserted by the adult bats several days

FIGURE 2.1

Locations of facilities and Pteropus roosts visited in Brisbane environs

1 = Brisbane city centre
2 = University of Queensland St. Lucia Campus
3 = University of Queensland Veterinary Farm, Pinjarra Hills
4 = Indooroopilly Island camp
5 = Pine River camp
6 = Eagleby camp
7 = Beenleigh camp
8 = Mt. Flinders camp

Several other roosts located on the Moreton Bay Islands and elsewhere in the district were not visited during this study.



previously at Mossman, North Queensland (Fig. 1.2) in December, 1981 and again in November 1983, when a small number were also shot. Adult male and juvenile *P. scapulatus* were collected by shooting in a winter camp located in brigalow scrub near Bauhinia Downs, central Queensland (Fig. 1.2) in June 1982. Juveniles also were caught here by shaking from trees.

2.1.2 Maintenance in captivity

From January 1982, bats were housed in a timber-framed chicken-wire 8 x 8 x 2.5 m cage at the University Farm at Pinjarra Hills (Fig. 2.2 A). A 2nd cage, 9 x 3 x 3 m, of tubular steel covered with galvanised 400 mm chain-mesh was built in January 1982, so that up to 90 bats could be housed comfortably with sufficient room for flight (Fig. 2.2 D). Bats were kept in these cages until January 1985. The mainstay of their diet was condemned fruit, obtained from the Brisbane Markets at Rocklea. Although unfit for human consumption, the fruit in general was of good quality and proved nutritionally adequate. In summer, this included papaws, stonefruit, grapes, mangoes, bananas, rockmelons and avocadoes, whereas in winter apples and pears, supplemented with purchased bananas, were the major items. This was rationed at one 9 litre bucket of chopped fruit per 10 animals daily, and was hung from the roofs of the cages in buckets about 1/3rd full and widely separated to prevent dominant males denying access to less aggressive individuals. To avoid the risk of hypocalcaemia and tetany in bats maintained only on fruit (Buckland-Wright & Pye, 1973), calcium was supplemented as skimmed milk powder, 200 g/l of water for every 10 bats. For extra calories sucrose 100 g/l was added, and vitamin supplementation provided as Avi-Drops (Medical Research [Marketing] Pty Ltd, North Ryde, NSW) 3 ml/l. The milk was poured into plastic containers fixed around the sides of the cages about 60 cm below the roof (Fig. 2.2 A).

FIGURE 2.2

Maintenance of captive bats

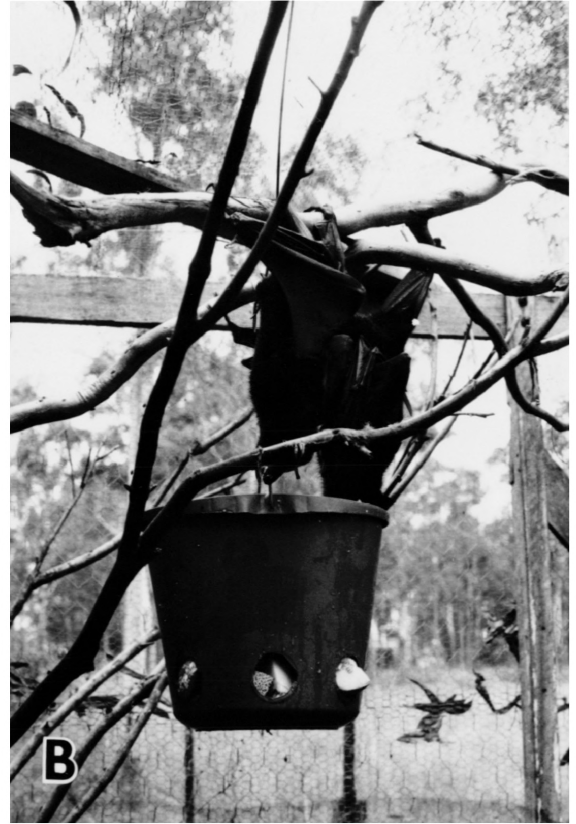
A. Adult *P. poliocephalus* and *P. alecto* in original timber-framed chicken-wired cage, previously used for maintenance of *Miniopterus schreibersii*. Note denuded cultivated fig tree in lower left foreground, ducks on floor and plastic milk container on far wall.

B. Adult male *P. alecto* feeding. Holes in buckets allowed easier access to food for less dominant bats, as well as drainage during wet weather. Buckets were left in cages overnight and removed in the mornings.

C. Mother *P. poliocephalus* nursing natural pup plus adopted pup in small cage used for 24 h faecal collection and worm recovery.

D. Specifically-built iron-framed chain-mesh wire cage for maintaining growing juveniles and bats individually infected.

A


B

C

D

Because the cages had earthen floors, several ducks were kept in each to eat the large amounts of fruit dropped by the bats. Fruit and native blossom trees were planted in each cage but were rapidly destroyed by bats eating the foliage (Fig. 2.2 A).

For closer observation and collection of Toxocara eggs, mother-young pairs and juveniles were kept in 60 x 45 x 45 cm wire cages (Fig. 2.2 C) in the laboratory at the St. Lucia campus, and fed fruit and milk rationed as above. In the last 2 years of the study, infected juveniles were maintained on reconstituted skimmed milk (with sugar and vitamins) fortified with an equal quantity of Digestelact (Sharp Laboratories Pty Ltd, Artarmon, NSW) in an attempt to reduce diarrhoea, after the early finding that the introduction of fruit may have resulted in the spontaneous passage of worms. Orphaned juveniles (caught without their mothers) were also kept in small cages and initially fed the fortified milk formula through a syringe fitted with a rubber teat made from fine rubber tubing (for bicycle tyre valves), being given about 20% of their body weight daily as formula divided over 4 to 6 feeds. Older juveniles learned within several days to lap milk from a plastic container, whereas those under 2 weeks of age required hand feeding often for 2 weeks or longer. The younger juveniles also preferred to hang onto old towels or stuffed socks rather than the wire roof of the cage. The cages were left open during the day to allow the bats to climb about and flap their wings. Juveniles strong enough to fly (older than 6 weeks) which had lost their gut worms, either spontaneously or after treatment with piperazine, were transferred to the large cages at the farm, in the hope that they would later reproduce for future experimental studies.

2.1.3 Blood sampling

The most reliable and efficient means of collecting blood samples in quantity (1 ml or more) was by cardiac puncture with a 23 gauge needle

under ether anaesthesia. The ether was applied on a soaked cottonwool pad fitted into a plastic mask, and produced rapid anaesthesia. However, excessive dosing or prior agitation sometimes resulted in the unpredictable cardiorespiratory arrest in adult bats.

2.1.4 Tissue larval recovery

In the initial studies (Appendix 1), all large organs were examined for Toxocara larvae. Freshly dead adults were weighed and their ulnar lengths measured prior to dissection. Intestines were opened longitudinally in normal (N) saline (8.5 g sodium chloride/1 l distilled water) and examined macroscopically for parasites. They were then minced with scissors and placed into 100 ml 2 % trypsin solution (20 g trypsin, 8 g sodium chloride, 10 g sodium hydrogen carbonate in 1 l water). From each animal, the liver, lungs, kidneys, spleen, heart, brain, diaphragm and portions of pectoral muscle were minced finely with scissors, placed into 50 ml trypsin solution and incubated overnight (18 h) at 37 ^{o}C. Each sample was then poured through gauze, centrifuged and the supernatant discarded. An equal volume of 10% formalin solution was added to each sediment, which was later examined for larvae in a grid-marked glass tray by stereomicroscopy. In pregnant females, the mammary glands, uteri and placentae, and the foetal lungs, hearts, livers and intestines, were treated similarly.

Consequent to early findings, from 1982 onwards, only livers of adult bats, plus the mammary glands of lactating and pregnant females, were examined for larvae.

From March 1983 onwards, trypsin solution was replaced with N saline. Organs were minced with scissors, then incubated overnight at 37 ^{o}C in N saline over a double-layer of 8-ply gauze supported by nylon mesh or bird-wire suspended in containers of appropriate size, i.e. 600 ml for large

organs such as adult livers, 80 ml for mammary glands. This method will be subsequently referred to as "saline separation". To the sediment in each container an equal volume of boiling water was added, to heat-kill any larvae, followed immediately by sufficient 10% formalin to give a final concentration of approximately 3% formalin. This enabled large numbers of tissues samples to be processed at one time and examined later.

Initially, the intestines of young bats were first syringed out with N saline, following which the gut was opened longitudinally and the mucosa scraped into saline, with washings and scrapings then being examined for worms. The gut wall, lungs, livers, brains and muscle samples were digested in trypsin. In October 1982, the intestines of young bats were opened in saline and left overnight at 37 ^{o}C, which freed the larvae from the mucosa and mucus effectively but also damaged the cuticles. Subsequently, intestines of juveniles were examined by dissecting directly in saline under the stereo microscope, then leaving overnight in saline over gauze at 5 ^{o}C for later re-examination. Leaving the intact animal, or the intestines in N saline, overnight at 5 ^{o}C did not damage the larval or adult worms in the intestine and helped to liquefy the viscous contents.

2.1.5 Faecal examination

To detect *Toxocara* eggs in individual bats, faecal samples were obtained by rectal swabbing, using a pledget of cottonwool tightly twisted onto the end of an orange stick. The bat, usually a juvenile, was relaxed and immobilized by being wrapped in a towel after folding its legs up over its abdomen and its wings around its body. The cottonwool was moistened in N saline, then very slowly pushed into the anus until the sphincter relaxed, following which it was inserted about 2 cm into the rectum, twisted gently a few times then slowly withdrawn. This procedure did not distress the bats, which showed no signs of discomfort. Depending on the quantity and consistency of faeces obtained, the swab was streaked onto a

dry glass slide or mixed in a drop of water on a slide then examined microscopically under a coverslip.

2.1.6 *T. pteropodis* egg collection

Infected individuals or groups of bats were kept in cages at the bottom of which were placed galvanised steel trays. These trays were removed daily at 0800 - 0900 hours, household detergent/tapwater solution was added and then they were scraped and brushed with a paintbrush. The faecal suspension was poured through a bank of sieves, with pore sizes of 700 um, 420 um, 160 um and 50 um (replaced by 63 um after the first 2 years). The larger meshes sifted out fibrous material when the bats were eating fruit. The fine particulate matter trapped in the final sieve was backwashed with a jet of tapwater from a washbottle into a large plastic container, then allowed to settle in a 1 l cylinder for 2 - 3 h. The supernatant was discarded by aspiration and the sediment transferred to a 100 ml (or larger) beaker to settle for another hour, after which usually the top 80 ml was aspirated leaving 20 ml containing all the sedimented eggs.

Eggs were counted at x 40 magnification in 4 x 0.05 ml aliquots of stirred sediment, under coverslips on glass slides, allowing total numbers to be calculated.

Several samples of eggs were obtained by removing the uteri from gravid female *T. pteropodis*. These eggs were suspended in N saline and total numbers calculated as above.

2.1.7 Embryonation of eggs

Eggs recovered from juveniles on a fruit diet were heavily contaminated with fruit pulp. Some of this "sludge" was smeared onto microscope slides left at room temperature, some onto leaves of palm,

eucalypt, avocado and mango trees and some soaked into filter paper. Specimens on all thse substrates were soaked in tapwater daily and examined microscopically for embryonic development.

Faecal sediment containing eggs was mixed with activated charcoal granules and stored, for the embryonation of eggs, in 15 cm Petri dishes kept in a desiccator with water to maintain humidity. From 1984 onwards, eggs for experimental infections were collected from juveniles fed exclusively milk, which greatly reduced the volume of sediment, allowing higher concentrations to be used in experimental infections.

Eggs collected from gravid female worms were stored in 0.1 N sulphuric acid in 10 cm Petri dishes and examined microscopically daily for embryonation. Larvae were expressed from eggs by pressing gently on the coverslip.

2.1.8 Recovery of infective eggs

After a minimum of 3 weeks storage, a quantity of charcoal estimated to contain sufficient eggs for a particular experiment was mixed into water in a screw-top jar, shaken vigorously for about 30 s, then poured through a 160 um sieve. The charcoal was discarded and the filtrate left to settle for 1 - 2 h. The numbers of infective eggs in the sediment were calculated as described previously. An egg was considered infective if its larva moved or looked viable and adequately developed (*T. pteropodis* larvae in eggs were generally much less active than those of *T. canis*). The required concentration of eggs was obtained by adjusting the suspension volume by adding water or aspirating more supernatant. Eggs for parenteral inoculation were rinsed in N saline then in 10% sodium hypochlorite for 1 h prior to injection.

2.1.9 Recovery of worms

Worms were obtained from dead juveniles as described above. Frequently adult, and sometimes immature, worms were passed by juvenile bats, or after the administration of 300 mg piperazine citrate in milk formula. These worms were collected from the floor of the cage, immersed in saline for several hours, rinsed twice, fixed in Berland's fixative (5% formaldehyde in 95% glacial acetic acid) (Gibson, 1979) for several minutes or hot 3% formalin in saline (70 ^{o}C) and stored in either 6% formalin or 5% glycerine in 70% alcohol. A small number of selected worms from bats passing fertile, infertile or mixed eggs, were dissected alive in saline for microscopic examination of uterine contents.

2.1.10 Bats from overseas

In February 1984, several adult *Pteropus tonganus* were shot on Rarotonga and Mangaia, in the Cook Islands, which represent the most easterly penetration of the Megachiroptera. These were dissected and examined for larvae (saline separation) by myself at the New Zealand Government's Agricultural Research Station at Totokoitu, Rarotonga. Another collection was later organised by the Station's director, Mr John Campbell, who forwarded the specimens in formalin. Specimens of *P. tonganus* were also collected in Niue and forwarded by Mr Misi Nicholas.

On Christmas Island, in the Indian Ocean south of Java, 20 suckling *Pteropus natalis* (= *melanotus*) were collected and examined by Dr Chris Tidemann. Dr Karl Koopman, of the American Museum of Natural History, New York, provided 26 alcohol-fixed juvenile Megachiroptera of several species from Africa and Asia for dissection. Juveniles of several species from the collection of the British Museum of Natural History were dissected in May 1987 (details in Chapter 7) and type specimens of *Ascaris cynonycteridis* from a *Rousettus* species in Burma were examined in the Genoa Museum of Natural History in June 1987.

2.2 TOXOCARA CANIS AND T. CATI

Adults of these 2 species were obtained from dogs and cats destroyed at the Animal Shelter, Yeerongpilly, or dissected in the autopsy room of the University of Queensland Veterinary School.

Some adult worms were cleaned and fixed as described for *T. pteropodis*. Eggs were dissected from several gravid females and embryonated on charcoal. Larvae were recovered from the tissues of experimentally-infected animals as described for *T. pteropodis*.

2.3 EXPERIMENTAL ANIMALS

2.3.1 Bats

Some juvenile bats which had been found free of infection or had passed their worms were infected with eggs of either *T. pteropodis* or *T. canis*, fed with milk formula, by syringe.

Adults and juveniles kept in the large flight cages were given *T. pteropodis* eggs mixed into milk and sprinkled on fruit. When young were born, the mother-pup pairs were brought back to the laboratory, kept in individual cages in an air-conditioned room and maintained on fresh fruit and milk formula. Aborted foetuses and stillborn pups were examined for tissue larvae, whereas young which died were examined for intestinal worms. Some mothers of dead young bats were killed for study by the intrathoracic injection of 1 ml (325 mg) sodium pentobarbitone ("Lethobarb"), which caused death within 5 - 10 s.

2.3.2 Mice

Various inbred strains and outbred Quackenbush mice were obtained from the Central Animal Breeding House of the University of Queensland. They were kept in appropriate cages, cleaned regularly, in the air-conditioned mouse room of the Department of Parasitology, and given free access to

pelletised commercial food and water.

Calculated infective egg doses were administered in 0.3 - 0.5 ml water intragastrically via a 1 ml syringe with a "stomach tube" fashioned from a blunted 18 G needle. Mice were killed by cervical dislocation under ether anaesthesia, and larvae were recovered from their livers by saline extraction.

2.3.3 Other experimental animals

Infections were studied in a variety of animals, the details of which are given in Chapter 8.

2.4 HAEMATOLOGICAL STUDIES

Most blood samples were examined in the Department of Parasitology, and a small number were analysed in the diagnostic laboratory of the Department of Veterinary Pathology at the University of Queensland. A thin blood smear was made at the time of collection, air-dried, fixed in methanol and stained with Giemsa, using standard techniques. The rest of each sample was deposited into an EDTA-coated 5 ml plastic screw-topped bottle. Total red and white cell counts were determined in a Coulter Counter Model ZF (Coulter Electronics Ltd, Hertfordshire, England) which was calibrated for each species, and maintained and operated according to the instruction manual. For red cell counts, blood was diluted 1:50,000 (2 µl blood/100 ml) in Isoton-2 (Coulter Electronics) and for white cell counts, 1:500 (20 µl in 10 ml) in Isoton-2 followed by Zaponin (Coulter Electronics) to lyse the red cells.

2.5 HISTOLOGICAL EXAMINATION

2.5.1 Tissues

Small pieces of various tissues were fixed and stored in buffered 10% formalin, processed by routine techniques, embedded in paraffin wax, sectioned at 5 µm (unless otherwise stated) and stained with H & E.

2.5.2 Worms

Larval and adult worms from faeces or gastrointestinal tracts were washed in N saline then fixed in 3% formalin/saline at 70 °C, replaced from 1985 onwards by Berland's fixative. All specimens were stored in 5% glycerine/ethanol (5% glycerine, 70% ethanol, 25% water). For drawing and description, larvae were cleared in chlorlactophenol and examined microscopically. For examination of cephalic structures, fixed larvae were immobilized in glycerine jelly, "heads" cut off with a scalpel and positioned under a suspended coverslip. Larger worms were cleared in creosote. For serial sections, adults were treated as tissue specimens, described above, cut at 10 µm and stained with H & E. Serial transverse sections of the anterior ends of 3 adults were examined, as well as those of the tails of 2 adult males and 2 females. Longitudinal sections of the anterior and posterior ends of 1 male were also studied.

2.6 SCANNING ELECTRON MICROSCOPY (SEM)

Anterior and posterior ends from 3 fixed adult males and 2 females, stored in 5% glycerine/ethanol, were dehydrated in an ascending series of alcohols to amyl acetate, dried in a critical point drier, mounted on stubs and sputter-coated with gold. A Philips 505 and a Cambridge Stereoscan 600SEM were used for viewing.

CHAPTER 3

NATURAL INFECTIONS IN *PTEROPUS POLIOCEPHALUS*

3.1 INTRODUCTION

Natural infections were studied most extensively in *P. poliocephalus*, this being the most common flying fox in south-east Queensland.

3.2 RESULTS

3.2.1 Adult males

Findings from adult male bats are shown in Tables 3.1 & 3.2. All bats in Table 3.1, and most in Table 3.2, were from the Beenleigh camp. The remainder in Table 3.2 were from Eagleby, except 2-79 and 2-80, which were shot at Mt. Flinders. The first 3 carcasses examined had been frozen prior to digestion and therefore are excluded from further consideration and statistical analysis. Thus, of 26 males examined between August and November 1981 (Table 3.1), 14 (54%) harboured 3rd-stage larvae in their livers. Larvae were not found in brains, hearts, kidneys, lungs, spleens or skeletal muscle. The 2 smallest, weighing 445 and 450 g, were yearlings (Nelson, 1965b). The others weighed 540 - 865 g. Numbers of larvae recovered ranged 1 - 455. Excluding those free of infection, body weight correlated positively with number of larvae (r = 0.45). Although not all larvae could be measured, there was considerable variation in their mean lengths (595 - 710 µm) and length ranges (overall range 505 - 820 µm).

From 1982 onwards, tryptic digestion followed by sieving and cold fixation was replaced by saline separation with hot fixation; results are shown in Table 3.2. Every bat in this 2nd group harboured larvae in its liver, with numbers recovered ranging 1 - 306. Mean lengths of larvae ranged 595 - 780 µm, and the overall length 510 - 940 µm.

TABLE 3.1

Numbers and dimensions of Toxocara pteropodis larvae recovered from adult male Pteropus poliocephalus using tryptic digestion and cold fixation (measurements in µm)

Autopsy No.	Date	Weight (g)	Number of larvae in liver recovered/measured	Larval length $\bar{x} \pm$ S.D. µm (range)	Larval width $\bar{x} \pm$ S.D. µm (range)
1-1*	24.07.81	835	0	-	-
1-2*	24.07.81	860	0	-	-
1-3*	24.07.81	715	0	-	-
1-16	17.08.81	820	56/54	710 ± 57 (570 - 820)	27 ± 2.7 (22 - 34)
1-17	17.08.81	605	0	-	-
1-18	17.08.81	850	8/8	595 ± 33 (530 - 630)	30 ± 4.5 (26 - 38)
1-19	17.08.81	855	0	-	-
1-20	17.08.81	810	20/11	680 ± 49 (600 - 750)	26 ± 2.5 (24 - 34)
1-21	17.08.81	870	455/44	670 ± 61 (505 - 770)	-
1-22	17.08.81	875	36/27	635 ± 56 (555 - 770)	27 ± 3.4 (22 - 31)
1-27	7.10.81	865	88/0	-	-
1-28	7.10.81	780	22/0	-	-
1-29	7.10.81	675	7/0	-	-
1-30	7.10.81	705	10/0	-	-
1-31	7.10.81	750	29/0	-	-
1-32	7.10.81	710	3/0	-	-
1-33	7.10.81	750	16/0	-	-
1-34	7.10.81	550	1/0	-	-
1-35	7.10.81	450	0	-	-
1-39	14.10.81	700	0	-	-
1-44	14.10.81	580	0	-	-
1-45	14.10.81	760	0	-	-
1-46	14.10.81	540	0	-	-
1-47	14.10.81	570	0	-	-
1-48	14.10.81	800	0	-	-
1-62	5.11.81	725	2/0	-	-
1-63	5.11.81	710	0	-	-
1-64	5.11.81	520	0	-	-
1-65	5.11.81	445	0	-	-

* Frozen prior to dissection

TABLE 3.2

Numbers and dimensions of *Toxocara pteropodis* larvae recovered from adult male *Pteropus poliocephalus* using saline separation and hot fixation (measurements in µm)

Autopsy No.	Date	Weight (g)	Number of larvae in liver recovered/measured	Larval length $\bar{x} \pm$ S.D. (range) µm	Larval width $\bar{x} \pm$ S.D. (range) µm
1-102	27.03.82	810	18/18	715 ± 66 (605 - 895)	-
1-103	27.03.82	850	121/121	680 ± 78 (510 - 845)	-
1-104	27.03.82	n.e.	4/4	630 ± 85 (510 - 745)	-
2-1	21.09.82	770	5/5	715 ± 46 (665 - 775)	32 ± 3.8 (29 - 39)
2-3	21.09.82	700	1/1	780	31
2-8	21.09.82	690	14/0	-	-
2-9	4.10.82	615	5/0	-	-
2-10	4.10.82	710	6/5	605 ± 50 (565 - 690)	-
2-12	6.10.82	710	24/0	-	-
2-25	14.10.82	740	17/0	-	-
2-26	14.10.82	690	30/0	-	-
2-27	14.10.82	680	3/3	705 ± 21 (680 - 725)	-
2-28	14.10.82	710	18/17	620 ± 52 (580 - 725)	-
2-29	14.10.82	650	6/1	715	34
2-30	14.10.82	630	36/36	720 ± 77 (600 - 940)	31 ± 4.0 (25 - 43)
2-31	14.10.82	660	306/0	-	-
2-32	14.10.82	660	5/0	-	-
2-63	14.02.83	870	6/6	595 ± 49 (540 - 665)	-
2-64	14.02.83	625	7/7	635 ± 33 (610 - 710)	-
2-67	14.02.83	700	67/66	680 ± 47 (585 - 780)	-
2-69	22.03.83	920	121/118	700 ± 39 (615 - 815)	-
2-72	22.03.83	850	53/40	780 ± 50 (670 - 895)	-
2-73	22.03.83	800	44/44	780 ± 48 (650 - 895)	-
2-79	16.06.83	700	94/0	-	-
2-80	16.06.83	665	65/65	745 ± 63 (630 - 925)	-
5-22	3.03.86	915	44/11	715 ± 56 (620 - 820)	

n.e. - not examined

From Tables 3.1 & 3.2, the overall positive rate for adult male bats was 40/52 (77%). The numerical dispersal of hepatic larvae from these bats is represented in Fig. 3.1. The mean number of larvae recovered from bats in Table 3.1 (29 $\pm$ 87) was not significantly different from that of Table 3.2 (43 $\pm$ 63). Combining data from both tables, and applying the Spearman Rank Correlation Coefficient, a positive correlation appears between the number of larvae recovered and the host weight ($P < 0.001$). However, the same test applied only to data in Table 3.2 is inconclusive ($P < 0.2$). By the same test, no correlation is shown between body weights and larval lengths or between larval numbers and lengths.

Mean lengths are generally higher in Table 3.2, which also includes the longest larvae. However, considering only bats from which 5 or more larvae were measured, the difference in the average of mean lengths between Tables 3.1 ($\bar{x}$ = 658 $\pm$ 40) and 3.2 ($\bar{x}$ = 688 $\pm$ 58) were not significant (Student's t-test, $P < 0.2$). Mean larval lengths varied 595 - 780 µm, with considerable ranges in some individuals, the largest being 600 - 940 µm (bat 2-30, Table 3.2). No seasonal pattern in mean lengths was discernible. The length dispersal of larvae from several heavily-infected bats was of a normal distribution, e.g. bats 2-67, 2-69 and 2-80 in Figure 3.2. However, larval lengths from bat 1-103, killed on 27 March, 1982, distributed bimodally (Fig. 3.2). Their number and mean length were comparable with those from 2-67 and 2-69, but length SD and range were greater (see Table 3.2).

In Table 3.1, mean maximal body widths varied 26 - 30 µm, and the overall width range was 22 - 38 µm.

In Table 3.2, fewer widths were measured, and ranged 22 - 43 µm overall. In general, longer larvae were wider, but with many exceptions, even in larvae from the 1 bat. In Table 3.1, the shortest larvae (bat 1-18) also were the widest.

FIGURE 3.1

Intensity of hepatic infection with larval T. pteropodis in naturally-infected population sub-groups of P. poliocephalus

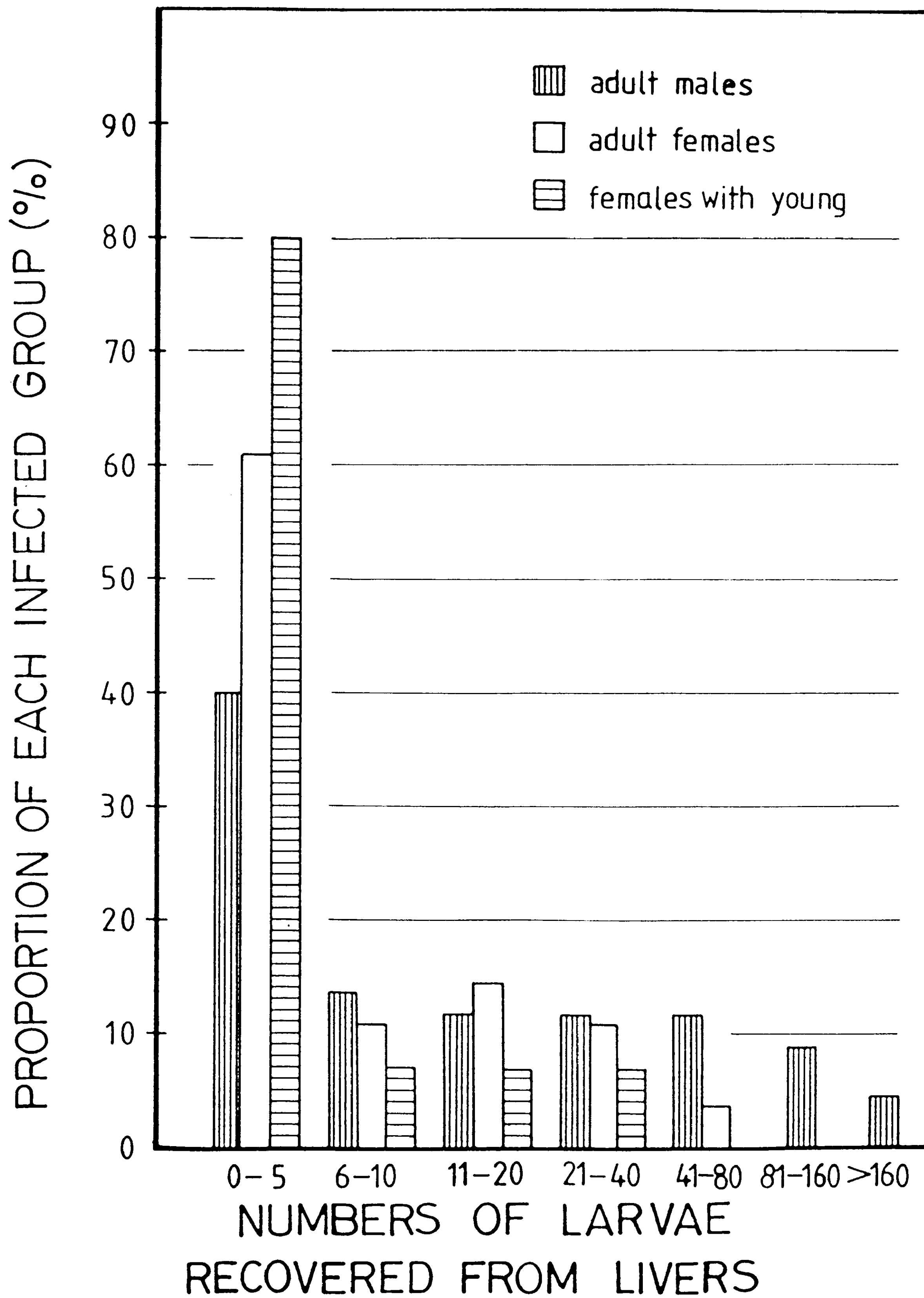
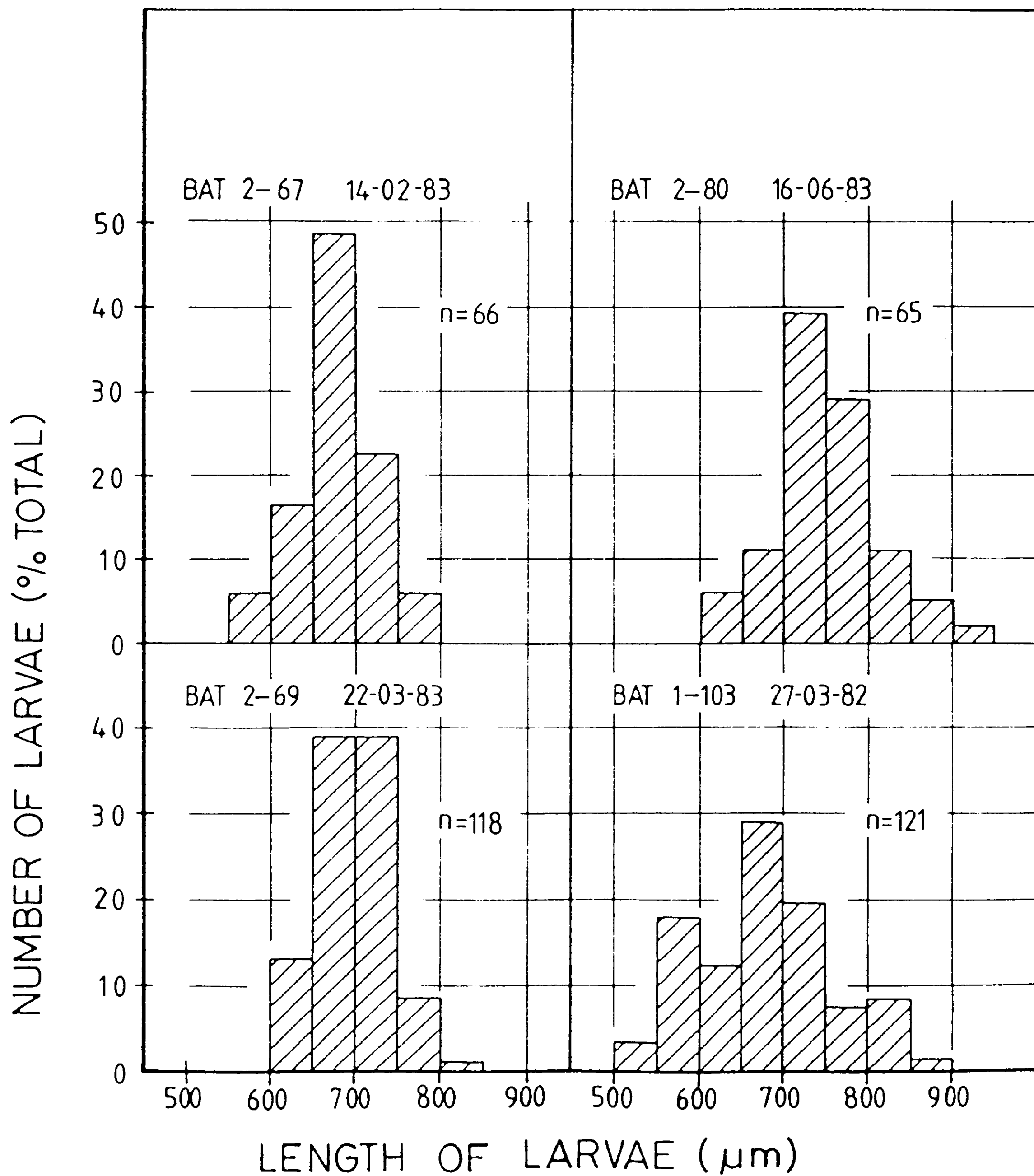


FIGURE 3.2

Length distribution of T. pteropodis larvae recovered from livers of four naturally-infected adult male P. poliocephalus



3.2.2 Adult females

Findings from 36 adult females examined between July 1981 and February 1983 are presented in Table 3.3. The first 6 (1-4 to 1-9) were frozen prior to digestion and are excluded from further consideration. As with adult males, saline separation and hot fixation were introduced in 1982 (from bat 2-2 onwards). The smallest 2 weighed 420 and 450 g and were immature yearlings (Nelson, 1965b). Of the remaining 28, 24 were pregnant (weight range 590 - 920 g), 3 had never been pregnant (as determined by appearance of external genitalia, mammary glands and uterus) and 1 had been pregnant previously (1-43). Larvae were found only in livers (from 1982 only livers and mammary glands were examined), except for 1 large larva (1.39 x 0.041 mm) recovered from a mammary gland of bat 2-21, which also had 6 larvae in its liver. Larvae were not found in any foetuses (weight range of specimens near term: 67 - 130 g). Two livers (bats 2-13 and 2-15) were not examined, so that of the other 28, 17 (61%) yielded larvae. Of the 13 digested in trypsin, 6 (46%) yielded larvae, whereas 11 of 15 (73%) treated by saline separation were positive. This difference was not statistically significant ($\underline{z}$ = 1.46, $\underline{P}$ < 0.20). The highest yield from 1 adult female was 53 larvae (bat 2-2).

Hepatic larval lengths ranged 550 - 975 µm, and widths, 20 - 44 µm. Too few were measured to correlate mean lengths and widths. From bats in which 4 or more larvae were recovered, the mean of average lengths of cold fixed larvae was 715 $\pm$ 36 µm, and of hot fixed larvae 702 $\pm$ 52 µm, these being not different statistically. Using the Spearman Rank Correlation Coefficient, a positive relationship was found between numbers of larvae recovered and host weights ($\underline{P}$ < 0.01). There was no correlation between larval lengths and weights of bats ($\underline{P}$ < 0.5) or between larval lengths and numbers recovered ($\underline{P}$ < 0.2).

TABLE 3.3

P. poliocephalus: adult females

Autopsy No.	Date	Weight (g)	Reproductive status	No. of larvae in: liver	mammary glands	Larval length (µm) x̄ ± S.D. (range)	Larval width (µm) x̄ ± S.D. (range)
1-4*	24.07.81	740	pregnant	0	0	-	-
1-5*	24.07.81	590	pregnant	0	0	-	-
1-6*	24.07.81	675	non-pregnant	0	0	-	-
1.7*	24.07.81	425	nulliparous	0	0	-	-
1-8*	24.07.81	695	pregnant	0	0	-	-
1-9*	24.07.81	790	pregnant	0	0	-	-
1-10	17.08.81	680	pregnant	3	0	775 ± 36 (740 - 810)	35 ± 8.5 (27 - 44)
1-11	17.08.81	790	pregnant	31	0	735 ± 58 (610 - 850)	27 ± 2.2 (23 - 31)
1-12	17.08.81	740	pregnant	12	0	690 ± 67 (550 - 770)	29 ± 3.6 (26 - 35)
1-13	17.08.81	705	pregnant	4	0	675 ± 58 (635 - 760)	23 ± 3.4 (20 - 30)
1-14	17.08.81	775	pregnant	0	0	-	-
1-15	17.08.81	670	pregnant	0	0	-	-
1-23	7.10.81	700	pregnant	14	0	700 ± 46 (650 - 770)	26 ± 2.0 (23 - 28)
1-24	7.10.81	520	nulliparous	0	0	-	-
1-41	14.10.81	700	pregnant	6	0	-	-
1-42	14.10.81	560	nulliparous	0	0	-	-
1-43	14.10.81	580	non-pregnant	0	0	-	-
1-60	5.11.81	450	nulliparous	0	0	-	-
1-61	5.11.81	420	nulliparous	0	0	-	-
2-2	21.09.82	750	pregnant	53	0	635 ± 52 (560 - 750)	-
2-4	21.09.82	740	pregnant	7	0	740 ± 95 (575 - 840)	-
2-5	21.09.82	750	pregnant	0	0	-	-
2-6	21.09.82	760	pregnant	1	0	820	40
2-7	21.09.82	690	pregnant	33	0	675 ± 45 (590 - 750)	-
2-13	6.10.82	860	foetus (79g)	n.e.	0	-	
2-15	6.10.82	800	foetus (97g)	n.e.	0	-	
2-16	6.10.82	840	foetus (92g)	2	0	930 ± 44 (885 - 975)	-
2-17	6.10.82	750	foetus (73g)	0	0	-	-
2-21	14.10.82	590	foetus (75g)	6	1	740 ± 65 (680 - 855)	-
				larva in mammary gland		1390 x 41	
2-22	14.10.82	640	foetus (83g)	1	0	715	-
2-23	14.10.82	625	foetus (67g)	0	0	-	-
2-40	17.10.82	920	foetus (105g)	22	0	630 ± 40 (565 - 720)	-
2-41	17.10.82	770	foetus (93g)	0	0	-	-
2-45	17.10.82	830	foetus (100g)	14	0	720 ± 59 (655 - 805)	-
2-53	17.10.82	845	foetus (130g)	13	0	775 ± 59 (660 - 845)	-
2-65	14.02.83	600	nulliparous	1	0	635	23

* frozen prior to dissection   n.e. - not examined

3.2.3 Adult females with young

Findings from 42 mother-young pairs in which either or both were autopsied between October 1982 and January 1984 are presented in Table 3.4. Only livers and mammary glands of adult females (with 12 exceptions) were examined, and the intestines and lungs of their pups. The neonate of 1 (2-66) was missing, while a pup (3-25) with patent infection was kept alive. Of 30 livers examined, 16 (53%) yielded larvae. In the uterus of bat 2-39, shot while giving birth, a 2nd apparently normal 30 g foetus was present. In 3 bats shot in-partum (2-37, 2-38 and 2-39), larvae were found in livers but not in mammary glands or pups. In 34 of the 42 (81%) mother-young pairs in Table 3.4 larvae were recovered from maternal livers, mammary glands, juvenile intestines or a combination of these. In couples 3-2 the mother and in 1-40 and 1-57 the pups were not examined; these are excluded from further analysis. It is assumed that the missing young of mother 2-66 was infected because of the length of 3rd-stage larvae remaining in the maternal liver indicates these had been acquired in the previous seasons. Hence, of 39 valid pairs, the pups in 35 (90%) either harboured intestinal larvae, or would have developed gut infection if larvae in maternal livers and/or mammary glands eventually passed into the milk.

Maternal hepatic larvae ranged 555 - 905 µm in length, and mean lengths 655 - 805 µm. Considering only bats from which 4 or more hepatic larvae were recovered, the difference between mean larval lengths from adult females (Table 3.3) ($\bar{x}$ = 701 ± 44 µm) and those with pups (Table 3.4) ($\bar{x}$ = 733 ± 47 µm) was not significant ($P < 0.2$, Student's t-test).

Larvae from mammary glands ranged in length from 585 µm (bat 2-50) to 1.39 mm (bat 2-21, Table 3.3). Although some mammary larvae were considerably longer than the largest from livers, others were shorter (bats 2-14, 2-34, 2-50 and 3-16).

Table 3.5 includes all pups autopsied which harboured developing *Toxocara* larvae in their intestines, or from whose mothers larvae had been

TABLE 3.4

P. poliocephalus: females with young

Autopsy No.	Date	Maternal weight (g)	Young weight (g)	Numbers of larvae in: maternal liver	mammary glands	baby gut	Larval lengths (um) liver $\bar{x} \pm$ S.D. (range)	mammary glands $\bar{x} \pm$ S.D. (range)
1-25	7.10.81	550	80	0	0	0	-	-
1-26	7.10.81	640	80	0	0	0	-	-
1-37	14.10.81	655	100	0	6	0	-	845 ± 85 (745 - 955)
1-40	14.10.81	690	n.e.	0	0	n.e.	-	-
1-49	15.10.81	680	75	0	1	1	-	705
1-55	5.11.81	560	99	0	0	6	-	-
1-56	5.11.81	610	85	0	0	4	-	-
1-57	5.11.81	660	n.e.	0	0	n.e.	-	-
1-58	5.11.81	590	n.e.	0	0	6	-	-
1-59	5.11.81	600	n.e.	0	0	6	-	-
2-11	6.10.82	700	97	6	1	3	730 ± 27 (715 - 785)	1240
2-14	6.10.82	720	91	2	4	0	700 ± 40 (680 - 735)	785 ± 118 (660 - 940)
2-18	14.10.82	600	58	0	0	0	-	-
2-19	14.10.82	680	86	1	3	0	595	1090 ± 84 (975 - 1165)
2-20	14.10.82	625	108	8	0	8	705 ± 45 (635 - 760)	-
2-34	17.10.82	650	80	1	1	0	805	630
2-35	17.10.82	615	80	0	0	10	-	-
2-36	17.10.82	710	75	0	0	0	-	-
2-37*	17.10.82	870	100	1	0	0	870	-
2-38*	17.10.82	830	105	37	0	0	655 ± 42 (555 - 750)	-
2-39*	17.10.82	860 (30g foetus in uterus)	92	5	0	0	730 ± 33 (655 - 825)	-
2-46	28.10.82	725	100	n.e.	n.e.	6	-	-
2-47	28.10.82	750	92	0	1	0	-	1020
2-48	28.10.82	675	91	n.e.	n.e.	1	-	-
2-49	28.10.82	815	90	n.e.	n.e.	6	-	-
2-50	28.10.82	800	73	1	2	0	795	660 ± 74 (585 - 730)
2-51	28.10.82	790	125	n.e.	n.e.	11	-	-
2-52	28.10.82	645	117	n.e.	n.e.	3	-	-
2-56	29.10.82	n.e.	118	n.e.	n.e.	10	-	-
2-57	29.10.82	n.e.	102	n.e.	n.e.	2	-	-
2-59	2.11.82	n.e.	95	n.e.	n.e.	3	-	-
2-60	2.11.82	n.e.	106	n.e.	n.e.	30	-	-
2-61	2.11.82	n.e.	124	n.e.	n.e.	2	-	-
2-66	14.02.83	700	-	12	0	-	690 ± 22 (640 - 720)	-
3-2	3.10.83	515	55	n.e.	n.e.	0	-	-
3-16	9.11.83	580	76	31	1	0	805 ± 53 (645 - 905)	775
3-19	25.11.83	575	80	2	0	0	765 ± 11 (755 - 775)	-
3-20	25.11.83	580	92	5	0	0	775 ± 36 (720 - 820)	-
3-21	25.11.83	540	68	12	0	8	775 ± 62 (715 - 905)	-
3-22	25.11.83	645	108	3	0	3	775 ± 76 (680 - 860)	-
3-23	25.11.83	500	77	n.e.	n.e.	1	-	-
3-25	25.01.84	670	n.e.	1	0	passing eggs	755	-

* in-partum

TABLE 3.5

Number and size of *T. pteropodis* larvae recovered from intestines of young *P. poliocephalus* correlated with estimated age and maternal infection

Autopsy No.	Weight grams	Estimated age	Numbers of larvae in maternal liver	Numbers of larvae in mammary gland	Numbers of larvae in baby gut	Lengths x widths (mm) of larvae in baby gut	Mean length (mm) ± S.D.
2-21	75	foetus	6	1	0	(pre-natal)	-
2-39	92	0 hrs	5	0	0	(in partum)	-
2-37	100	0 hrs	1	0	0	(in partum)	-
2-38	105	0 hrs	37	0	0	(in partum)	-
3-16	76	<3 hrs	31	1	0	(placenta attached)	-
2-50	73	<6 hrs	1	2	0	(milk in gut)	-
2-34	80	<6 hrs	1	1	0	(gut empty)	-
1-49	75	1-2 days	0	1	1	0.81 x 0.037	0.81
2-14	91	2-3 days	2	4	0	-	-
2-19	86	5-8 days	1	3	0	-	-
2-35	80	1-2 weeks	0	0	10	1.32 x 0.03 1.36 x 0.04 1.46 x 0.025 1.74 x 0.04 1.79 x 0.035 1.91 x 0.05 2.23 x 0.07 2.23 x 0.07 2.66 x 0.095 3.11 x 0.08	1.98 ± 0.58
3-3	82	1-2 weeks	n.e.	n.e.	1	2.91 x 0.073	2.91
2-49	90	1-2 weeks	n.e.	n.e.	7	2.35 x 0.09 3.34 x 0.15 3.62 x 0.16 4.00 x 0.13 4.60 x 0.11 4.85 x 0.15	3.79 ± 0.90
2-48	91	1-2 weeks	n.e.	n.e.	1	2.37 x 0.10 (moulting)	
2-47	92	1-2 weeks	0	1	0	-	-
2-11	97	1-2 weeks	6	1	3	1.40 x 0.033 1.47 x 0.035 1.50 x 0.036	1.46 ± 0.42
1-37	100	1-2 weeks	0	6	0	-	-
3-21	68	2-3 weeks	12	0	8	11.5 x 0.17 11.6 x 0.19 12.8 x 0.19 18.8 x 0.25 19.5 x 0.23 21.9 x 0.34 22.8 x 0.35 25.4 x 0.33	18.0 ± 5.43
1-58	75	2-3 weeks (died 10 days after capture)	0	0	6*	6.23 x 0.12 7.98 x 0.15 10.0 x 0.19 10.3 x 0.30 13.1 x 0.28	9.52 ± 2.31
3-23	77	2-3 weeks	n.e.	n.e.	1	18.2 x 0.24	18.2
3-19	80	2-3 weeks	2	0	0	-	

(continued over)

Autopsy No.	Weight grams	Estimated age	Numbers of larvae in maternal liver	mammary gland	baby gut	Lengths x widths (mm) of larvae in baby gut	Mean length (mm) ± S.D.
3-18	90	2-3 weeks (died 1 week after capture)	n.e.	n.e.	9	23.1 x 0.39	
						24.2 x 0.39	
						24.4 x 0.39	
						26.1 x 0.47	
						27.5 x 0.47	27.7 ± 3.45
						29.1 x 0.42	
						31.2 x 0.53	
						31.3 x 0.53	
						32.3 x 0.51	
2-59	95	2-3 weeks	n.e.	n.e.	3*	2.78 x 0.06	2.87 ± 0.09
						2.95 x 0.06	
2-46	100	2-3 weeks	n.e.	n.e.	7	2.63 x 0.07 (moulting)	
						3.51 x 0.11	
						3.57 x 0.13	
						4.72 x 0.15	4.14 ± 1.07
						4.85 x 0.12	
						5.53 x 0.16	
2-57	102	2-3 weeks	n.e.	n.e.	2	2.20 x 0.048	2.69 ± 0.49
						3.18 x 0.058	
2.60	108	2-3 weeks	n.e.	n.e.	30*		13 females
						7.80 x 0.13	
						to 9.85 x 0.17	9.10 ± 0.60
							8 males
						6.29 x 0.10	
						to 9.64 x 0.19	7.99 ± 1.16
					males &	females combined	8.68 ± 1.04
2-20	108	2-3 weeks	8	0	8	1.24 x 0.03	
						1.26 x 0.04	
						1.47 x 0.04	
						1.59 x 0.04	1.56 ± 0.24
						1.59 x 0.05	
						1.62 x 0.05	
						1.72 x 0.05	
						1.98 x 0.05	
2-56	118	2-3 weeks	n.e.	n.e.	10	3.62 x 0.081	
						3.78 x 0.074	
						4.01 x 0.074	
						4.18 x 0.067	
						4.36 x 0.074	4.45 ± 0.72
						4.52 x 0.083	
						4.52 x 0.10	
						4.54 x 0.11	
						4.71 x 0.10	
						6.24 x 0.11	
1-59	75	3-4 weeks (died 15 days after capture)	0	0	6*	5.33 x 0.09	
						6.88 x 0.13	
						12.4 x 0.23	9.40 ± 3.35
						13.0 x 0.23	
1-56	85	3-4 weeks	0	0	4*	6.69 x 0.16	
						7.75 x 0.18	7.22 ± 0.53
3-20	92	3-4 weeks	5	0	2	45.8 x 0.64	
						51.3 x 0.63	48.6 ± 2.75

(continued over)

TABLE 3.5 continued

Autopsy No.	Weight grams	Estimated age	Numbers of larvae in maternal liver	mammary gland	baby gut	Lengths x widths (mm) of larvae in baby gut	Mean length (mm) ± S.D.
1-55	99	3-4 weeks	0	0	6*	7.34 x 0.18 7.48 x 0.15 8.15 x 0.13 9.63 x 0.17 10.3 x 0.18	8.58 ± 1.18
3-22	108	3-4 weeks	3	0	3	43.8 x 0.60 58.5 x 0.82 61.5 x 0.82	54.6 ± 7.73
2-52	117	3-4 weeks	n.e.	n.e.	3*	3.06 x 0.09 4.19 x 0.08	3.63 ± 0.57
2-61	124	3-4 weeks	n.e.	n.e.	2	2.69 x 0.09 6.12 x 0.06	4.41 ± 1.72
2-51	125	3-4 weeks	n.e.	n.e.	11*	3.38 x 0.11 3.40 x 0.11 4.00 x 0.11 4.81 x 0.12 5.40 x 0.14 6.23 x 0.12 7.00 x 0.14 7.17 x 0.17	5.17 ± 1.43
1-75	120	5-6 weeks (died 3 weeks after capture)	n.e.	n.e.	2	58.6 x 0.88 71.2 x 1.21	64.9 ± 6.30 (infertile eggs in uterus)
1-93	150 (emaciated)	6-8 weeks	n.e.	n.e.	11	8.36 x 0.15 28.9 x 0.36 45.0 x 0.58 60.6 x 0.91 62.3 x 0.75	males 41.0 ± 20.33
						11.0 x 0.22 45.8 x 0.52 57.0 x 0.72 61.5 x 0.90 62.1 x 0.88 68.7 x 0.80	females 51.0 ± 19.20
						males & females combined	46.5 ± 20.3
3-25	220	6-8 weeks	1	0	n.e.	(passing eggs)	

* not all larvae measured

n.e. - not examined; M - male; F - female

recovered. They are presented in order of increasing age, this being estimated in most cases on the basis of body and head size, healing of the umbilical scar, patency of the umbilical vein, distribution of body hair and development of teeth, and general behaviour if obtained alive. Orphans which had been held in captivity for varying periods prior to autopsy generally failed to gain weight. A few which did thrive were kept alive and are not included in this sample. Gut larvae were not found in pups in the first 24 h pp, even though 2 had suckled (3-16 and 2-50) and larvae were present in the mammae of their mothers. One larva was found in the gut of pup 1-49, aged 1 - 2 d, whereas pups 2-14 and 2-19, aged between 2 and 8 d, were both free of infection even though larvae were found in the maternal livers and mammary glands. Most pups aged 1 - 2 weeks harboured intestinal larvae. Of 7 in their 3rd week of age, only 1 whose mother harboured hepatic larvae (bat 3-19) was free of infection.

In only 1 (2-21) was a larva recovered from mammary glands prenatally. During birth, larvae were not recovered from mammary glands (bats 2-37, 2-38 and 2-39), but were found in other bats killed from 6 h to well into the 2nd week pp. Until 6 h pp, all infected mothers harboured larvae in their livers, but beyond this time there was a decline in hepatic larval numbers. In 1 (2-35), both liver and mammae were free of larvae by the 2nd week, whereas in others, larvae were still present in livers when mammary glands were clear (bats 3-21, 3-19, 2-20, 3-20, 3-22 and 3-25). The highest number of hepatic larvae in such a case was 12, from bat 3-21 whose offspring harboured 8 gut larvae, 11.5 - 25.4 mm in length. One larva (755 x 30 µm) was recovered from the liver of mother bat 3-25, whose mammae were clear and whose 6 - 8-week-old offspring had a patent infection. One pup (2-47) aged 1 - 2 weeks was not infected but from its mother only a single mammary larva was recovered. Larvae were not found in the mammary glands of mother bats whose young were older than 2 weeks, although several were

not examined.

Assuming that all the mammary glands not examined were free of larvae, and that all larvae recovered from the mammaries examined would have eventually established in the offsprings' intestines, then the expected average worm burden of pups older than 5 d was 6.0 $\pm$ 5.7 (range 1-30). In the 21 infected pups older than 2 weeks or whose mothers' mammaries were free of larvae, i.e. those which had attained their full complement of gut worms, the mean worm burden was 6.9 $\pm$ 6.1.

Many gut larvae were damaged during dissection and could not be measured. The smallest (810 µm, bat 1-49, aged 1 - 2 d) was well within the range of mammary and hepatic larval lengths, yet the largest mammary larva (1.39 mm, bat 2-21) was recovered pre-natally. Lengths of gut larvae varied widely within individual bats, being most marked in bat 1-93 (range 8.36 - 68.7 mm). Mean lengths of larvae from bats of the same age also varied widely (e.g. 1.46 - 3.79 mm in the 1 - 2 weeks age-group, and 1.56 - 27.7 mm in the 2 - 3 weeks group). There was a weak correlation (r = 0.30) between the length range of gut larvae, as a fraction of mean larval length, and the numbers of larvae found in an individual animal.

Except for bats 2-60 and 1-93, the sexes of larvae are not indicated in Table 3.5. In bat 2-56, 7 of the larvae were female and the remaining 3 indeterminate. In many instances, larvae were damaged or internal features not sufficiently clear for identification of genital tract structures. In total, 51 female and 28 male gut worms could be identified.

Many intestinal larvae, particularly in the series 2-, had damaged and ballooning cuticles, so that it was impossible to detect moulting. In only 2 larvae, 2.37 mm (bat 2-48) and 2.63 mm (bat 2-46) long, did a clearly double cuticular layer indicate moulting. One juvenile (bat 1-75, estimated age 5 - 6 weeks) harboured 2 female worms, 1 of which (length 71.2 mm) contained infertile eggs (mean diameter 85 $\pm$ 6 µm, n = 10) in the uterus and several in the vagina. The faeces of this bat were not

examined. None of the worms in bat 1-93, aged probably 6 - 8 weeks, was sexually mature.

3.2.4 Patent infections in suckling juveniles

Most suckling juveniles, some with mothers, were caught in summer camps at Pine River, Indooroopilly Island and Eagleby. Sixteen which were found dead or injured were autopsied. A number were kept in small cages so that all worms passed spontaneously or after piperazine treatment could be collected.

From January 1982 to March 1986, 119 suckling juveniles were examined by rectal swab and the data are presented in Table 3.6. Eggs of *T. pteropodis* were found in 60; an additional juvenile passed 2 male worms spontaneously after capture (18 January, 1985) and a male worm was recovered from another at autopsy (3 March, 1986). Hence, at least 62 of these 119 were infected (52%), not including those swabbed rectally which may have harboured only male worms. Of the 60 with patent infection, 10 (17%) were passing exclusively infertile eggs, and 1 was passing a mixture of fertile and infertile eggs in a ratio of approximately 2:1. Of the 76 juveniles examined during the months December-February inclusive, 49 (64%) were infected, whereas of the 43 obtained in March, only 13 (30%) were positive. This reduction in relative prevalence of infection amongst older juveniles was significant (Z = 3.56, P < 0.001). In the final sample (March, 1986), representing the oldest suckling juveniles examined (mean weight 438 $\pm$ 44 g), only 1 live male worm was recovered from the 10 bats. The average age of these bats, all of which were shot at Eagleby, was approximately 5 months. Milk was found in the intestines of each, interspersed with boluses of vegetable matter comprising fibrous material, seeds, stamens and pollens, as well as numerous small insects. Findings from 9 of these juveniles are shown in Table 3.7. Between 1 and 7 hepatic

TABLE 3.6

Patent infections in and recovery of adult *Toxocara pteropodis* from suckling juvenile *Pteropus poliocephalus*

Date	Number examined	Weight range	Number infected	Status of eggs	Number of worms recovered
7.01.82	2	136 - 200	2	fertile	?
12.01.82	2	194 - 197	2	fertile	?
12.01.82	7	n.e.	3	2 fert/1 infert	?
8.03.82	1 X	325	0	-	-
8.03.82	1 X	355	1	fertile	2F + 1M
8.03.82	22	n.e.	9	7 fert/2 infert	12F + 7M
16.03.82	1	n.e.	1	infertile	2F
31.03.82	1	211	1	fertile	?
31.03.82	7	n.e.	0	-	-
14.01.83	1	365	1	fertile	?
14.01.83	2	262 - 285	0	-	-
17.01.83	1	353	1	fertile	2F + 1M
17.01.83	1	265	1	infertile	2F
27.01.83	1	383	1	fertile	1F + 1M
27.01.83	2	260 - 290	0	-	-
27.01.83	1	283	1	infertile	3F + 1M
1.02.83	1 X	360	1	fertile	4F + 1M
3.02.83	1	302	1	infertile	1F
20.01.84	5	n.e.	5	4 fert/1 infert	1F
20.01.84	1	n.e.	1	fertile	7F + 2M
20.01.84	3 X	206 - 230	0	-	-
25.01.84	6	n.e.	4	fertile	?
11.12.84	9	n.e. (age 5-10 wks)	8	fertile	?
18.01.85	13*	n.e. (age 10-14 wks)	5**	fertile	14F + 9M
24.01.85	8*	200 - 250	1	fertile	
6.01.86	7	age 3 months	7	fertile	
6.01.86	2	age 3 months	2	infertile	26F + 14M
6.01.86	1	age 3 months	1	mixed	
3.03.86	10 X	315 - 475	1	-	1M

* these bats were kept in the same cage
** 1 additional bat with clear rectal swab later passed 2 male worms

F - female; M - male; X - autopsy; ? - not determined; n.e. - not examined

TABLE 3.7

Findings in livers of nine suckling *Pteropus poliocephalus* collected 3 March 1986

Weight (g)	Sex	No. larvae recovered	Length of larvae mean ± SD (μm)	Length range (μm)
476	male	0	-	-
*455	male	0	-	-
415	male	0	-	-
455	female	0	-	-
447	female	0	-	-
445	male	1	465	-
440	male	2	565 ± 4	560 - 565
460	female	2	655 ± 45	610 - 700
467	male	7	630 ± 43	580 - 695

* 1 adult male *T. pteropodis* in gut

larvae were recovered from 4, ranging 465 - 700 µm in length. In 2 of this group, hymenolepid tapeworms were found high in the small intestines and were provisionally identified as Pseudoligorchis magnireceptacula by Dr Malcolm Jones.

Entire worm burdens were recovered from 36 bats (Table 3.6). These comprised 77 adult female worms and 28 adult males, giving an average burden of 2.14 females and 1.06 males per infected bat, or a total burden of 3.2 adult worms per bat.

The spontaneous expulsion of adult worms from older juveniles was observed frequently in the laboratory, both in natural and experimental infections, and is examined in detail in Chapter 5. Furthermore, adult worms were found on the ground beneath the roost in mangroves at Pine River on 9 March, 1984 as well as on the floor of the P. poliocephalus camp at Gordon, Sydney (Rhys Puddicombe, pers. comm.).

3.3 DISCUSSION

For the purposes of this study, it is assumed that the P. poliocephalus population of south-east Queensland is stable and relatively homogeneous, with individuals moving freely amongst their various summer and winter camps. This is in keeping with the findings of Nelson (1965b). Likewise, it must be assumed that infection rates do not vary appreciably from year to year, otherwise the sample sizes from different locations in different seasons would be too small for meaningful analysis.

The accuracy of techniques for the recovery of nematode larvae from tissues has never been evaluated reliably, and is considered in more detail in the section on experimental infections (Chapters 4 & 8). Prior to March 1982, tryptic digestion of minced tissues was used followed by sieving then centrifugation of the sediment. The different infection rates between male bats depicted in Tables 3.1 & 3.2 (54% vs 100%) suggests this technique was less sensitive than saline separation in recovering small numbers of

larvae; the average numbers recovered from infected bats were comparable in both groups, whereas an overall mean, including bats found uninfected, would show a significantly lower recovery from the 1st group. From Table 3.2 it appears that all adult male P. poliocephalus carry hepatic larvae. It is not clear how reliably the numbers of larvae recovered reflect those actually present in tissues, but presumably there would be a direct relationship under similar experimental conditions. Some tissue sediments, such as those of lungs and brain, were very clear and much easier to examine than livers. Individual livers varied, perhaps reflecting fat content; some sediments flocculated, which facilitated detection of larvae, whereas others were of the consistency of muddy water and very difficult to examine. Complete examination of the sediment from one adult male liver required up to 8 h of attentive work, so undoubtedly very light infections may have been missed. From many infected bats, all larvae after counting were removed from sediments for later drawing and measuring. Re-examination of such sediments often revealed up to an additional 10% larvae which had been missed. The lower hepatic infection prevalence in adult females than in female-young pairs (Section 3.2.5) may simply reflect an inefficiency of tissue digests in detecting light infections compared with the greater reliability of identifying intestinal larvae. Therefore, a finding of "0" cannot guarantee freedom from hepatic infection, so that in the distribution histogram (Fig. 3.1) the first group includes bats with "0-5" larvae recovered. This frequency distribution of hepatic larvae is very similar to the highly aggregated ("over-dispersed") pattern observed in intestinal nematode infections of humans (Anderson, 1986), in which most individuals harbour few parasites while few harbour heavy loads. The dispersal was greatest in males, presumably because their larvae were trapped indefinitely, with seasonal increments, whereas in females they were depleted with each new offspring. This would also explain the

slightly narrower numerical range in females with suckling pups than in those without, as well as the proportions of each group with very light infections.

Of 39 mother-young pairs, 35 (90%) were infected (Section 3.2.3 & Table 3.4); this was significantly higher than the hepatic infection rate of 17/28 (61%) in adult females (Section 3.2.2 & Table 3.3) ($P < 0.01$, $Z = 2.816$, $v = 65$). Likewise, the prevalence of infections in suckling juveniles was significantly lower than in mother-young pairs. Of 119 suckling juveniles swabbed rectally, 60 had patent infections, with 10 passing only infertile eggs, i.e. harbouring only female worms. Assuming an equal number had only male worms and were undetected, the true prevalence of Toxocara in this group was $\frac{60 + 10}{119}$, i.e. 59%. Even this amended result is significantly lower than the mother-young rate ($P < 0.001$, $Z = 3.563$, $v = 156$). If the true mother-young rate is assumed to lie between that actually found (35/39) and the rate in females (17/28), i.e. $\frac{35 + 17}{39 + 28} = 78\%$, then the difference between this figure and the amended higher rate for suckling young (59%) is still significant ($P < 0.01$, $Z = 2.613$, $v = 184$). This means that larvae must be lost from these infected mother-young units, either in transit from mother to pup, or after arrival in the juvenile intestine.

The comparable larval lengths in Tables 3.1 and 3.2 indicate little difference between cold and hot fixation. However, larvae from cold formalin, often grossly contorted, were more difficult to measure. The mean hepatic larval length was independent of host size or the number of larvae recovered. With livers weighing up to 40 g (approximately 5% of total body weight), the numbers of larvae must have been insufficient to induce a "crowding phenomenon", inhibiting each other's growth. Variation in lengths of nematode larvae may depend on the methods used in fixation and examination (Fagerholm & Lovdahl, 1984). A small number of observations indicated that the time larvae were stored in fixative (up to

2 years) or kept in lactophenol before measuring (up to 2 days) did not affect their lengths.

The marked ranges in larval lengths and variations in mean lengths therefore indicate that larvae grow after entering the liver. In T. canis infections in mice, larvae do not grow and their lengths vary less (Sprent, 1958). Likewise, larvae of T. canis undergoing somatic migration in dogs do not grow and demonstrate a much narrower length range (Sprent, 1958; Noda, 1958). Larval length distributions from 3 adult males (Fig. 3.2) collected in late summer (2-67 and 2-69) and winter (2-80), were approximately normal, whereas one examined in late summer (1-103), demonstrated a bi-modal pattern, with a wider length range and an earlier first peak (550 - 600 μm). These bats were selected because they had sufficient larvae for such an analysis. It is likely that in bat 1-103 a recently-acquired infection was superimposed on an older one, the first peak representing the cohort of new larvae. By winter these larvae may have attained a length plateau.

Larval growth may also explain the wide range in lengths of hepatic larvae from the infected suckling juveniles (Table 3.7). The bat with the single larva 465 um long probably was infected shortly prior to examination, whereas the others presumably had been infected longer.

Larval body widths probably are much more susceptible to influences such as osmotic variations and coverslip pressure and therefore give little indication of the "true" diameters of living nematodes.

The finding of larvae in mammary glands but not foetuses or placentae indicates that this nematode exclusively undergoes transmammary transmission. With 1 exception, larvae did not reach the mammary glands until after parturition, some within hours. In bat 2-21 (Table 3.3), the large mammary larva may have migrated several days before birth, or perhaps was a relict from the previous season. Some mammary larvae were much longer than

any found in livers, but most were of the same size and 1 was only 585 µm (2-50, Table 3.3). Hence, length does not appear to be a major factor governing larval migration to the mammary glands, where some may be temporarily arrested and grow considerably. The finding of an intestinal larva only 810 µm long (bat 1-49) indicates that some larvae traverse the mammaries quickly, without significant growth.

From Table 3.5, it appears that all transmammary migration of larvae was completed within 2 weeks of birth, assuming the age estimates were reliable. Beyond this time, all mammary glands, but not all livers, were free of larvae (bats 3-21, 3-19, 2-20, 3-20, 3-22 and 3.25). The shortest hepatic larva in this group was 635 µm long (bat 2-20); this was not a recent acquisition, as shown subsequently in studies of larval growth (Table 4.5 & Fig. 4.1). These larvae must have been in the livers from at least the previous season, but failed to undertake the transmammary passage.

The stimulus for larvae to leave the liver is probably an endocrine change preceding parturition, either the appearance or disappearance or a rapid change in levels of one or more circulating hormones, and may even be the same stimulus which initiates uterine contractions in the bat. Possibly all hepatic larvae are mobilized, those passing through the mammary glands being arrested there by chemotactic factors, perhaps associated with suckling, and the remainder returning to the liver. Alternately, only some may leave the liver to circulate until reaching the mammaries. The mothers with most residual hepatic larvae had heavily infected offspring; 1 whose pup was not infected had only 2 larvae in her liver. This is consistent with either supposition.

Once in the pup's intestine, larvae grew rapidly. The considerable range in lengths of larvae within infected young, and the demonstration that this range increased with age and the numbers present indicated that this length dispersal probably was influenced by the numbers, with the

shorter ones growing more slowly. One explanation is that larvae did not traverse the mammaries in a group, but trickled through over many days, as shown in the infected pups less than 2 weeks old whose mothers still harboured mammary larvae (Table 3.5). However, in bat 1-93 (Table 3.5), aged 6 - 8 weeks with patent infection, the shortest gut larva was only 8.36 mm long. Assuming this larva had crossed the mammaries at the latest possible time, i.e. 2 weeks pp, then over at least 4 weeks it had grown less than some larvae in pups aged 2 - 3 weeks. This is discussed in detail in Chapter 4.

The fall in mean intestinal worm burden from 6.9 in pre-patent infections (Section 3.2.3) to 3.2 in suckling juveniles harbouring adult worms (Section 3.2.4) indicated that a significant proportion of developing intestinal larvae did not mature, and probably were expelled faecally.

The impression that female worms grew faster than males was supported by the findings in bat 2-60 and 1-93 (Table 3.5) in which mean lengths of female worms were 14% and 24% longer than males respectively. If shorter worms were more susceptible to spontaneous expulsion, this would explain why more female worms than males were recovered from juvenile bats with patent infections (Section 3.2.4).

Infections in juveniles may be patent at 5 - 6 weeks, and worms are eventually lost spontaneously, by about 5 months. From initial observations of captive animals (Appendix I) it seemed that weaning to a fruit diet precipitated worm loss, but further observations showed the explanation was not so simple (Chapter 5). As the juveniles examined in early March 1986 (Table 3.7) were virtually free of gut infection and as peak births were observed to occur in October 1985, this means that *T. pteropodis* eggs were released into the environment for a maximal period of approximately 4 months. That some of those juveniles (4/9) already harboured hepatic larvae indicates exposure to infection early in life.

These bats were already foraging, so their infections may have been acquired in the camps or their feeding grounds. This is analysed in more detail in Chapter 5.

CHAPTER 4

EXPERIMENTAL INFECTIONS AND OBSERVATIONS IN PTEROPUS POLIOCEPHALUS

4.1 INTRODUCTION

Findings from naturally-infected bats helped considerably in identifying the key features of the life-cycle of T. pteropodis. However, elucidation of the mode of infection, larval migration routes, and worm development in relation to host growth, required experimental investigations. Also examined were the haematological and histological responses to infection, dispersal of larvae following the parenteral inoculation of infective eggs, and the distribution of T. canis larvae in flying fox tissues. Difficulties in procuring and maintaining live animals restricted the extent of these experiments.

4.2 EXPERIMENTAL PROCEDURES AND RESULTS

4.2.1 Larval distribution in an adult bat (from Appendix 1)

An adult female of body weight 720 g, whose pup was patently infected, was fed 500 infective eggs daily for 10 days. One day after the last dose, it was killed with intraperitoneal pentobarbitone and its organs examined for larvae by tryptic digestion.

No larvae were recovered from the lumen of the gastrointestinal tract or the wall of the stomach, duodenum or upper third of the intestine, 1 was recovered from the middle third and 12 from the lower third of the intestine (mean larval length 425 $\pm$ 25 μm, range 380-460 μm). None was found in the mesentery or mesenteric lymph nodes. From the liver, 194 were recovered (mean length 445 $\pm$ 40 μm, range 365-525 μm). Larvae were not found in lungs, brain, adrenals, kidneys, pancreas, heart, skeletal muscle or mammary glands.

4.2.2 Larval migration in juvenile bats

Larval migration was followed in 5 bats aged 2 - 4 months maintained on a diet of fruit and milk. Four were each fed 20,000 embryonated *T. pteropodis* eggs which had been kept in charcoal for one month, while a 5th was fed 2,000 eggs. All previously had patent gut infections. The 240 g male had been born in captivity, and was aged 55 d at the time of infection. Bats were killed with intracardiac pentobarbitone under ether anaesthesia at the times indicated in Table 4.1, and their tissues examined for larvae by saline separation. Tracheae were not minced but slit open longitudinally and left immersed in saline at 37 ^{o}C for 18 h for larvae to migrate. The findings are summarised in Table 4.1; the sequence of organs listed reflects the presumed migratory pathways of larvae. In the bat killed 6 h pi, small petechiae were visible throughout the small intestinal mucosa, more numerous distally. The intestine of this animal was divided into 4 equal lengths and examined for larvae; the results are:

	1st quarter	2nd quarter	3rd quarter	4th quarter
contents	0	17	32	110
wall	0	85	430	620

While being anaesthetised, this bat passed about 3 g liquid faeces containing approximately 300 free *Toxocara* larvae, as well as empty eggshells and unhatched embryonated eggs. Larvae were recovered only from the intestines, mesentery and mesenteric nodes. Most were in the intestinal wall, with increasing numbers distally (the short colon cannot be distinguished from the ileum in these animals without opening the gut). One larva was found in the stomach wall, and a small number in the mesentery and nodes. In the bat killed 2 d pi, there were small numbers of larvae throughout the lumen, but more in the walls of the gastrointestinal tract. Several were found in the mesentery and nodes, 26 in the lungs, 1

TABLE 4.1

Numbers of *T. pteropodis* larvae recovered from tissues of experimentally infected juvenile *P. poliocephalus* at varying intervals after ingestion of eggs

Sex	F	F*	F	F**	M
Weight (g)	350	280	215	390	240
Egg dose	2 000	20 000	20 000	20 000	20 000
Time killed p.i.	6 hrs	2 days	4 days	7 days	40 days
Stomach					
contents	0	50	0	0	0
wall	1	134	0	0	0
Intestine					
- Proximal					
contents	17	11	3	0	0
wall	85	27	29	0	0
- Distal					
contents	142	21	36	0	0
wall	1 050	157	121	0	0
Mesentery	3	3	32	3	0
Mesenteric lymph nodes	2	9	49	3	0
Lungs	0	26	264	2	0
Trachea	0	1	0	0	0
Kidneys	0	0	2	0	0
Brain	0	0	2	0	0
Liver	0	3 100	7 700	12 500	750

* Numerous unidentified mites found in lung digest

** Two adult male and one female *T. pteropodis* found in upper small intestine

in the trachea and 3,100 in the liver. At 4 d pi, larvae were still present throughout the gut. Macroscopically, fine pale spots were discernible over the liver surface, and numerous petechiae in the lungs. Almost 8,000 larvae were recovered from the liver, and the greatest numbers from lungs, mesentery and nodes were recovered at this time. Two were found in the kidneys and 2 in the brain, but none in the trachea. At 7 d pi, the gastrointestinal tract was free of larvae, but 6 were found in the mesentery and nodes. The liver was densely mottled, and lungs covered with petechial haemorrhages. Only 2 larvae were found in the lungs, but from the liver, an estimated 12,500 were recovered. This bat harboured 3 adult _T. pteropodis_, which had been missed by rectal swab, in the upper intestine. In the bat killed on day 40, larvae were found only in the liver, which appeared finely mottled without discrete puncta.

Lengths of these larvae are shown in Table 4.4. Only approximately the first 40 counted were drawn and measured. Hepatic larvae at 40 d pi were longer than at 2 d pi ($P < 0.001$).

4.2.3 Parenteral inoculation of _T. pteropodis_ eggs

Six weaned juveniles, all with previous patent infections, were given subcutaneous (sc) injections of embryonated eggs under ether anaesthesia. The doses, indicated in Table 4.2, were administered in 0.5 ml sterile saline into the interscapular regions.

Periods after which autopsies were performed and larval recoveries are shown in Table 4.2, with organs again listed to indicate presumed migration routes.

At 30 h after injection, numerous fine petechiae were present in the lungs and many larvae were recovered from the lungs and liver. Three were found in the trachea, and over 200 were recovered from the gastrointestinal tract, predominantly in the walls. At 3 d pi, the lung petechiae were coalescing, and the maximal number of pulmonary larvae was recovered at

TABLE 4.2

Numbers of T. pteropodis larvae recovered from tissues of juvenile P. poliocephalus inoculated subcutaneously with embryonated eggs

Sex	F	F	F*	F**	NR	M
Weight (g)	210	280	330	300	NR	300
Egg dose	30,000	5,000	20,000	5,000	5,000	5,000
Time pi	30 hrs	3 days	5 days	7 days	10 days	20 days
Lungs	750	830	380	91	30	2
Trachea	3	2	8	3	0	0
Liver (% total dose)	660(2.2)	45(0.9)	1,040(5.2)	280(5.6)	770(15.4)	400(8.0)
Brain	1	20	35	3	3	0
Kidneys	0	28	83	2	6	0
Stomach:						
contents	1	0	0	0	1	0
wall	38	2	14	1	0	1
Intestine:						
- Proximal						
contents	1	0	1	0	1	0
wall	12	2	7	0	0	1
- Distal						
contents	69	3	1	0	2	0
wall	94	4	7	0	0	1
Mesentery + abdominal lymph nodes	1	NR	14	NR	0	0

NR - not recorded
* One larva found in spleen
** One adult male T. pteropodis found in intestine

TABLE 4.3

Recovery of larvae from tissues of juvenile P. poliocephalus fed 10 000 embryonated T. canis eggs

Sex & Weight (g)	Days p.i.	Liver	Lungs	Brain	Skeletal muscle	TOTAL
Female 270	7	110	83	520	1,200	1,920
Female 240	28	90	2	850	1,100	2,070
Female 355	90	50	1	890	800	1,830

this time. Considerable numbers were also found in the brain and kidneys, but very few in the intestine. In the bat killed at 5 d after 20,000 eggs, the pulmonary petechiae were coalescent and there was patchy consolidation throughout both lungs. Again, considerable numbers of larvae were recovered from the brain and kidneys, and most of those in the gut were within the walls. The mesentery and abdominal nodes yielded 14.

At 7 d after 5,000 eggs, most of the punctate pulmonary haemorrhages were starting to resolve, although several appeared fresh (Fig. 4.6 B). Fewer larvae were found in the lungs. Three were in the trachea, with similar numbers in the brain and kidneys. Only 1 was found in the alimentary tract, in the stomach wall. Subsequently, none was found in the trachea and the numbers in organs other than the liver declined. At the injection site 7 d pi, considerable numbers of viable larvae were found within eggs. At 10 d pi, only an occasional motile larva was seen either within the fibrinous exudate or in an egg.

The lengths of some of these larvae are shown in Table 4.4. At 7 d pi, larvae in the trachea were longer than those in the lung ($P < 0.005$). At 10 d, larvae in the liver were longer than those in lungs ($P < 0.001$).

4.2.4 Infection of juvenile *P. poliocephalus* with *T. canis*

Infective larvae of *T. pteropodis* and those of *T. canis* are almost identical in size (Chapter 6). To determine whether size alone influenced the distribution of larvae in tissues, *T. canis* infection in bats was studied.

Three juvenile females aged about 4 months, all previously infected with adult *T. pteropodis*, were each fed 10,000 embryonated eggs of *T. canis*. One was killed 7 d, the 2nd 4 weeks and the 3rd at 3 months pi, and their livers, lungs, brains and skeletal muscles examined for larvae by saline separation. Portions of skeletal muscle from limbs, trunk, head and

TABLE 4.4

Lengths of larvae recovered from bats infected orally and subcutaneously with T. pteropodis eggs and orally with T. canis eggs

Time p.i.	Organ	No. larvae recovered	No. larvae measured	Mean length (μm) ± S.D.	Range in length (μm)
T. pteropodis - oral inoculation					
6 hrs	proximal gut wall	85	40	405 ± 23	360 - 455
2 days	distal gut wall	157	45	425 ± 19	375 - 475
2 days	liver	3,100	45	455 ± 20	405 - 495
7 days	liver	12,500	53	465 ± 28	410 - 530
40 days	liver	750	43	530 ± 45	455 - 635
T. pteropodis - subcutaneous inoculation					
7 days	lungs	91	34	425 ± 21	385 - 470
"	trachea	3	3	475 ± 26	445 - 490
"	stomach	1	1	470	-
"	brain	3	3	450 ± 23	425 - 470
"	kidneys	2	2	410 ± 17	395 - 420
10 days	lungs	30	30	420 ± 35	360 - 475
"	liver	770	44	465 ± 29	400 - 525
20 days	liver	400	32	485 ± 35	430 - 550
T. canis - oral inoculation					
7 days	brain	520	36	430 ± 13	400 - 455
90 days	brain	890	33	435 ± 14	415 - 465

neck, estimated to represent 20 - 40% of the total muscle mass, were processed and total larval numbers calculated from those recovered. Findings are shown in Table 4.3 and lengths of larvae in Table 4.4.

Total numbers recovered from the 3 bats were remarkably similar, accounting for about 20% of the egg dose. At 7 d pi, 63% of larvae were recovered from skeletal muscle, and only 6% from the liver. At 3 months pi, those from skeletal muscle represented 43% of the total, the brain 48% and liver only 3%.

4.2.5 Longevity and growth of hepatic larvae

In addition to the studies already mentioned, larvae from livers of other experimentally-infected juvenile and adult bats were also measured. Some were exposed to unknown doses, when eggs were added to their food. In such cases, it was not certain that all the bats in a cage became infected, as in 3 hepatic larvae were not found. Others were fed calculated doses of eggs; in these the numbers of larvae recovered varied from 4% to 63% of the egg dose (Table 4.5). Excluding the exceptionally high figure of 63%, the mean recovery rate was 12.5% $\pm$ 4.4%. Proportions were unrelated either to doses or interval pi.

Studies on larval recovery and growth were extended over 3 years. In the 1st year (1982), most larvae were fixed in cold 10% formalin, but subsequently in hot saline/3% formalin. Lengths of all larvae measured are shown in Table 4.5 and, of those from bats exposed to only a single infection, plotted in Figure 4.1. Despite individual variations, it is clear that larval lengths increased within several days of infection, a mean length plateau of about 600 µm was reached 40 - 60 d pi, and there was a slight continuing increase in prolonged infections. The bat examined 24 months pi was an adult female which, despite having produced 2 infected offspring in the interim, still yielded 85 hepatic larvae 565 - 790 µm in length. It appears, from Table 4.5 and Figure 4.1, that hepatic larval

TABLE 4.5

Lengths of *T. pteropodis* larvae recovered from livers of *P. poliocephalus* at varying intervals after experimental infection

Source*	Egg dose	Time pi	Number of larvae recovered	Number of larvae % dose	Number of larvae measured	Length (µm) of larvae mean ± S.D.	Length (µm) of larvae range
Eggs embryonated 10 w			-	-	49	425 ± 25	360 - 470
Juvenile F	2,000	6 h	(gut contents)		40	405 ± 23	360 - 455
Juvenile M	1,500	1 d	120	8	31	410 ± 21	370 - 455
Juvenile M	1,500	2 d	160	11	35	410 ± 20	370 - 450
Juvenile F	20,000	2 d	200	-	45	425 ± 19	375 - 475
Juvenile F	20,000	2 d	3,100	16	45	455 ± 20	405 - 495
Juvenile F	20,000	7 d	12,500	63	53	465 ± 20	410 - 530
Juvenile M	1,000	15 d	120	12	15	475 ± 23	425 - 515
Juvenile F	?	3 w	?	-	48	460 ± 40**	380 - 525
Adult F	?	3 w	?	-	50	480 ± 35**	395 - 545
Juvenile M	20,000	6 w	750	4	43	530 ± 45	455 - 635
Juvenile F	?	6 w	?	-	70	600 ± 30**	520 - 670
Juvenile	?	6 w	?	-	54	525 ± 40**	440 - 590
Juvenile	?	3,6 w	?	-	52	520 ± 42**	430 - 620
Juvenile M	400	7 w	41	10	28	590 ± 48**	495 - 695
Juvenile F	5,000	9 w	770	16	51	560 ± 47	465 - 680
Juvenile F	?	3,9 w	?	-	54	515 ± 36**	450 - 600
Adult F	?	3,6,9 w	?	-	27	570 ± 94**	440 - 750
Juvenile F	500	10 w	48	10	48	560 ± 56**	440 - 645
Juvenile F	500	10 w	63	13	50	655 ± 48**	555 - 750
Adult M	?	8,10 w	?	-	32	595 ± 53**	475 - 670
Yearling M	1,000	5 m	200	20	48	590 ± 43	480 - 665
Juvenile F	2,000	8 m	250	13	63	595 ± 33**	515 - 680
Adult F	1,000	8 m	150	15	24	625 ± 70	530 - 795
Yearling M	1,000	10 m	190	19	37	655 ± 43	585 - 750
Adult M	1,000	12 m	140	14	35	655 ± 47	507 - 740
Adult M	1,000	18 m	72	7	32	665 ± 48	530 - 750
Adult F	?	24 m (2 offspring infected)	85	-	40	675 ± 55	565 - 790
Adult M	?	27 m	300	-	26	745 ± 43	675 - 845

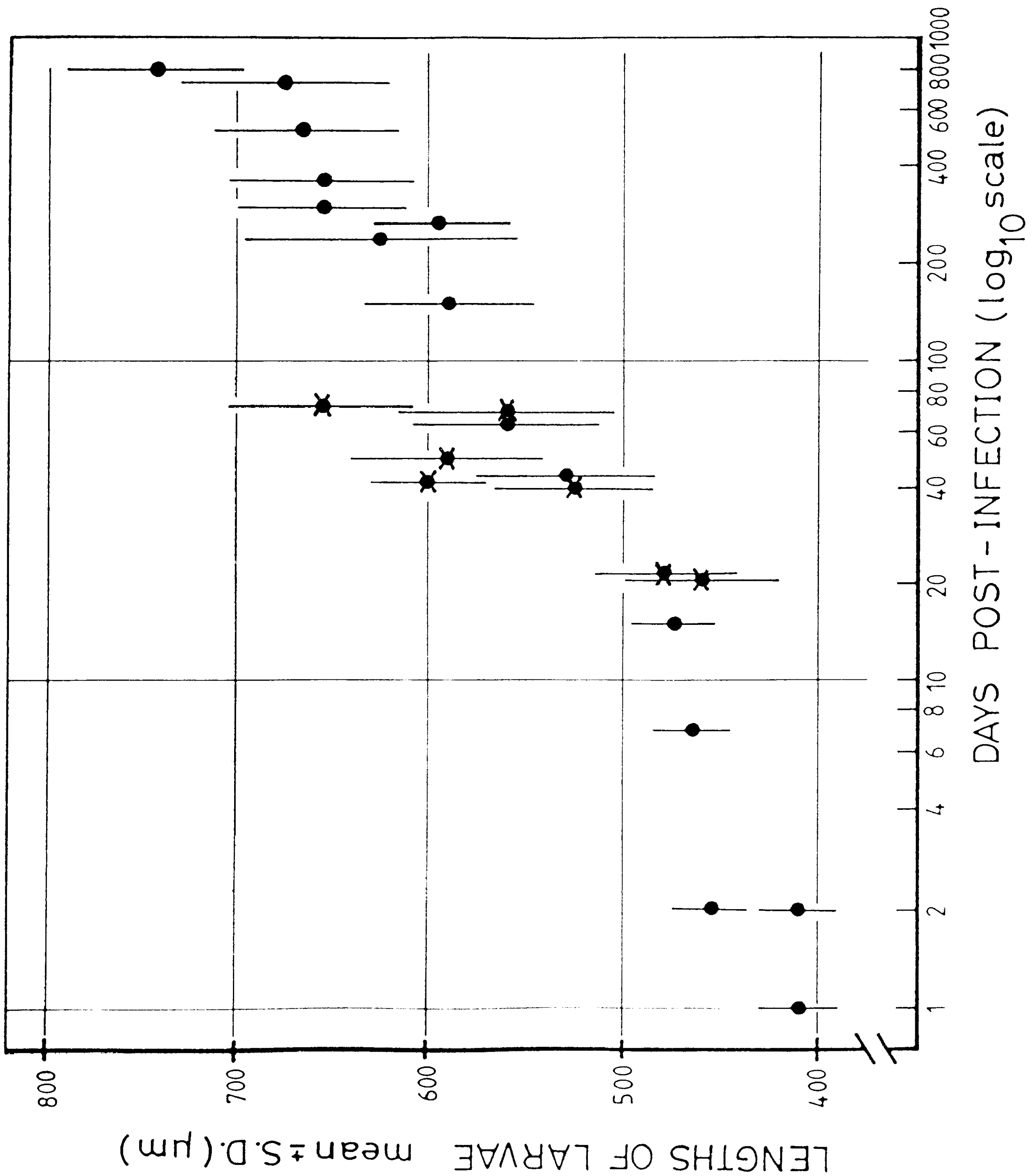
* liver unless otherwise stated
** fixed in cold formalin

M = male F = female
m = month w = week

FIGURE 4.1

Mean lengths of T. pteropodis larvae (vertical bar = ± SD) recovered at various intervals from livers of experimentally-infected juvenile and adult P. poliocephalus receiving only a single dose of infective eggs (note: time at Log_{10} scale)

✱ = fixed in cold formalin



growth was not related to their numbers and cold-fixation did not consistently produce shorter larvae.

4.2.6 Maternal transmission of infection

Up to 25 wild-caught adult female P. poliocephalus were kept in a flight cage with 3 adult males and a smaller number of P. alecto. In January-March of 1982, 1983 and 1984, infective T. pteropodis eggs were added to their food and milk 3 times at 3-week intervals. Maximal copulatory activity occurred in April 1982, June 1983 and May 1984, so from about 4 months after these times the females were closely observed for the presence of neonates. As soon as a newborn young was detected, the pair were transferred to smaller cages for close observation.

There was only 1 birth in 1982, on 30th October. Mother and pup were killed 6 d later, and the maternal mammary glands, liver and intestines, and the pup's gastrointestinal tract, liver and lungs, were examined for larvae. The mammary glands were poorly developed, and the pup, whose intestine contained little milk, weighed only 55 g. Four larvae (1 damaged) were recovered from its gut, but none from liver or lungs. From the mother, 9 were recovered from the liver, none from the gut and none from 1 mammary gland (the other was fixed in formalin for histology). The following summer, 5 young were born, and killed with their mothers (with 1 exception) at times pp indicated in Table 4.6. The hypertrophied and congested mammary glands of these females were dissected out before abdomens were opened, instruments rinsed well between each step, and pups' intestines always examined last. From the 1st 3 mothers (9, 16 and 23 days pp), 1 mammary gland was fixed in buffered 10% formalin, and the other minced for saline separation. From the female killed at 41 days pp, both glands were examined for larvae. The mother of the 49-d-old juvenile was kept for breeding. The livers and lungs of all pups were subjected to

saline separation, and intestines opened and examined in saline under a stereo-microscope. The findings are presented in Table 4.6. Larval numbers in maternal livers were extrapolated from numbers recovered from 25-50% samples of sediments. Larvae were not recovered from the liver or lungs of any pups. The 6th mother, returned to the flight cage, subsequently produced another infected offspring, and when killed 56 d pp, yielded 85 hepatic larvae.

Mammary gland and liver histology are presented in Section 4.4.3.

From November 1984 onwards, mothers with neonates were left undisturbed in the flight cage until about 30 d pp, when they were transferred to individual cages for faecal studies (Chapter 5).

From 1 to 18 intestinal larvae were found in the pups (Table 4.6). Of worms which could be identified, 24 were female and 26 male. Insufficient bats were examined for statistical analysis, but offspring of heavily infected mothers harboured most worms. Mean lengths of hepatic larvae were not obviously related to their numbers. The single mammary larva in the mother killed 41 d pp probably was a contaminant; this larva was smaller than other mammary larvae recovered, and much shorter than those in the pup's intestine.

Fertile eggs were found in the faeces of the 41-d-old juvenile, and the uteri and spermathecae of the 3 largest worms were packed with eggs and spermatozoa. The 49-d-old pup was passing both fertile and infertile *T. pteropodis* eggs. Its longest female worm (93.5 mm) contained fertile eggs and spermatozoa; an 81.3 mm female worm contained infertile eggs in the undivided uterus, with spermatozoa and fertile eggs higher up in the uterine branches and spermathecae, and the other 81.3 mm female had only infertile eggs with no spermatozoa. Only in the 60.6 mm male were mature spermatozoa found.

Toxocara eggs were present in the faeces of the mothers of the last 2 juveniles, but worms were not found in the intestines of the mother killed

TABLE 4.6

Numbers and lengths of *T. pteropodis* recovered post-partum from experimentally infected adult female *P. poliocephalus* and their offspring

Days post partum	6	9	16	23	41	49
Weight of young (birth weight)	55 g (?)	94 g (95 g)	91 g (93 g)	115 g (70 g)	120 g (?)	132 g (?)
No. worms in gut	4	8	16	1	18	9
Length (mm) & sex of gut worms	1.15 F 1.49 M 1.56 M (+ 1 damaged)	1.17 F 1.25 F 2.08 F 2.21 F 2.45 M 3.16 M 3.30 M 3.59 M	1.31 ? 1.71 F 1.78 M 1.94 F 1.98 M 2.02 F 2.34 F 2.38 F 2.47 F 2.49 M 2.58 F 2.66 F 2.94 F (+ 3 damaged)	2.83 F	6.92 F 9.53 M 10.8 M 15.0 M 16.0 M 16.2 M 16.7 M 19.6 M 19.6 M 20.3 M 20.3 M 55.2 M 59.1 M 63.5 M 66.9 M 80.4 F 87.8 F 91.7 F	8.83 M 13.9 M 34.0 F 40.4 F 41.9 F 60.6 M 81.3 F 81.3 F 93.5 F
$\bar{x} \pm$ S.D. (mm)	1.40 ± 0.18	2.41 ± 0.90	2.20 ± 0.45	2.83	37.5 ± 29.8	50.6 ± 30.4
No. larvae in mammary glands	0 (1 gland)	3 (1 gland)	2 (1 gland)	0 (both)	1* (both)	N.E.**
Lengths (μm) of mammary larvae	-	800 925 975	730 1180	-	675	-
No. larvae in maternal liver:						
- recovered	9	150	1000	130	500	85
- measured	9	31	50	63	33	40
Length $\bar{x} \pm$ S.D. (μm)	740 ± 44	715 ± 53	720 ± 47	700 ± 50	685 ± 44	675 ± 55
Range	685 - 825	620 - 835	620 - 830	590 - 845	605 - 785	565 - 790

* probable contaminant (see text)

** mother returned to cage - produced infected offspring the following season, then was killed 56 days post partum

41 d pp. The mother of the 49-d-old juvenile produced an infected offspring the following summer (which harboured 2 adult female and 1 adult male worm at patency), after which 85 larvae were recovered from her liver.

4.2.7 Growth of intestinal larvae

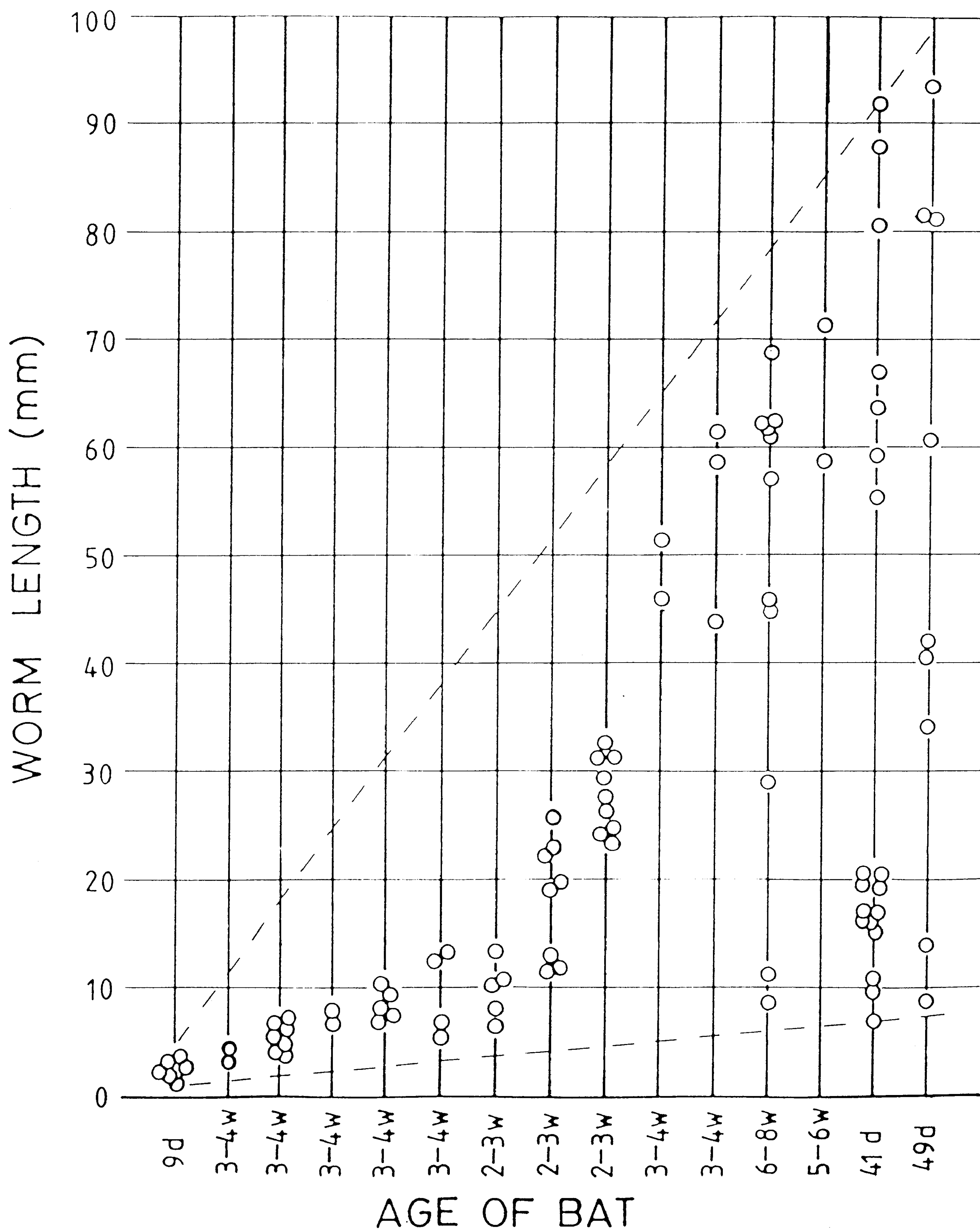
Lengths of larvae from pups born to experimentally-infected mothers are shown in Table 4.6. In the 6 d, 9 d and 16-d-old pups, the shortest larvae were similar in length, but the longest was in the 9-d-old pup. In the 41-d-old juvenile, lengths ranged 6.92 - 91.7 mm, and were clustered in 2 distinct groups (Fig. 4.2), comprising smaller larvae 6.92 - 20.3 mm, and long worms 55.2 - 91.7 mm. In the 49-d-old bat, a similar pattern was apparent. The lengths of these larvae, and some representative samples from naturally-infected pups, are presented in Figure 4.2, ranked in order of increasing size of the longest worm found in each bat. In older bats with 9 or more larvae, the larvae clustered into long and short groups about the 50 mm line, with the smallest larvae remaining at remarkably short lengths.

4.2.8 Infection of suckling young _P. poliocephalus_ with hepatic larvae

In late October 1984, 3 live orphaned bats aged 1 - 2 weeks, recovered from dead mothers on power lines, were maintained on milk formula and each given 10 mg mebendazole in milk. Two days later they were fed live _T. pteropodis_ larvae, recovered in saline from the liver of a female bat infected 8 months previously. Larvae were administered in warm milk and glucose via syringe. The young bats were then killed at intervals, and their intestines, livers and lungs examined. Larvae were not found in intestines or lungs, but were recovered from livers as follows:

FIGURE 4.2

Length dispersal of T. pteropodis from intestines of P. poliocephalus ranked in order of increasing maximal length (based on data from Tables 3.5 and 4.6). Ages of naturally-infected animals are estimates (w = weeks) and those of experimentally-infected juveniles are precise (d = days).



Larval dose	Days after infection	No. larvae recovered	Lengths of larvae (μm) mean ± S.D.	range
35	4	3	550 ± 25	525 - 570
30	6	3	570 ± 39	550 - 615
53	18	14	635 ± 43	555 - 695

Of the 150 larvae recovered from the donor bat's liver, the mean length was 625 ± 70 μm (range 530 - 795 μm).

4.2.9 Induction of pseudo-pregnancy

In March, 1982, a 5-month-old female bat was fed 2,000 infective T. pteropodis eggs. Three months later it was transferred to the care of Dr L. Martin in the Department of Physiology and Pharmacology, where it was given subcutaneous injections of medroxyprogesterone acetate, 5 mg in 0.1 ml saline, on 17.6.82, 1.7.82, 14.7.82 and 9.8.82. Five months after the first injection, 1 mg depot oestrodiol valerate in oil was injected subcutaneously weekly for 4 weeks. The bat was killed 6 months after the first progesterone injection, and 9 months pi. Approximately 250 Toxocara larvae (mean length 595 ± 33 μm, n = 33) were recovered from the liver, but none from the mammary glands.

4.3 HAEMATOLOGICAL RESPONSES TO T. PTEROPODIS INFECTION

A preliminary study in 1 adult female (Appendix 1) indicated a rise in circulating leukocyte and eosinophil levels following ingestion of infective eggs. Haematological responses to this infection in the natural host were futher studied in captive animals. All were infected orally. Results from bats sampled only once are shown in Table 4.7. The juveniles all had previous patent infections terminated spontaneously or after piperazine treatment. Except for the mother of the 4-month-old female, all the adults had been caught over the preceding 3 years, most as patently-

TABLE 4.7

Haematological indices of P. poliocephalus exposed orally to T. pteropodis infections. Adult males and females had been captured as juveniles and reared in captivity in the same cage.

Age when infected/ sex of animal	Egg dose	Time after infection	Haemo- globin g/l	Red cells $x10^6/mm^3$	White cells $x10^3/mm^3$	Neutro- phils %	Eosino- phils %	Lympho- cytes %	Mono- cytes %	Baso- phils %
4 m.o. F	0	10 d after passing gut worms	14.0	7.1	16.3	74	0	5	21	0
same	2000	7 d	16.5	8.2	14.0	26	21	21	31	1
mother of above	0	-	-	8.2	9.3	29	1	35	35	0
5 m.o. F	3000	3 d	15.6	9.1	25.9	35	19	36	10	0
5 m.o. M	1000	6 d	14.8	9.6	27.3	34	15	40	1	0
5 m.o. M	1000	6 d	12.0	7.8	30.0	36	17	40	7	0
5 m.o. M	1000	6 d	16.4	10.4	21.6	49	10	32	8	1
5 m.o. M	1000	6 d	15.5	10.0	29.1	44	10	37	9	0
2 m.o. M	20000	40 d	15.1	-	27.8	54	22	20	4	0
adult M	1000	9 mths	-	-	13.4	32	12	52	4	0
adult M	1000	9 mths	-	-	21.6	30	14	54	2	0
adult M	1000	9 mths	-	-	26.8	20	6	70	4	0
adult M	1000	9 mths	-	-	13.1	22	11	62	5	0
adult F	0	-	-	-	18.3	29	8	59	4	0
adult F	0	-	-	-	24.3	34	6	55	5	0
adult F	0	-	-	-	18.4	48	1	49	2	0
adult F	0	-	-	-	11.6	37	6	54	3	0
adult F	0	-	-	-	9.2	15	5	79	1	0
3 m.o. F	10000 T. canis eggs	3 mths	13.0	-	27.9	35	12	34	19	0

m.o. = months old

infected juveniles. The only bat without detectable circulating eosinophils was the 4-month-old female. By far the highest absolute eosinophil count, $6,100/m^3$, was found 40 d pi in a juvenile fed 20,000 eggs.

In the group of adult females which had not been experimentally infected, the mean blood eosinophil level was $850 \pm 520/mm^3$ (range $180 - 1,460/mm^3$). In the adult males bled 9 months pi, the mean eosinophil level of $1,920 \pm 640/mm^3$ (range $1,440 - 3,020/mm^3$) was higher (t-test, $P < 0.02$). In the 5-month-old males bled 6 days p.i., the mean absolute eosinophil level was $3,570 \pm 1,120/mm^3$ (range $2,160 - 5,100/mm^3$) which was higher than that of the adult male group ($P < 0.05$). Total white cell counts were not significantly different between the adult male and non-infected female groups, but were higher in the 5-month-old male group ($P < 0.05$).

The leukocyte profile of the juvenile infected with *T. canis* eggs was within the range of those infected with *T. pteropodis*.

Experimentally-infected mothers and their pups (Section 4.2.6 & Table 4.6) and 1 naturally-infected pair, were also investigated haematologically, with findings shown in Table 4.8 and Figure 4.3. In the first 3 weeks pp, mothers had higher total white cell counts than their offspring, but this pattern reversed later. Absolute eosinophil counts peaked at 16 d, both in the pup ($6,100/mm^3$) and mother ($20,500/mm^3$) then declined to very low levels at 120 d. In all pairs, maternal eosinophilia exceeded that in the pup, yet in both it followed the same trend.

In the final study of this series, 3 3-month-old juveniles were each fed 5,000 *T. pteropodis* eggs. They had been caught 6 weeks previously and found to be free of intestinal infection. Blood was taken at times indicated in Figure 4.4, which shows total leukocyte and eosinophil responses. Prior to infection, total white cell counts ranged $5,100 - 9,200/mm^3$. They

TABLE 4.8

Haematological indices of experimentally-infected adult female P. poliocephalus and offspring (see section 4.2.6 and Table 4.6)

	Days pp	Haemoglobin g/dl	Red cells $x10^6/mm^3$	White cells $x10^3/mm^3$	Neutrophils %	Eosinophils %	Eosinophils Total $x10^3/mm^3$	Lymphocytes %	Monocytes %
M	9	-	9.8	31.0	48	9	2.79	33	10
O	9	-	9.0	12.0	37	12	1.44	47	4
M	16	16	8.9	31.5	25	65	20.5	8	2
O	16	17.1	8.4	20.3	37	30	6.1	30	3
M	23	-	9.0	20.0	16	52	10.4	29	3
O	23	-	8.4	16.0	21	32	5.12	45	2
M	41	-	7.0	9.1	30	19	1.73	50	1
O	41	-	7.6	27.1	46	5	1.34	43	6
M*	120	-	8.2	9.3	29	1	0.09	35	35
O*	120	14.0	7.1	16.3	74	0	0	5	21

* Naturally infected (age estimated) - transposed from Table 4.7

M = mother; O = offspring

FIGURE 4.3

Peripheral blood eosinophil levels in 5 separate experimentally-infected <u>P. poliocephalus</u> mother-young pairs at various intervals post-partum.

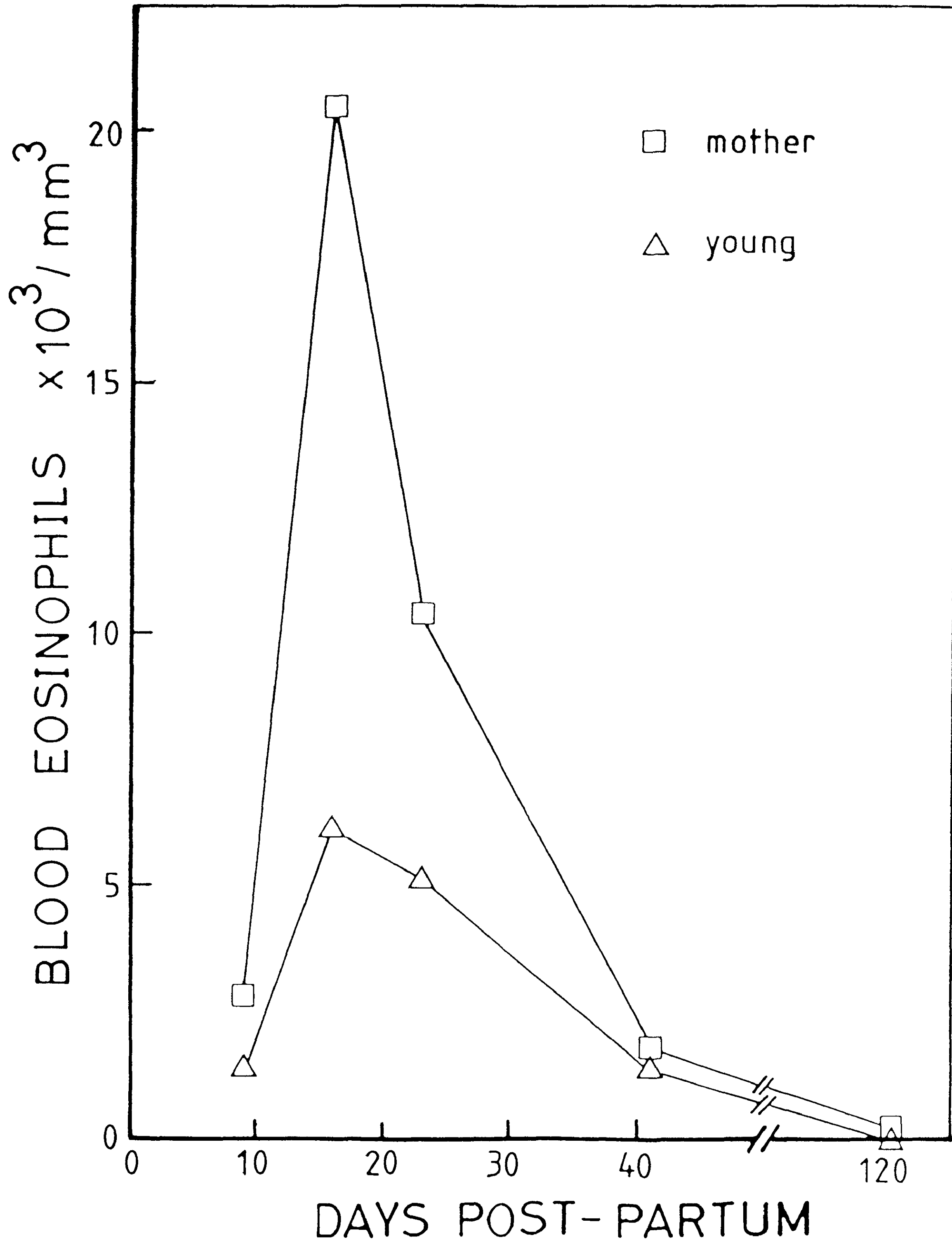
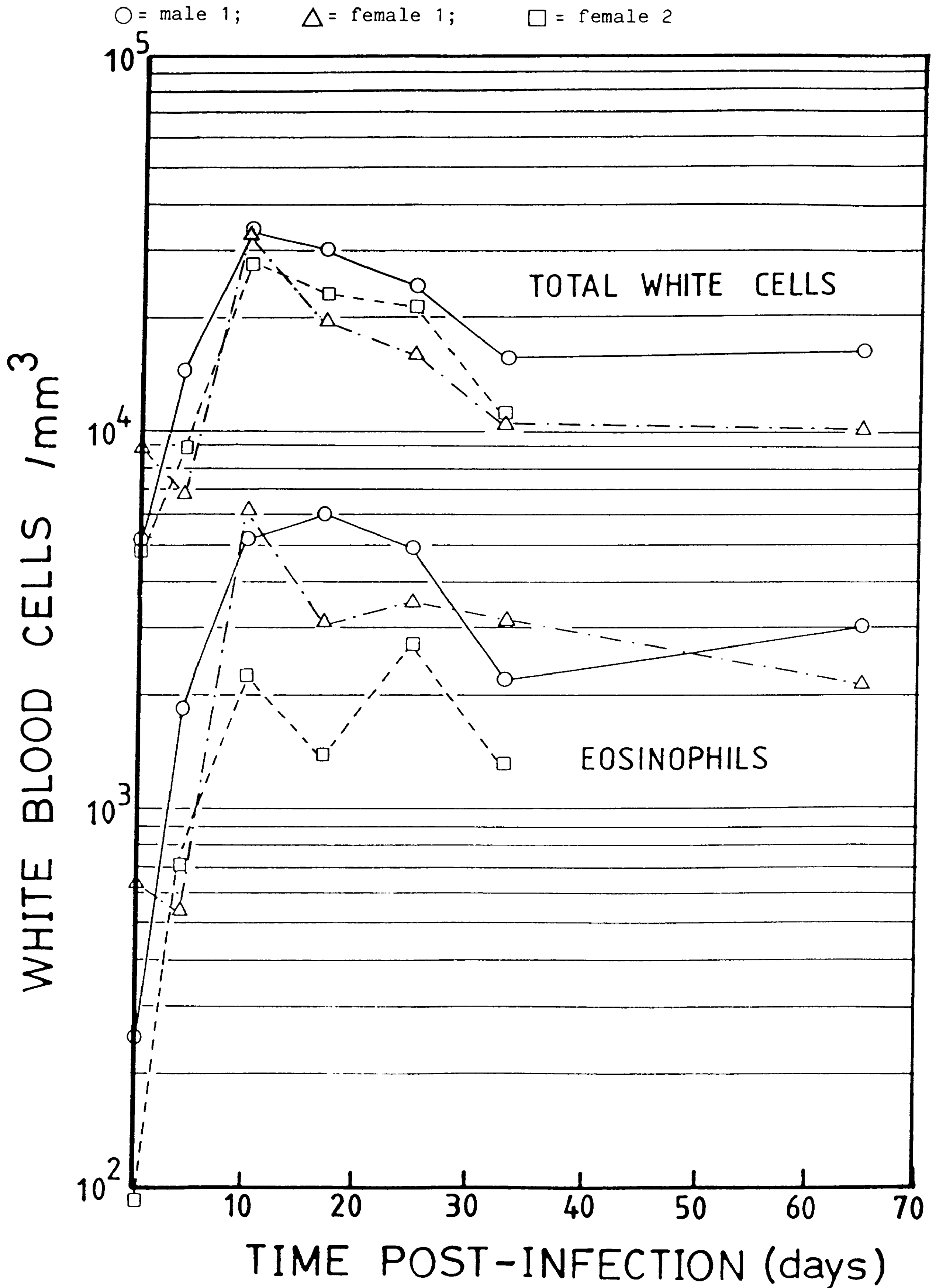


FIGURE 4.4

Peripheral blood total white blood cell counts and eosinophil levels following oral administration of 5,000 infective T. pteropodis eggs to 3 juvenile P. poliocephalus.

○ = male 1; △ = female 1; □ = female 2



rose to a peak at 10 d pi then declined to a plateau at 33 d, after which they remained higher than at pre-infection levels. Female 2 died unexpectedly at 60 d pi and was not bled.

Absolute eosinophil counts generally paralleled the total white cell responses. Pre-infection levels ranged from 0 - 640/mm^3. In male 1 and female 2, a marked rise had occurred by 4 d pi. Levels peaked in the 2 females at 10 d (2,250 - 6,140/mm^3) and in the male at 17 d (5,980/mm^3). In the females there was a 2nd peak at day 25, following which eosinophilia declined to a sustained plateau. Lymphocytes accounted for the bulk of other leukocytes, although there was a mild neutrophilia, while monocyte levels generally remained at 2 - 3%, never exceeding 10% of the total. Basophils were not seen in any films.

4.4 HISTOLOGICAL RESPONSES TO INFECTION

4.4.1 Non-pregnant bats

4.4.1.1 Intestine

Six h pi in the 4-month-old juvenile fed 2,000 eggs, fine petechial haemorrhages, more numerous distally, were evident in the mucosa of the intestine. Larvae were found in sections taken from the distal half of intestine, being most numerous in the terminal quarter. Most were in the submucosa, with some apparently in lymphatics. These larvae were usually in zones of cellular disruption and haemorrhage heavily infiltrated with neutrophils and eosinophils. Usually, overlying villi were disrupted, with heavy cellular infiltration and loss of epithelium. Often, larvae were found in damaged villi heavily infiltrated with inflammatory cells. Neither larvae nor evidence of damage or inflammation were found in the muscle layers of the gut. In a female bat fed 3,000 eggs and examined 3 d pi, larvae were not seen in gut sections but numerous subserosal haem-

orrhages were evident, being most concentrated in the rectum. The intestines at later stages of infection were not examined histologically.

4.4.1.2 Lymph nodes

The mesenteric nodes of all bats dissected appeared normal macrocopically. Those only of the bat killed 48 h pi were sectioned. More larvae were seen in histological sections than indicated by the number recovered by saline separation. Larvae were found at all levels, from the lymph follicles to the subcapsular sinus, some in normal lymphatic tissue and others within foci of disrupted cells infiltrated with eosinophils.

4.4.1.3 Lungs

From 2 to 14 d pi, occasional subpleural petechial haemorrhages were seen in bats fed large egg doses. However, lung sections from bats at 2 and 4 d pi were normal. At 7 d pi (20,000 egg dose; 2 larvae recovered), there were numerous petechiae; in sections, red blood cells, lymphocytes and eosinophils were found in the bronchi.

4.4.1.4 Livers

Progressive changes in liver histology were studied in detail. Tissue responses varied with time, and there were individiual differences. The intensity of infection did not seem to affect the nature of focal lesions but did influence the extent of more diffuse inflammatory changes in the liver. Histologically, larvae generally were more numerous than indicated by numbers recovered in saline, but occasionally they were not found in sections of livers from which 50-100 or more had been recovered.

Macroscopic changes in the liver did not appear until 4 d pi and were well-established at 7 d pi. At 6 h pi no larvae or inflammation were observed. At 24 h (1,500 egg dose, 120 larvae recovered), one 16 μm diameter larva was found in 50 sections, surrounded by a collection of

neutrophils, lymphocytes, eosinophils and a small number of macrophages. Several adjacent hepatocytes appeared damaged with denser, more basophilic cytoplasm. At 2 d pi (20,000 eggs; 3,100 larvae), most larvae were within small inflammatory foci of neutrophils, eosinophils, lymphocytes and histiocytes. Around several portal tracts were barely perceptible infiltrates of lymphocytes. At day 3 pi (3,000 eggs; 340 larvae), small scattered foci of damaged hepatocytes with intense eosinophil infiltration were seen throughout the sections, many surrounded by a layer of grossly vacuolated hepatocytes ("hydropic degeneration"). Such foci, occasionally with larvae, were adjacent to or incorporated portal triads, and dense eosinophilic infiltration continued asymmetrically into "draining" portal tracts. Most of these foci were small, but several were extensive. In 70 sections, only 2 larvae were seen "free" in the tissues with accompanying fine eosinophilic "trails" remote from inflammatory foci. Blood in hepatic veins contained many eosinophils, and cuffing with eosinophils and lymphocytes was evident around some portal tracts.

The inflammatory pattern at 4 d pi (20,000 eggs; 7,700 larvae) was different. Numerous foci ("microabscesses") of neutrophils, eosinophils, lymphocytes and fewer histiocytes (epithelioid cells) were scattered throughout the liver. Larvae were usually within the vicinity of such foci, to which they were connected by trails of inflammatory cells. Swelling of hepatocytes at the periphery of these microabscesses was not conspicuous.

The liver at 6 d pi (1,000 eggs; 25 larvae) showed changes progressing from those in bats examined at 2 and 3 d pi. Acute microabscesses with necrotic cores and intense inflammatory infiltrates, predominantly of eosinophils, had grown in size to 400-500 µm across, with a thicker and more distinct surrounding zone of grossly vacuolated hepatocytes (Fig. 4.5 A). With apparently expanding edges and epithelioid cells, these young

FIGURE 4.5

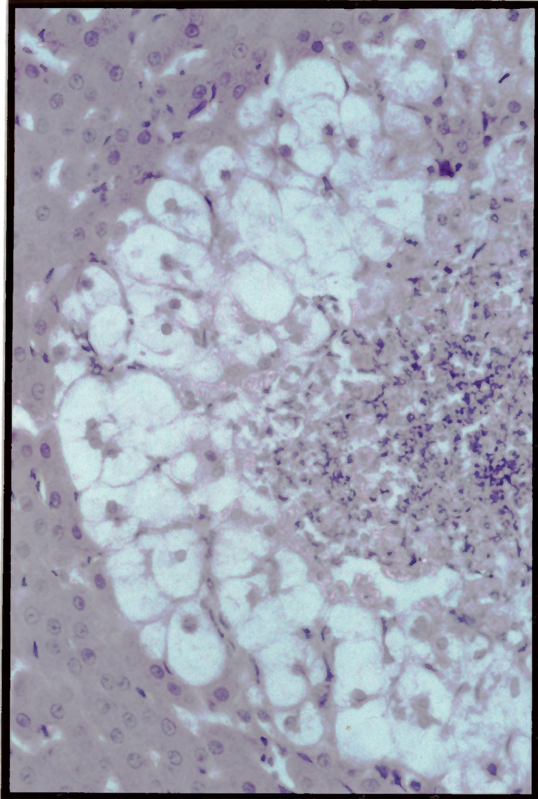
Liver histology of *P. poliocephalus* 6 days following ingestion of 1,000 *T. pteropodis* eggs

A. Zone of grossly vacuolated hepatocytes ("hydropic degeneration") at edge of eosinophilic microabscess. (magnification x 300)

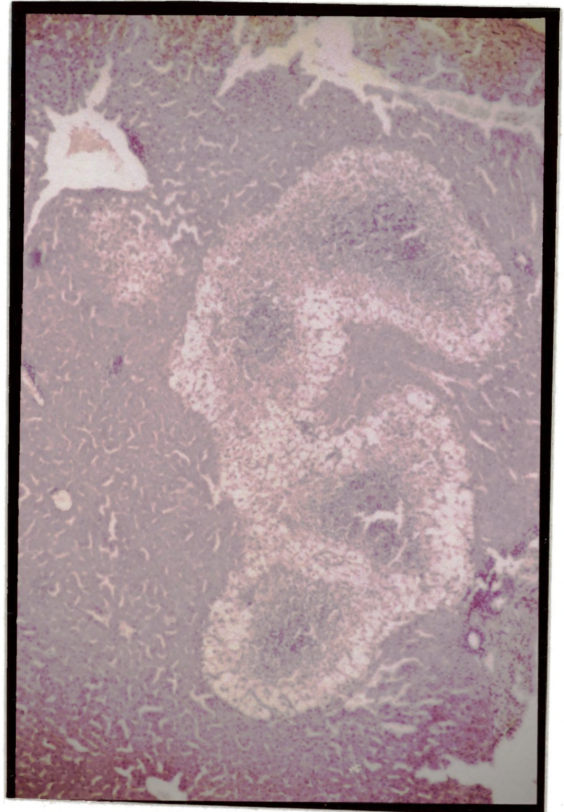
B. Coalescing microabscesses forming early stellate greanuloma. Figure A taken from lower pole of this lesion. (magnification x 60)

C. Young stellate granuloma contiguous with draining portal tract, densely infiltrated with eosinophils and lymphocytes. (magnfication x 150)

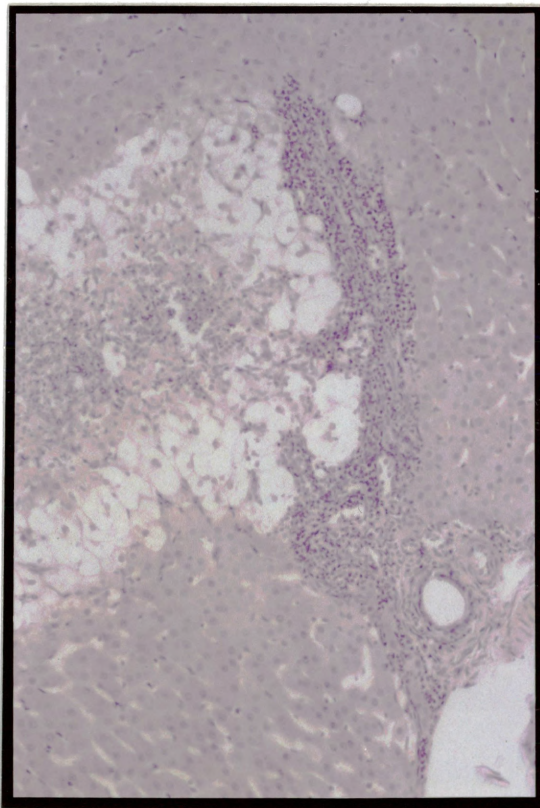
D. Confluence of portal tracts showing asymmetrical infiltration of eosinophils, lymphocytes and small numbers of epithelioid cells around bile ductules and hepatic arterioles. (magnification x 300)



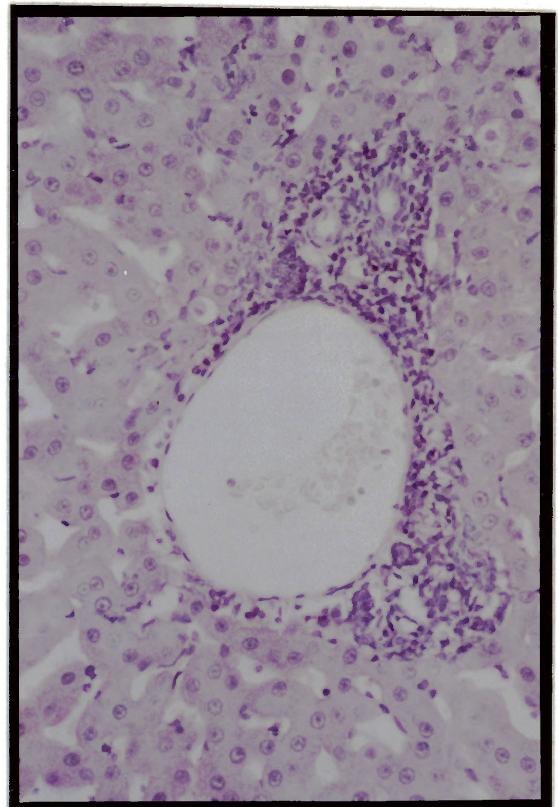
A



B



C



D

granulomas in many areas were coalescing into stellate formations (Fig. 4.5 B). When followed through serial sections, such lesions were contiguous with dense, asymmetrical eosinophil infiltration around portal tracts (Fig. 4.5 C). Despite the intensity of these focal inflammatory changes, no larvae were found in 50 serial sections examined. Focal asymmetrical periportal cuffing with eosinophils and lymphocytes was found remote from lesions throughout the sections (Fig. 4.5 D).

At 7 d pi (20,000 eggs; 12,500 larvae) the liver was densely mottled with pale puncta. The histological pattern was remarkably similar to the previous bat, despite the much heavier infection. Numerous larvae were found in sections, most adjacent to or within the young granulomas (Fig. 4.6 A). Several appeared "free", although some of these were found at the extremities of inflammatory trails. Epithelioid cells near the necrotic cores of some foci were fusing into multi-nucleated giant-cells (MNGCs) (Fig. 4.6 A) whose nuclei were dispersed either peripherally (Langhans-type) or in central clusters (foreign-body-type). In serial sections, a transition from 1 form to the other was often found. Small, scattered dense foci of eosinophils and lymphocytes were evident (Fig. 4.6 A), with asymmetrical cuffing around some portal tracts and increased numbers of eosinophils and plasma cells in the hepatic sinusoids. No larvae were found in sections of the bat killed 28 d pi (1,000 eggs; 14 larvae); liver histology was essentially normal except for many small lymphocytic foci, with fewer eosinophils, mainly centred on portal triads and occasionally associated with disrupted hepatocytes.

At day 40 (20,000 eggs; 750 larvae) the liver was finely mottled but distinct lesions were not discernible histologically. Larvae were found "free" with "trails" of eosinophils, macrophages and lymphocytes to portal tracts. A granuloma, which contained a larva, had no MNGCs in the epithelioid cell layer surrounding a necrotic core densely infiltrated with eosinophils and lymphocytes. Several more typical large granulomas were

seen, of average diameter 300 µm and up to 600 µm long, with MNGC layers around necrotic, eosinophilic, amorphous cores. These did not contain larvae, which were often found nearby as close as 80 µm. About 3 times as many larvae as granulomas were found in 100 sections. In addition, there were scattered nebulous collections of eosinophils and epithelioid cells, with occasional MNGCs, and patchy periportal infiltration.

In a bat found dead 49 d pi (dose unknown; larvae not counted), several larvae were found, some "free" without accompanying inflammation and others with trails leading to diffuse clusters of lymphocytes, eosinophils and histiocytes. No granulomas were found and the liver looked normal macroscopically. In another bat, found dead 32 weeks pi (dose unknown; 100 larvae recovered), larvae were adjacent to small granulomas which had necrotic, infiltrated cores, outer layers of hydropically vacuolated hepatocytes and intermediate layers of MNGCs. Periportal cuffing in subcapsular regions was associated with fine fibrosis and "dimpling" of the liver capsule.

In the "pseudo-pregnant" bat (Section 4.2.8), 9 months pi (dose 2,000 eggs; 250 larvae), 4 larvae in 60 serial sections were each intimately associated with granulomas smaller than those seen in earlier infections. Periportal infiltration with eosinophils and lymphocytes was also less marked. In some granulomas, MNGCs appeared to be phagocytosing eosinophils and releasing degenerating cytoplasm into the core of the lesion.

An adult male bat infected 12 months (1,000 eggs; 140 larvae) was autopsied as late as 16 h post-mortem. Many larvae were found in sections, all "free" in tissues without associated inflammation. Several granulomas were found, up to 800 µm in maximal diameter, with central, necrotic cores infiltrated with inflammatory cells, intermediate zones of epithelioid cells and outer layers of vacuolated hepatocytes.

4.4.2 Juvenile bats inoculated subcutaneously

4.4.2.1 Livers

At 30 h pi, larvae were seen within sinusoids amongst disrupted hepatocytes and with trails of neutrophils, lymphocytes and occasional eosinophils connecting to adjacent portal venules. At 5 d pi, the picture was similar, with more eosinophils and the presence of macrophages. Numerous early granulomas comprised cores of necrotic hepatocytes infiltrated with lymphocytes, epithelioid cells and few neutrophils and eosinophils, and were surrounded by grossly distended, vacuolated hepatocytes. Intense infiltration of epithelioids, polymorphs and lymphocytes had developed around adjacent portal veins. At 7 d pi, granulomas were larger and more sharply demarcated. Eosinophils were more numerous, and periportal cuffing was confluent with inflammation around granulomas. At day 10 larvae were found "free" in tissues, associated with fine inflammatory trails. Epithelioid cells within some granulomas were coalescing into MNGCs (Fig. 4.6 C) and over 50% of inflammatory cells were eosinophils. In sections, granulomas appeared to be of 2 types (described further in 4.4.3.1). The liver at 20 d pi demonstrated patchy, dense, asymmetrical periportal infiltration with eosinophils, macrophages and lymphocytes. Several MNGC granulomas were found, with outer layers of vacuolated hepatocytes. The only larva in 40 sections was not in such a lesion.

4.4.2.2 Lungs

The lungs at 30 h pi looked normal macroscopically, but larvae were found in alveoli, capillaries and in transit between these compartments, associated with septal and sometimes alveolar haemorrhages.

At day 5, subpleural petechial haemorrhages were numerous, and larvae were found within extensive consolidated zones heavily infiltrated with

FIGURE 4.6

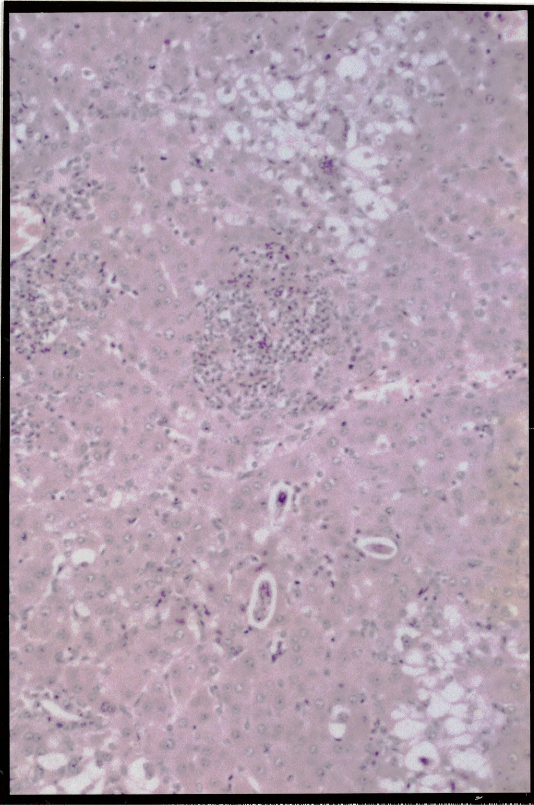
Tissue changes in _P. poliocephalus_ experimentally-infected with _T. pteropodis_

A. Liver histology 7 days post-infection in bat fed 20,000 eggs (12,500 larvae recovered) showing larva in lower half of picture amongst scattered inflammatory foci. Note young multi-nucleated giant cell within collection of vacuolated hepatocytes near top centre. (magnification x 150)

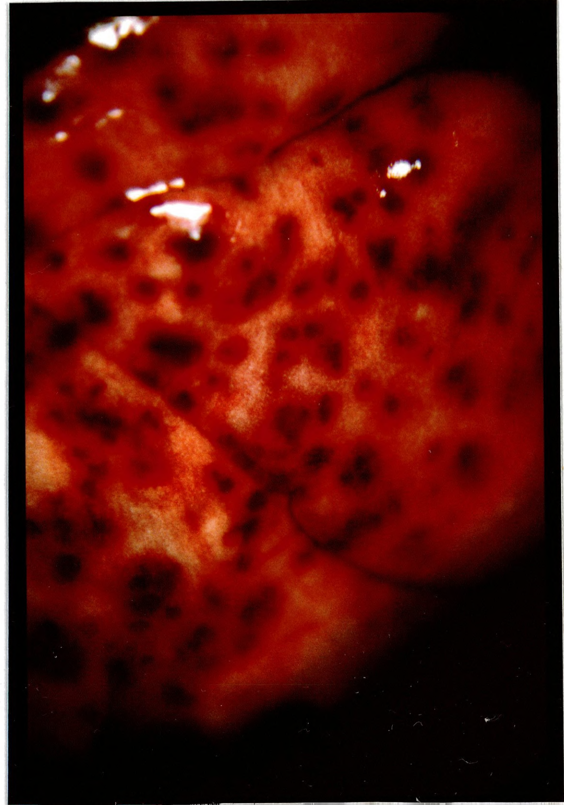
B. Macroscopic view of lung 7 days after subcutaneous injection of 5,000 eggs, showing petechial haemorrhages at different stages of evolution. Small, bright red lesions presumably are fresh, whereas larger, dark lesions are in early resolution with haem breakdown. (magnfication x 5)

C. Liver histology 10 days following subcutaneous injection of 5,000 eggs, showing bipolar granuloma. Core at upper pole of lesion is microabscess of predominantly eosinophils, surrounded by epithelioid cell layer and outer vacuolated hepatocyte layer 2-3 cells thick. Core at lower pole of liquefied, necrotic cells surrounded by coalescing MNGCs and thinner outer vacuolated hepatocyte layer. Intermediate zone, not seen here, found in serial sections. (magnfication x 60)

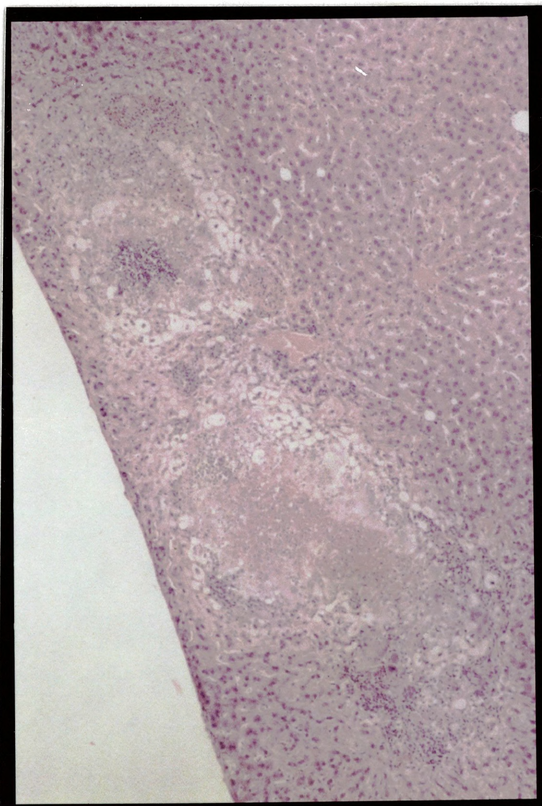
D. Liver histology of adult female 16 days post-partum, showing large bipolar granuloma. "Type 1" lesion at upper pole, and "type 2" at lower pole (see Section 4.4.3.1). (magnification x 30)



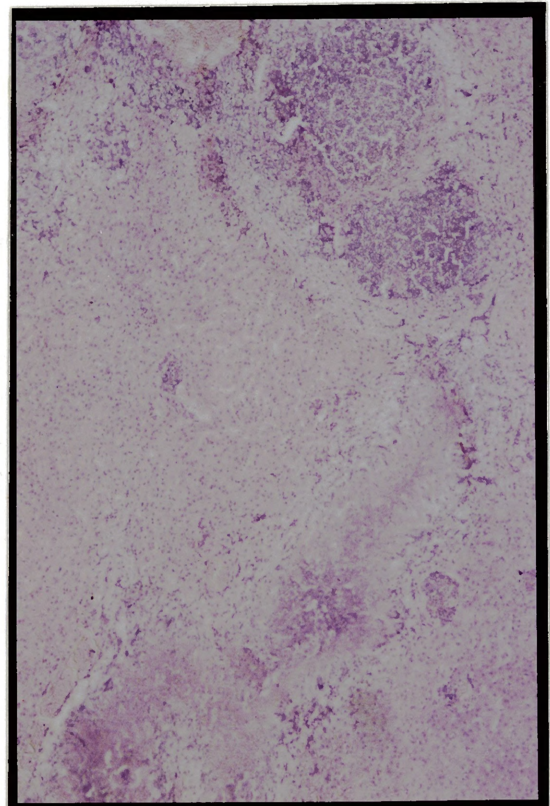
A



B



C



D

epithelioid cells, lymphocytes, neutrophils and eosinophils. Patchy, dense inflammatory infiltrates surrounded blood vessels traversing these zones, and blood and inflammatory cells were seen within bronchi. Some larvae were found in relatively normal patent alveoli. At 7 d pi (Fig. 4.6 B), patchy haemorrhagic consolidation was denser, more sharply demarcated, and heavily infiltrated with epithelioid cells and eosinophils. The cores of these lesions were necrotic, and fibroblasts had invaded the peripheries. Larvae were not found in 80 sections. The extensive petechial haemorrhages at day 10 were more diffuse and, histologically, the picture was resolving, with minimal patchy consolidation, interstitial thickening and infiltration mainly by macrophages and neutrophils. Eosinophils were numerous within blood vessels but not in the tissues. Larvae were not found in 35 sections. At 20 d pi, haemorrhages had resolved but several consolidated patches were found, densely infiltrated with eosinophils, macrophages and fibroblasts, with early collagen deposition.

4.4.3 Post-parturient females

4.4.3.1 Livers

Sections from livers of experimentally-infected post-parturient bats (Table 4.6) were examined.

At 9 d pp (egg dose unknown; 150 larvae recovered) small collections of eosinophils and lymphocytes and several inflammatory "trails" were scattered throughout the liver. A small focus of swollen hepatocytes with an inflammatory core was found "draining" into an adjacent portal tract. In 50 serial sections, only 1 larva was found, within a blood clot in the lumen of a hepatic vein.

The liver at 16 d pp (dose unknown; 1,000 larvae) demonstrated florid changes, with numerous large granulomas, up to 2 mm long, morphologically forming a spectrum between 2 extremes, here designated "types 1 & 2". All

had a core of necrotic hepatocytes, an intermediate layer of epithelioid cells and an outer layer of swollen, vacuolated hepatocytes, and were infiltrated predominantly with eosinophils. The "type 1" lesion (Figs 4.6 D & 4.7 A) had a large, occasionally haemorrhagic, core intensely infiltrated with eosinophils and a relatively thick, but often incomplete, outermost layer of vacuolated hepatocytes. The "type 2" lesion (Figs 4.6 D & 4.7 B) had a thinner outer vacuolated layer, a necrotic, eosinophilic, amorphous core, and an intermediate layer comprising a dense syncytium of MNGCs. In intermediate types of lesions, cores comprised degenerating inflammatory cells, MNGCs were sparse and small in the epithelioid cell layer, and the outer vacuolated layer thicker. In serial sections these lesions were found to occur at opposite poles of elongated, serpiginous, "bipolar" granulomas (Fig. 4.6 D). Only 2 larvae were found in 40 sections, both within type 1 lesions (Fig. 4.7 A).

The changes in the liver examined 23 days pp (dose unknown; 130 larvae) were far less spectacular. Dense eosinophil, lymphocyte and histiocyte infiltrates were present around some portal tracts, and foci of these cells surrounded by a 1-2 cell layer of vacuolated hepatocytes were scattered throughout the liver. These foci were always confluent with a periportal inflammatory cuff. Only 1 larva was seen, lying along an eosinophil "trail" connecting a portal vein to such a focus. At 6 weeks pp (dose unknown; 500 larvae) patchy eosinophilic periportal cuffing was evident, with occasional typical MNGC granulomas as found in non-parturient bats. Larvae were not found.

4.4.3.2 Mammary glands

In the first few days pp, mammary glands were poorly developed and produced little milk, but after 1 week were intensely vascularised and hypertrophied, up to 5 cm diameter and 3 mm thick (compared with about 1 mm in the non-lactating gland). Sectioning and staining of the gland fixed

FIGURE 4.7

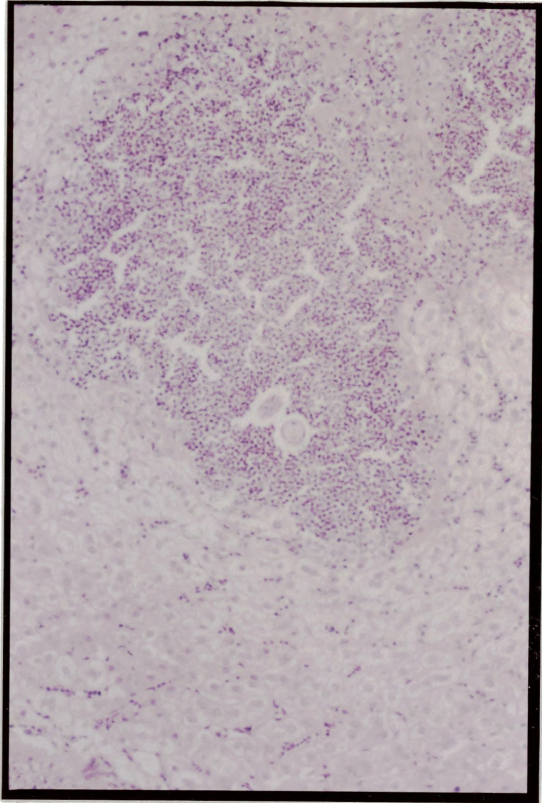
Histological findings in post-parturient adult *P. poliocephalus* infected with *T. pteropodis* and juvenile infected with *T. canis*

A. Maternal liver 16 days post-partum (same bat as in Fig. 4.6 D) showing early "type 1" lesion containing larva (centre). (magnification x 150)

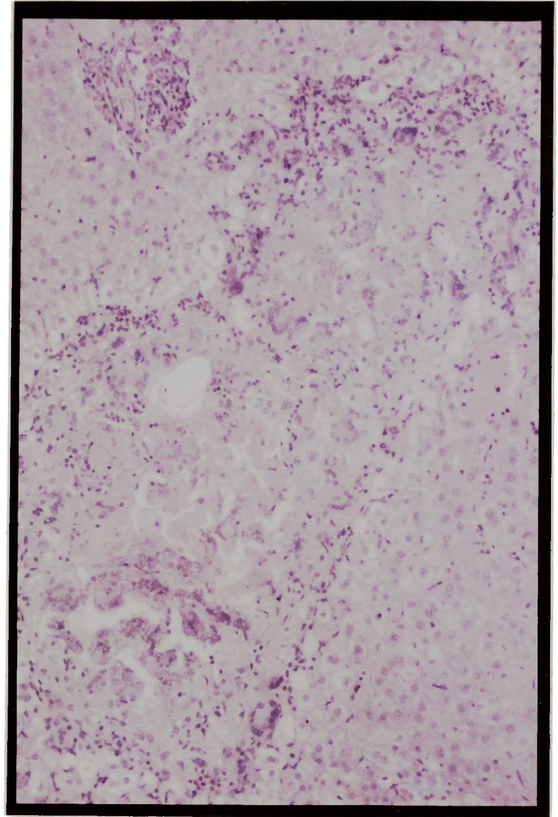
B. Same liver as in A, showing advanced "type 2" lesions with extensive intermediate MNGC layer and thin outer layer of vacuolated hepatocytes. (magnification x 150)

C. Evolving granuloma 7 days following infection with 10,000 *T. canis* eggs, showing large, necrotic core infiltrated with eosinophils and lymphoctyes, intermediate epithelioid cell layer and outer zone of vacuolated hepatocytes. Cystic lesion towards bottom is merocyst of *Hepatocystis pteropi*. (magnification x 30)

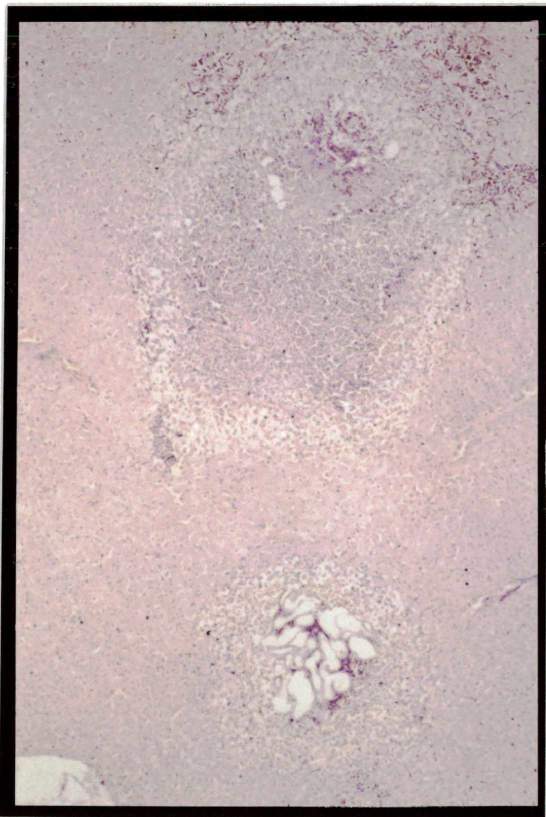
D. Granuloma in grey matter of cerebral cortex 90 days after infection with 10,000 *T. canis* eggs, containing 2 distinct types of giant-cells. (magnification x 600)



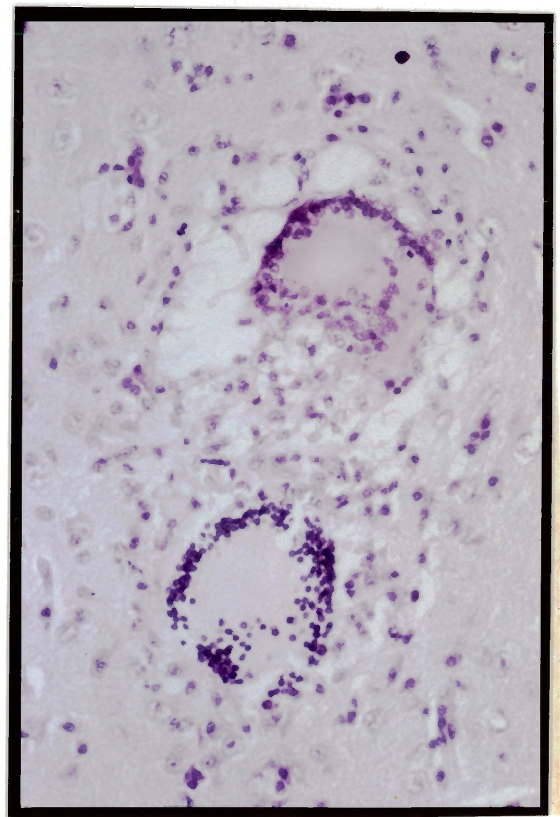
A



B



C



D

6 d pp proved unsatisfactory and impossible to interpret histologically. The gland removed 9 d pp was cut into segments which were serially sectioned at 7 µm. Every 5th slide of sections (about 6 per slide) was stained with H & E, and examined until an adequate picture was established, and 2 larvae found. At 9 d, the glandular tissue was up to 2.5 mm thick. Most lobules appeared normal, but many were segmentally infiltrated with eosinophils and lymphocytes, some quite densely. Ductules from normal lobules contained milk globules with few cells, whereas those draining infiltrated lobules contained numerous eosinophils and plasma cells. In most heavily infiltrated lobules, large eosinophilic microabscesses were found (Fig. 4.8 A & D). One lobule contained a 22 µm diameter larva within a large haemorrhagic microabscess packed with red blood cells, infiltrated peripherally with eosinophils and surrounded by compressed acini (Fig. 4.8 C & D). There was little inflammation peripherally in this lobule. Another larva, 27 µm diameter, was within a microabscess in a lobule more densely infiltrated with eosinophils, lymphocytes, plasma cells and histiocytes (Fig. 4.8 A & B). Adjacent normal lobules were noticeably less infiltrated. Several other lobules contained resolving microabscesses. These were infiltrated less with eosinophils and lymphocytes, but more with histiocytes and fibroblasts, and muliplying acinar epithelial cells apparently were replacing destroyed tissues.

The mammary gland at 16 d was thicker (3 mm), and acini more distended with milk so that the epithelium was flattened. Slide preparation was much less satisfactory and more difficult to interpret presumably owing to high lipid content, so few sections were examined. No larvae, but many resolving microabscesses, with some undergoing fibrosis, were seen.

FIGURE 4.8

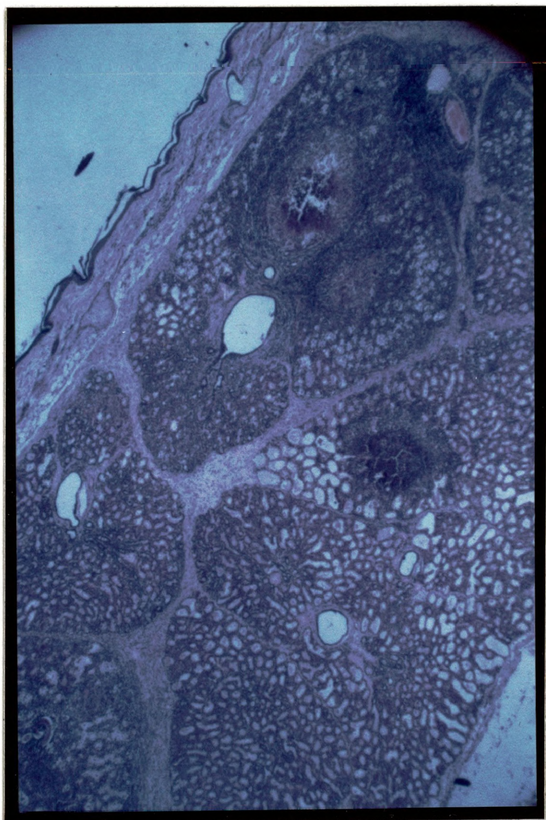
Sections of mammary gland (cut at 7 µm) of adult female *P. poliocephalus* 9 days post-partum

A. Section through full thickness of gland showing acini with central ductules and eosinophilic microabscesses at different stages. Note 27 µm diameter larva (bottom left corner) in lobule more diffusely infiltrated with eosinophils and lymphocytes.
(magnfication x 30)

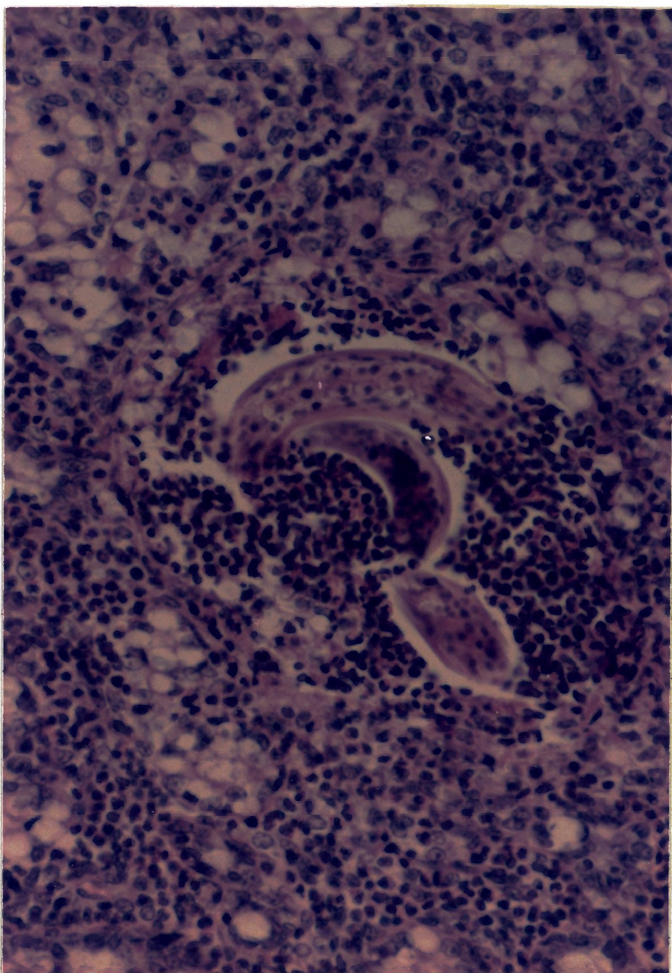
B. Same larva as in A, apparently within acinus and surrounded by intense eosinophilic inflitrate.
(magnification x 300)

C. Same larva as in D.
(magnfication x 300)

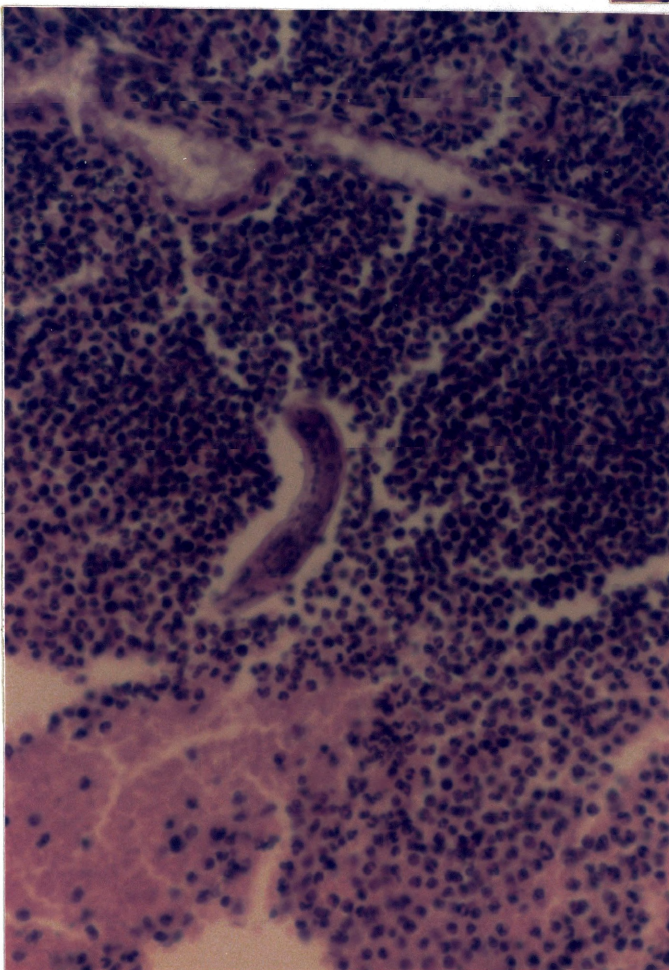
D. Acute eosinophilic microabscess with necrotic haemorrhagic core containing 22 µm diameter larva. Note minimal inflammatory infiltrate in lobule away from lesion.
(magnfication x 60)



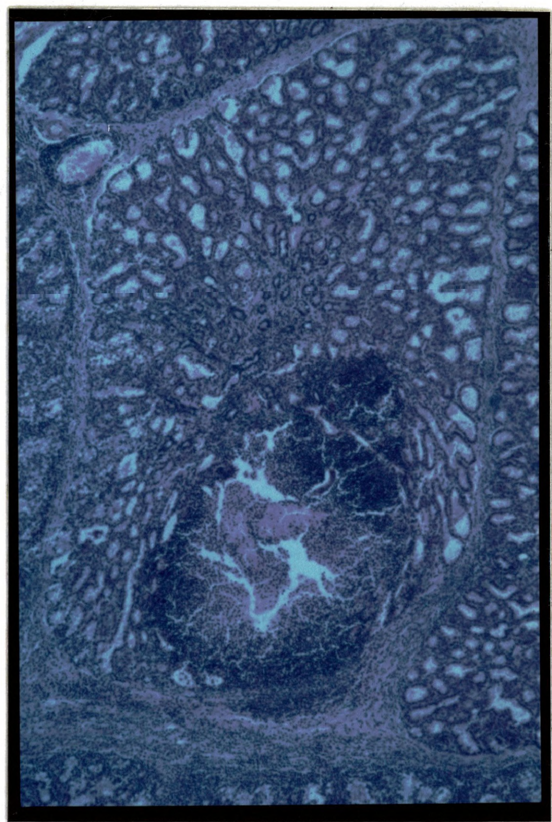
A



B



C



D

4.4.4 Suckling juveniles with intestinal infections

The livers, but not intestines, of infected suckling juveniles aged 23 and 41 d (Section 4.2.6) were sectioned. In the younger bat an increase in sinusoidal eosinophils was evident, but otherwise histology was normal. At 41 d, in addition to generalized eosinophilia, there was infiltration with eosinophils, epithelioid cells and lymphocytes around every portal tract. In some areas increased numbers of fibroblasts were present, associated with surface "dimpling" when near the capsule. No larvae, granulomas or microabscesses were found in 50 sections.

4.4.5 *T. canis* infections in juvenile bats

Larvae of *T. canis* distributed more widely in the bats' tissues than did those of *T. pteropodis* (Table 4.3). At 7 d pi, evolving hepatic granulomas resembled those in *T. pteropodis* infections (Fig. 4.7 C), with dense lymphocyte, plasma cell and eosinophil infiltrations around draining portal venules. Larvae were not seen in 50 serial sections. Several merocysts of *Hepatocystis pteropi* were found in this liver (Fig. 4.7 C). Lung sections demonstrated small, patchy alveolar and interstitial haemorrhages infiltrated with eosinophils, lymphocytes, plasma cells, macrophages and neutrophils, but larvae were not seen. In the skeletal muscle, several lymphocytic-eosinophilic perivascular foci were found, but no larvae.

At 90 d pi, occasional hepatic granulomas were found with outer layers of vacuolated hepatocytes, cores of eosinophils, lymphocytes and epithelioid cells with occasional MNGCs, and perivascular cuffing along draining portal veins. No larvae were seen. The lungs were normal except for patchy interstitial thickening and fibrosis. In skeletal muscle, eosinophils and lymphocytes had infiltrated along interfascicular planes, and scattered collections of MNGCs were found in muscle tissue connecting

with linear eosinophil infiltrates. One large granuloma, composed of a partly necrotic MNGC core surrounded by a wide, dense zone of lymphocytes and epithelioid cells, contained a larva. In the brain, larvae in both grey and white matter were mainly "free" in the tissues, but usually associated with fine inflammatory trails. Others were found beside or within granulomas comprising lymphocytes and neuroglial cells. Giant cell systems were common and of 2 distinct types, often within the same lesion. One consisted of larger, vesicular nuclei, whereas the other type had smaller, denser nuclei packed peripherally in paler cytoplasm (Fig. 4.7 D). Eosinophils were not seen in any of these neural lesions, and only occasionally in the subarachnoid space and meninges, which were infiltrated with lymphocytes.

4.5 DISCUSSION

Experimental studies generally confirmed the conclusions derived from naturally-infected bats and led to a more detailed understanding of the life-cycle.

Adult and juvenile bats were readily infected by ingesting embryonated eggs which, in view of flying fox dietary preferences, indicates it exceedingly unlikely that intermediate hosts are involved. Ingested eggs hatched throughout the intestine, but many were passed intact in the faeces. Both in the one adult female and in the juveniles most larvae penetrated the alimentary tract wall distally. The gut of *Pteropus* is one long tube, without caecum or appendix, and it is impossible without opening the gut to locate the indistinct junction between ileum and colon. The large intestine represents only 5 - 10% of the total length, and the histological appearance suggests that the entire mucosa has an absorptive function, extending almost to the anus (Tedman & Hall, 1985). Rapid intestinal transit and relatively slow hatching of *T. pteropodis* eggs

probably accounts for the distal penetration of larvae. In mice and guinea pigs (Chapter 8), most larvae also penetrate the ileum, caecum and proximal colon. Mice, considered resistant to A. suum infection, were rendered susceptible by giving morphine sulphate with an intragastric egg dose (Larsh, 1950), indicating that A. suum eggs had insufficient time to hatch in their usually rapid transit through the mouse intestine. In pigs, A. suum eggs hatch and larvae penetrate in the stomach and upper small intestine (Douvres et al., 1969) presumably because of the relatively slower passage of contents.

Histologically, larvae were most numerous in sections of the distal gut. The location of larvae in tissues must be interpreted with caution owing to post-mortem migrations (discussed in Chapter 8). The inflammation and localised tissue disruption at 6 h pi was remarkable, possibly reflecting immune pre-sensitization by the recent patent intestinal infection in that bat. Most larvae were in the submucosa, but none in the deeper muscle layer. The inflammation and damage in villi indicates that they were penetrated by larvae, either directly from the lumen or perhaps after initial entry to the submucosa via the crypts. In mice, T. canis larvae were found in normal villi by Burren (1968), with inflammation in the underlying submucosa. This may have reflected post-mortem migration.

The finding of larvae in mesenteric lymph-nodes indicates that some enter the lymphatic system, where their transit may be retarded as suggested by their presence within inflammatory infiltrates. The greater number found in sections than in "digests" may indicate destruction of some larvae in lymph nodes. Those found in the mesentery were also probably within lymph vessels, as their smaller numbers paralleled those in lymph nodes and presumably larvae in the portal vein would have passed through the mesentery quickly.

In the adult female fed 5,000 eggs over 10 days, larvae were found only in the liver, whereas in juveniles given single doses of 20,000, small

numbers were found elsewhere. Most conspicuous was the female 4 d pi from which 264 pulmonary larvae were recovered. In view of the marked propensity of larvae to accumulate in the liver, probably all of these larvae had reached the lungs via the lymphatics, a conclusion supported by the high recovery of larvae from the mesenteric nodes of this animal. It is possible that such massive egg doses cause gut mucosal inflammation and lymphatic dilatation, predisposing to larval entry into lymphatics which would not occur under natural conditions. The 4 larvae found in the kidneys and brain of this bat undoubtedly were blood-borne migrants which had traversed the lungs, and the one larva from the trachea at 2 d pi shows that occasionally this route may be used. Absence of larvae from peripheral organs at 7 d pi, when 12,500 were in the liver, may indicate that the few dispersed by the circulation traversed capillary beds and eventually arrived in the liver, or else perished in other tissues. In dogs fed _T. canis_ eggs, larvae are much less organ-specific (Noda, 1958). In cats, the distribution of _T. cati_ larvae in tissues has not been fully elucidated. In pigs fed _A. suum_ eggs, most larvae pass via the portal vein to the liver, then through the lungs to the trachea. A very small proportion penetrate mesenteric lymphatics (Roberts, 1934), and possibly reach the lungs, although Douvres et al. (1969) considered that most in lymphatics were not capable of proceeding. However, at least some _T. pteropodis_ larvae passing through lymphatics probably reach the lungs.

Eggs were inoculated subcutaneously to determine the fate of larvae should they hatch. Subcutaneous inoculation of nematode eggs has been used for vaccination. Soulsby (1957) stated, without giving supporting data, that "a minority" of _A. suum_ eggs hatch when inoculated into guinea pigs, and that few of the released larvae migrated to the lung. Such larvae may have influenced his results more than he realised; at least with _T. pteropodis_ a significant proportion of eggs hatch in the subcutaneous

depot. The tissue dispersal of these larvae indicates that, in many, hatching is delayed. This was confirmed by the finding of unhatched viable larvae at the injection site 10 d pi. Findings after subcutaneuous and intraperitoneal inoculation of T. pteropodis eggs in rats and mice are similar (Chapter 8).

After subcutaneous injection of eggs, larvae probably distribute passively via the circulation, arriving first in the lungs then other tissues in proportion to the blood supply, with the liver functioning as a very efficient final "trap". This was supported by data in Table 4.2. As early as 30 h pi, over 2% of the dose was in the liver, although a larger number was negotiating the lungs. A small proportion of those reaching the lungs underwent tracheal migration, while the vast majority followed the "somatic" route, as indicated by larvae in brain and kidneys. Skeletal muscle was not sampled in view of its bulk and earlier findings that significant numbers of larvae do not enter this tissue, but undoubtedly larvae in small numbers would have been found there if sought. It is not possible to conclude whether larvae in "distant" organs such as brain and kidney are arrested or continue recirculating until finally lodging in the liver. The onset of immunity may retard larvae in their transit through various tissues, as occurs with T. canis in the livers of immunized mice (Brindley et al., 1985). Larvae found in the gastro-intestinal tract may have arrived via either the tracheal or circulatory route. Considerable numbers were already present throughout the tract at 30 h pi, mainly distally, and more were found in the wall than in the lumen. Probably these had travelled via the circulation, as such large numbers are unlikely to have arrived via tracheal migration and penetrated the mucosa so rapidly. Furthermore tracheo-oesophageal migrants presumably would have penetrated the mucosa more proximally, as they would not be delayed by having to hatch from eggs as occurs in oral infections.

Apportioning larvae to gut "wall" and "contents" may be misleading. Numerous sloughed villi were found in gut washings, so larvae in these would be included in "contents". Larvae in crypts, but not yet within the mucosa, are unlikely to be washed out by rinsing and would be counted as "wall" larvae. Bias in favour of "contents" numbers would be more likely following sc inoculation, with circulating larvae traversing villous capillaries, whereas the opposite would occur in oral infections with larvae penetrating from the lumen enmeshed in crypts and between villi. Furthermore, post-mortem efflux of larvae from gut wall into lumen may have contributed to the higher numbers in the distal gut contents, which were processed a significant time after the proximal gut.

Larvae commenced growing soon after hatching (Table 4.4). At 7 d after sc injection, larvae recovered from the trachea were longer than those in lungs, indicating a delayed passage from capillaries to airways. The shorter lengths of larvae in the brain and kidneys, although a small sample, supports the assumption that they had traversed the lungs more rapidly. Likewise, the length difference between pulmonary and hepatic larvae on day 10 (liver larvae on day 7 were not measured) suggests those in the lungs were later hatchlings.

Pulmonary transit was traumatic, but before day 7 petechial haemorrhages were far fewer than larvae recovered, indicating that not all these larvae had ruptured capillaries. Perhaps only those perforating alveolar walls caused haemorrhage. Histologically, most larvae were found within haemorrhages, but many were also seen within relatively normal alveoli (these may have migrated post-mortem). By 20 d after sc injection, virtually all larvae had reached the liver, except for a few stragglers in the lungs and gut wall.

The hepatic numbers from day 5 onwards (5 - 15% of egg dose, Table 4.2) were comparable with those in orally-infected bats (12.5% mean, Section 4.2.5). Errors in counting larvae and fluctuating intestinal hatch

rates could have accounted for some variation in hepatic larval recovery (Table 4.5), but the major factor may have been difficulty in assessing egg "infectivity". Larvae of T.pteropodis were very sluggish within eggs, yet motility was the only reliable criterion of viability (see Section 5.9.2). Many eggs containing apparently normal larvae were counted as infective, when such larvae may have been dead. Alternately, infective larvae may have been considered non-viable if not moving over several minutes' observation. Expressing the larvae from eggs often did not help resolve this difficulty, and interfered with dosage calculations.

Larvae commenced growing within 2 days of hatching and continued steadily for about 2 months, then slowed markedly but continued for up to 27 months. Despite variations in mean larval lengths, in each bat the length distribution was approximately normal and the length range was of the order 100-200 µm, increasing with duration of infection. Mean length differences at the same stage of infection may have been real or resulted from fixation technique. Several small studies (e.g. Section 7.1.2.1) confirmed that there was not much difference between fixation in cold or hot formalin, whereas live larvae immobilized in chlorolactophenol were longer. As larval lengths were not significantly altered by hot or cold fixation, or by time kept in formalin, it must be assumed that these variations were real. Egg doses, numbers of larvae recovered, and size and sex of bat may be influential, but no correlations were found.

Immunity retards the growth of A. suum larvae in pre-sensitized guinea pigs (Soulsby, 1961) and pigs (Kelly & Nayak, 1964). Crandall & Arean (1964) showed that A. suum larvae within diffusion chambers in the peritoneal cavities of immune and non-immune mice survived equally well, but failed to grow in immune mice. All these studies on A. suum larvae in immunized hosts were carried out over several weeks. Larvae of T. pteropodis grow more slowly, which would allow time for immunity to develop

in the infected bat. Many larvae measured were from juveniles not exposed previously to hepatic infection, although an earlier gut infection may have induced resistance to larvae in tissues. In mice, _T. pteropodis_ larvae also grow but not as long as in bat livers (Chapter 8). Immunizing mice with low-dose oral infections, and immunosuppressing mice with steroids and cyclophosphamide, did not affect larval growth (Prociv & Brindley, unpublished).

Neither the larvae of _T. canis_ nor _T. cati_ grow in the tissues of paratenic hosts or the somatic sites of their definitive hosts (Sprent, 1956; 1957; Noda, 1958; Appendix VII). Larvae of several other ascaridoids, such as _Baylisascaris procyonis_ and _B. columnaris_ do grow in rodents and other paratenic hosts (Tiner, 1953; Sprent, 1953; Kazacos et al., 1981), which possibly are true intermediate hosts for these nematodes (Sprent, 1953). _T. pteropodis_ does not utilize intermediate hosts, yet its larvae do grow in the tissues of mice and bats. Furthermore, _T. pteropodis_ larvae in bat lungs and livers and mouse livers, fed to mice, again infected livers (Appendices IV & V). This "paratenic tendency" also was demonstrated in neonatal bats fed live larvae from an adult bat liver (Section 4.2.8) in an attempt to establish patent infection. Perhaps their artificial milk diet was unfavourable for larval development in the gut, but more likely the larvae are "programmed" to develop in the gut only after passage through the lactating mammary gland.

The migration of _T. canis_ in bats was similar to that in mice and guinea pigs (Brindley et al., 1985; Appendix VII). Larvae passed through the liver to lungs and other tissues, accumulating ultimately in skeletal muscle and brain. Newly-hatched larvae of _T. pteropodis_ and _T. canis_ are virtually identical in size (Chapter 6), so that the hepatic arrest of _T. pteropodis_ cannot be attributed to their dimensions. Their accumulation in the liver after parenteral infection demonstrates an ability to pass through the pulmonary capillaries. This marked hepatotropism of _T._

pteropodis is therefore not determined by larval size. While larval "migration" through the circulation is passive, recognition of liver tissue and arrest in the sinusoids reflect goal-directed behaviour. The small numbers undergoing tracheal migration after oral or sc infection may have been capable of penetrating the gut mucosa, but their numbers were negligible.

Larvae recovered from neonatal bats fed larvae from the liver of an adult female infected 8 months previously (Section 4.2.8) tended to be smaller than those in the female liver. Perhaps only the shorter larvae were able to penetrate the gut, following which they commenced growing again in the neonatal liver. Were this the case, then the shorter larvae in livers infected long-term (Table 4.5) may have been "retarded", perhaps being suppressed by host immunity or inhibitory secretions from other larvae. Release from such inhibition could allow further growth. This would mean that in an adult female given only a single egg dose, the mean length of hepatic larvae should increase with each succeeding pregnancy. Although this would be difficult to test, the captive bat with offspring in two consecutive years (adult female, 24 months p.i., Table 4.5) did in fact harbour some of the longest hepatic larvae. However, this simply may have reflected the duration of her infection.

Studies in infected mother-young pairs gave a clearer indication of intestinal larval growth and confirmed that not all hepatic larvae pass through the mammary glands post-partum. The shortest larvae from pups at 6, 9 and 16 d were all about the same length, indicating that they had all been in the intestine for about the same period of time and that therefore transmammary passage had continued over at least 10 days (6 - 16 d pp). Presumably the 2 mammary larvae at 16 d were capable of passing into the milk. The absence of mammary larvae at 23 d pp is inconclusive. This bat's pup had only one intestinal larva, yet the mother still harboured 130

hepatic larvae. The impression from the mammary histology (at 9 d pp) was that more larvae were present than recovered by saline separation, so perhaps some larvae had not been detected. However, the gut larva at day 23 was already 2.83 mm, indicating its presence there for a considerable time. The single larva found in the mammary sediment at 41 d was probably a contaminant, in view of its length and the development of the respective pup's intestinal larvae. That occasional larvae may arrive in the mammaries before birth is suggested by a single finding (pregnant bat 2-21, Table 3.3, with a 1.39 mm larva), but most do so in the first 2 weeks. Transit may be rapid, allowing little growth before reaching the pup's intestine (e.g. 810 µm gut larva, bat 1-49, Table 3.5), while in others delay may allow growth in the gland (e.g. bat 2-21, Table 3.3; bat 2-19, Table 3.4; bat 16 d pp, Table 4.6). The largest mammary larva was 1.39 mm long. It is not possible to determine whether such large larvae are capable of passing into the milk. In the mammary gland at day 9, the lobule containing the smaller larva was less infiltrated with inflammatory cells than that with the larger larva. Had both larvae arrived in the gland at the same stage of development, the larger one must have been present longer, to account for its size and cellular response. Almost certainly the resolving microabscesses in glands at 9 and 16 d pp represented tissue damage by larvae that had traversed. The variable inflammation may have reflected either time elapsed since larval transit or the speed of larval transit from arterioles into milk ducts.

The florid histological findings in the livers of post-parturient bats, very similar to those in juveniles inoculated sc, may indicate that all hepatic larvae are mobilized about the time of parturition, releasing their "hold" in the liver temporarily and then "re-settling" to initiate fresh acute inlammatory responses. Their circulation through tissues would be governed by relative blood flows, so more larvae would return to the liver than pass through the mammary glands. Others may simple "burrow

around" in the liver without entering a hepatic venule in which to escape. The larva found within the hepatic vein lumen of the mother bat 9 d pp may have just entered the circulation, but it could also have migrated post-mortem. Larvae which returned to the liver (or perhaps had never left it) could survive another year or longer to infect subsequent offspring.

In dogs, maternal transmission of T. canis is usually transplacental, and larvae "stored" in maternal tissues invade the foetus after the 42nd day of gestation (Douglas & Baker, 1965). Larvae from eggs fed to the dam during pregnancy take at least 2 weeks to reach the foetus, again only after the 42nd day of gestation. A few larvae from eggs fed at the time of conception undergo transmammary transmission, being found in the milk from 5 to 13 d pp (Stoye, 1976). Infection of the bitch at mid-pregnancy with 10,000 T. canis eggs resulted in the passage of 29% of the dose as larvae to pups, less than 5% of these through the milk (Burke & Roberson, 1985b). Infections in later pregnancy led to an increasing proportion of larvae passing through the milk (Stoye, 1976; Burke & Roberson, 1985b). Larvae appeared in the milk at 4 d pp, peaked to 200/day in the 2nd week and continued until 20 d pp. Larvae in the bitch probably are "stored" mainly in the kidneys, liver and skeletal muscle (Noda, 1958; Griesemer et al., 1963). This is supported by histological findings in naturally-infected dogs of larvae in many tissues, particularly kidneys, lungs, livers and skeletal muscle (Barron & Saunders, 1966). Tissue larvae of T. canis in adult dogs do not grow (Noda, 1958; Sprent, 1958), unlike those of T. pteropodis in bat livers. In in vitro culture, T. canis larvae likewise do not increase in length (Dr W. Nicholas, pers. comm.) whereas those of T. pteropodis do grow (Dr J.W. Mak, pers. comm.). However, following transplacental migration of T. canis to the foetal liver, it appears that rapid growth commences. Augustine (1927) found T. canis larvae 0.7 - 0.9 mm long in the liver of a newborn pup whose dam had been infected 33 d

previously. The growth rate of these larvae would have exceeded that of T. pteropodis in bats, which at 33 d pi averaged 550 µm (Fig. 4.1). Furthermore, T. canis continue to grow rapidly on passage through the neonatal lung (Sprent, 1958; Webster, 1958).

Thus, with T. canis in dogs, it appears that physiological changes about mid-pregnancy trigger not only larval migration from tissue repositories, but also rapid larval growth. These larvae have a predilection to "disembark" in the placenta and, to a lesser extent, the mammary glands. The greater uptake by the placenta may simply reflect its relative blood supply. Larvae from infections acquired in later pregnancy also are "trapped" preferentially in the placenta but increasingly in the mammaries as well, which presumably become hypervascularised towards term. It may be that larval egress in milk is purely "accidental". Larvae entering the mammaries can be detected in milk or the pup's intestine, whereas, those in other tissues remain "captive" within the host, and circulate until they finally bed down in tissue stores. If larvae remain in such a state of flux for a certain period after infection, then the nearer to parturition that eggs are fed to the bitch, the more larvae will be found in the milk. Post-partum, larvae have no choice but to invade mammary glands or return to tissue stores, where a considerable proportion is retained, as subsequent litters also develop patent infections (Douglas & Baker, 1965). These residual larvae do not grow in the tissues of the bitch, so entry to the foetus may be a prerequisite for growth. It is possible that T. canis larvae commence growth in the mammary glands, but as at this stage they would be much shorter than those in foetal livers, there may still be a need for the tracheal migration which occurs in pre-natally infected pups to enable larvae to mature further prior to establishing in the gut.

In bats, T. pteropodis larvae arrive in the mammary glands shortly before, during or after parturition. Some move rapidly into the milk to

infect the pup within 2 d of birth (bat 1.49, Tables 3.3 and 3.4), while others may pass as late as 16 d pp. If all larvae grew at the same rate, the relative length differences should diminish with time, i.e. the range and standard deviations of lengths in proportion to mean length should grow smaller. The dispersal pattern (Fig. 4.2) showed the reverse to occur. This pattern may have been biased by the spontaneous loss of some shorter larvae, or by overlooking some small larvae, which were more difficult to find, but such errors would have narrowed the dispersal range. Previously (Table 3.4), female worms were shown to grow faster than males and in early infections, equal numbers of males and females were found. However, in patent infections, females predominated, e.g. 77 mature female and 38 male worms were recovered from naturally-infected bats (Section 3.2.4), and the average worm burden in naturally-infected juveniles fell from 6.9 in pre-patent infections to 3.2 in patent infections (Section 3.2.3). Hence, more males are lost than females. Once several mature, the remainder are inhibited and voided in the faeces, so that females are selected for survival by their faster growth. This predominance of females in patent infections occurs with other ascaridoids. Noda (1956) found that *T. canis* larvae in pre-natally infected pups were of comparable lengths until 15 d pp, after which females started to grow longer than male larvae. From dogs younger than 38 d, he recovered 356 female and 377 male worms. In older dogs, he found 140 females and 97 males. Furthermore, in older dogs he often found a population of smaller larvae in the presence of mature worms, which he concluded were post-natally acquired; in fact, they may have been retarded larvae from the original brood. Greve (1971) reported the spontaneous loss of larvae from the intestines of experimentally-infected pups, and showed that female larvae grew faster than males. Refuerzo & Albis-Jimenez (1954) found 32 adult female and 20 male *T. vitulorum* spontaneously expelled by cattle calves, and 172 females and 85 males voided by buffalo calves. Shoho (1970) reported a 38-d-old

calf spontaneously passing 22 female and 8 male *T. vitulorum*. Jung (1954), in 7 cases of massive infection with *A. lumbricoides*, recovered a total of 665 female and 417 male worms. These patients all lived in areas of continuing *Ascaris* transmission, yet all their worms were at the same stage of development. Jung concluded that "new broods of larvae fail to mature until the older worms are almost totally eliminated", suggesting that the presence of adult worms inhibited the development of larvae. Beaver (1952) observed a similar pattern with *A. suum* in pigs.

The mechanisms involved in suppressing development and perhaps leading to the expulsion of immature ascaridoids are not clear. Similar phenomena are well known with trichostrongyle infections in domesticated animals and have been the subject of intensive research. One possibility is that larger or adult worms release inhibitory substances which retard development in less mature forms. This was first suggested by Bremner (1956), but has been largely ignored, even though pheromones are coming to be recognised as of great importance in nematode behaviour and perhaps development (Bone, 1982a,b). In her studies of thelastomid (oxyuroid) nematodes in cockroaches, Zervos (1986) found that, regardless of the egg dose administered, at the time of patency the cockroach harboured a single male and only 1 or 2 females. She concluded that "in cockroaches, infrapopulations are not regulated by host immune responses ... [but] a density- and sex-dependent parasite-mediated chemical interference competition reduces infection intensity". This type of feedback inhibition could explain many of the examples considered by Michel (1974) in his comprehensive review of arrested development in nematodes.

Although in many cases endocrine and seasonal factors undoubtedly influence the maturation of "arrested" nematode larvae in the intestine, the most widely held views implicate the immune response. The immunology of gastrointestinal nematode infections is complex and poorly understood,

and it seems that each host-parasite system studied has its unique features. With the trichostrongylids in sheep, cattle and other herbivores, the immune response to a large larval load can expel adult worms from the intestine (Michel, 1974; Wakelin, 1978). In mice, adult Trichuris muris and Nematospiroides dubius are resistant to the host immune response whereas larvae are damaged and expelled (Miller, 1986). A similar pattern to this may occur in ascaridoid infections (see Discussion, Chapter 5). However, a massive dose of A. suum eggs fed to pigs precipitates such an aggressive immune response to migrating larvae that they are all lost through the faeces, sometimes without successfully establishing a patent infection (Schwartz, 1959; Douvres et al., 1969). In piglets fed lower doses of eggs, the number of adult worms establishing is inversely related to the dose (Roneus, 1971; Andersen et al., 1973). With high doses, larvae appeared in the faeces as early as 7 d pi and elimination in some pigs continued beyond patency at 51 d (Schwartz, 1959). In massive A. suum infections, the piglets' diet may influence the successful establishment of adult worms, and milk may be unfavourable (Kelley et al., 1958). These massive-dose experiments probably have little relevance to natural situations, where pigs are exposed to continuous low doses of eggs (Andersen et al., 1973) and it is usual to find small numbers of adults of the same brood (Beaver, 1952).

Ascariasis is more prevalent in children than in adults (Beaver, 1952; Spillman, 1975; Martin et al., 1983), which may reflect age-resistance or exposure-risk. In areas of high prevalence and continuing exposure, average A. lumbricoides burdens are low, indicating resistance to re-infection, yet a high level of tolerance is reflected by the paucity of acute symptoms (Spillman, 1975). Studies of T. canis in dogs (Noda, 1956; Greve, 1971), already discussed, also indicate that adverse gut factors may inhibit the development of larvae and facilitate their elimination.

There is no tissue phase with T. pteropodis in juvenile bats, so immunity may take longer to develop, although colostrum from the infected mother may contain specific antibodies and other factors which influence the immune response. As T. pteropodis inhabits the intestine over a considerable period, developing larvae which have not matured before critical dietary, physiological or immunological changes ensue may be incapable of further growth and are expelled; the resistant adult worms survive and reproduce. Some of these metabolic and physiological changes in the intestine may be induced by the worm (Castro, 1982; Mettrick, 1986).

Despite numerous reports of infections in "abnormal" experimental mammals, few systematic studies have been published on the haematological and histological changes induced by ascaridoids in their definitive hosts. Furthermore, other ascaridoid larvae either pass rapidly through tissues (e.g. A. suum in pigs) or are widely dispersed in the organ systems of large hosts, such as T. canis in dogs and T. cati in cats. Changes in other experimental animals, to be seen in perspective, must be compared with responses in the normal definitive host.

There is no baseline data on the "normal" haematological profile of Australian flying foxes. Lewis (1977), in a limited study of adult Pteropus giganteus, found high red cell numbers ($7 - 10 \times 10^6/mm^3$), high average leukocyte counts ($7.1 - 27.0 \times 10^3/mm^3$) and no eosinophils. The mean haemoglobin was 13.4 ± 2.2 g/dl (range 11.8 - 16.7). Haemoglobins and red cell counts in the present study were similar, and were not influenced by T. pteropodis infection. It was impossible to find bats known to be non-infected to determine "normal" white cell counts; even pups harbouring gut larvae manifested blood eosinophilia (Table 4.8). The only bat without circulating eosinophils was a 4-month-old female which had spontaneously passed gut worms 10 d previously (Table 4.7). Perhaps all these animals had other unidentified infections causing blood eosinophilia, but this seems unlikely in the experimental neonates (Table 4.8).

Hepatic infection with _T. pteropodis_ larvae elicited a total white cell and eosinophil response (Fig. 4.4). The rise in eosinophils in the 1st week, a single or double peak over the next 2 weeks and then a gradual decline in levels, without a return to the baseline, is typical of patterns seen in many animals acutely infected with various ascaridoid species, including dogs infected with _T. canis_ (Noda, 1958; Greve, 1971) and pigs with _A. suum_ (Roneus, 1971). Noda (1958) fed high doses of _T. canis_ eggs to dogs aged 40 d - 2 years, which may have been naturally-infected, and observed an initial slight fall in peripheral eosinophils followed by a peak at one week, with considerable individual variation. The intravenous administration of _T. canis_ larvae to helminth-free beagles produced eosinophil peaks at 18 - 28 days, depending on the dose, with lower peaks in older dogs (Oshima, 1976). This type of eosinophil response implies an immunological reaction, as increased eosinophil production and mobilization largely depends on the activation of lymphocytes and macrophages (Weller & Goetzl, 1979).

Blood eosinophilia in humans occurs in a variety of clinical conditions of which a major group is tissue helminthic infections (Beeson, 1980). The eosinophil is a major effector cell in the destruction of larval helminths in tissues, such as _T. spiralis_ and _Schistosoma_ (Gleich & Loegering, 1984). If hepatic larvae of _T. pteropodis_ are present in virtually all adult male _P. poliocephalus_ then persistent eosinophilia should be a common feature of wild populations, as was shown by the adult male group 9 months pi (Table 4.7). However, the non-infected adult females, caught as suckling juveniles, also manifested blood eosinophilia, albeit at lower levels. They were unlikely to harbour significant numbers of _T. pteropodis_ larvae, so there must be another explanation for their high eosinophil counts. Similarly, the high baseline (day 0) eosinophilia in female 1 (Fig. 4.4), free of intestinal worms, requires explanation.

Perhaps this bat had been infected earlier with gut larvae which it passed spontaneously. The other 2 juveniles in that experiment had levels below 300/mm^3, which in humans (Beeson, 1983), pigs (Roneus, 1971), dogs (Greve, 1971) and mice (Wakelin & Donachie, 1983), would be considered in the normal range.

The decline of circulating eosinophil levels in the presence of persisting hepatic infection in bats indicates the attainment of dynamic equilibrium or a degree of tolerance. In tissue sections, intense eosinophilia was found in all late infections, so the lower blood levels more likely reflect a balance between continuing bone marrow production, blood transport and tissue destruction of eosinophils. The circulating half-life of eosinophils in the rat is 8 - 12 hours, and in the human about 2 hours (Weller & Goetzl, 1979); in bats it is likely be of the same order. Augmented marrow production is required for continuing raised blood levels. In pigs fed *A. suum* eggs, after the initial peak, circulating eosinophil levels return to their baseline at about 30 d pi (Roneus, 1971), reflecting the clearance of larvae from liver and lungs. Eosinophilia was not prolonged in pigs which developed patent gut infections. In guinea pigs infected with larvae of *T. canis* and *T. cati* which remain viable indefinitely, blood eosinophil levels persist above normal, but in those infected with *T. pteropodis* larvae, which perish within 6 weeks pi, eosinophil levels gradually return to normal (Appendix VII). In bats the levels declined, but did not return to normal.

It is generally assumed that eosinophilia is a feature of tissue infections with metazoan parasites, but not when helminths are confined to the gut lumen (Beeson, 1980); yet, in infected neonatal bats (Fig. 4.3), a transient peripheral eosinophilic response corresponded with the development of *T. pteropodis* larvae in the intestine. Tissue invasion by these larvae was not found and there was no indication of temporary development in the gut wall, as occurs with *Toxascaris leonina* (Sprent,

1959). Weller & Goetzl (1979) concluded that tissue immobilization of particulate antigens stimulated T-lymphocyte-mediated eosinophil responses both locally and systemically. This is consistent with the eosinophilic response in mice to *N. dubius*, which undergoes larval development in the intestinal wall. However, soluble *Limulus* haemocyanin administered in Freund's complete adjuvant to cyclophosphamide-treated mice (to inhibit suppressor T-cells) also elicited a marked eosinophilic response in mice (Wakelin & Donachie, 1983), indicating that soluble antigens could stimulate eosinophilia. Perhaps antigens secreted by developing *T. pteropodis* in young *P. poliocephalus* are absorbed through the gut to evoke the response. This would also explain the diffuse interstitial eosinophilic infiltrates in the livers of the juveniles 23 and 41 d old (Section 4.4.4). Once the nematodes matured, either a reduction in their secretions, development of host tolerance or diminished gut "permeability" to soluble antigens led to involution of the eosinophilia. Oshima (1976) did not detect eosinophilia in helminth-free beagles fed adult *T. canis* to produce patent infection.

Alternatively, antigens or other factors in mothers' milk, absorbed without inactivation by the pups, may have been responsible for the juvenile eosinophilic responses, which remarkably paralleled those in their mothers, albeit at lower levels. Higher numbers of eosinophils are found in the blood and inflammatory exudates of neonatal animals (Beeson, 1980), perhaps as a result of a graft- versus-host or other immune reactions. In the infected flying fox pairs the close correlation between eosinophilia in mothers and pups suggests a response to larval migration. The maternal eosinophilia may have been a response to re-activated hepatic larvae, or a manifestation of transiently suppressed host tolerance of these larvae. However, uninfected control animals were not studied so this may be a normal phenomenon in the puerperium and completely unrelated to *T.*

pteropodis.

The histological response of the bat liver to *T. pteropodis* larvae cannot be compared with other ascaridoid infections in definitive hosts. Barron & Saunders (1966) reported incidental findings in dogs with infections of indeterminate duration. Most *T. canis* larvae were found in kidneys perhaps because there they were associated with macroscopic lesions, whereas in other tissues granulomas were found unexpectedly. In ascarid-naive dogs inoculated subcutaneously with *T. canis* larvae, Greve (1971) described the hepatic lesions as miliary foci which were "typical verminous granulomas", many of which lacked larvae. In ascarid-naive pigs fed *A. suum* eggs, larval passage through livers produced tracks of cellular disruption and inflammation which gradually resolved, leaving "small granulomatous foci" in interlobular septa, with some MNGCs (Copeman, 1971). Secondary infections elicited more intense and diffuse inflammatory reactions, with focal infiltrates of eosinophils, histiocytes, lymphocytes and MNGCs. Repair and resolution were associated with interstitial and capsular fibrosis. Copeman concluded that the primary infection elicited a response to mechanical damage, whereas the secondary infection stimulated a hypersensitivity reaction. In secondary infections, Kelly & Nayak (1964) found arrested larvae in pig liver associated with "chronic focal interstitial hepatitis".

Hepatic *T. pteropodis* infections offer a unique opportunity to monitor the evolution and perhaps resolution of granulomatous reactions to persisting larvae. Changes in the first few days were confined to the entry paths of larvae into the liver, characterised by foci of damaged hepatocytes lightly infiltrated with neutrophils, lymphocytes and macrophages. Eosinophils, scarce in the first 2 days, rapidly became the dominant cell type. By 3 d pi, eosinophils and other cells were forming dense, discrete foci, and trailed from these lesions to infiltrates along, but not encircling, adjacent portal veins. This distribution, easily

attributed to "larval tracks", possibly represents infiltration around lymphatics draining antigens released by larvae. These aggregations continued asymmetrically around portal veins of increasing size. The other drainage route for larval antigens, the hepatic sinusoids and venules, probably allows greater diffusion so that the inflammatory response has a widely dispersed component. Such periportal infiltrates were found in almost all infected bats, with time becoming less intense and highly focal. In some livers from which larvae were recovered, but in sections of which neither larvae nor typical granulomata were found, these asymmetrical perivascular infiltrates of eosinophils, lymphocytes and epithelioid cells were readily evident.

By 3 d pi, the hepatocyte layer surrounding inflammatory foci was grossly pale and swollen, although the nuclei were normal. This outer layer of "hydropically degenerate" hepatocytes, which resembled the ballooned hepatocytes of acute viral hepatitis (Robbins et al., 1984b), was maximally developed around granulomas by 7 d pi but was still present in the liver at 12 months. This vacuolar swelling is probably a reaction to larval products. Staining of several sections with periodic acid Schiff's reagent (PAS) showed no glycogen in these hepatocytes. By the time these sections were examined microscopically, no wet-fixed tissues were available (all had been blocked in wax) so it was impossible to stain for lipid. However, the features were similar to those of fatty "degeneration" (Robbins et al. 1984a) and possibly these hepatocytes had been distended with lipid.

The hallmark of the granuloma is the distinct aggregation of macrophages and their derived epithelioid cells and MNGCs (Unanue, 1978). Distinct granulomas had developed by 6 d pi, and MNGCs were evident at day 7. As monocytes move from the periphery to the centre of granulomas, they become highly activated macrophages (epithelioid cells) (Unanue, 1978).

Their cytoplasm contains more enzymes stored within lysosomes and becomes increasingly eosinophilic. Fusion of epithelioid cells leads to MNGC formation through the mediation of lymphokines (Postlethwaite et al., 1982) although nuclear division may also be involved (Unanue, 1978).

Infectious granulomas have all the features of T-lymphocyte-mediated immunity, and require a high density of specific antigens in a physical form allowing persistence in a tissue focus (Unanue, 1978). Necrosis within granulomas depends on the number of organisms, their virulence and the development of an immune response. With non-replicating antigens, such as schistosome eggs, granulomas eventually regress without necrosis (Unanue, 1978). Perhaps Toxocara larvae secrete potent antigens, which substitute for high numbers of virulent micro-organisms, persist (or are continuously secreted), cause necrosis in the core of the lesions and probably induce the changes in surrounding hepatocytes. In the early lesion, the core was heavily infiltrated with eosinophils and other cells, and surrounded by epithelioid cells. With time, a virtually syncytial layer of MNGCs had formed, some containing over 1,000 nuclei, and the core became eosinophilic and acellular. MNGCs ingested material, including clumps of eosinophils, and their central cytoplasm, often containing nuclei, contributed to the degenerate core. Such highly active granulomas seemed to be attempting to wall off and destroy an irritant in the core. In bats in early infections or post-partum, these granulomas became elongated and bipolar. The young pole, with a cellular necrotic core surrounded by epithelioid cells, was associated with larvae, whereas the old pole, comprising MNGCs and an amorphous core, may have been resolving.

In all bats, it was exceptional to find larvae within granulomas. Often, larvae were found nearby, but occasionally they were remote (over 100 µm away) or not seen. There is no doubt that larvae are capable of significant migration in tissues following death of the host (Chapter 8). In bats whose livers were fixed hours after death, larvae were further from

granulomas or more were "free" in tissues. However, even in livers fixed within minutes of death, larvae were usually outside granulomas. Perhaps the slow diffusion of formalin, which is highly irritant to larvae (pers. obs.), stimulates egress from granulomas. Alternatively, a larva may move in a "stepwise" fashion, i.e. periodically stopping, then moving on; this may account for the "bipolar granulomas". With time, the lesions become less florid, perhaps reflecting host tolerance or larval quiescence. In bats, never was a degenerate larva found within a granuloma. Presumably, dead larvae are engulfed by granulomas. Surprisingly, no significant fibrosis developed in any livers, even 12 months pi.

In the post-parturient female bats, the finding of larvae associated with infiltrates typical of early infections supported the impression of larval reactivation, discussed earlier.

Not all bats developed focal granulomatous lesions. The response in the liver 4 d pi, with numerous scattered microabscesses, was distinct. At 49 d pi, larvae were found in sections, some "free" from inflammation (this bat had died some time prior to fixation), and the infiltrates of lymphocytes, eosinophils and histiocytes were more nebulous. The inflammatory "trails" may have represented antigen drainage pathways, or perhaps post-mortem these cells could still adhere to larvae and were dragged through the tissues. Likewise, the maternal liver 23 d pp had no typical granulomas but smaller, more scattered foci. This poorly "focussed" reaction, without the development of giant-cell granulomas, seems unrelated to egg dose or larval numbers, and may represent individual variability in responses to larvae. As blood eosinophil responses, dependent on immune and bone marrow functions, vary amongst different mouse strains (Wakelin & Donachie, 1983), it is likely that eosinophilia, and probably inflammatory reactions, also vary amongst individuals. Variations in immune responsiveness may influence the rate and frequency of larval

stepwise movements, affecting in turn the focal concentration of secreted antigens, the intensity and duration of local responses and the drainage of antigens to produce more diffuse inflammation.

Livers of bats infected subcutaneously reacted similarly to those infected orally, except in the early stages when the higher proportion of larvae with acute inflammatory trails indicated their "staggered" arrival. Absence of granulomas from lung sections suggested that larvae had passed rapidly, although several haemorrhagic necrotic foci did progress to fibrosis.

The histological changes in the livers of flying foxes were quite different from those found in all other experimental animals studied (unpublished), and the outer layer of ballooned hepatocytes appearing around inflammatory foci may be unique.

The histological response to _T. canis_ in bats obviously differed because of the different organ distribution of these larvae. Well-developed young granulomas had developed in the liver by 7 d pi, and were identical with those of _T. pteropodis_, with an outer layer of ballooned hepatocytes induced by larval products or the host response. Again, this feature has not been reported previously in other _T. canis_ infections. MNGCs had not formed at 7 d, but were present in the few hepatic granulomas at 90 d pi. The lung changes were consistent with larval passage. The large granuloma found in the skeletal muscle at 90 d pi was similar to those in mice (Burren, 1968). Diffuse interstitial eosinophilia has been described in the muscles of mice infected with _T. canis_ by Kayes & Oakes (1978), who reported 2 types of granulomas in later infections (4 - 5 weeks pi), "young" and "old", and concluded that larvae occasionally leave older lesions to set up new foci elsewhere. They described lipid-laden epithelioid cells in the cores of their granulomas 10 weeks pi, but these were quite distinct from the ballooned hepatocytes reported here. In these old infections, Kayes & Oakes found larvae in most granulomas.

In the brain, 90 d pi with *T. canis*, most "free" larvae may have left their inflammatory foci post-mortem, but others were closely associated with focal granulomas. The 2 different types of giant cells were distinct entities, showing no fusion or intermediate stages in serial sections. Presumably one or both types formed from neural phagocytes (microglial cells) and they may have been of different ages. In mice infected with *T. canis*, such granulomatous foci do not develop in the brain (Sprent, 1955; Burren, 1968), and this appears to be also the case in guinea pigs (Prociv, unpublished). Likewise, as in the bat, there was no eosinophilic infiltrate in these experimental hosts, nor did eosinophils occur in the subarachnoid space, despite blood eosinophilia. However, in pigs, *T. canis* larvae elicited an aggressive granulomatous response, with perivascular eosinophil-lymphocyte cuffing and intense eosinophilic infiltration of the meninges (Done et al., 1960). MNGCs formed in the spinal cord, but Done et al. did not report these in the cerebrum. Furthermore, larvae could not be recovered by digestion from pigs' brains after 52 d pi, indicating that they were destroyed by the granulomatous reaction, whereas in mouse brains larvae survive indefinitely. In the bat the granulomatous reaction to *T. canis* was less intense, and many larvae survived at least 90 d.

Neuropathological changes in primates infected with *T. canis* have been variable. In baboons several months pi, "numerous necrotic tracks infiltrated with epithelioid cells and giant cells were found in sections of the brain" (Aljeboori et al., 1970). In cynomolgus monkeys (*Macaca fascicularis*) 7 months after single and multiple infections, perivascular cuffing and granulomas of two types were evident (Glickman & Summers, 1983) - "simple" foci of densely packed histiocytes, and larger complexes with central cores of MNGCs and foamy macrophages surrounded by mononuclear cells and eosinophils. Larvae, when found, were in complex lesions. Glickman & Summers did not clarify whether they examined random or serial

sections, as in their illustrations the "simple" granulomas could simply have been tangential sections of larger "complex" lesions. They also reported eosinophilic meningeal infiltration. In humans, however, there is doubt that toxocariasis induces eosinophilic meningitis. Kuberski (1979) claimed that T. canis had "not been proved to be responsible for a cerebrospinal fluid eosinophilic pleocytosis", and Fox et al. (1985) concluded that "it has never been shown to elicit eosinophilia in the cerebrospinal fluid". A recent case report implicating T. canis as the cause of eosinophilic meningitis in a healthy 11-year-old girl (Gould et al., 1985) is questionable on epidemiological and serological grounds. The human neural tissue response to T. canis infections may be more like that of flying foxes than of other primates!

Sprent (1955) concluded that tissue responses to larvae in the central nervous system depended on the nematode species, but clearly host factors are also important. He also speculated that "degenerative and cellular changes in the vicinity of the parasite only appear if the parasite has become quiescent before fixation. If the parasite is moving at the moment of fixation it may lie in apparently normal tissue, while extensive damage may be found in other parts of the central nervous system". This concept is considered in detail in Chapter 8.

In the following chapter, the development of T. pteropodis is described.

CHAPTER 5

EGG PRODUCTION, MORPHOLOGY, DEVELOPMENT AND DISPERSAL

5.1 INTRODUCTION

An appreciation of the infection risk of T. pteropodis to humans and other animals, and by the same token elucidation of its means of transmission to other bats, required quantitative data regarding the onset and duration of patency, egg production by female worms and environmental dissemination of eggs. Findings from experimental and field studies are reported here.

5.2 PRE-PATENT INTERVAL

Faeces of 4 young bats born in captivity were examined daily to detect the onset of patency. In 2, fertile eggs first appeared at 35 and 48 d pp respectively, while in the others, infertile eggs appeared at 37 and 42 d. In 1 of the latter bats (bat 2, Fig. 5.1), fertile eggs appeared 23 d later, i.e. at age 60 d, whereas in the other a solitary female worm was expelled spontaneously after 4 weeks of infertile egg production.

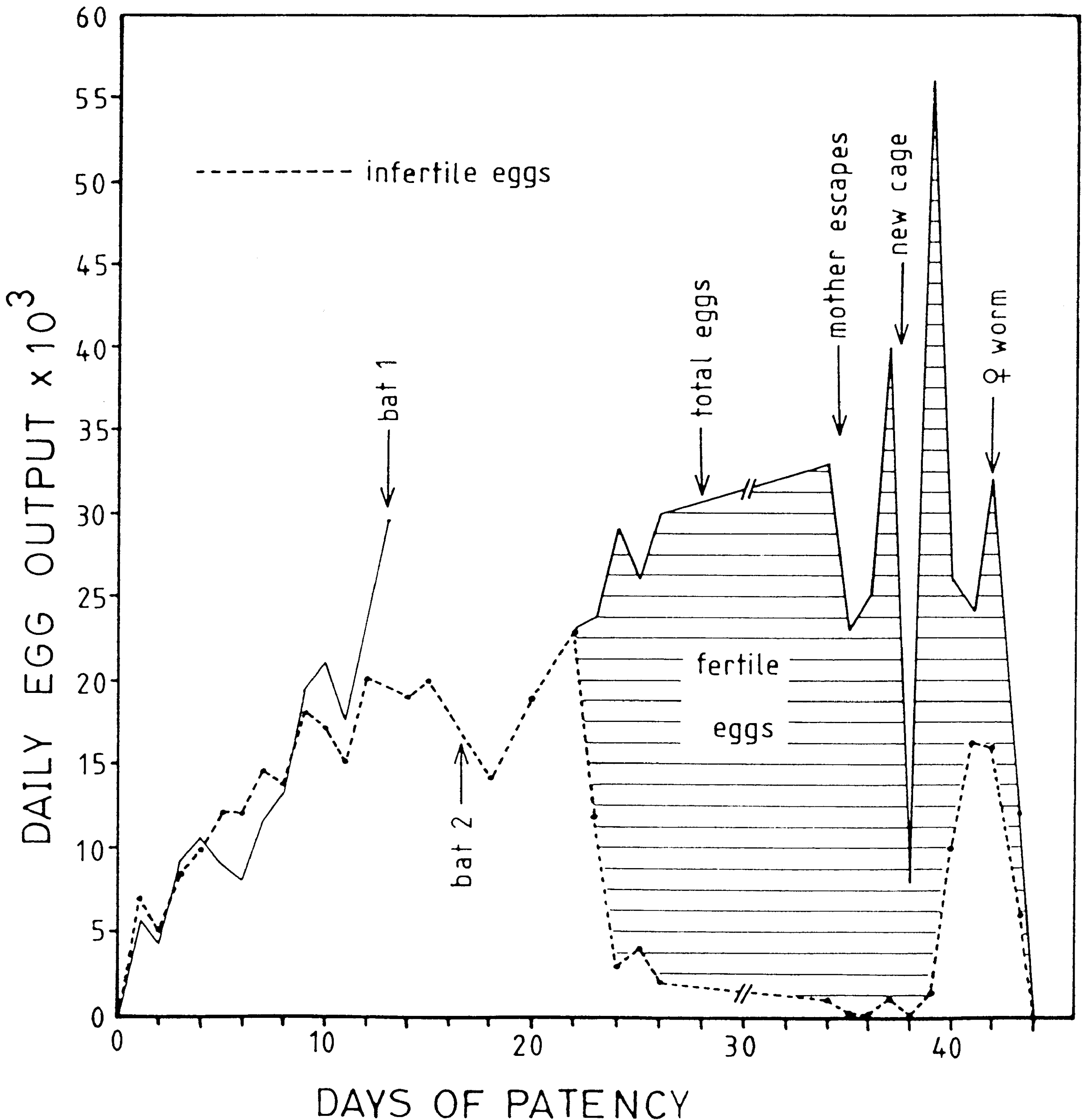
5.3 EGG OUTPUT IN EXPERIMENTAL INFECTION

The above 4 bats were kept with their mothers which were given a fortified milk/sugar/vitamin diet. Daily egg outputs were determined but in 2 the data were deemed unreliable and disregarded when a barely detectable, but significant, leak was noticed in the sieve which had been used in egg recovery. The findings from the remaining 2 animals are shown in Figure 5.1. Eggs first appeared in the faeces of bat 1 at 48 d, and of the 5,800 eggs passed in the 1st 24 h, 88% were fertile. Of the 4,200 collected over the 2nd 24 h, 86% were fertile, as were all 9,000 collected on the 3rd day. From then on, fertile eggs constituted 93 - 100% of the

FIGURE 5.1

Twenty-four hour faecal output of T. pteropodis eggs from 2 juvenile P. poliocephalus born to experimentally-infected mothers

Bat I commenced passing fertile eggs at age 48 days. Bat 2 commenced passing infertile eggs at 37 days and continued voiding exclusively infertile eggs for 22 days, when the first fertile eggs appeared. Outputs over the period days 26 - 33 were not measured. On the evening of day 34, its mother escaped and on day 37, Bat 2 was moved to a different cage in another room. A male worm was found in the cage on the morning of day 39, and on day 42 one female worm was passed.



total collected daily. Collections from this animal ceased at day 13, but it continued to produce eggs until a female worm was spontaneously passed on day 19. Bat 2 commenced passing infertile eggs at age 37 d, and over the next 22 d all eggs were infertile. On day 23 of patency, 50% of the 23,000 eggs collected were fertile, as were 89% on day 24. Collections were interrupted from day 26 until day 34, when 97% of the 33,000 eggs were fertile. Following the escape of its mother, there was a moderate fall in output. On day 37, transfer to a new cage in another room caused a temporary reduction in food intake and faecal output. At the same time, bananas were introduced to the diet, and on day 39 a male worm was found in the cage. The following day, the proportion of infertile eggs increased markedly. On day 42, a female worm was voided which contained mainly infertile eggs in its uterus and no spermatozoa in the spermathecae. Administration of piperazine citrate (300 mg) on day 44 did not result in the passage of any more worms.

5.4 EGG OUTPUT FROM NATURALLY-INFECTED BATS

In the late summer-autumn periods of 1982 - 1985, large series of egg collections from groups of wild-caught infected juvenile P. poliocephalus enabled the calculation of daily egg outputs per female worm. Typical examples follow:

a) Consecutive daily outputs of fertile eggs from 1 juvenile: 42,000; 36,000; 35,000; 30,000; 55,000; 40,000 (1 female worm expelled); 50,000; 15,000; 18,000; 18,000; 21,000 (2nd female worm expelled); 4,200; 0 (piperazine - 1 male worm passed).

b) Consecutive daily outputs of fertile eggs from 1 juvenile: 23,000; 27,000; 30,000 (1 female worm passed); 5,000; 0; piperazine - 1 male worm passed.

c) Daily outputs from 1 juvenile passing infertile eggs: 62,000; 59,000 (piperazine - 2 female worms).

d) Daily outputs from a group of 4 juveniles: 130,000; 150,000; 140,000; (2 female worms in faeces); 70,000; 80,000 (1 male worm in faeces); 80,000 (1 female in faeces); 87,000 (1 male in faeces); 70,000; 42,000 (1 male in faeces); 5,000 (1 female in faeces); (piperazine - 1 female + 3 males: i.e. a total of 5 female and 6 male worms).

e) One group of 3 juveniles passing 79,000 - 92,000 eggs/d (mean 84,000/d) later expelled 4 female worms.

f) One group of 8 juveniles passing 280,000 - 300,000 eggs/d later passed 14 female worms.

From these data, the mean daily egg output of a female T. pteropodis was calculated to be $25 \pm 5.0 \times 10^3$ (range $18 - 32 \times 10^3$), with some daily fluctuation.

Two female worms producing fertile eggs, expelled after piperazine treatment, were dissected in saline and their uteri removed and comminuted. By counting eggs in a measured aliquot of suspension, it was estimated that the worms contained 45,000 fully-developed fertile eggs, i.e. approximately 22,500/female.

5.5 INFERTILE EGG OUTPUT

The daily production of infertile eggs was comparable with that of fertile eggs. Both bats in Figure 5.1 presumably harboured single female worms. In bat 1, a male had matured earlier, so all eggs were fertile from the outset, whereas in bat 2, the male worm was immature in early patency so all eggs were infertile, yet output levels were very similar. One wild-caught juvenile passing 55 - 65,000 infertile eggs/d expelled 2 females but no males after piperazine administration.

Spontaneous voiding of male worms from groups of infected juveniles was usually followed by a noticeable rise in infertile egg output over the following 48 h, i.e. 2 x 24 h faecal collections. On 2 occasions, while

fertile egg output was being monitored from individual juveniles, male worms were passed spontaneously and infertile eggs appeared over the following 24 h (30% and 50% of totals). In the 2nd 24 h collections effectively all eggs of 1 were infertile as were the majority in the other (bat 2, days 39 - 41 in Fig. 5.1), with little change in the total numbers produced. In both cases the female worms were later dissected and found not to contain spermatozoa in their uteri or spermathecae (Fig. 5.2 G). In 1 of these bats a rectal swab, taken 24 h after a male worm was found on the cage floor, produced 89% infertile eggs (177/198). The uterus of the single female worm, passed after piperazine treatment, was devoid of spermatozoa and contained only infertile eggs.

Another juvenile, passing 100% fertile eggs, died unexpectedly. At autopsy, 1 female worm was found in the stomach, with a female and male in the duodenum. In both females, the uteri were full of fertile eggs, yet almost no spermatozoa were present in the spermathecae of the first.

In the late summers of 1983 - 1985, groups of infected juveniles produced gradually increasing numbers of infertile eggs, with gradually declining total outputs, which could not be explained on the basis of worm loss. This occurred in bats in captivity for several weeks, as well as recently caught animals, late in the season, i.e. in bats 3 - 5 months old. The uteri of female worms from these juveniles contained varying mixtures of fertile and infertile eggs, plus spermatozoa, although the latter were present in smaller numbers than usual (Fig. 5.2 H & I). Although some of these worms seemed to produce normal total egg numbers, in others there was a marked decline, e.g. 1 bat passing 7,000 eggs/d, of which 70% were infertile, voided 2 female worms and 1 male after piperazine administration.

5.6 SPERMATOZOA OF *T. PTEROPODIS*

Spermathecae from worms releasing fertile eggs invariably contained spermatozoa (Fig. 5.2 A - E) whose structure was readily discernible.

FIGURE 5.2

Uterine contents from adult T. pteropodis passed by P. poliocephalus with patent infections (scale bar in D)

A. Spermatozoa, mounted in 0.85% saline, from spermathecae of freshly-passed worm producing normal, fertile eggs. Conspicuous, eccentric, large, refringent lipid-like body occupies most of cytoplasm, and early pseudopodial extensions evident.
(bar = 50 µm)

B. Same preparation as in A, 5 minutes later, showing extensions of pseudopodia.
(bar = 50 µm)

C. Same field as in B, 3 minutes later. Processes of adjacent central spermatozoa later appeared to fuse and movement ceased.
(bar = 50 µm)

D. Intact worm cleared in creosote, showing distal uterus packed with fertile eggs and spermatozoa.
(bar = 200 µm)

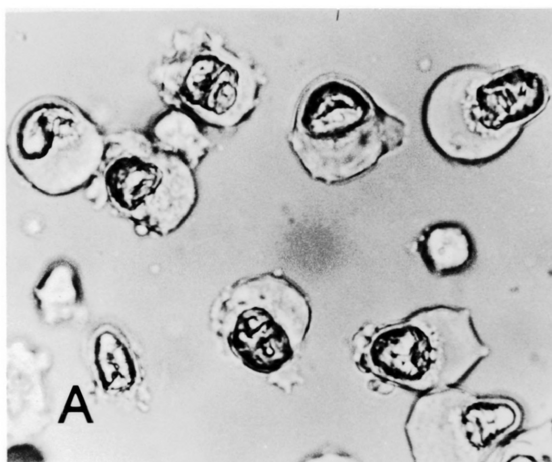
E. Spermatheca of female worm releasing fertile eggs, near junction with oviduct. Numerous motile spermatozoa, and apparently fertilized oocytes with early outer uterine shell layer but no separation of granules to form inner layers (see Section 5.9).
(bar = 200 µm)

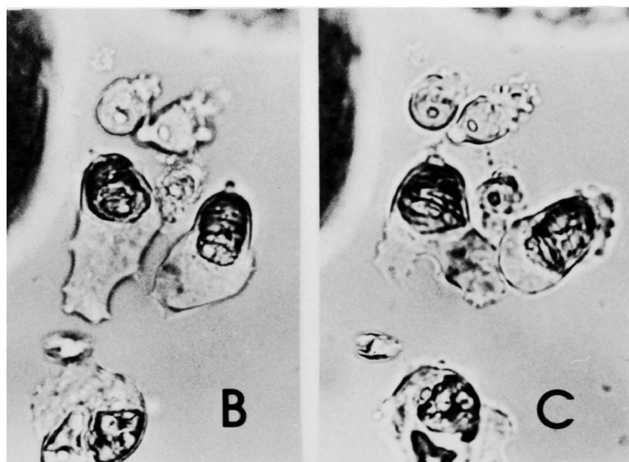
F. Transverse section spermatheca showing fertile eggs at varying stages of cytoplasmic organization and shell formation.
(bar = 200 µm)

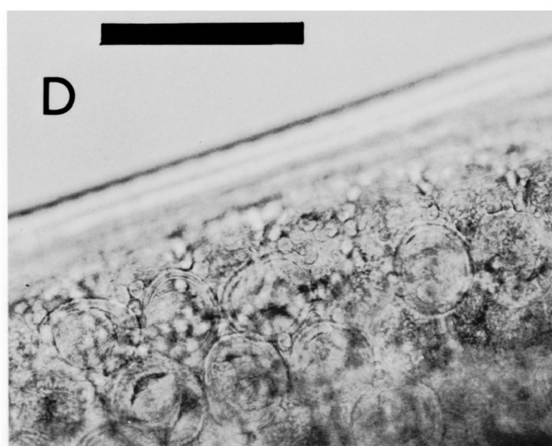
G. Spermatheca of female worm recovered 30 hours after male spontaneously passed by bat. Most eggs infertile, one at right upper corner distended (see Fig. 5.5 and Section 5.9.2 for details). No spermatozoa present.
(bar = 200 µm)

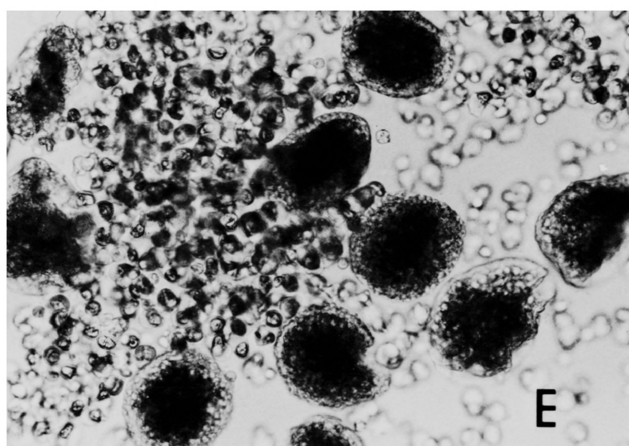
H. Distal uterus of "senescent" worm passing mixture of fertile and infertile eggs. Note distended and typical infertile eggs, occasional fertile eggs, and intermediate forms with apparently normal shell layers but granular and vacuolated cytoplasm.
(bar = 200 µm)

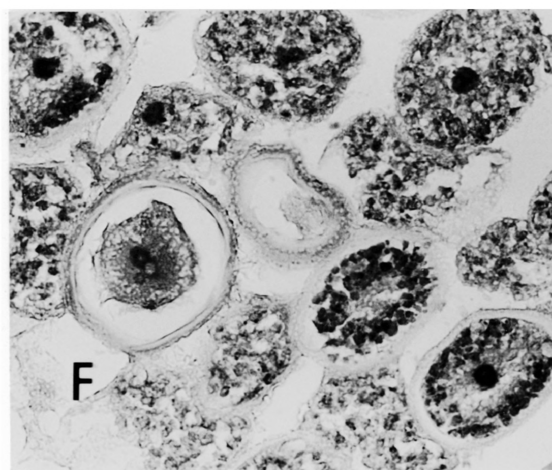
I. Spermatheca of worm in H, showing spermatozoa amongst eggs. Worm was found dead, and spermatozoa were not mobile.
(bar = 100 µm)

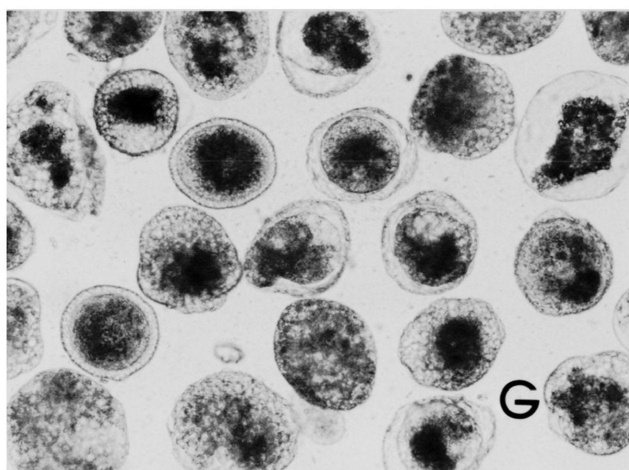

A

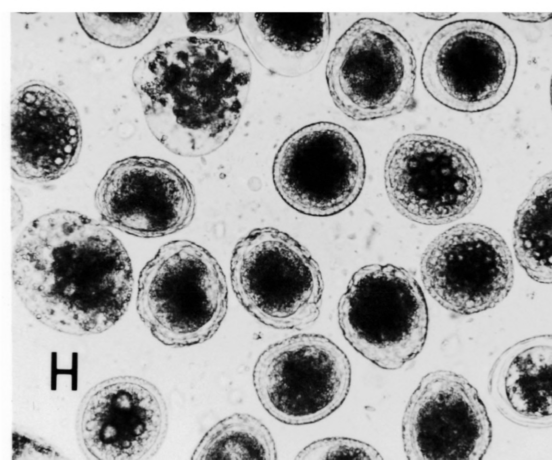

B
C

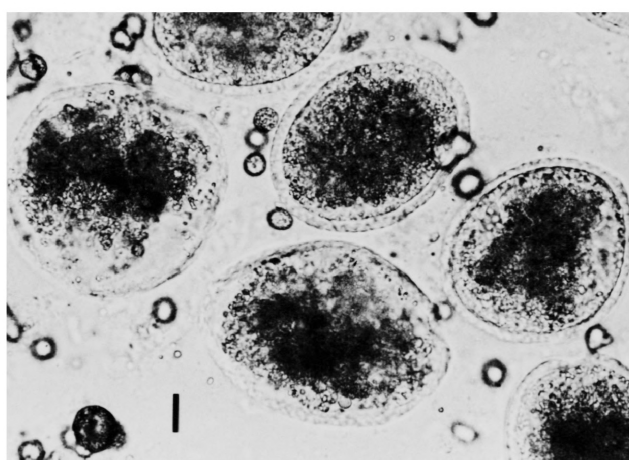

D


E


F


G


H


I

When the female worms were fresh and active, the spermatozoa were motile (Fig. 5.2 A, B & C). Their morphology was very similar to that of experimentally activated spermatozoa from seminal vesicles of A. suum described by Foor & McMahon (1973). Within each was an eccentric, "large, refringent lipid-like body" which occupied most of the cell (Fig. 5.2 A - C), and cytoplasmic pseudopodia formed. These pseudopodia often projected extensively (Fig. 5.2 B & C), although the spermatozoa did not show any progressive movement. Spermatozoa from females producing a mixture of fertile and infertile eggs were fewer in number than but identical in appearance to those from worms releasing exclusively fertile eggs.

5.7 FAECAL EGG CONCENTRATION

T. pteropodis eggs were most concentrated in the faeces of newly-caught suckling juveniles whose diet comprised mainly milk, although many were also passing pollen grains (Fig. 5.6 A). The numbers of eggs per gram (epg) were calculated by emulsifying weighed quantities of faeces in water and counting eggs in a known volume. Levels of over 5,000 epg were common. In one 100 mg faecal sample, 1,596 eggs were counted, i.e. 16,000 epg.

With the introduction of a fruit diet, concentrations often fell below 100 epg, depending on the fruit. Wide varations in egg concentrations in faecal pellets from the same bat indicated that worms released eggs in "pulses" rather than continuously.

Mothers groomed their offspring frequently, ingesting the entire daily faecal output of the very young pups. In the 1st month of life, young on mothers were never seen defaecating spontaneously. The mother stimulated elimination of faeces and urine by regularly licking the pup's perineum. As the pups commenced hanging separately from their mothers, inversion and independent faecal expulsion was then observed frequently, but often the mother induced defaecation while a pup suckled. In these mothers, eggs

were readily detected in rectal swabs. Captive juveniles were promiscuous "mutual-groomers", frequently licking each other's ano-genital regions, as well the cage floor and sides, particularly where milk had spilt. In this way uninfected bats were occasionally mistakenly assumed to harbour worms on the basis of a positive rectal swab. In such cases the eggs were often embryonating and in very low concentrations, sometimes too low for accurate calculation but usually well below 1,000 epg.

5.8 SPONTANEOUS LOSS OF ADULT WORMS

From early observations (Appendix I), it was suspected that the introduction of fruit to an exclusively milk diet precipitated the spontaneous loss of worms, but further investigation revealed a more complex picture. General observations were that:

a) younger bats retained their worms, whereas older infected juveniles caught later in a season sometimes expelled over 50% of their worm burdens within 24 - 48 h of capture;

b) spontaneous loss of adult worms could occur at any time, but was more frequent in later infections;

c) introduction of fruit to the diet often precipitated expulsion of some worms, including immature adult stages, often within hours of ingestion;

d) occasional bats, particularly in earlier infections, retained their worms for up to 3 weeks after commencing a fruit diet;

e) suckling bats retained their worms considerably longer than orphaned juveniles on artifical milk;

f) even on an exclusively milk/sugar/vitamin diet, all worms eventually were expelled spontaneously;

Almost all worms when passed were alive and motile. Exit could be precipitous, or occur over an hour or more, with the worm protruding from the anus, retracting and reappearing before final expulsion. Some juveniles seemed oblivious of the event, whereas others (or their mothers)

would pull the worm out with their teeth or chew it. Usually, worms were recovered from faeces on the cage floor, in faeces, but at times were found suspended on the side of the cage, stuck onto a bat's fur or even up to 30 cm from the cage, presumably having been flung there by the bat's wing-flapping. On several occasions it was evident that a worm had been passed, e.g. by the sudden cessation of egg output, or a sudden change from fertile to infertile egg production, yet no trace could be found; possibly the worm had been eaten by the pup or its mother.

In early February 1983, 2 male worms were obtained by piperazine treatment of a pup which earlier had spontaneously passed 2 females. The males were "resuscitated" in buffered glucose/saline for 2 h then inserted into the upper intestine of another juvenile passing only infertile eggs, by laparotomy and enterotomy under halothane/N_2O/O_2 anaesthesia (performed by Wendy Blanchard of the Veterinary Clinic). Post-operative recovery and progress were uneventful, the pup suckling its mother. One live male worm was passed 24 h later, but the other was never recovered. The bat did not pass Toxocara eggs for 10 d, when it resumed production of exclusively infertile eggs for another 6 d (up to 10,000/24 h) and then ceased completely. Piperazine treatment 1 week later failed to expel any worms.

The spontaneous loss of adult worms under natural conditions is recorded in Section 3.2.4.

5.9 MORPHOLOGY OF T. PTEROPODIS EGGS

5.9.1 Fertile eggs

Typical bile-stained fertile eggs passed in juvenile bat faeces were ovoid to spheroid (Fig. 5.3 A - G), often slightly irregular in outline with a finely pitted outer uterine shell layer (Figs 5.3 B, 5.4). The size of 25 randomly selected eggs was as follows: mean maximum diameter 104 $\pm$ 8 µm (range 88 - 125 µm); mean minimum diameter 89 $\pm$ 7 µm (range 80 - 110 µm).

FIGURE 5.3

Fertile eggs of *T. pteropodis* recovered from faeces of naturally-infected juvenile *P. poliocephalus* (scale bars in C & I)

A. Typical egg, showing condensed central cytoplasmic mass with distinct membrane surrounded by fluid-filled space. Three inner shell layers discernible at upper pole (see Section 5.9.1 for details), with thick outer uterine coat. (bar in C = 50 µm)

B. Same egg as in A, showing pits in uterine layer. (bar in C = 50 µm)

C. Freshly-passed decorticate egg. Inner cytoplasmic mass has not yet contracted. Two clear layers of shell are distinct - inner chitinous and outer vitelline layers. Very thin uterine layer best seen at upper pole. (bar = 50 µm)

D. Irregular-shaped egg after first embryonic cleavage, showing two distinct inner layers with very thick uterine layer. (bar in C = 50 µm)

E. Freshly-passed egg with double outer uterine coat. (bar in I = 50 µm)

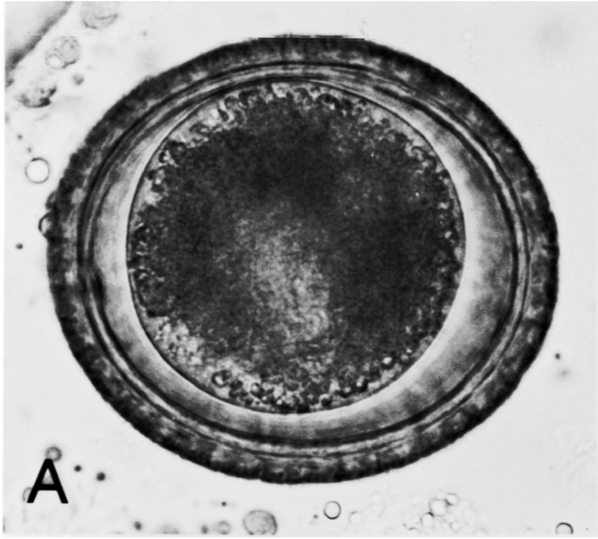
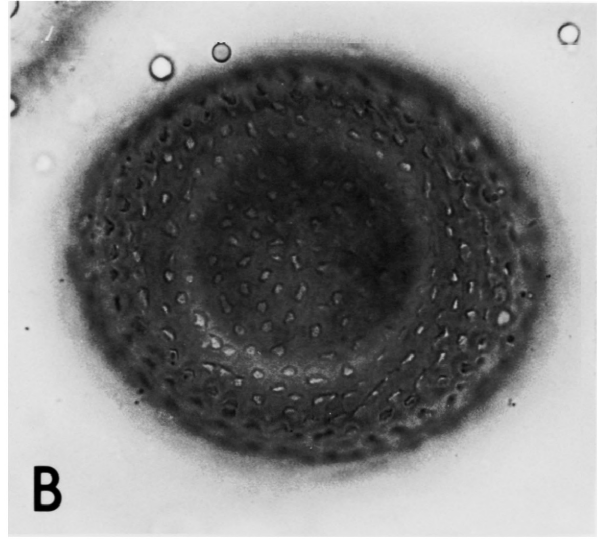
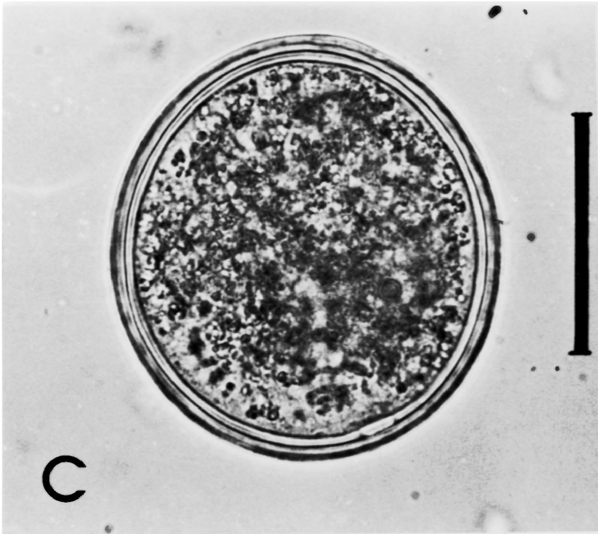
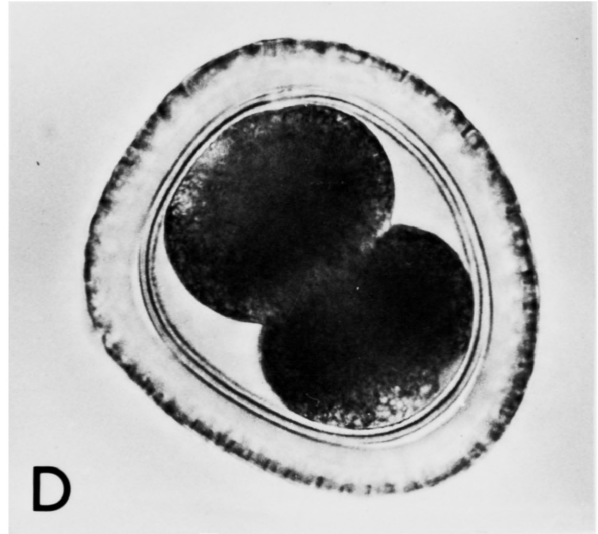
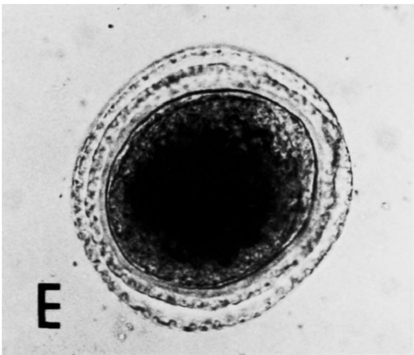
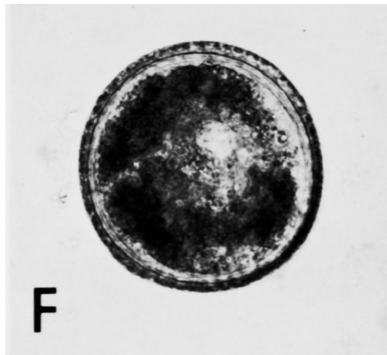
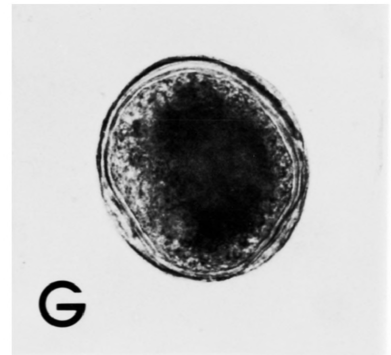
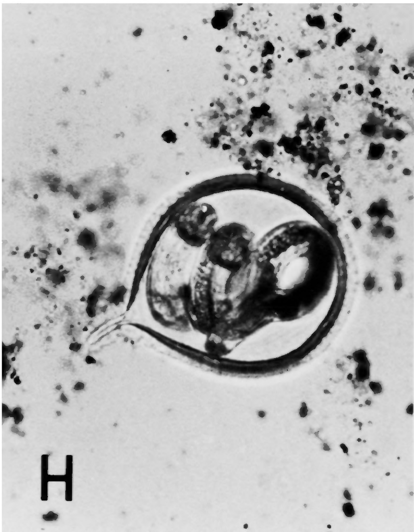
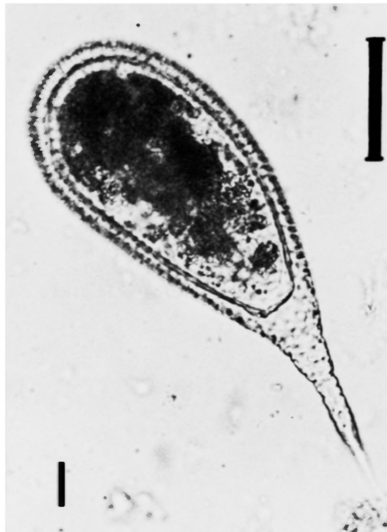
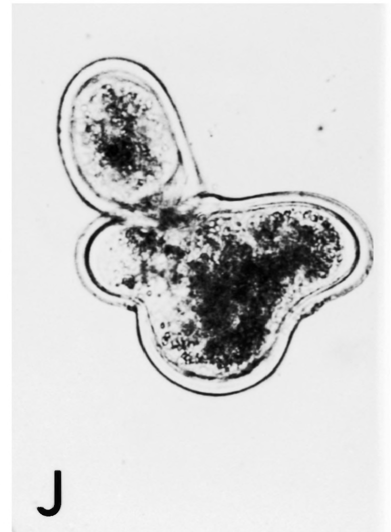
F. Freshly-passed egg with thin uterine coat. (bar in I = 50 µm)

G. Freshly-passed egg with unevenly deposited uterine coat. (bar in I = 50 µm)

H. Embryonated egg from charcoal culture with tail-like appendage. (bar in I = 50 µm)

I. Freshly-passed elongated egg with uterine layer extended as "tail". (bar = 50 µm)

J. Freshly-passed lobulated egg presumably fertile because of well-formed shell layers and texture of internal mass. (bar in I = 50 µm)


A

B

C

D

E

F

G

H

I

J

Many apparently fertile or embyonating eggs were less regular in outline, some occasionally of bizarre shapes (Fig. 5.3 H, I & J). When freshly passed from the female worm, the cytoplasmic mass filled the entire egg, but after several hours it condensed, with the formation of a clear, fluid-filled perivitelline space between the 1-cell embryo and the shell layer (Fig. 5.3 A). Up to 4 shell layers could be discerned (Fig. 5.3 A, C & D) corresponding with those found in A. suum eggs by Foor (1967): a fine, inner lipid layer; a thicker, clear, chitinous layer; an equally thick outer vitelline layer; a pitted outermost layer of variable thickness. The fine pits, uniformly distributed over the outer surface, showed subtle variations in shape and size (Figs 5.3 B & 5.4 D & F). Occasionally the uterine layer was very thin or absent (Fig. 5.3 C, F & G) or duplicated (Fig. 5.3 E). Eggs passed in faeces were at the 1-cell stage (Fig. 5.6 A), but had proceeded to 2-cells within 24 h. Occasionally, eggs at an advanced stage of development were passed, and those in the faeces of mother bats were usually at the 2-4-cell stage.

Fertile eggs maintained their shape and appearance regardless of the medium in which they were examined, i.e. faeces, saline, formalin, 0.1N sulphuric acid or water. Eggs passed by juveniles of different bat species, P. alecto, P. conspicillatus and P. scapulatus (Chapter 7), were all similar and within the same size range as those from P. poliocephalus.

5.9.2 Infertile eggs

Infertile eggs appeared in 3 different circumstances:

i) from initial patency, in bats which did not harbour male worms or in which female worms matured first (in which case, eggs became fertile when the male matured;

ii) within 24 h of spontaneous loss of the only male worm from a bat passing fertile eggs, in which case all eggs were infertile usually by

48 h;

iii) in captive bats, after variable periods of patency, when a gradually increasing proportion of eggs produced were infertile (Fig. 5.5 G).

Infertile eggs varied in appearance from almost normal (Fig. 5.5 H & I) to grossly bizarre and almost unrecognisable, with wide variation in shape and appearance of eggs even from the one worm. Sizes of 34 randomly selected infertile eggs were as follows:

mean length 100 ± 8 µm (range 89 - 118 µm)

mean width 84 ± 10 µm (range 67 - 108 µm)

The following features were diagnostic (not all were present in any 1 egg):

i) shape irregular;

ii) uterine layer present, but usually thin, with pits irregular in size and distribution (Fig. 5.5 A, C, E, F & G);

iii) inner shell layers not discernible (Fig. 5.5 A, B, E, F, K, L & M);

iv) "embryo" indistinct irregular mass of variable size (Fig. 5.5 A, B, D, F, G, K, L & M);

v) cytoplasm containing numerous "oily" vacuoles of variable size and yellow-stained if passed in juvenile bat faeces Fig. 5.5 A, B, D, K & L);

vi) cytoplasmic material occasionally condensed into dense, amorphous dark mass without distinct membrane (Fig. 5.5 E, G & H);

vii) in tapwater or other hypotonic media, often herniated asymmetrically through uterine layer (Fig. 5.5 E & F).

In the faeces of bats passing infertile eggs, often large (up to 200 µm diam.), thin-walled clear spheroid bodies containing clumps of fine granules were seen (Fig. 5.5 M). These were later determined to be unfertilized oocytes which had swollen grossly (Fig. 5.2 G, H & I).

FIGURE 5.4

Scanning electron micrographs of fertile eggs from uterus of adult female *T. pteropodis*

A. Low power view showing shape variation (scale bar = 100 µm)

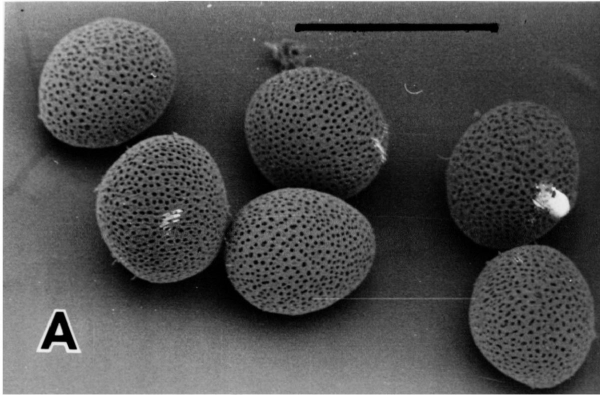
B. Two typical eggs (scale bar = 50 µm)

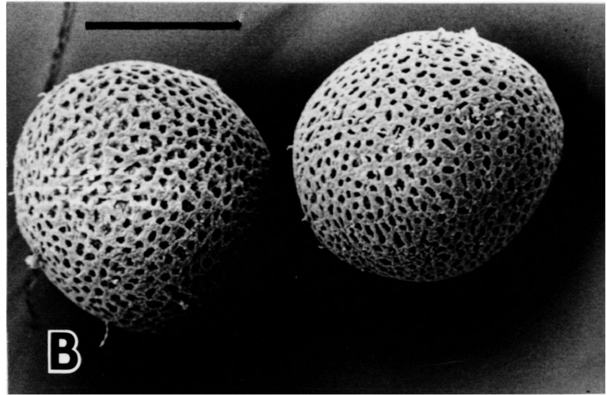
C. Egg with coarsely pitted uterine layer (scale bar = 20 µm)

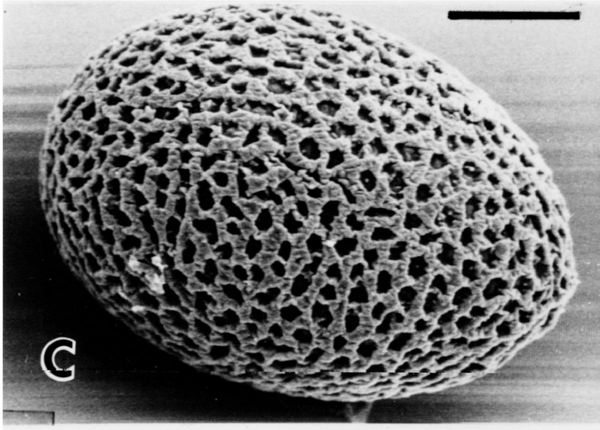
D. Surface pits of egg in C (scale bar = 4 µm)

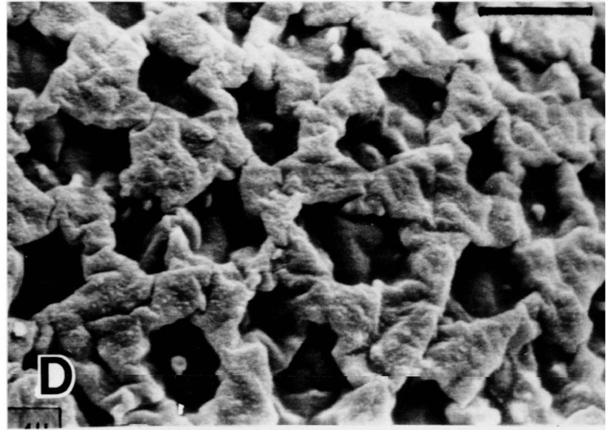
E. Egg with finely pitted uterine layer (scale as in C)

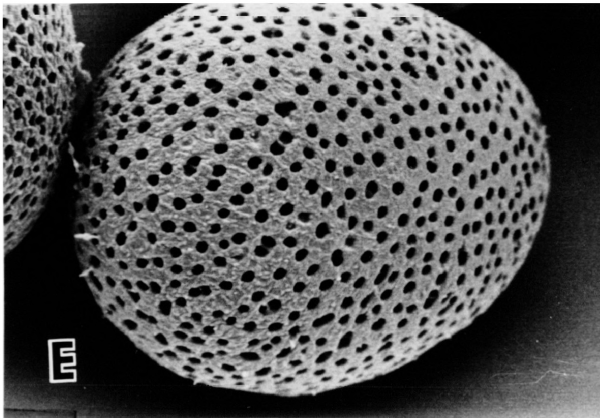
F. Surface pits of egg in E, showing pits to be narrower and deeper than in D (scale as in D)

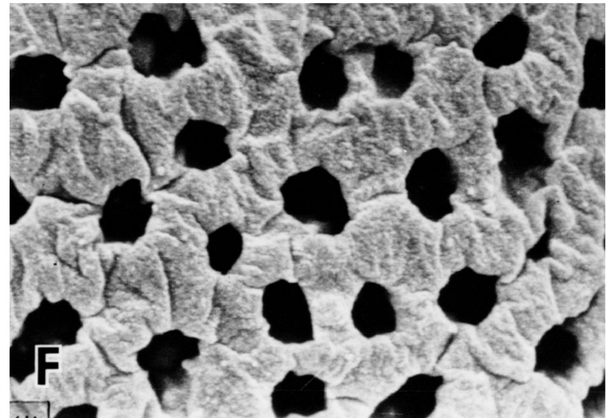

A


B


C


D


E


F

Often infertile eggs of normal shape and external appearance (Fig. 5.5 H) were passed by bats known not to harbour male worms, and could be diagnosed only after careful examination of the "embryo" and search for all the egg-shell layers. Occasionally a thin layer was present immediately beneath the uterine coat (Fig. 5.5 D & H).

5.10 DEVELOPMENT OF EGGS

5.10.1 Conditions for embryonation

Eggs of *T. pteropodis* embryonated on various substrates with little difference in rates of development. Good aeration was essential. Most eggs in faeces comprising mainly fruit pulp failed to develop. After 2-3 d under such conditions, attempts to improve oxygenation by spreading the faeces, adding dilute hydrogen peroxide (0.01%) or mixing into activated charcoal failed to promote further development, and the embryos, mostly at the 1-cell stage, appeared to be dead.

To determine the survival of eggs under different conditions, concentrated faeces of infected bats on a fruit diet were "painted" onto glass microscope slides left open to the room air, onto leaves of palm, eucalypt, avocado and mango trees, and onto green and ripe mangoes and papaws. Some eggs were mixed into activated charcoal stored in 150 mm Petri dishes and others stored in 0.1 N sulphuric acid in Petri dishes to a depth of 5 mm. Eggs were scraped from the fruit and leaves daily for microscopic examination, and at least 200 eggs from the charcoal and sulphuric acid cultures were examined daily to determine rates of embryonation. The effects of desiccation on development and survival were not studied. The following observations were made during summer when average humidity was high (relative humidity 66% at 9 am, 58% at 3 pm), and room temperatures ranged 20 - 30 ^{o}C:

i) fewer eggs developed with increasing organic matter content

FIGURE 5.5

Infertile eggs of T. pteropodis recovered from faeces of juvenile P. poliocephalus (scale bar in B)

A. Typical irregular-shaped egg, with condensed, coarsely granulated cytoplasmic mass not bound by membrane, and without distinct shell layers other than irregular uterine coat. (bar = 50 μm)

B. Typical egg in which central mass dispersed and containing large refringent lipid-like granules. (bar = 50 μm)

C. Same egg as in B, showing coarse, irregular surface pitting. (bar = 50 μm)

D. Egg from bat not harbouring male worm. Inner mass vacuolated, with dense core. Thin, irregular, clear layer underlies outer uterine coat. (bar = 50 μm)

E. Infertile egg washed from faeces in tapwater, showing "blow-out" at upper pole, possibly covered with remnants of uterine coat. Inner mass has distinct outline. (bar = 50 μm)

F. More advanced "blow-out" of egg suspended in water. Inner mass has less distinct outline than in E. (bar = 50 μm)

G. Mixed fertile and infertile eggs from rectal swab of bat harbouring "senescent" worms. (bar = 250 μm)

H. Egg from rectal swab of bat not harbouring male worm. Outer, uterine layer appears normal, and overlies thin, presumably chitinous, layer. Dense inner mass appears abnormal (cf. Fig. 5.3A) and irregular outline suggests not membrane-bound. (bar = 50 μm)

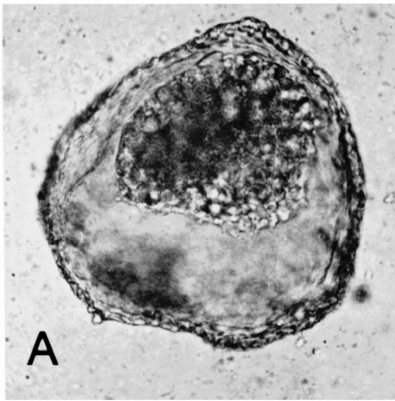
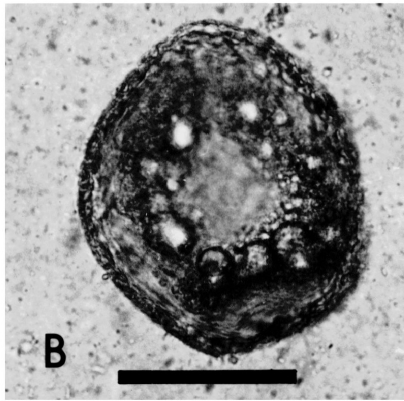
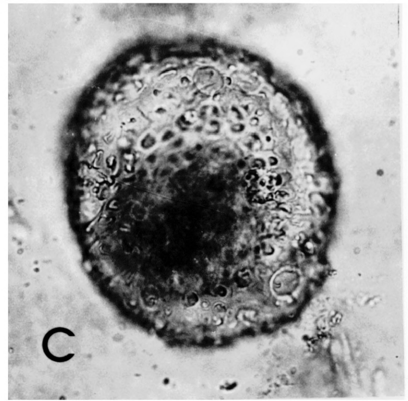
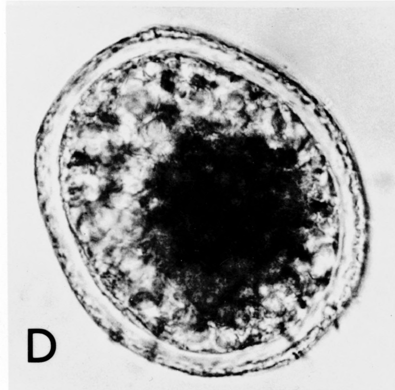
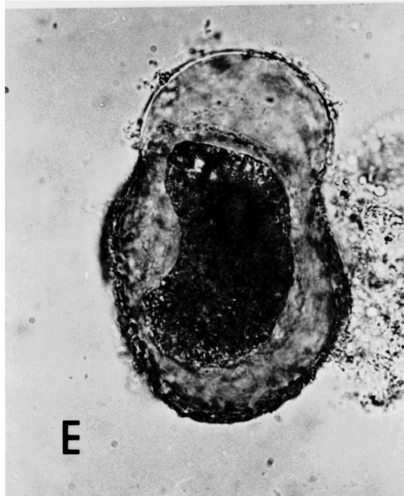
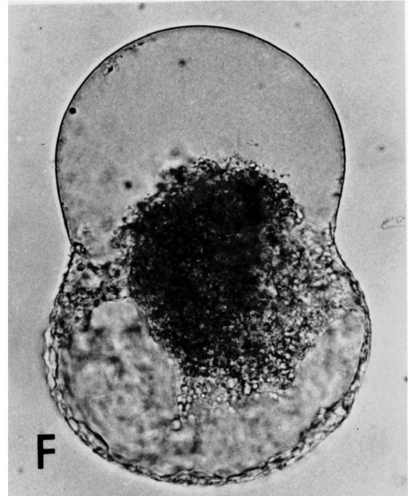
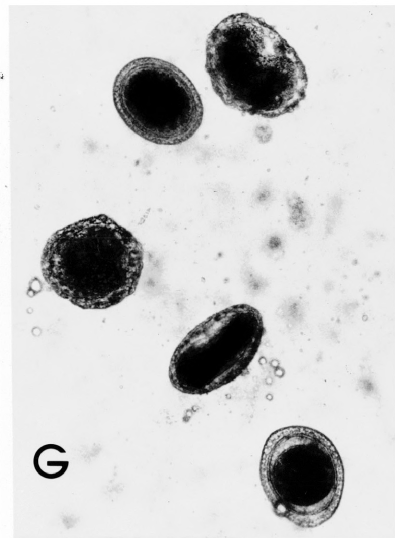
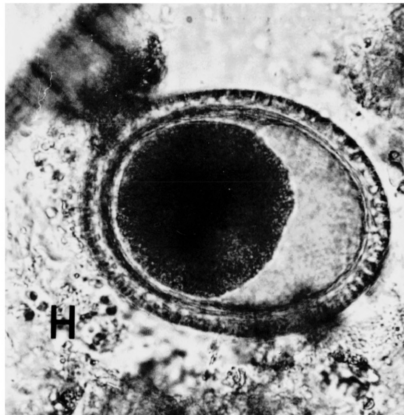
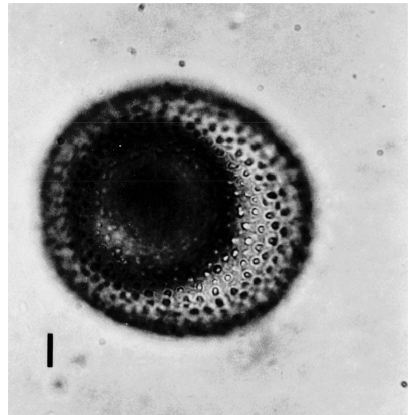
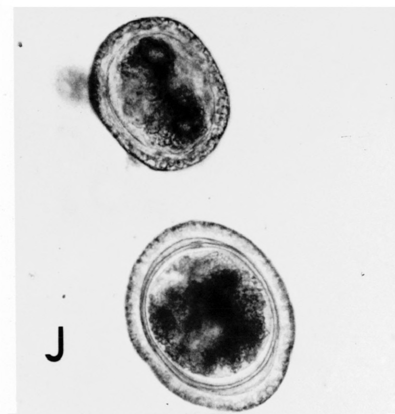
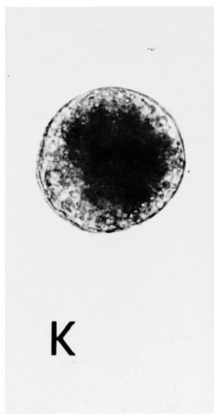
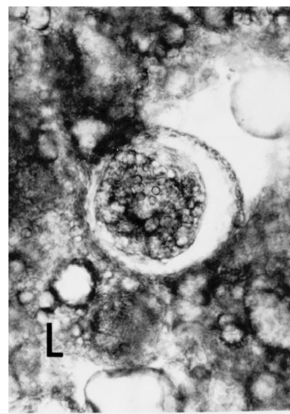
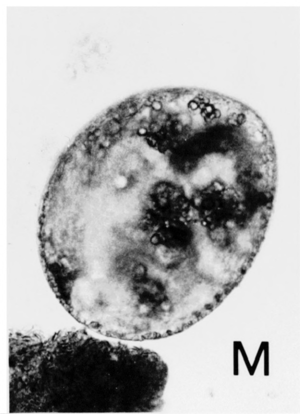
I. Same egg as in H, showing normal surface pitting. (bar = 50 μm)

J. Unusually small infertile egg with normal fertile egg in faeces of bat passing almost entirely normal fertile eggs. (bar = 100 μm)

K. Decorticate, infertile egg, with thin uterine layer evident along left border. (bar = 100 μm)

L. Egg (rectal swab) in which outer uterine coat does not have distinct inner surface. Size indicates that swelling has not occurred. (bar = 100 μm)

M. Infertile egg washed in tapwater from charcoal culture showing gross diffuse distension with remnant uterine layer stretched over surface. (bar = 100 μm)


A

B

C

D

E

F

G

H

I

J

K

L

M

(fruit pulp) and presumably bacterial contamination. Most eggs stayed at the 1-cell stage and those which developed often died before larvae had formed;

ii) with adequate aeration from the outset, development proceeded at almost the same rate regardless of the substrate;

iii) optimal development, i.e. maximal numbers of eggs embryonating completely, occurred in 0.1 N sulphuric acid, but fungal overgrowth in most of the Petri dishes was a problem. Many eggs in sulphuric acid had a green-stained outer shell layer, but embryonated normally.

5.10.2 Embryonation and infectivity of eggs

Eggs from 24 h faecal collections did not develop synchronously. To observe eggs at the same stage, 45,000 recovered from the uteri of 2 female worms were stored in 0.1 N sulphuric acid in a Petri dish. At the intervals shown in Table 5.1, 200 eggs were examined and calculated doses of eggs containing motile, apparently infective, larvae were administered intragastrically to pairs of mice as shown in the table. Development of these eggs, and recovery of hepatic larvae from the mice 7-10 d pi, are also shown in Table 5.1.

Larval development in these eggs was synchronous. At 6 h after removal from the uterus, 21% of embryos were at the 2-cell stage; at 24 h, 57% were at the 5-8-cell stage. The proportion at the 1-cell stage from this time onward remained remarkably constant, and from the 3rd day all of these looked abnormal, although less than half had the features of infertile eggs. By the 4th day, most embryos (77%) had reached the "tadpole" stage (Fig. 5.6 C), with barely perceptible movement. Degenerating embryos, 2% of the total, were first noticed at this time. Initially they appeared to be morula to gastrula stages which had developed erratically and started to break down, with cells of irregular sizes. Subsequently many degenerate forms were motile larvae with grotesque

TABLE 5.1

Development of *T. pteropodis* eggs in 0.1 N sulphuric acid at room temperature (20 - 26°C)
(numbers = % of total)

Time in culture:	1-cell	2-cell	4-cell	5/8-cell	Morula	Blastula	Gastrula	Tadpole stage	Immature larva	Advanced larva	Degenerate forms	No. "infective" eggs fed to 2 mice	% recovered as hepatic larvae
6 hrs	79	21	0	0	0	0	0	0	0	0	0	-	-
24 hrs	5	5	33	57	0	0	0	0	0	0	0	-	-
2 days	4	0	0	7	89	0	0	0	0	0	0	-	-
3 days	4	0	0	0	7	40	49	0	0	0	0	-	-
4 days	8	0	0	0	0	0	13	77	0	0	2	-	-
5 days	4	0	0	0	0	0	0	28	60	0	8	-	-
6 days	6	0	0	0	0	0	0	0	29	31	9	-	-
7 days	4	0	0	0	0	0	0	0	0	80	16 (60% motile)	-	-
8 days	4	0	0	0	0	0	0	0	0	73	23 (50% grotesque)	2,000	0
9 days	5	0	0	0	0	0	0	0	0	75	20 (30% grotesque)	1,400	0
10 days	5	0	0	0	0	0	0	0	0	82	13 (20% grotesque)	220	0
11 days	6	0	0	0	0	0	0	0	0	76	18 (10% grotesque)	600	
12 days	4	0	0	0	0	0	0	0	0	76	20	600	2
13 days	6	0	0	0	0	0	0	0	0	76	18	520	5
20 days	7	0	0	0	0	0	0	0	0	71	22	900	15
36 days	9	0	0	0	0	0	0	0	0	67	24	280	23
90 days	8	0	0	0	0	0	0	0	0	63	29	500	5
150 days	7	0	0	0	0	0	0	0	0	59	34	1,300	2.9
180 days	3	0	0	0	0	0	0	0	0	59	38	400	0.5
230 days	9	0	0	0	0	0	0	0	0	31	60	1,000	0

malformations (Fig. 5.6 E & F). These first appeared on day 7, making up 10% of all embryos, rose to a peak of 12% on day 8 (when other degenerate forms comprised a further 11% of the total), 3% on day 10 and then gradually disappeared. By day 20, all degenerate forms appeared to be disintegrated non-motile larvae.

"Advanced" larvae, which could not be distinguished from infective larvae, first appeared at 6 d. However judging by larval recovery from mouse livers, these were not infective until day 11 (larvae are described in Chapter 6). Increasing larval numbers (in proportion to egg dose) were recovered until a peak of 23% on day 36, following which infectivity declined. At 3 months, the dose recovery was 5%, at 5 months 2.9% (38/1300), at 6 months only 2 larvae after a dose of 400 eggs and, at 7 months, no larvae from 1,000 eggs. By this time, only 31% of eggs contained apparently normal larvae, but no movement was detected over periods of 5-10 minutes. Some larvae had loose sheaths which had wrinkled (Fig. 5.6 H), a feature not observed in younger eggs. On expression, these were found to be dead. Nevertheless, a small number of larvae as late as 7 months were motile when expressed but were very sluggish compared with those from fresher eggs.

Eggs in faecal sludge which had been smeared onto leaves were also recovered and fed to mice. Two larvae were recovered from a mouse fed 110 eggs which had been on leaves for 10 d. After 6 weeks on mango leaves eggs were still infective to mice (3% recovery), having been exposed to dappled sunlight (about 8 h daily) and a total rainfall of 250 mm, but those on leaves beyond this time were not infective (Appendix I).

One batch of eggs embryonated in charcoal was stored at 5 ^{o}C. After 4 months, 3% of a 1,000 egg dose was recovered from mouse livers, and at 5 months these eggs were not infective.

FIGURE 5.6

Intraovular development of *T. pteropodis*

A. Rectal swab of wild-caught *P. poliocephalus* passing exclusively fertile eggs. Note condensed inner cytoplasmic mass in most eggs, fat droplets and numerous pollen grains (two arrowed)

B. Two gastrulae in 0.1 N sulphuric acid culture at day 3, showing synchronous development

C. Motile late "tadpole" stage on day 5 in acid culture (scale as in H)

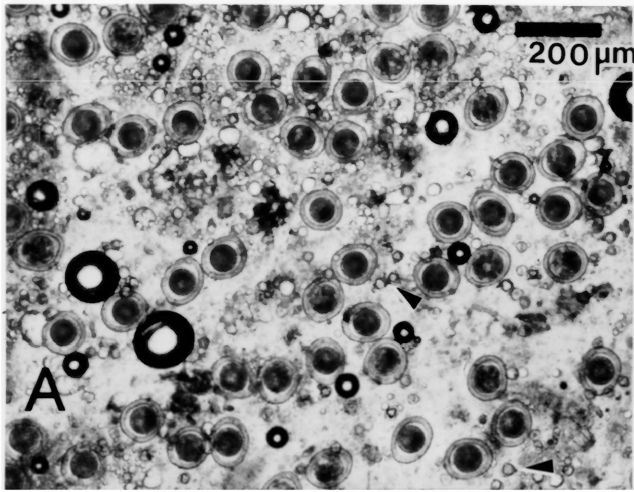
D. "Immature" larva (early first stage) on day 6 in acid culture (scale as in H)

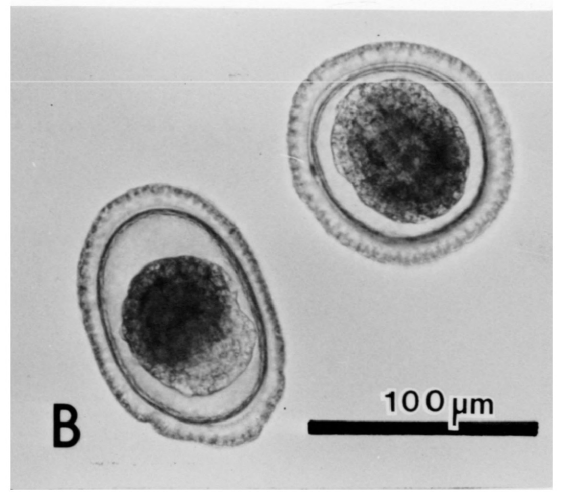
E. Motile but apparently degenerating larva, day 7 (scale as in H)

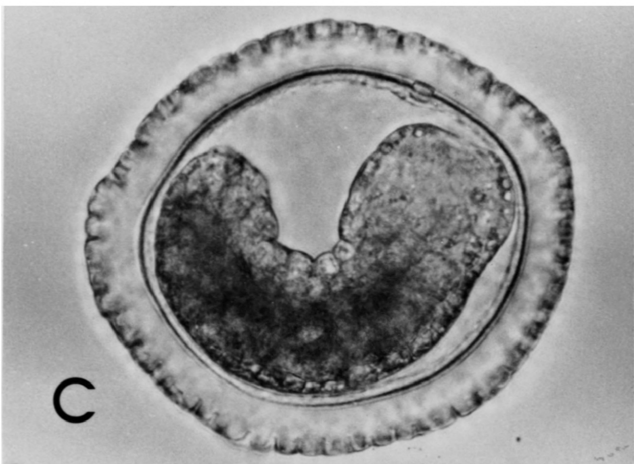
F. Motile, malformed, degenerating larva, day 7 (scale as in H)

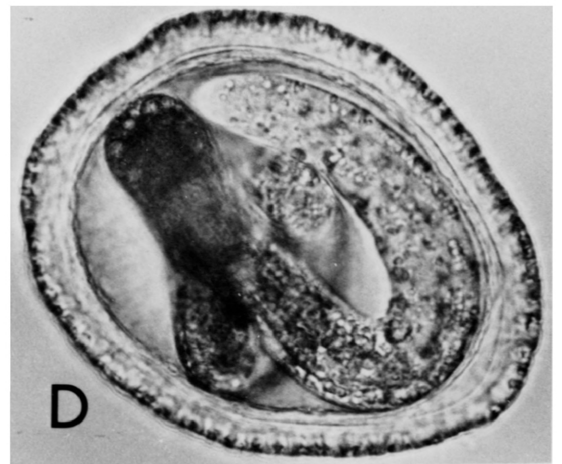
G. "Mature", infective larva after 6 months in acid culture. Loose cuticular sheath not apparent while in egg. Excretory pore prominent (arrow) (scale bar in H = 40 μm)

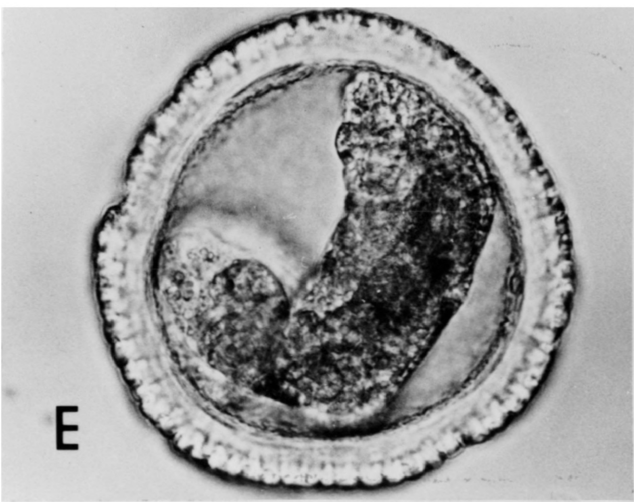
H. Dead larva in egg after 10 weeks in acid culture. Cuticle lifted from hypodermis (arrow), egg shrunken

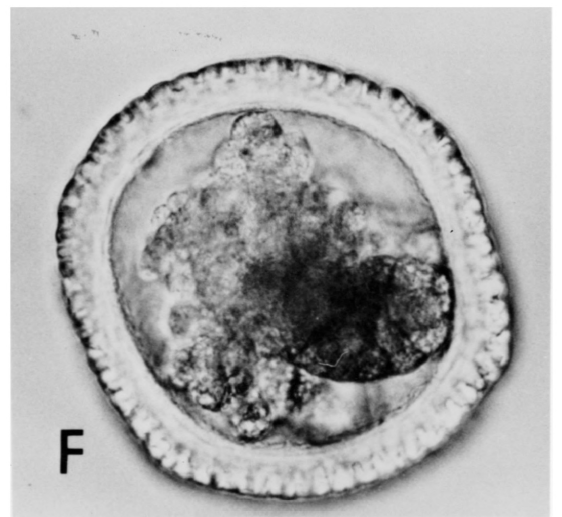

200 µm
A

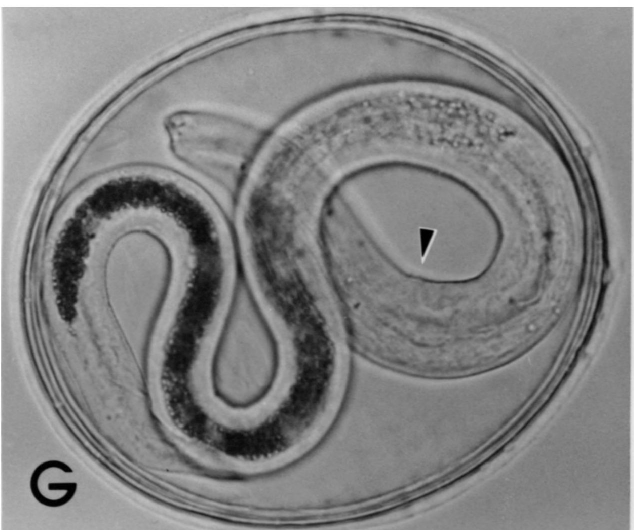

100 µm
B

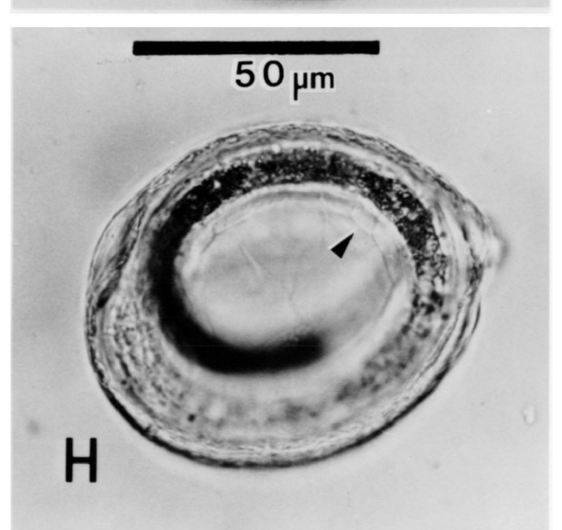

C


D


E


F


G


50 µm
H

5.11 ENVIRONMENTAL DISTRIBUTION OF *T. PTEROPODIS* EGGS

Mangrove leaves contaminated with bat faeces were collected from beneath the Pine River camp in January and February 1983 as described in Appendix III. Approximately 48,000 eggs were recovered from 200 leaves, i.e. an average of 240/leaf. About 10% were fully embryonated, 20% infertile and the remainder degenerate. Of another 100 contaminated leaves examined individually, 12% carried worm eggs (6/50 in January and 6/50 in February). Hence, the number of eggs per infected leaf was estimated at 2,000, of which 200 were potentially infective.

In February 1982, 20 green mangoes, apparently contaminated with bat faeces, from domestic trees about 1 km from Indooroopilly Island were examined and an estimated 18 eggs recovered (Appendix III). Eggs were not found on 3 papaws.

In January 1983, masses of tomato-like seeds carpeted the ground beneath the nursery camp at Pine River, and to a lesser extent at Beenleigh and Eagleby. Some of these seeds planted in nursery pots eventually grew into *Solanum hispidens*. Hence, on 17 January 1986, 1,500 berries were picked from dense stands of *S. hispidens* in a paddock at Keperra, 12 km from Indooroopilly Island and 15 km from the Pine River camp. Flying foxes had been seen in the vicinity at night, but not a single *Toxocara* egg was found in the washings of these berries.

On 23 December 1985, 40 mangoes were collected from home gardens in Townsville under the supervision of Mr Peter Foxwell, Chief Health Surveyor, Townsville City Council, and air-freighted to Brisbane. *Toxocara* eggs were not found on any of these.

On 23 January 1986, 27 mangoes picked from trees visited by bats about 1.5 km from the Indooroopilly Island camp were found to be free of *Toxocara* eggs. The following day, another 19 mangoes from a tree 300 m from that camp were also free of eggs. On the same day, 1,200 cocos palm (*Arecastrum romanzoffianum*) nuts, collected from under trees 400 m from the camp and

known to be visited by bats, were soaked overnight in detergent solution. A 5 ml aliquot of the 500 ml concentrated sediment did not contain Toxocara eggs.

On 3 February 1986, 500 g of Ficus obliqua fruit picked from trees visited by bats 2 km from Indooroopilly Island were found to be free of eggs.

On 4 February 1986, 43 mangoes picked from 2 trees 1 km from the Indooroopilly camp were free of eggs.

On 18 February 1986, about 1,200 figs were picked from 3 Ficus macrophylla in which mothers and juveniles had been seen feeding nightly for at least the preceding 2 weeks. Again, eggs were not found in the washings. Four representative clusters of figs in one of these trees were photographed from directly above to determine the cross-sectional surface of fruit in relation to total area. By measuring the diameters of figs in the photograph, it was calculated that fruit represented 1.7% (1/60) of the total horizontal aspect presented to particles (i.e. faecal pellets) falling from above.

Summarising, Toxocara eggs were not found on any seeds or fruit of trees frequented by bats, other than mangoes. From a total of 149 mangoes, from trees in 5 different locations visited by bats in the right season, only a few eggs were recovered from a sample of 20 mangoes in 1 tree.

5.12 DISCUSSION

The pre-patent interval in young P. poliocephalus infected with T. pteropodis is quite variable, ranging 35 - 48 d after birth in the 4 animals studied. This variation very likely reflects the protraction of larval passage through the mammaries as shown earlier. Fertile eggs in the faeces of a 35-d-old bat means that a male worm was already mature. The rapid conversion from infertile to fertile eggs in another juvenile aged

60 d indicates the male worm in this case took 25 d longer to mature than in the previous bat. Assuming equal rates of development, this male must have entered the bat's intestine 25 d later, which means that larvae undergo transmammary migration over a longer period than assumed earlier.

Related ascaridoids show varying pre-patent intervals, which probably depends on the developmental stage of the infecting larva and the migration route followed. Eggs of T. canis appeared in the faeces of naturally-infected pups 23 d old (Sprent, 1957). Noda (1956) found the pre-patent interval in pre-natally infected pups to be 21-27 d. In beagle pups infected orally with eggs, Dubey (1978) reported a pre-patent period of 30-35 d. Cats passed T. cati eggs 56 d after an oral dose, and the pre-patent period after eating infected mice is likely to be similar (Sprent, 1956). T. vitulorum eggs appeared in the faeces of calves 14-42 d old (Refuerzo & Albis-Jimenez, 1954) and in buffaloes 21 - 28 d old (Mia et al., 1975). In humans, the pre-patent interval for A. lumbricoides is 8-12 weeks (Vogel & Minning, cited by Beaver et al., 1984).

From the onset of patency, daily egg production in T. pteropodis increased gradually, perhaps reflecting growth of female worms or their genital tracts, and attained a plateau level after about 2 weeks. T. canis attain their maximal size when pups are 50 - 60 d old (Noda 1956), which is more than 3 weeks after the onset of egg production. In pigs, A. suum eggs appear when female worms are 17.5 cm and males 14 cm long (Schwartz, 1959), yet these worms grow to 39 and 24 cm respectively.

The average steady-state egg output of a female T. pteropodis is about 20,000 - 30,000/d, even without copulation. Despite few studies in other nematode species, Foor (1983) generalized that the egg output is directly correlated to size of the adult female. Dubey (1967) estimated that a female T. cati, slightly smaller than T. pteropodis (Section 6.3), produces 19,000 - 24,000 eggs/d with marked fluctuations in output. The adult female A. lumbricoides, about 10 times the size of T. pteropodis, produces

about 240,000 eggs/d, fertile or infertile, with considerable daily variation probably due to varying host faecal output (Brown & Cort, 1927; Sinniah, 1982). The output of T. canis, slightly larger than T. pteropodis (Section 6.2.6), is not known. According to Douglas & Baker (1965), a female T. canis may produce 200,000 eggs/d, but they produced no data in support of this figure which may be erroneous. Sprent & English (1958) reported faecal outputs up to 15,000 epg in some pups. Assuming these to be maximal levels, and that dogs may harbour more than 50 T. canis (pers. obs.), then in a pup passing 25 g faeces/d this would give an output per female worm approaching that of T. pteropodis. In children infected with A. lumbricoides, there is evidence of diminishing egg output per female worm with increased intestinal worm burden (Martin et al., 1983). In view of the small numbers of adult female worms in infected bats (average 2.14 per infected bat, maximum 4 - Section 3.2.4), it was not possible to confirm a density-dependent reduction in egg output, and this has not been reported with other Toxocara species.

Elimination of T. pteropodis eggs was not continuous, with fluctuating faecal concentrations and varying 24 h outputs. Occasionally rectal swabs were negative in bats with patent infections. Such fluctuations may have been due to intermittent discharge of eggs from the worms, or to changes in food intake which altered host intestinal motility and faecal fibre and fluid content. Mother bats confused the findings by ingesting their pups' faeces. When mother-pup pairs were kept together, often most eggs on the cage floor had developed beyond the 1-cell stage, and juvenile faecal droppings were not seen, indicating that all the eggs recovered had passed through the mother's intestine. When its mother escaped, bat 2 (Figure 5.1) appeared to produce less eggs in the following 24 h, perhaps because the mother carried off a sizable "reservoir" of eggs in her intestine. When moved to a new cage shortly afterwards, this juvenile stopped eating

for 1 day, passing very little faeces. With resumption of feeding and normal faecal output the following day there was an excessive 24 h output of eggs. Patent infection was never found in mother bats, unlike the common finding of adult T. canis in post-parturient bitches, which appear susceptible to infection from larvae in their pups' faeces (Sprent, 1961).

Variable rates of embryonation in eggs collected over 24 h periods may be partly explained by recycling through mothers, and also through intestines of juveniles. Some juveniles, found later by piperazine treatment or autopsy to be uninfected, were passing eggs, but usually these were developing and must have been ingested from the cage or by mutual grooming. Eggs dissected from female worms embryonated synchronously.

The rapid reduction in fertile egg output, in some cases to zero, with unchanged total numbers, within 24 - 48 h of the loss of male worms, indicates that copulation normally occurs at least once daily. The average of 23,000 fertile eggs recovered from 2 females, approximates the 24 h output. Spermatozoan stores are presumably of a similar order. Alternatively, spermatozoa remain effective for only one day, or perhaps "unused" spermatozoa are flushed from the uterus with the fertile eggs.

It is not surprising that spermatozoa of T. pteropodis resemble those of another ascaridoid, A. suum. Perhaps their amoeboid motility indicates that nematode spermatozoa migrate along uterine walls and oocyte membranes prior to fertilization. Foor & McMahon (1973) showed that in A. suum, spermatozoa are activated by secretions from the glandular vas deferens (ejaculatory duct) presumably during copulation. Female T. pteropodis producing fertile eggs always contained spermatozoa in varying numbers, whereas those releasing exclusively infertile eggs were always totally depleted. "Senescent" females producing diminishing numbers of increasingly infertile eggs, sometimes contained large numbers of spermatozoa. It is not known if spermatozoa become ineffective in late patency, or whether the frequency of copulation diminishes. However, from

the findings in younger female worms following expulsion of a male, it would appear that in senescent females there is defective fertilization of oocytes. Older spermatozoa may not be able to reach oocytes before the outer uterine shell is deposited, rendering the egg inpenetrable.

Male worms were surgically implanted into the intestine of a bat harbouring only females to determine the interval before fertile eggs appeared. The total cessation of egg production was unexpected. Perhaps the anaesthetic agents used inhibited oogenesis. Being late in the season, possibly the female worms were already senescent. Worms producing exclusively infertile eggs presumably become senescent as do those producing fertile eggs. The resumption of egg production 10 d later, at low levels, and its termination after 6 d are inexplicable as were the fate of the female worm and implanted males.

The phenomena of diminishing fecundity and spontaneous expulsion of adult worms are likely to be related and distinct from that of intestinal larval loss (Chapter 4). Various mechanisms may be involved. Host diet may affect parasite physiology and biochemistry (Lee, 1982). Host nutrition influences the egg output of several parasitic nematodes (Anya, 1976), but this has not been evaluated in ascaridoids. Perhaps in captive bats, a deficiency of essential nutritients produced reproductive abnormalities in their worms. However, worm loss occurred sooner and more readily in bats caught late in summer than earlier. This suggests that loss of adult worms was "normal" and unrelated to captivity, as did the finding of expelled worms in the field and the natural diminution in prevalence of patent infections in older juvenile bats (Chapter 3).

Damage to worms, manifesting initially through rapidly turning-over genital cells,may result from the cumulative effects of host immunity. In many parasitic nematodes, host resistance mediated via the immune system may cause diminishing egg output (Anya, 1976). In one of the best-studied

model systems, Nippostrongylus braziliensis in rats, adult worms are damaged during a primary infection (Wakelin, 1984). There is an early reversible decline in egg output with the appearance of tissue damage, but worms expelled from the gut are alive and active. Responsible mechanisms probably include mucosal inflammation, release of amines by degranulating mast cells, increased efflux of antibodies into the gut lumen, increased mucus production and other as yet undetermined consequences of host immunity. In guinea pigs, suppression of eosinophilia with anti-eosinophil serum increases susceptibility and diminishes immunity to Trichostrongylus colubriformis (Gleich et al., 1979).

However, in ascaridoid infections the situation is likely to be different. In patent A. lumbricoides infections in humans, hosts tolerate adult worms and immunity is not sterilizing; parasite numbers are controlled apparently by acquired resistance to migrating larvae (Pawlowski, 1978). According to Wakelin (1984), "there is evidence suggesting that immunity [against adult A. lumbricoides] is non-existent". Anderson (1986) strongly supports this, as well as the concept that some individuals are predisposed to heavy infections regardless of immune status, and reports that senescent A. lumbricoides produce less eggs.

Spontaneous expulsion of ascaridoid adults has been described. It is common in humans with A. lumbricoides and occurs regularly in individuals who are repeatedly acquiring patent infection (Otto, 1930) suggesting that worm senescence rather than host immunity is responsible. Anderson (1986) concludes that the worm's longevity is less than 1 year. With T. canis, which is highly prevalent in pups but not in dogs older than 6 months (Sprent, 1958), Noda (1956) reported that naturally-infected pups commence losing worms at about 1 month, although some worms "remain in the hosts until about 2 months after birth". Working with ascarid-naive dogs, Dubey (1978) concluded that spontaneous worm loss was not a manifestation of host "age-resistance". Expulsion of T. cati occurs frequently in cats (Dubey,

1967), although here a limit to patency has not been clearly defined. In cattle and buffalo calves infected with *T. vitulorum*, natural worm expulsion commences as early as 37 d after birth and very few animals remain infected by 1 year of age (Thienpont & de Keyser, 1981). Gupta (1986) found 82% buffalo calves infected, with a maximal limit of patency of 60 d.

It is likely, therefore, that the spontaneous loss of adult *T. pteropodis* from young flying foxes results from worm aging rather than immunity, facilitated by hastened intestinal transit associated with the changing host diet.

Morphologically, the eggs of *T. pteropodis* are similar to those of other *Toxocara* species, the major difference being their large size. According to Warren (1971a), *T. vitulorum* eggs range 76-95 µm x 65-85 µm; *T. canis* 66-85 x 64-77 µm and *T. cati* 65-77 µm in diameter. Cellular cleavage and embryogenesis in *T. pteropodis* followed the typical pattern seen in other nematodes as outlined by Bird (1971). Infective larvae of *T. pteropodis* developed earlier (11 d) than in *T. canis* and *T. cati*, which at room temperature (20-25 $^{\circ}$C) required 2 - 3 weeks to attain infectivity (pers.obs.) and at 30 $^{\circ}$C take at least 11 d (Okoshi & Usui, 1968). At 26 $^{\circ}$C, eggs of *T. vitulorum* are not infective before the 11th d; below 10 $^{\circ}$C they fail to embryonate, and above 30 $^{\circ}$C they develop rapidly but degenerate (Irfan & Sarwar, 1954).

The egg-shell layers of *T. pteropodis* correspond with those described by Foor (1967) for *A. lumbricoides*. In comparison with *T. canis*, the eggs of *T. pteropodis* possess a distinctly thicker shell. The diameter of the internal space is similar in both species, which is in keeping with the almost identical sizes of newly-hatched larvae of these 2 species (Chapter 6). The outer proteinaceous uterine coat of *T. pteropodis* is particularly thickened, but in many eggs the vitelline layer, immediately subjacent to

the uterine layer, is thicker as well (Fig 5.3 D). This thickened shell is likely to be an adaptation for survival in the environment.

The susceptibility of ascaridoid eggs to desiccation is well-known. Otto (1929) found that *A. lumbricoides* eggs would develop within the temperature range 7-38 ^{o}C, and those of *T. canis* at 6-40 ^{o}C. The survival range for embryonated eggs was wider, but above 50 ^{o}C larvae died rapidly. Within the developmental range, drying led to early death of embryos within their shells. By spreading egg suspensions on glass slides, Otto found that, at 22 ^{o}C in a relative humidity of 50%, eggs of *Parascaris equorum* embryonated, but not those of *A. lumbricoides* or *T. canis*. By increasing the humidity, he found that *T. canis* was more resistant than *A. lumbricoides*. At humidities approaching 100%, development and survival were maximal. Okoshi & Usui (1968) found that *T. canis* and *T. cati* larvae survived in their shells at 40 ^{o}C for only 3 days. However, as no provision was made for maintaining humidity in their experiments, it is likely that desiccation rather than temperature destroyed the embryos. According to Wharton (1979), desiccation is a major hazard of the terrestrial environment. He found that the rate of *A. suum* egg development was temperature-dependent and was not influenced by humidity. However, at low humidities, eggs collapsed from desiccation before development was completed. In a dry atmosphere, water loss from these eggs rose exponentially with temperature, suggesting that the permeability of the shell increased with temperature. In a later review of nematode egg-shell structures, Wharton (1980) reiterated that a membrane permeable to oxygen must also be permeable to water vapour, and speculated that the proteinaceous layer was responsible for minimizing desiccation. The function of pores, or pits, in this layer, was to permit adequate oxygen diffusion while minimizing water evaporation. Virtually all ascaridoids of terrestrial hosts produce eggs with a pitted outer layer, which has been well-illustrated by SEM for *A. lumbricoides*, *A. suum*, *T. canis* and *T. cati*

by Ubelaker & Allison (1975) and for Bayliscaris procyonis, B. transfuga, P. equorum, T. canis and A. suum by Kazacos & Turek (1983). Ascaridoids of aquatic hosts, completing their life-cycles entirely in water and therefore not exposed to desiccation, should have no need for a pitted outer egg-shell layer. Indeed, eggs of Anisakis marina have a thin smooth shell (van Thiel, 1966) and this appears to be the case with related species.

The morphology of infertile eggs of T. pteropodis can be explained by the formation of shell layers. Developing oocytes of A. lumbricoides contain numerous lipid droplets and refringent granules (Foor, 1967). When fertilization occurs, a dense vitelline layer separates from the cytoplasm, and beneath it a clear zone appears which becomes the 3 µm-thick chitinous layer. At the same time, an external uterine layer begins to deposit and an inner lipid (ascaroside) layer, distinct from the cell membrane, begins to form from the coalescence of extruded refringent granules. The uterine layer gradually increases in thickness. The formation of all layers other than the uterine is triggered by sperm penetration of the oocyte, and does not proceed in unfertilized eggs. Later, Foor (1983) reported that a thin chitinous layer may appear at the margin of infertile Ascaris eggs. This is consistent with the finding of a thin membrane in some infertile eggs of T. pteropodis just beneath the uterine layer (Fig. 5.5 D & H). Foor (1983) showed that oocytes which progressed beyond the uterine-oviduct junction could not be fertilized, so that even under normal circumstances a small proportion of eggs would be infertile. This was seen with T. pteropodis.

The localised bulging ("blow-out") of infertile eggs immersed in water (Fig. 5.5 E & F), occasionally leading to gross swelling of the entire egg (Fig. 5.5 M), is almost certainly an osmotic effect, and indicates that it is not the uterine coat which protects against water influx, or provides mechanical strength, but the underlying layers which have not formed in these infertile eggs. De-corticate fertile eggs were never observed to

swell in this fashion. This aspect of shell layer function has not been investigated for ascaridoid species and casts doubt on the practice of de-corticating infective eggs with sodium hypochlorite and detergents for experimental hatching.

The occasional finding of grossly deformed, fertile eggs of T. pteropodis is not without precedent. A variety of grotesquely-shaped eggs, some embryonating normally, have been reported for T. vitulorum (Refuerzo et al., 1954). Douglas & Baker (1965) published photographs of "aberrant" eggs of T. canis, which in fact appeared to be infertile, concluding that deformed eggs were incapable of embryonation. In the present study it was not unusual to find mis-shapen eggs of T. pteropodis containing motile larvae, but it is not known if they could hatch. The shape of the egg in Fig. 5.3 J suggests that the outer proteinaceous coat was not applied directly by uterine cells but precipitated from secretions in the uterine lumen. This would explain the variation in surface pit sizes; the early thin shell may have wide shallow pits, which narrow and deepen as the uterine layer accretes (Fig. 5.4 D & F).

The fate of T. pteropodis eggs in the environment, crucial to the acquisition of infection by adult bats, warrants a detailed consideration of their dispersal. Juveniles commence passing eggs when they are being left behind in camps at night while the adult bats fly off to feed. During this period, faeces containing eggs are deposited in the camp environs. After returning at dawn, the mothers groom their offspring and so ingest much of their pups' egg output. Adult bats defaecate frequently while resting in the camp through the day, and void a considerable volume of faeces just prior to departing on their evening flights (pers. obs.), so the camp environment is very heavily contaminated with faeces. Hence, many eggs ingested by nursing mothers would be deposited in the roost, but undoubtedly some would be carried out on the nocturnal feeding flights. Dissemination of these residual eggs in the wider environment would be

influenced by the maternal diet. With a high fluid/fibre intake (e.g. cultivated fruit) intestinal transit could take less than 20 minutes (pers. obs.; Tedman & Hall, 1985). However, the bulk of eggs in the mother's gut would have been deposited in the vicinity of the camp. Contamination of fruit and blossom trees would be highest close to the camp and decline with increasing distance. From about 6 - 8 weeks of age juveniles commence foraging with their mothers, so the milk component of their diets then gradually diminishes. Adult worm losses commence about this time, although some juveniles continue passing eggs perhaps for as long as 2 - 3 months. Again, the highest faecal egg concentrations would be found while pups suckled during the day as milk produces minimal faecal bulk (pers. obs.). At night, on a fibrous diet, epg levels would be much lower. It is not known what effect a blossom diet, perhaps more important to flying foxes than fruit, has on faecal bulk and intestinal transit time, as this has not been studied in *Pteropus*.

In the Moreton Bay figs examined (*F. macrophylla*) fruit accounted for less than 1.7% (1/60) of horizontal-plane cross-sectional area. As this fruit is relatively dry, and feeding bats spit out most of the seeds and fibre whilst feeding (pers. obs.), only small volumes of juice actually enter the intestine. Hence, whilst eating figs, a bat is likely to defaecate perhaps once every hour. This was observed in captive animals. Assuming that 5 mother-juvenile pairs visit such a tree each night, and each juvenile defaecates only once while feeding there, the chance of a faecal pellet falling onto a fig is only 5 x 1/60, i.e. 1 in 12. This means that after 12 nights of such visitations one fig would become contaminated, and there is only a 50% chance that such faeces will contain fertile eggs. Furthermore, most figs at any time are green, so a considerable period may elapse before such a fig is ready for consumption. On trees such as palms, e.g. *A. romanzoffianum*, a highly favoured food

source (pers.obs.), the bats invert away from the fruit cluster to defaecate so contamination of fruit is even less likely.

The almost total absence of Toxocara eggs from the various fruits examined supports these considerations and suggests fruit is not the vehicle of infection. Furthermore, when eating mangoes, bats do not chew the skin, but tear off strips and eat beneath it (pers. obs.) thus reducing their chances of ingesting any Toxocara eggs on the surface. The situation on Palm Island in November, 1979, when mangoes were heavily contaminated (Moorhouse, 1982) could have been unique. The mango trees on the island are centrally clustered in the human settlement, less than 1 km from the P. alecto camp. As there is little alternative food at that time of year, the mangoes are subjected to heavy depredation by adult and juvenile bats aged 2 - 3 months (pers. obs.), but this need not implicate the mangoes in infection transmission.

The unavoidable conclusion is that larval hepatic infection of adult (and occasionally juvenile) bats is acquired in camps. Under the roost site, 12% of mangrove leaves were contaminated with T. pteropodis eggs, with an average of about 200 infective eggs per leaf. Levels of contamination would diminish higher in the trees, as flying foxes space themselves both vertically and horizontally in their camps. Early in this study, it was suspected that adult bats acquired infection by active coprophagy, on the basis that this would also satisfy their vitamin B_{12} requirments (Appendix IX). However, despite repeated attempts, convincing evidence of this behaviour was not observed. Nevertheless, many opportunities for the accidental ingestion of faeces were witnessed. In heavy rain, the bats wrapped their wings around their bodies and hung tightly, but during lulls in the storm there was much agitation as they "walked" along branches, frequently grooming themselves and licking drops of water from leaves and branches. Their claws, heavily coated with wet faeces from the branches, were used to "comb" the fur, and were then

cleaned by licking. Shed fur, ectoparasites, faeces and other adherent matter was thus ingested. This behaviour during rainfall seems the most likely explanation for infection acquisition, and would also contribute to the bats' vitamin B_{12} needs. Furthermore, bats roosting higher would be less exposed to infection, and those at low levels to the heaviest infections. Almost without exception, P. alecto roosted higher, often directly above, P. poliocephalus, in camps in south-east Queensland. When P. scapulatus joined these camps, they occupied peripheral regions and ventured into low sites directly under P. poliocephalus (Fig. 7.4 C). This may explain the very low prevalence of infection in southern P. alecto and the unusual features of T. pteropodis infections in P. scapulatus (Chapter 7).

The low proportion of eggs on mangrove leaves containing motile larvae probably reflects the adverse environmental conditions. In the laboratory, too dense a layer of fluid or faeces inhibited embryonic development and promoted larval degeneration, presumably because of unfavourable redox conditions. The opacity of bat faeces would protect eggs from ultraviolet radiation, but direct sunlight could destroy the larvae by heat and desiccation. The outer pellicle forming on the sticky faecal pellet may protect eggs immediately beneath the surface from drying yet allow sufficient oxygenation for development. High temperatures may also destroy many eggs, but a significant proportion on mangrove leaves would be in shade for most of the day. On the leaves of fruit trees, infectivity did not persist beyond 6 weeks; in the harsh environment of the flying fox summer camp it is unlikely many eggs would survive longer, even though in the laboratory occasional eggs suvived over 6 months.

Nevertheless, T. pteropodis is highly successful. Considerable egg losses are compensated by intense localised environmental seeding. In a summer camp such as at Indooroopilly Island, each year perhaps 20,000 young

P. poliocephalus are born (pers. obs.). If half of these each carried 1 fertile female worm, then for a period of about 3 months every summer 200 million eggs would be shed daily into a mangrove forest covering a land area of about 5 hectares, giving a cumulative density of 40 eggs/cm^2. The advantages of rapid embryonation in a hostile environment are obvious. However, there is little to be gained by the eggs remaining infective for more than a few months, as the bats disperse to their winter camps in autumn. There also would be little to gain by the worm's persisting in juvenile bats beyond this time, for, as the juvenile bat is weaned to an increasingly herbivorous diet, accelerated intestinal transit, to which the bat intestine is highly adapted (Tedman & Hall, 1985), may impose nutritional austerity upon the worm. Furthermore, over winter there is so little rainfall that transmission of the infection would be seriously compromised. Hence, the worm has adopted the strategy of "hibernating" as larvae in the livers of adult female flying foxes.

In effect, *T. pteropodis* is a soil-transmitted nematode, the "soil" in this case being deposited along tree branches and on leaves. The eggs show special adaptation, in their shell structure, to resist desiccation, and their limited infectivity is consistent with the short season of transmission.

The development of larvae is described in the following chapter.

CHAPTER 6

THE DEVELOPMENT OF *T. PTEROPODIS*

6.1 INTRODUCTION

Further understanding of the life-cycle of this nematode required detailed information on its developmental stages. Morphological changes in larvae as they mature in eggs to infectivity were observed, as well as changes in larvae passing through bat livers and mammary glands, and establishing in juvenile intestines leading to patent infections. The somatic, or 3rd-stage, larva is described in considerable detail, being the stage most likely to be found in human tissues should *T. pteropodis* prove to be zoonotic. Morphological features are compared with those of *T. canis* and *T. cati*, since these are the most likely to be confused with *T. pteropodis*, and were the only other available members of the genus.

6.2 OBSERVATIONS

6.2.1 Larval development in the egg

Eggs from *T. pteropodis* uteri were kept in 0.1 N sulphuric acid at room temperature (20 - 26 ^{o}C) (Section 5.10.2). When larvae had developed in embryonated eggs they were expressed daily in tapwater, in 0.85% saline, in 1.7% saline and in 2.5% saline to investigate osmotic effects.

By day 5, 60% were recognisable as 1st-stage larvae (Fig. 5.6 D). A cuticle at first was not apparent, lips were not formed and internal organs not visible. The body was packed with coarse refractile granules, and the only discernible features were the paler anterior end and the pointed tail. They disintegrated when expressed from eggs.

By day 6, most larvae were covered in a recognisable cuticle, and a small number were successfully expressed without being disrupted (Fig. 6.1A)

A). Although internal structures were indiscernible, the oesophageal and caudal regions were clearly delineated by a much lower density of granules than typified the intestinal region (Figs 6.1 B & C). Early lips in some larvae were clearly visible (Fig. 6.1 D) and in 10% of 100 larvae expressed, the cuticle over the lips was lifted as a small bleb (Fig. 6.1 E). In some, there appeared to be a double layer of cuticle to this bleb (Fig. 6.1 F) indicating the beginning of the 1st moult.

On day 7, most expressed larvae were still too damaged for examination, but about 70% of intact larvae possessed an oral bleb or a loose cuticle (Figs 6.1 G & H) indicative of moulting. The larvae on day 7 could not be distinguished from 1st-stage larvae, but presumably some had completed the 1st moult.

On day 8, several larvae were seen with distinct double-layered cuticles (Fig. 6.2 A), and about 10% (5/48) appeared to have 2 layers of loose cuticle overlying the normal cuticle (Fig. 6.2B). This was usually most evident at the anterior end. The double loose sheaths were seen on larvae expressed in tapwater and saline of different concentrations.

On day 9, 2 distinct layers of loose cuticle were seen in 7% (4/56) of expressed larvae, at either or both extremities (Fig. 6.2 C, D & E). In some, the nerve ring and the outline of the oesophagus were apparent (Fig. 6.2 D).

On day 10, larvae showing evidence of a 2nd moult, with loose double cuticular sheaths, comprised 3% (2/61) of those expressed intact from eggs (Fig. 6.2 F), whereas on day 11, such larvae accounted for only 1% (1/76) of the total (Fig. 6.2 G). They were not seen beyond this time.

On day 10, the first "mature"-looking larvae appeared, typical of the infective stage. Most of these were quite active, compared with those from older eggs, and were enclosed in a very loose cuticular sheath (Fig. 6.2 H), a feature of most infective larvae beyond this time (Fig. 6.3 A). The

FIGURE 6.1

Larvae expressed from T. pteropodis eggs embryonating 6-7 days in 0.1 N sulphuric acid at room temperature, photographed live in saline solutions

A. Day 6, first-stage larva in 0.85% saline

B. Tail region of same larva as in A (scale as in C)

C. Cephalic region of same larva

D. Day 6. Lips of first-stage larva (d = dorsal lip)

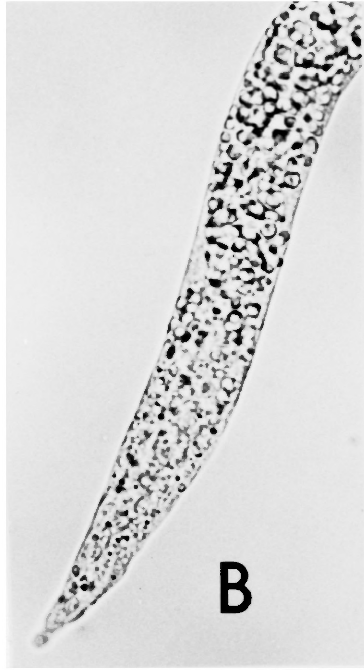
E. Day 6. Cuticular bleb at lips (scale as in C)

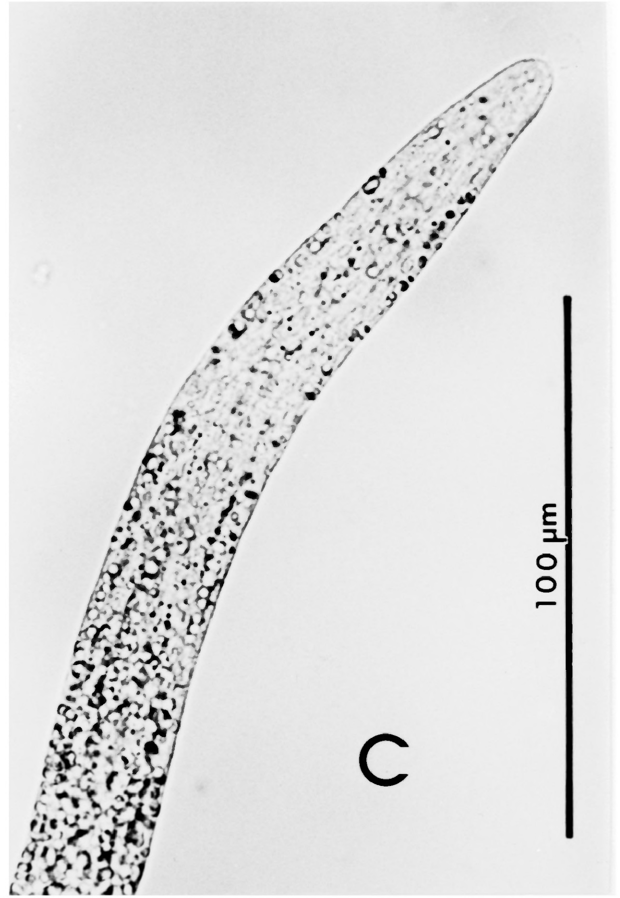
F. Day 6. Larva (450 x 25 um), in 0.85% saline, with a second cuticular layer (arrowed) overlying the oral bleb (scale as in D). Note barely discernible cuticular striation lower right corner.

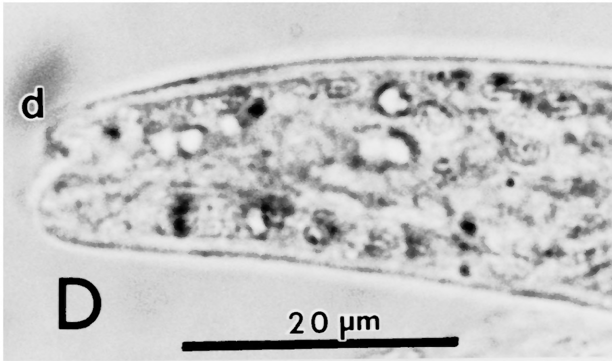
G. Day 7. Larva expressed in 2.5% saline. Cuticle lifting along body (arrows). First moult.

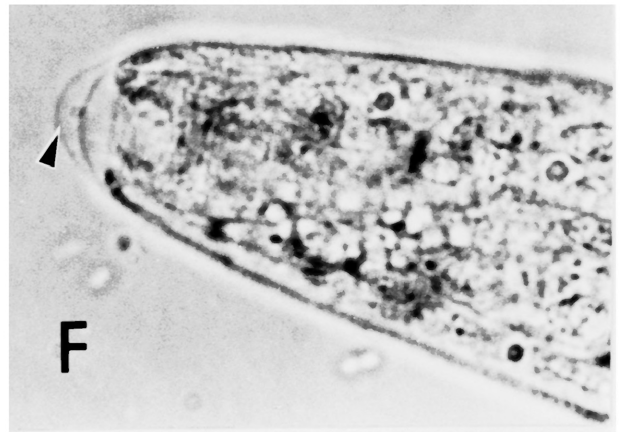
H. Day 7. Larva possibly with damaged anterior end, expressed in 2.5% saline. First moult.

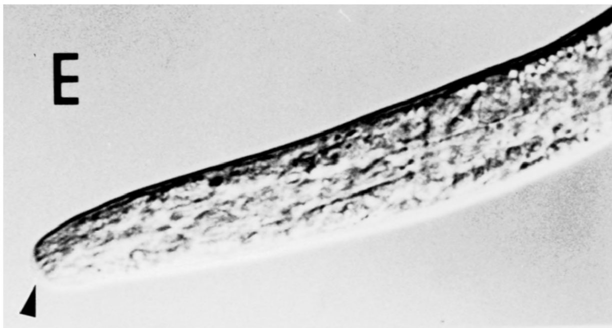
200 µm
A

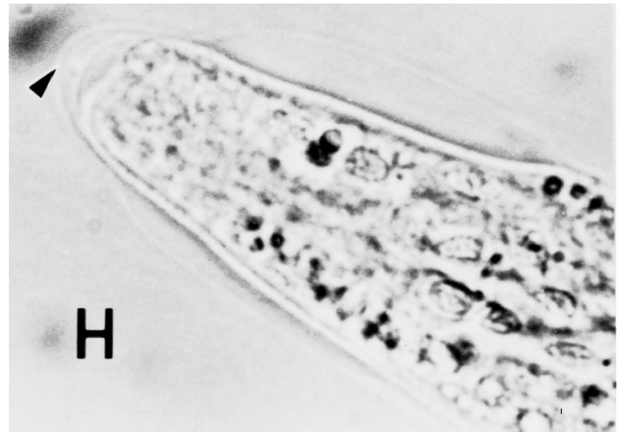

B


100 µm
C


d
D
20 µm


F


E


H

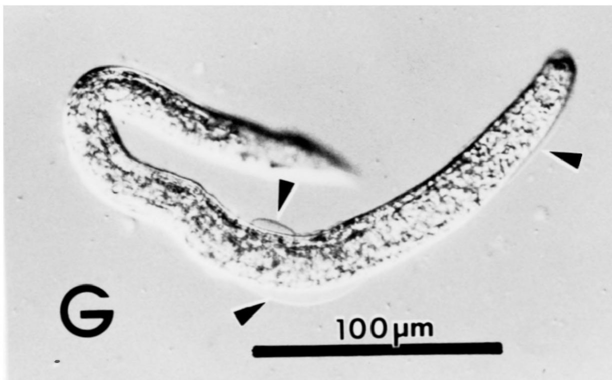

G
100 µm

FIGURE 6.2

Larvae expressed from *T. pteropodis* eggs embryonating 8 - 11 days in 0.1 N sulphuric acid, photographed live in saline solutions (unless otherwise stated)

A. Day 8. Larva with distinct double cuticle (arrows). Scale as in G.

B. Day 8. Larva with oral bleb, a second cuticular layer at mouth (arrow) and apparently a third outermost layer (arrow). Scale as in E.

C. Day 9. Larva expressed in 1.7% saline. Normal cuticle covered by 2 outer layers (arrows). Second moult. Fine hypodermal striations just perceptible in mid-body region. Scale as in G.

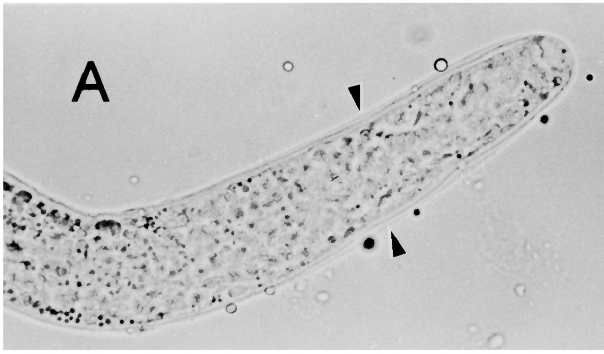
D. Day 9. Larva in 0.85% saline, second moult. Three cuticular layers clearest at arrow. Scale as in G. (n.r. = nerve ring).

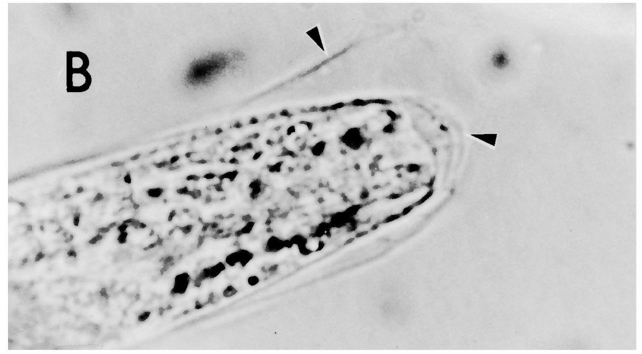
E. Day 9. Larva in 2.5% saline, second moult. Two loose cuticular sheaths in caudal region.

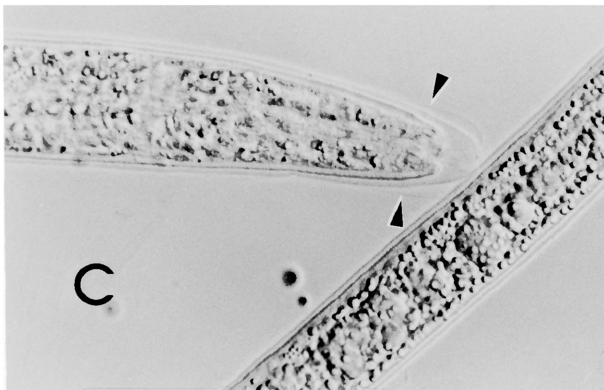
F. Day 10. Larva in 2.5% saline. Double-layered cephalic sheath (outer layer arrowed). Scale as in E.

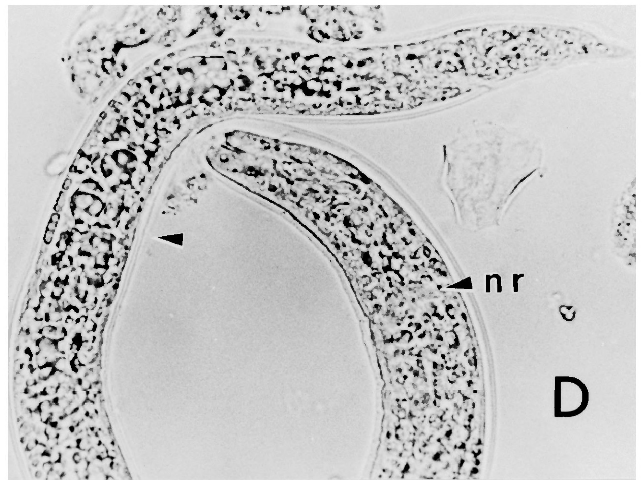
G. Day 11. Larva in 2.5% saline. Double-layered caudal sheath. Arrow at tip of tail cuticle. Second moult.

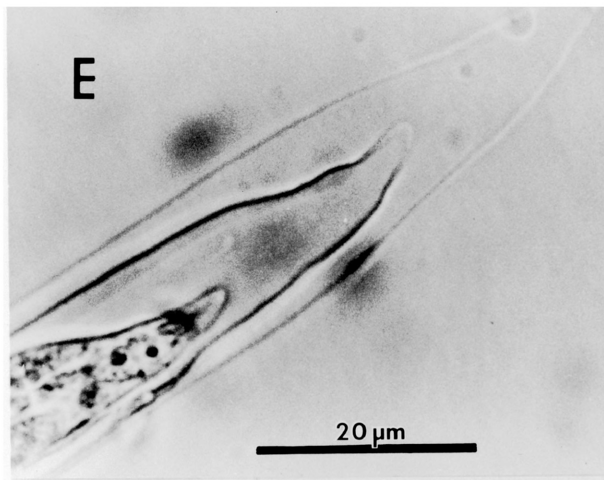
H. Day 10. Infective third-stage larva expressed in tapwater. Loose cuticular sheath. Ventral labial protuberance well-developed, oesophagus buckled, excretory pore clear (arrow), gut region granular. Scale as in G.

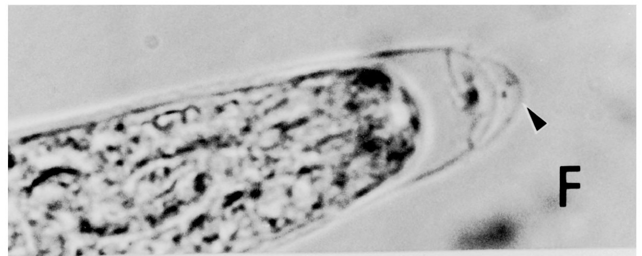

A

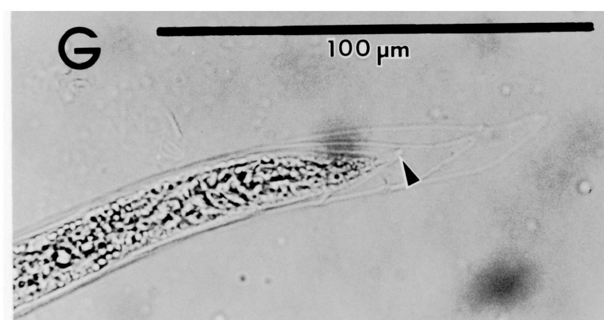

B

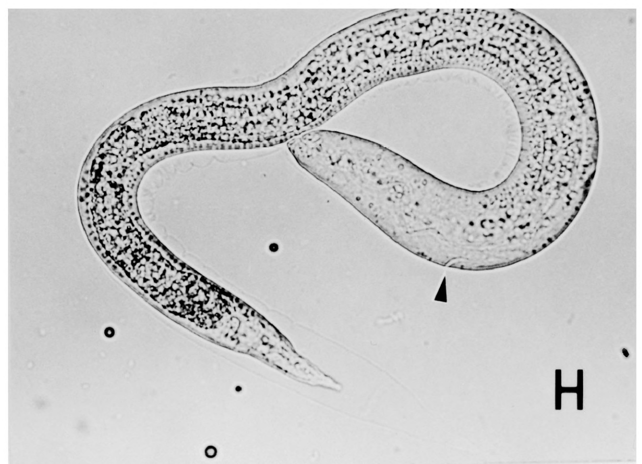

C


nr
D


E
20 µm


F


G
100 µm


H

intestinal region was still coarsely granular, with most granules apparently in the gut cells. The oesophageal region was clearer, with the ventriculus barely discernible in living specimens. The oesophagus was distinctly buckled, particularly in the vicinity of the nerve ring, and the excretory pore and canal were conspicuous. At the lips was a prominent ventral cuticular thickening. The tail was tapered and pointed, with a slight pre-terminal constriction. The genital primordium, 10 - 13 µm long, comprised apparently 3 cells and lay ventrally about midway along the intestine. Other structures were difficult to identify in living specimens, but in formalin-fixed larvae, the excretory nucleus was seen on the left side ventro-lateral to the ventriculo-oesophageal junction, and the nucleus of the dorsal oesophageal gland occupied the posterior third of the dorsal segment of the ventriculus. Between 7 and 13 intestinal cell nuclei could be discerned between the ventriculus and rectum. Fine cuticular striations (periodicity 0.6 - 1.0 µm) were evident at the caudal and cephalic regions of some larvae.

After 10 d embryonation, 51 intact larvae expressed from eggs then washed from the slide with 70% alcohol had a mean length of 350 $\pm$ 50 µm (range 220 - 450). The length of 43 larvae from eggs embryonated 30 d was 400 $\pm$ 40 µm (range 320 - 490).

Dimensions of 49 larvae expressed from eggs embryonated for 70 d and fixed in hot 3% formalin were:-

mean length: 425 $\pm$ 25 µm; range: 360 - 470 µm

maximal width: 17.5 $\pm$ 1.2 µm; range: 15 - 21 µm

mouth - genital primordium: 57.4 $\pm$ 1.3% body length;

range: 54.1 - 59.7%

6.2.2 Larval development in liver; comparison with *T. canis* and *T. cati*

The growth of hepatic larvae has been outlined in Chapter 4, with details in Fig. 4.1 and Table 4.5. Moulting was never observed. Only from the livers of 2 mice, killed 24 h pi, were live larvae recovered which had apparent loose cuticular sheaths. Immobilization by heat fixation, for photography, caused the sheaths to disappear. Attempts to repeat this observation, in mice and in bats, were unsuccessful. Often loose cuticles were seen on fixed larvae from both experimentally- and naturally-infected bats, but there was no consistency in time after infection or lengths of such larvae. Loose cuticles were seen as early as 3 d (Fig. 6.3 B) and as late as 1 year after infection, in larvae 440 - 750 µm long, and varied from a subtle to a gross degree, with almost the entire cuticle lifted off the larva. The affected larva never appeared quite normal, with indistinct internal morphology, and in most instances many (often more than 50%) larvae from a single liver were thus affected. Sometimes the ventriculus and intestine were separated, or gut disrupted, in these larvae.

As well as growth, and a reduction in granularity, there were other morphological changes in hepatic 3rd-stage larvae. The ventral labial protruberance and cuticular thickening gradually became less prominent and somatic muscle cell nuclei less conspicuous. The genital primordium grew slightly, to 12 - 17 µm in length, but still appeared to comprise 3 cells (Fig. 6.11 A).

At 5 d pi, intestinal cell nuclei in most larvae numbered 7 - 14 (median 10) (Fig. 6.3 C), but thereafter became indeterminable apparently as a result of rapid multiplication (Fig. 6.3 H). The nucleus of the dorsal oesophageal gland cell at 5 d pi occupied the posterior dorsal sector of the ventriculus, although in some larvae it was at mid-level, and by 50 d, it occupied the posterior left subventral sector (Fig. 6.3 D). The ventriculo-intestinal junction comprised a zone of 2 or 3 compressed cells (Figs 6.3 H, 6.4) and the lumen of the posterior ventriculus was

FIGURE 6.3

Third-stage larvae of T. pteropodis

. Infective third-stage larva expressed from egg in tapwater after one month in charcoal culture. Arrow at excretory pore.

. Formalin-fixed larva recovered from bat liver 3 days post-infection. Arrow shows subtle lifting of cuticle (pseudo-moulting).

. Same larva and same scale as in B, showing first 2 intestinal cell nuclei (arrow); v = ventriculus. Note cellular junctional structure between gut and ventriculus.

. Transverse section through posterior ventriculus of larva in liver of bat 50 days post-infection. Nucleus of dorsal oesophageal gland in left ventro-lateral position (d). Arrows indicate columns of excretory cell within right and left hypodermal cords. Scale as in B.

. Same larva and scale as in D, 20 µm posterior to ventriculus. Intestine (i) appears to comprise lumen surrounded by 3 longitudinal columns of cells. Excretory columns (c) in lateral cords; arrows indicate lateral alae of cuticle.

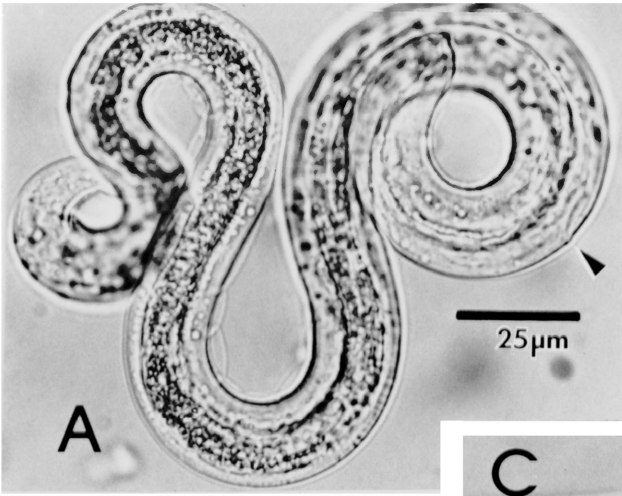
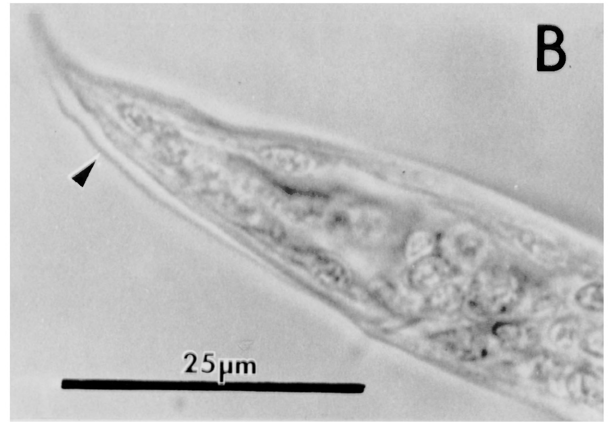
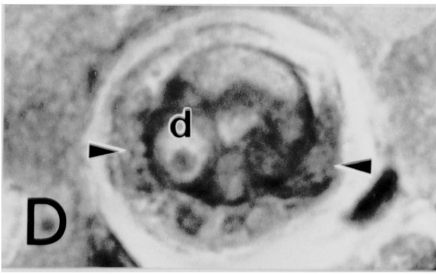
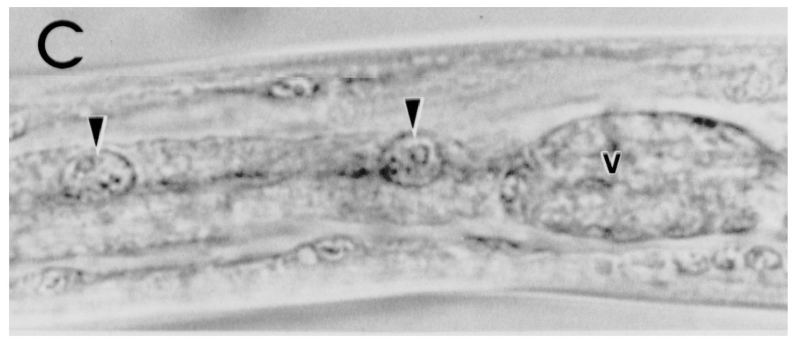
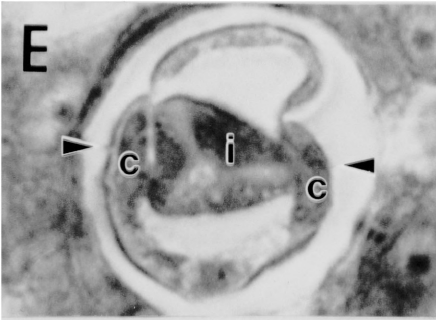
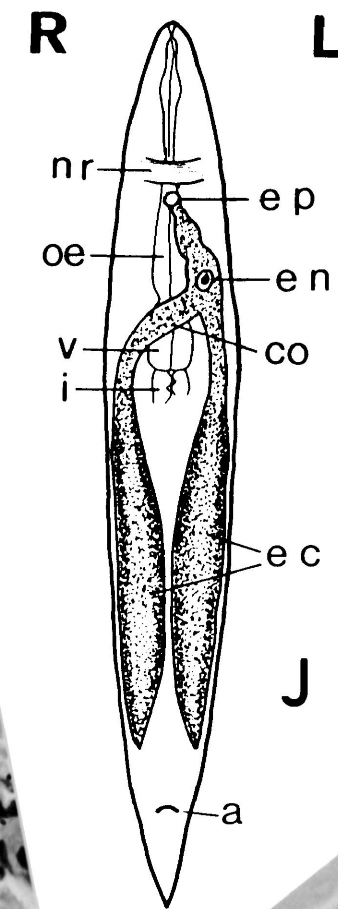
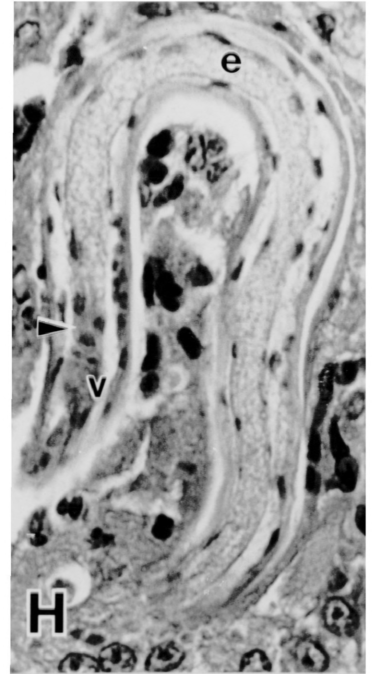
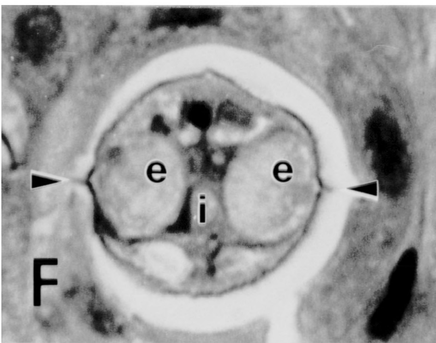
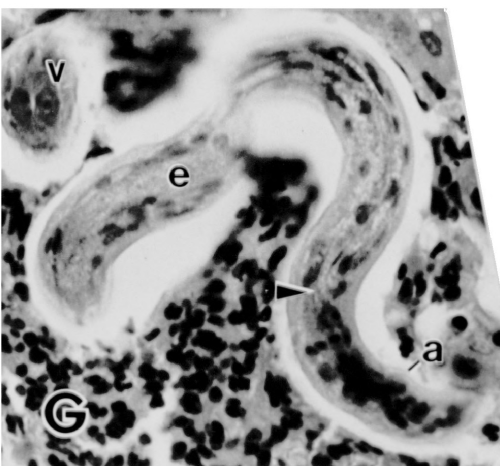
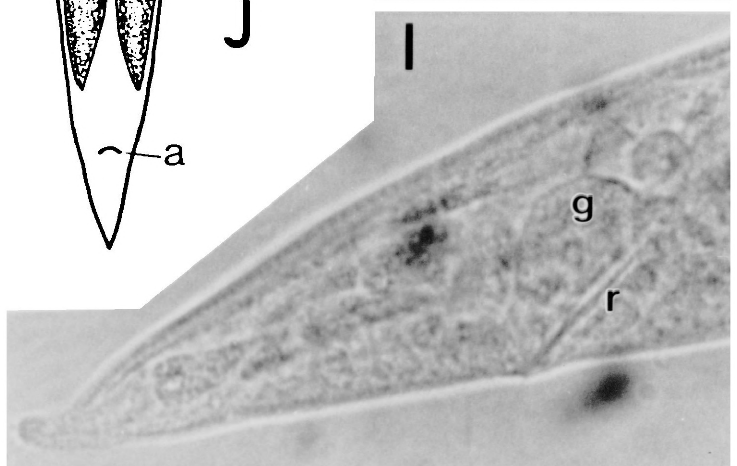
. Transverse section through mid-body region of larva in liver of bat 21 days post-infection. Excretory columns (e) grossly distend lateral cords and compress intestine (i). Scale as in B.

. Section of larva from bat liver 50 days post-infection. Pale, finely granular excretory columns (e) cut obliquely and longitudinally, with arrow indicating tapered termination 25 µm anterior to anus (a). Ventriculus (v) sectioned through adjacent nuclei of 2 subventral glands. Scale as in A.

. Section of larva from bat liver 21 days post-infection, showing excretory column in longitudinal section (e), lined with thin unicellular layer. First 3 gut cell nuclei (arrow) are evident just behind ventriculus (v), with 2 "compressed" nuclei in junctional zone. Scale as in A.

. Tail of formalin-fixed larva recovered from bat liver 6 months post-infection, showing particularly prominent dorsal rectal gland nucleus (g) above rectum (r). Note hypodermal striations along dorsal surface. Scale as in B.

J. Schematic representation of excretory cell of third-stage larva (not to scale); a = anus; co = excretory commissure; ec = excretory columns; en = excretory cell nucleus; ep = excretory pore; i = anterior intestine; nr = nerve ring; oe = oesophagus; v = ventriculus; R = right; L = left.


A
25µm

B
25µm

D
d

C
v

E
c
i
c

R
L
nr
ep
oe
en
v
co
i
ec
J
a

e
v
H

F
e
e
i

v
e
a
G

I
g
r

patent. The intestine just posterior to the ventriculus had a triradiate structure, the lumen apparently lined with the primordial bacillary layer (Fig. 6.3 E). Further back it was compressed between the lateral columns of the excretory cell (Fig. 6.3 F). For most of its length, the intestine was obscured by the relatively massive bulk of the excretory columns. Posteriorly, the intestine tapered sharply to its junction with the cuticularised rectum, which passed towards the anus between a prominent dorsal rectal gland cell and a less prominent ventral gland cell (Figs 6.3 G & I, 6.4 A & B). The dorsal rectal gland was much more prominent in some larvae than others (Fig. 6.3 I), and occasionally appeared to consist of 2 cells instead of one.

The excretory pore was conspicuous in live and fixed larvae, as was the excretory cell nucleus (Fig. 6.4 A & B). At 5 d pi, in most larvae the excretory nucleus was located ventrally to the ventriculo-oesophageal junction, although in about 20% it was beneath the ventriculus and in about 1% was actually located posterior to the ventriculus, by as far as 10 µm, but still on the left side and ventral to the intestine. With larval growth, the excretory nucleus moved forwards, to lie beneath and on the left side of the posterior oesophagus. The rest of the excretory system was difficult to examine, and findings (Fig. 6.3 J) are based on observations from numerous histological sections and the very occasional intact larvae in which these features were clear. The excretory pore opened through the cuticle ventrally in the midline just behind the nerve ring (Figs 6.3 J, 6.4 A, B & C). The canal passed back to join the median lobe of the excretory cell, which then continued into the main body of the cell, located ventrally left of the oesophagus and just anterior to the ventriculus. Beneath the anterior part of the ventriculus, the cell divided into 2, with the left column proceeding in the left lateral cord (Fig. 6.3 D) and the commissure joining the right cord. About 50 µm behind

FIGURE 6.4

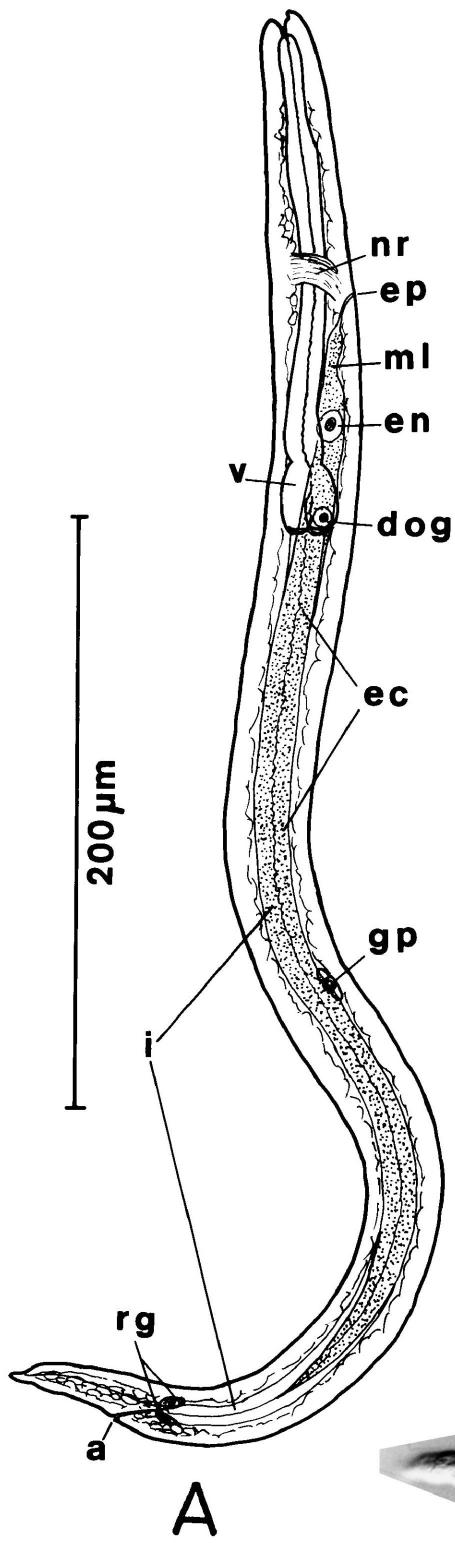
Typical third-stage *T. pteropodis* larva

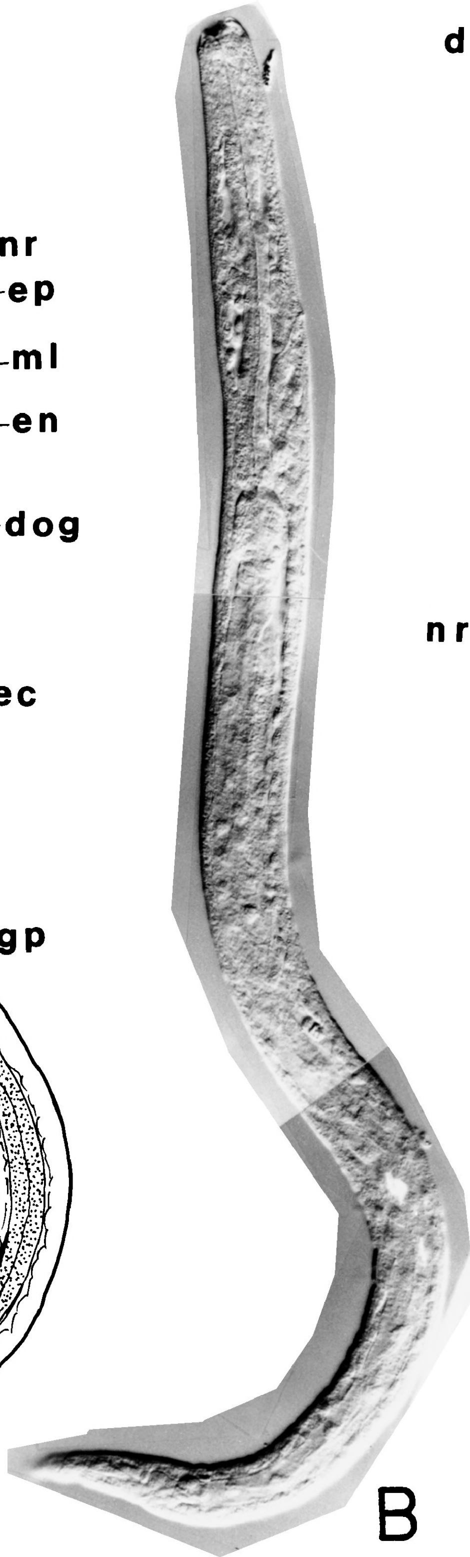
Third-stage larva, 590 µm long, recovered from liver of *P. poliocephalus* infected 6 months, showing extent of excretory system (finely stippled)

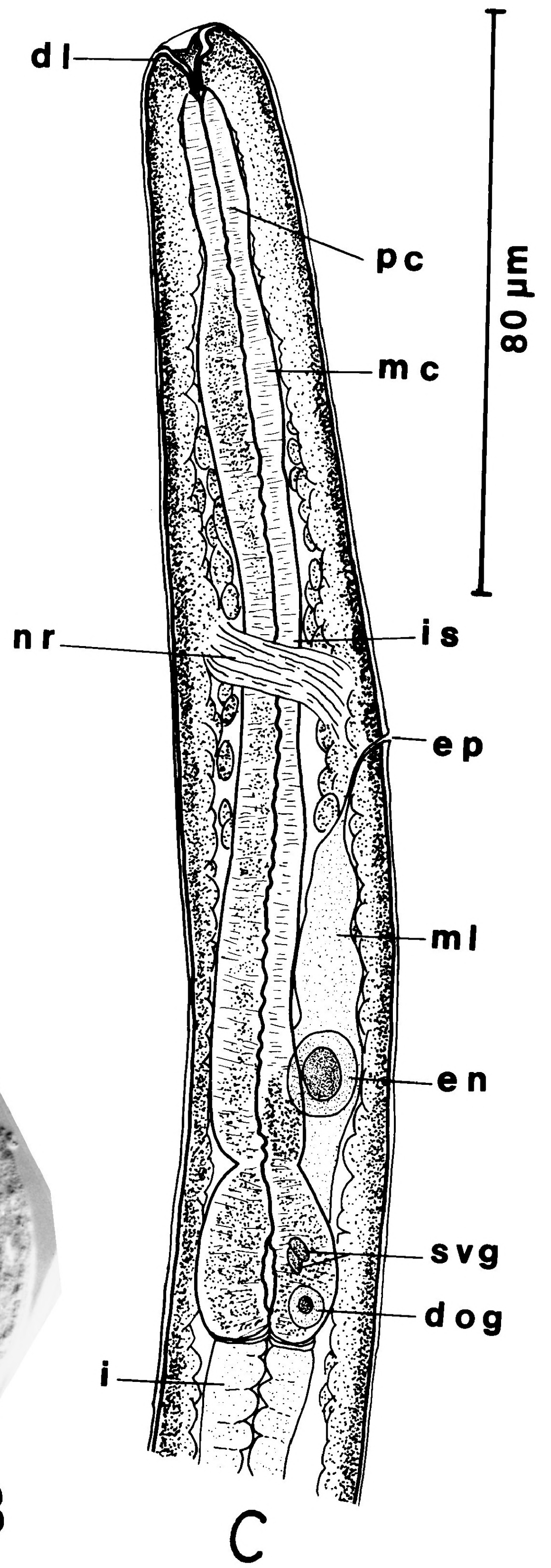
B. Composite photograph of same larva as in A.

C. Anterior segment of same larva as in A & B, showing details of lips, oesophagus and ventriculus. Excretory cell not represented beyond ventriculus. Secretory follicles of dorsal oesophageal gland are most densely aggregated in metacorpus and ventriculus, whereas those of subventral oesophageal glands extend only into terminal oesophagus.

a = anus
d l = dorsal lip
d o g = dorsal oesophageal gland nucleus
e c = excretory column
e n = excretory cell nucleus
e p = excretory pore
g p = genital primordium
i = intestine
is = oesophageal isthmus
mc = oesophageal metacorpus
m l = medial lobe excretory cell
n r = nerve ring
pc = oesophageal procorpus
r g = rectal gland cells
s v g = subventral oesophageal gland nuclei
v = ventriculus.


nr
ep
ml
en
v
dog
ec
200µm
gp
i
rg
a
A


B


dl
pc
80 µm
mc
nr
is
ep
ml
en
svg
dog
i
C

the ventriculus the columns commenced bulging into the pseudocoelom to compress the intestine, and they eventually tapered to a point in each lateral column anterior to the rectum (Fig. 6.3 G). In sections, the excretory columns appeared pale and granular, staining pink with H & E, and were lined with a thin unicellular layer (Fig. 6.3 F, G & H). In formalin-fixed specimens, numerous fine conspicuous dark granules were seen in the excretory columns of some larvae but not in others.

The major anatomical features of a T. pteropodis larva recovered from a bat 6 months pi are illustrated in Fig. 6.4 A, B & C. Measurements are presented in Table 6.1 and compared with tissue larvae of T. cati and T. canis. On superficial examination of intact fixed larvae, the 3 species appeared similar, except that T. cati seemed more slender and their tails tapered axially, whereas T. canis and T. pteropodis curved dorsally at the tip.

On SEM, larvae of the 3 species demonstrated only subtle differences. T. canis and T. cati, from brains and muscles of mice, respectively, were well-preserved, but despite repeated attempts, T. pteropodis from bat and mouse livers appeared distorted by fixation. However, sufficient detail was observed for comparative purposes.

T. pteropodis was markedly longer than T. canis and T. cati (Fig. 6.5 A, B & C). Lateral alae extended from behind the lips almost to the tail in each species, and appeared comparable in the 3 (Figs 6.5 A - C; 6.6 D - H) though perhaps a little less prominent in T. pteropodis (Fig. 6.6 H). Lips were obscured by the ventral cuticular thickening in all species, which was most prominent in T. cati (Fig. 6.5 H) and least in T. pteropodis (Figs 6.5 F & G). In T. canis and T. pteropodis, the buccal opening formed an equilateral triangle (Fig. 6.5 D & F), whereas in T. cati the inferior angle, at the junction of the "mandibles" (Fig. 6.5 E), was more acute, between 30 and 45° in the 4 specimens examined. In all 3 species, 2 subdorsal papillae, 2 subventral papillae and 2 lateral amphidial openings

TABLE 6-1

Key dimensions of Toxocara larvae recovered from tissues of experimentally-infected animals (measurements in μm)

Toxocara sp. Host Time p.i. Tissue	cati guinea pig 13 weeks muscle	canis bat 90 days brain	pteropodis mouse 7 days liver	pteropodis monkey 26 days liver	pteropodis bat 12 months liver	pteropodis bat mammary glands					
Number of larvae	31	33	27	35	35						
Total length (L)	455 ± 17 (430 - 505)	435 ± 14 (415 - 465)	465 ± 16 (420 - 495)	465 ± 16 (410 - 505)	655 ± 47 (505 - 740)	730	800	925	970	1180	1390
Maximal width	19 ± 0.7 (18 - 21)	22 ± 1.3 (19 - 24)	20 ± 1.7 (16 - 23)	19 ± 1.7 (16 - 23)	27 ± 2.2 (23 - 32)	31	35	35	32	35	41
Oesophagus length (LE)	135 ± 4.6 (128 - 145)	125 ± 3.5 (119 - 135)	108 ± 6.0 (90 - 113)	106 ± 10 (87 - 120)	120 ± 9.4 (95 - 138)	164	153	175	190	185	210
LE/L %	29.7 ± 1.2 (28 - 32)	28.5 ± 0.8 (27 - 30)	23.2 ± 1.2 (20 - 25)	22.7 ± 1.9 (20 - 26)	18.3 ± 1.2 (16 - 21)	22.5	19.1	18.9	19.5	15.7	15.1
Mouth to nerve ring (NR)	70 ± 3.5 64 - 78	69 ± 2.1 66 - 73	64 ± 3.9 51 - 71	65 ± 4.0 57 - 76	75 ± 6.1 64 - 89	89	87	99	95	107	106
NR/LE %	52 ± 2.3 (47 - 56)	55 ± 1.2 (53 - 57)	60 ± 3.2 (50 - 65)	61 ± 5.0 (53 - 67)	63 ± 2.9 (57 - 68)	54	57	57	50	58	50
Ventriculus length	19 ± 1.0 (18 - 21)	19 ± 1.0 (17 - 21)	20 ± 1.3 (18 - 23)	19 ± 1.5 (16 - 23)	25 ± 2.5 (21 - 30)	31	25	30	33	30	41
Mouth to genital primordium (GP)	268 ± 14 (248 - 297)	258 ± 10 (237 - 272)	268 ± 12 (240 - 287)	258 ± 14 (230 - 280)	368 ± 27 (330 - 429)	393	425	473	503	580	890
GP/L %	59 ± 1.4 (57 - 62)	59 ± 2.0 (54 - 62)	58 ± 1.7 (55 - 61)	56 ± 1.5 (54 - 59)	56 ± 1.5 (54 - 59)	54	53	51	52	49	64
Tail (anus to tip)	40 ± 2.5 (35 - 44)	37 ± 2.0 (34 - 43)	34 ± 1.4 (31 - 37)	34 ± 2.2 (31 - 37)	40 ± 3.0 (35 - 45)	43	42	48	48	51	57
Tail/L %	8.8 ± 0.5 (8.0 - 9.5)	8.5 ± 0.3 (7.9 - 9.2)	7.3 ± 0.3 (6.4 - 7.8)	7.3 ± 0.6 (6.0 - 8.1)	6.2 ± 0.5 (5.5 - 7.0)	5.9	5.3	5.2	4.9	4.3	4.1

FIGURE 6.5

Scanning electron micrographs of larvae of *T. canis*, *T. cati* and *T. pteropodis* recovered respectively from brains of mice, skeletal muscle of mice and livers of bats

A. *T. canis* third-stage larva showing right lateral ala and dorsal bend at tail-tip.

B. *T. cati* showing lateral ala and straight tail.

C. *T. pteropodis* showing right lateral ala and dorsal bend at tail-tip.

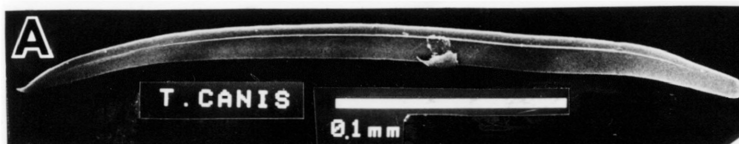
D. *T. canis* cephalic region, showing ventral cuticular thickening and protuberance with appearance of "mandible", 2 lateral amphidial openings (a), and four papillae (p).

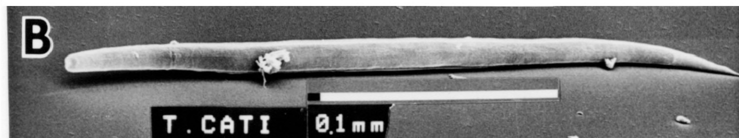
E. *T. cati* with structures as in *T. canis* (D), but more acute ventral angle in "mandible".

F. *T. pteropodis*, with oral opening similar to *T. canis* (D) but papillae (p) less prominent.

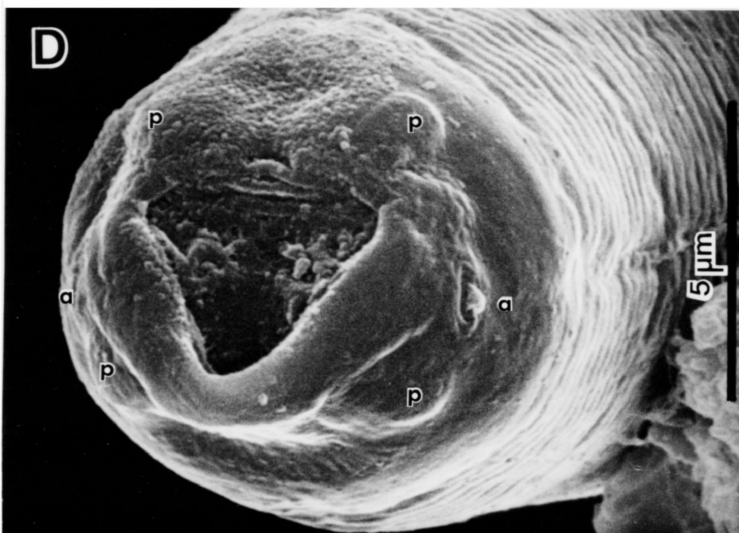
G. *T. pteropodis*, lateral view, showing less prominent ventral protuberance than in *T. cati* (H). Note amphidial opening and subventral papilla.

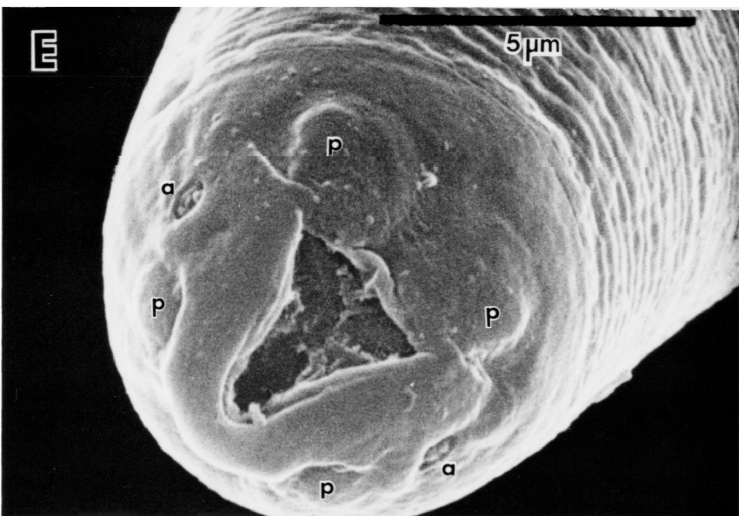
H. *T. cati*, showing prominent ventral protuberance, papillae (p) and right amphidial pore (a).

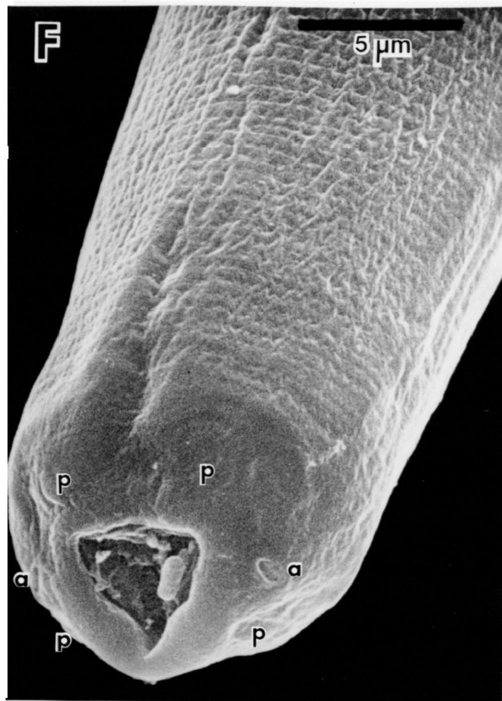

A
T. CANIS
0,1 mm

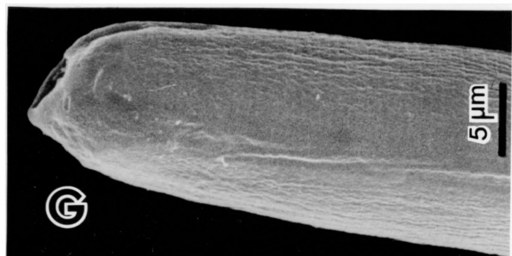

B
T. CATI
0,1 mm

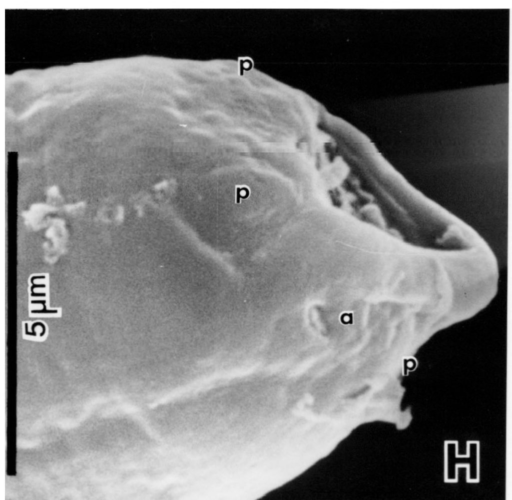
C
0,1 mm
T. PTER.


D
p
p
a
a
p
p
5 µm


E
5 µm
p
a
p
p
p
a


F
5 µm
p
p
a
a
p
p


5 µm
G


p
p
5 µm
a
p
H

were clearly seen, being least prominent in *T. pteropodis* (Fig. 6.5 D - H). Circumferential cuticular striations appeared just behind the papillae and extended over the entire body to the tip of the tail. Excretory pores were clear in the 3 species, varying in shape from circular to a transverse openings (Fig. 6.6 A - C). Tails varied between the species. The tip curved dorsally in *T. canis* and *T. pteropodis* with occasional exceptions, but terminated axially in *T. cati* (Figs 6.5 A - C; 6.6 F - H). The anus of *T. pteropodis* was nearer the tail than that of *T. canis* or *T. cati* (Fig. 6.6 F - H).

Light microscopic *en face* examination of the cephalic region of *T. pteropodis* showed the lip structures, papillae and amphids to be identical with that described by Sprent (1958) for *T. canis*.

Dimensions of larvae of the 3 species from different sources are given in Table 6.1. *T. pteropodis* were longer, although their lengths overlapped those of the other 2 species in early infections. In late infections, the shortest *T. pteropodis* larvae were equal in length to the longest *T. cati* larvae, which, however, did not exceed 505 µm. In early infections, *T. canis* were on average thicker, but there was a very broad overlap in the body widths of all 3 species. The oesophagus of *T. pteropodis* was the shortest, more so in proportion to body length, not overlapping with the range of these dimensions in other species. The oesophagus of *T. cati* was longer than that of *T. canis*, but not in relation to body length. The only other differential feature was the relatively short tail of *T. pteropodis*. As a fraction of total body length, this did not overlap with the other 2 species. In early infections, the position of the genital primordium was similar in all 3 species, but as *T. pteropodis* grew the genital primordium moved further back, occupying a fairly constant position in relation to body length. The relative positions of genital primordia in 100 *T. pteropodis* from long-term hepatic infections are represented in Figure 6.7.

FIGURE 6.6

Scanning electron micrographs of _T. canis_, _T. cati_ and _T. pteropodis_ third-stage larvae

A. _T. canis_, anterior end, showing ventral labial cuticular protuberance, subventral papillae, lateral alae and excretory pore (shown enlarged in inset).

B. _T. cati_, showing same features as in A.

C. _T. pteropodis_, excretory pore.

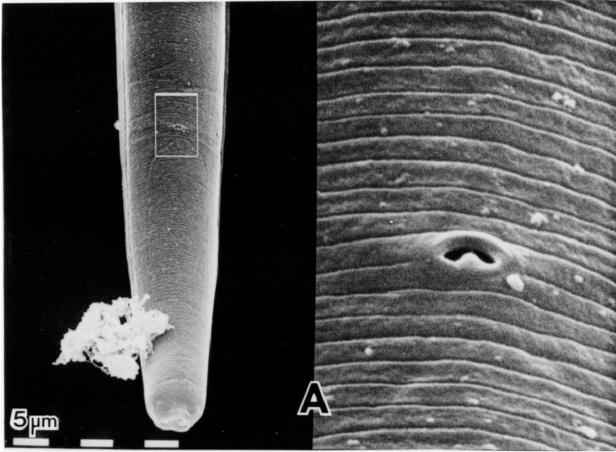
D. _T. canis_, lateral ala in mid-body region.

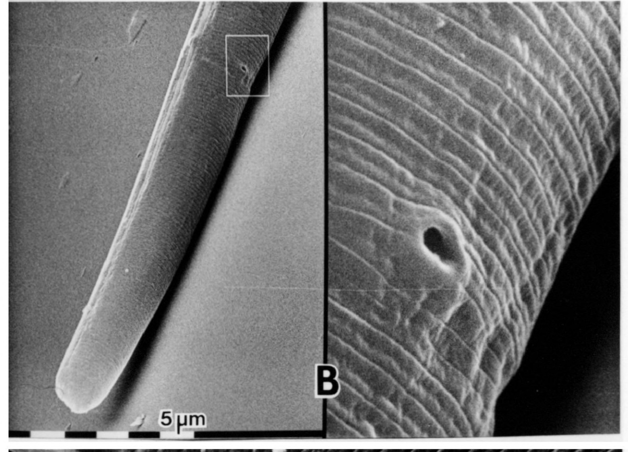
E. _T. cati_, lateral ala in mid-body region.

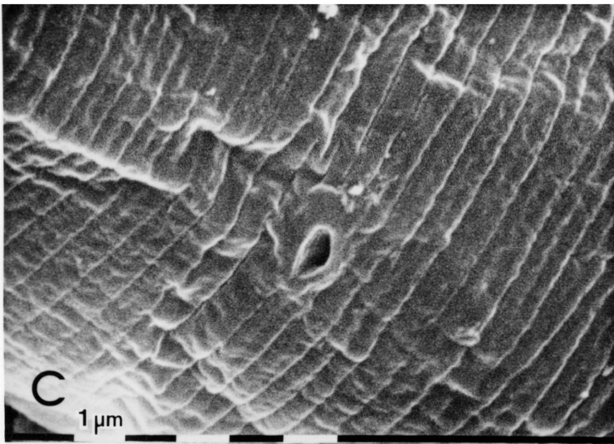
F. _T. canis_, ventral caudal region, showing termination of alae, dorsolateral bend in tail-tip, and anal opening (enlarged in inset).

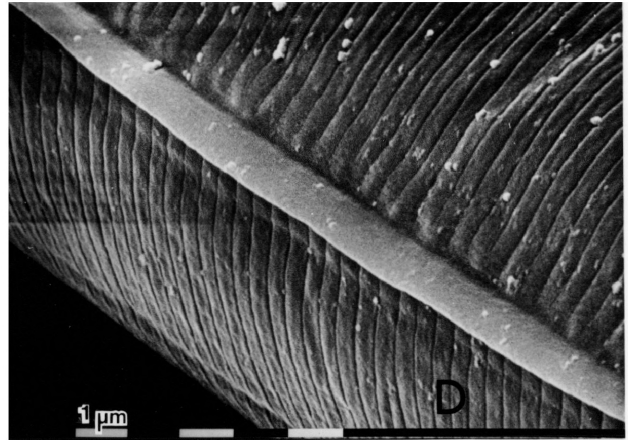
G. _T. cati_, ventral caudal region, showing termination of alae, anal pore (arrow) and straight tail-tip.

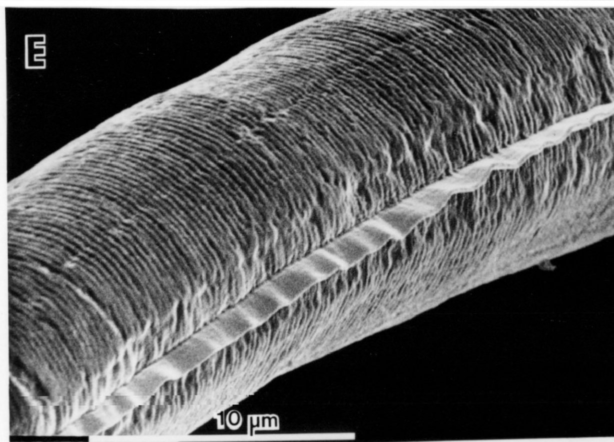
H. _T. pteropodis_, caudal region, showing anal pore (arrow), lateral alae and straight tail in this case.

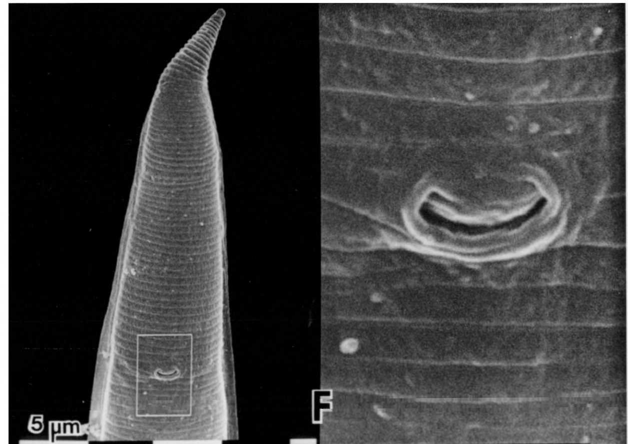

5 µm
A

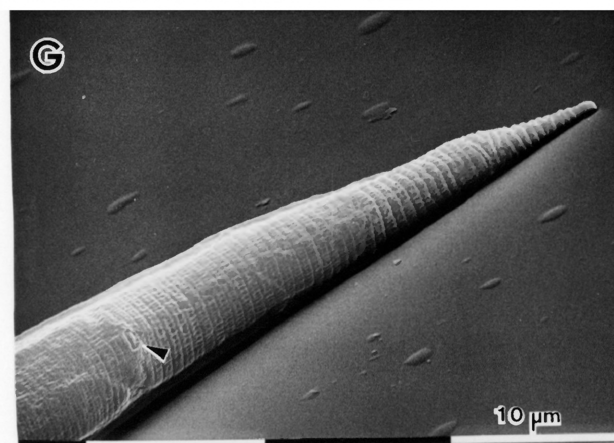

5 µm
B


C
1 µm


1 µm
D


E
10 µm


5 µm
F


G
10 µm

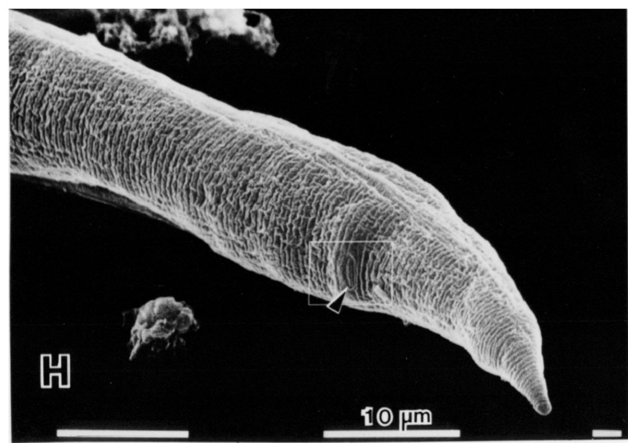
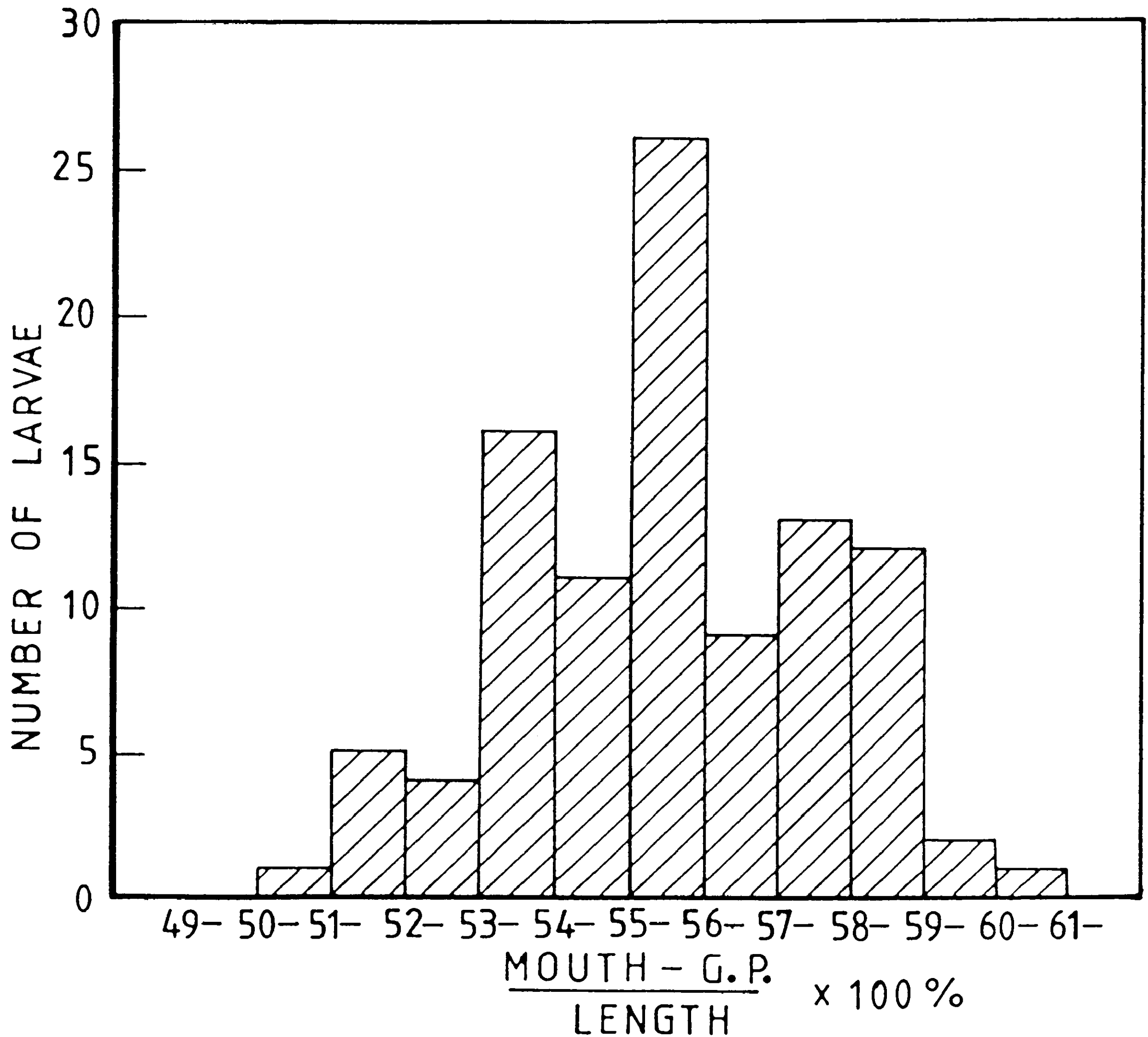

H
10 µm

FIGURE 6.7

Relative position of genital primordium (distance from mouth as fraction of body length) in 100 T. pteropodis larvae recovered from livers of adult female P. poliocephalus killed at 9, 16 and 23 days post-partum (mean larval length: 710 µm ± 50 µm S.D.; range: 620 - 845 µm).



They were located 50 - 60% of the body length from the mouth (mean position 55 ± 2.1%; median 55%). The mean length of these larvae was 710 ± S.D. 50 µm (range 620 - 845 µm), and there was no correlation between the positions of the genital primordia and the lengths of individual larvae.

6.2.3 Larval development in mammary glands

Larvae from mammary glands ranged in length from 630 µm (bat 2-34, Table 3.4) to 1.39 mm (bat 2-21, Table 3.3). The shorter of these (Fig. 6.8 A) were identical with those from livers and the longer similar to those found in the intestines of neonates (Fig. 6.8 B). Several were drawn in detail, and their dimensions are presented in Tables 6.2 and 6.3. In none was unequivocal moulting observed, although the cuticles of some larger larvae, even those incubated in saline, had lifted to give the appearance of moulting (Fig. 6.8 B, C & D).

6.2.4 Larval development in the intestine

In the second season of this study (October 1982), to facilitate the complete recovery of smaller larvae from the viscous gut contents, intestines of juvenile bats were incubated at 37 °C overnight in saline after the larger worms had been removed. All gut larvae 1 - 5 mm long which had been incubated overnight at 37 °C had lifted cuticles, which gave the appearance of moulting (Fig. 6.8 E, F & G), whereas those incubated overnight at 5 °C did not.

Measurements of some intestinal larvae are recorded in Tables 6.2 (males) and 6.3 (females), which also include several mammary larvae.

The shortest was a 3rd-stage larva (810 x 37 µm) from a naturally-infected bat (1-49, Table 3.5).

Distinct moulting from the 3rd to the 4th-stage was seen in only 3 larvae, 1.49, 2.37 and 2.63 mm long. Moulting from the 4th to the pre-adult stage (the term "adult" here will be used only for worms producing

FIGURE 6.8

Developing *T. pteropodis* larvae from mammary gland and juvenile intestine of *P. poliocephalus*

A. Third-stage larva from mammary gland 16 days post-partum (scale: bar = 200 µm)

B. Advanced third-stage larva from same mammary gland as in A, showing lifting cuticle at anterior end. (Scale: bar in A = 200 µm)

C. Anterior extremity of larva in B (scale: bar in A = 50 µm).

D. Same larva as in B, showing genital primordium and generalised lifting of cuticle (scale: bar in A = 50 µm).

E. Larva incubated at 37°C from intestine of naturally-infected juvenile 1-2 weeks old, showing pseudo-moulting (scale: bar in F = 200 µm)

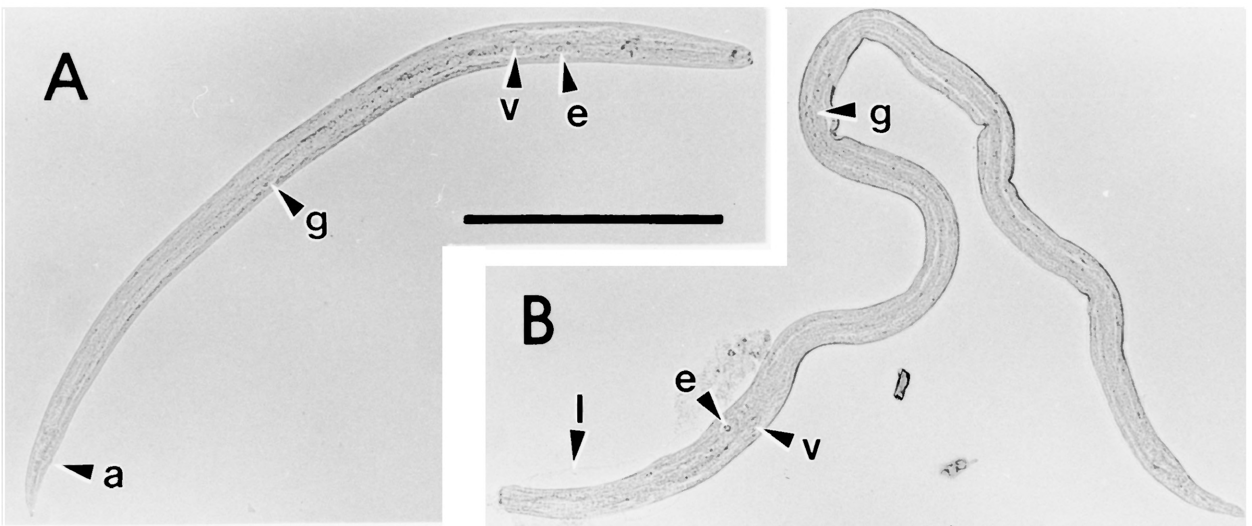
F. Larva from same bat as E (scale: bar = 200 µm).

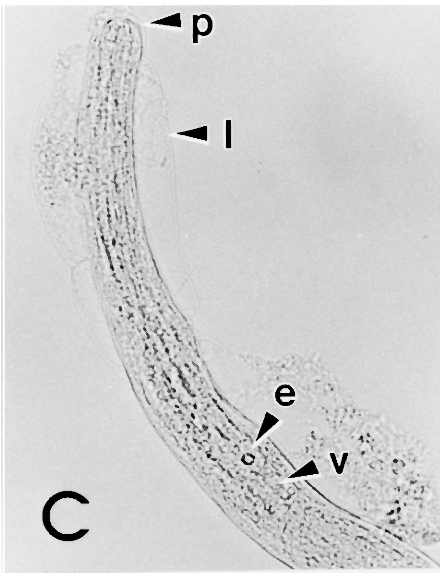
G. Caudal region of larva in F showing absence of distinct cuticular layer on body (scale: bar in F = 50 µm).

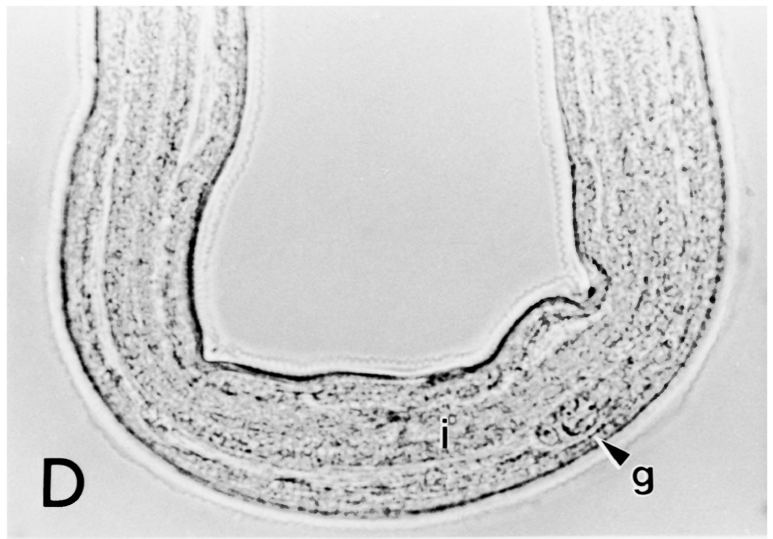
H. Fine cuticular striae underlying broad ridges in mid-body region of male larva (8.83 x 0.20 mm) from experimentally-infected bat aged 7 weeks (scale: bar in A = 50 µm).

I. Mid-body region of male larva (9.53 x 0.17 mm) from 6-week-old experimentally-infected bat, indicative of early moulting (scale: bar in A = 50 µm)

a = anus
e = excretory cell nucleus
g = genital primordium
i = intestine
l = lifting cuticle
p = labial papilla
v = ventriculus.

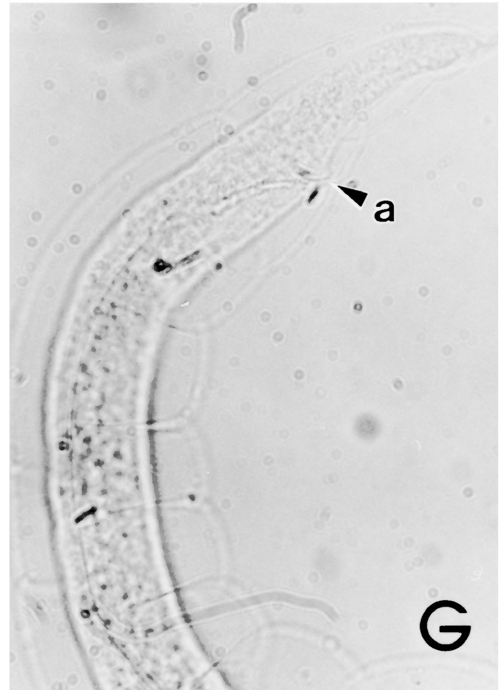

A
v
e
g
a
g
B
l
e
v

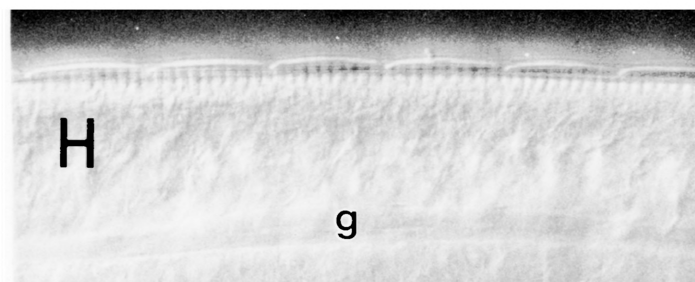

p
l
e
v
C


i
g
D

l
E

l
a
F


a
G


H
g

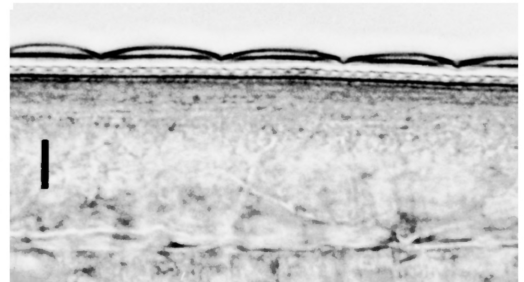

I

TABLE 6.2 Dimensions of male T. pteropodis larvae recovered from intestine of juvenile P. poliocephalus

Total length (L) (mm)	Maximal width (mm)	Oesophagus length (LE) (mm)	LE/L %	Mouth - nerve ring (NR) (mm)	NR/LE %	Ventriculus length (µm)	Ventriculus width (µm)	Width at ventriculus (µm)	Tail: cloaca - tip (mm)	Tail/L %	Cuticular stria (ventriculus) (µm)	Cuticular stria (pre-cloacal) (µm)	Mouth - anterior genital primordium (GP) (mm)	GP/L %	Genital tract length (mm)	Other features	Length spicules (µm)
*0.730	0.031	0.164	22.5	0.089	54	31	20	31	0.043	5.9	nd	nd	0.39	54	0.012		nd
*0.800	0.035	0.153	19.1	0.087	57	25	16	33	0.042	5.3	nd	nd	0.43	53	0.014		nd
0.810	0.038	0.154	19.0	0.082	53	28	20	20	0.049	6.1	nd	nd	0.42	52	0.015		nd
*0.925	0.035	0.175	18.9	0.099	57	30	24	33	0.048	5.2	nd	nd	0.47	51	0.022		nd
*0.970	0.032	0.190	19.5	0.095	50	33	19	30	0.048	4.9	nd	nd	0.50	52	0.026		nd
*0.975	0.032	0.164	16.9	0.102	62	25	19	32	-	-	1.6	nd	0.54	55	0.026		nd
*1.13	0.036	0.164	14.5	0.083	51	30	21	34	0.053	4.7	nd	nd	0.68	60	0.024		nd
*1.39	0.041	0.210	15.1	0.106	50	41	25	41	0.057	4.1	2.0	nd	0.89	64	0.029		nd
1.49	0.053	0.204	13.7	0.115	57	33	25	35	0.056	3.8	nd	2.1	0.96	65	0.025		nd
1.50	0.036	0.170	11.3	nd	-	30	26	36	0.060	4.0	3.0	2.9	0.72	48	0.044		nd
1.56	0.034	0.210	13.5	0.102	51	30	18	32	0.055	3.5	1.8	1.6	0.90	58	0.038		nd
1.78	0.043	0.213	12.0	0.131	62	40	26	40	0.066	3.7	2.4	2.4	1.08	61	0.023	(4 cells)	nd
1.79	0.038	0.213	11.9	0.119	56	31	26	38	0.060	3.3	nd	nd	0.88	49	0.050		nd
1.98	0.051	0.232	11.7	0.126	54	40	30	49	0.061	3.1	2.4	2.8	1.24	63	0.037	(7 cells)	nd
2.23	0.071	0.294	13.2	0.145	49	41	40	66	0.069	3.1	4.2	3.6	nd	-	nd		nd
2.45	0.063	0.272	11.1	0.137	50	45	40	59	0.061	2.5	2.9	3.4	1.70	69	0.180		nd
2.49	0.068	0.290	11.7	nd	-	42	35	66	0.074	3.0	3.2	3.1	1.72	69	0.077		nd
3.16	0.075	0.316	10.0	0.163	52	49	40	66	0.076	2.4	3.1	3.8	2.26	71	0.40		nd
3.18	0.058	0.301	9.5	0.163	54	46	32	53	0.071	2.2	nd	nd	2.29	72	0.072		nd
3.30	0.071	0.304	9.2	0.157	52	52	43	71	0.075	2.3	3.7	4.1	2.28	69	0.40		nd
3.40	0.190	0.315	9.2	nd	-	88	70	160	0.100	2.9	3.2	3.1	1.53	44	comp		160
3.59	0.088	0.350	9.7	0.170	48	56	42	78	0.079	2.2	3.7	4.5	2.37	66	comp		nd
6.23	0.120	0.570	9.2	0.210	38	76	47	93	0.082	1.3	5.6	9.7	nd	-	comp; CP		55
6.92	0.130	0.520	7.5	0.207	40	56	38	87	nd	-	4.9	nd	nd	-	nd		47
									[mid-body ridges: 11.7]								
8.03	0.150	0.610	7.6	0.240	39	82	63	130	0.110	1.3	7.3	nd	nd	-	nd; DA		nd
8.36	0.150	0.690	8.2	0.230	34	89	63	130	0.095	1.1	10.7	13.6	4.93	59	comp		94
8.83	0.200	0.640	7.2	0.230	36	93	67	180	0.108	1.2	nd	2.3	3.35	38	comp: DA		73
										[ridges:	-	16.2]					
9.53	0.170	0.650	6.8	0.270	42	107	66	130	0.091	0.95	3.0	2.3	4.85	51	comp		108
										[ridges:	9.8	15.5]					
10.8	0.160	0.685	6.3	0.265	39	91	77	154	0.086	0.80	2.8	2.2	5.16	48	comp		107
12.4	0.230	0.930	7.5	0.280	30	110	96	210	0.110	0.89	3.2	3.5	4.78	39	comp; DA; CP		150
13.1	0.280	0.900	6.9	0.330	37	130	125	250	0.140	1.1	2.8	4.5	4.43	34	comp; CA; CP		165
13.9	0.240	1.04	7.5	0.340	33	122	90	210	0.130	0.94	3.2	2.6	3.85	28	comp; CA; CP		155
16.0	0.190	1.00	6.3	0.320	32	135	80	160	0.114	0.71	3.6	3.0	4.09	26	comp; CA; CP		135
16.2	0.190	0.970	6.0	0.290	30	115	75	145	0.098	0.60	3.6	3.5	6.00	37	comp; CA; CP		137
16.7	0.240	1.03	6.1	0.355	35	135	105	200	0.130	0.78	4.1	3.2	4.90	30	comp; CA; CP		110
19.6	0.250	1.13	5.7	0.365	32	130	110	220	0.135	0.69	4.2	4.7	6.00	31	comp		170
19.6	0.250	1.26	6.4	0.385	30	155	110	220	0.140	0.71	4.5	3.8	6.43	33	comp; CA; CP		195
20.3	0.300	1.19	5.9	0.365	31	125	115	240	0.110	0.54	4.8	4.5	8.03	40	comp; CA; CP		195
20.3	0.260	1.10	5.4	0.350	32	170	120	220	0.126	0.62	4.5	3.7	5.90	29	comp; CA; CP		170
28.9	0.360	1.65	5.7	0.470	28	180	150	320	0.160	0.55	6.0	5.9	6.85	24	comp; CA; CP		250
41.9	0.690	2.06	4.9	0.560	28	225	145	395	0.190	0.45	7.0	10.3	9.71	23	ED 2.36;ED/L 5.6%		200
45.0	0.580	2.38	5.3	0.660	28	240	170	500	0.200	0.44	3.0	13.3	-	-	ED 2.22;ED/L 4.9%		420
60.6	1.00	2.50	4.1	0.770	31	310	225	610	nd	-	10.0	10.3	-	-	S		390
60.6	0.910	2.87	4.7	0.730	26	240	190	520	0.210	0.35	12.0	17.0	-	-	ED 2.89;ED/L 4.8%;S		510
62.3	0.750	2.09	3.3	0.570	27	190	190	470	0.230	0.37	6.6	14.5	-	-	ED 2.32;ED/L 3.7%;S		

* from mammary gland; nd - not discernible; comp - complete to cloaca; CP - caudal papillae evident; CA - caudal appendage discerned; DA - digitiform appendage discerned; ED - ejaculatory duct (mm); S - spermatozoa in vas deferens.

TABLE 6.3 Dimensions of female T. pteropodis larvae recovered from intestine of juvenile P. poliocephalus

Total length (L) (mm)	Maximal width (mm)	Oesophagus length (LE) (mm)	LE/L %	Mouth - nerve ring (NR) (mm)	NR/LE %	Ventriculus length (µm)	Ventriculus width (µm)	Width at ventriculus (µm)	Tail: anus - tip (mm)	Tail/L %	Cuticular stria (ventriculus) (µm)	Cuticular stria (pre-cloacal) (µm)	Mouth - anterior genital primordium (GP) (mm)	GP/L %	Genital tract length (µm)	Length undivided segment (µm)
1.15	0.032	0.170	14.8	0.090	53	26	21	31	0.046	4.0	1.4	1.5	0.53	46	26**	-
1.17	0.031	0.190	16.2	0.078	41	30	23	31	0.054	4.6	nd	nd	0.61	52	30**	-
1.17*	0.041	0.177	15.2	0.094	53	30	21	41	-	-	nd	nd	0.60	52	32**	-
1.18*	0.035	0.185	15.7	0.107	58	30	23	35	0.051	4.3	nd	nd	0.58	49	32**	-
1.25	0.033	0.180	14.4	0.099	55	33	23	33	0.063	5.0	nd	nd	0.65	52	26**	-
1.71	0.049	0.235	13.7	0.120	51	35	28	45	0.070	4.1	nd	2.3	0.86	50	39**	-
1.94	0.059	0.245	12.7	0.130	53	37	31	59	0.066	3.4	nd	2.8	0.94	48	30**	-
2.01	0.047	0.255	12.7	0.135	53	45	31	47	0.065	3.2	2.7	3.0	0.97	48	35**	-
2.02	0.059	0.255	12.6	0.140	55	44	28	52	0.051	2.5	2.6	2.7	0.95	47	30**	-
2.08	0.053	0.255	12.3	0.130	51	43	29	47	0.068	3.3	2.4	2.7	0.95	46	104**	-
2.20	0.048	0.240	10.9	nd	-	40	26	44	0.069	3.1	nd	nd	1.07	49	55	-
2.21	0.052	0.235	10.6	0.125	53	43	33	45	0.061	2.8	2.7	2.7	0.90	41	-	-
2.38	0.061	0.215	9.0	nd	-	40	35	58	0.066	2.8	2.9	2.7	1.16	49	-	-
2.47	0.060	0.285	11.5	0.150	53	42	28	59	0.077	3.1	3.0	3.5	1.08	44	56	44
2.58	0.063	0.325	12.6	0.160	49	47	37	63	0.073	2.8	3.0	3.8	1.20	46	59**	-
2.66	0.070	0.295	11.1	0.155	58	44	35	70	0.082	3.1	nd	3.8	1.09	41	30	-
2.66	0.093	0.310	11.7	0.145	47	45	45	91	0.086	3.2	3.7	nd	1.06	40	89	41
2.83	0.066	0.305	10.8	0.170	56	51	39	66	0.091	3.2	3.6	4.3	1.25	44	77	44
2.90	0.073	0.320	11.0	0.145	45	47	36	66	0.069	2.4	2.4	3.3	1.49	51	42	24
2.94	0.077	0.295	10.0	0.165	56	47	31	66	0.077	2.6	3.1	4.4	1.25	43	82	52
2.95	0.053	0.295	10.0	0.130	44	39	28	49	0.060	2.0	nd	nd	1.12	38	87	44
3.11	0.081	0.320	10.3	0.150	47	41	35	73	0.079	2.6	3.2	4.0	1.12	36	220	36
3.78	0.074	0.370	9.8	0.165	45	50	32	53	0.096	2.5	nd	nd	1.51	40	113	64
4.00	0.071	0.385	9.6	0.170	44	57	39	78	nd	-	nd	nd	1.67	42	425	170
4.01	0.150	0.550	13.7	0.180	32	73	62	140	0.130	3.2	7.1	11.7	1.45	36	240	115
4.36	0.074	0 420	9.6	0.170	40	60	39	71	0.085	1.9	nd	nd	nd	nd	nd	nd
4.52	0.076	0.445	9.8	0.190	43	43	35	71	0.092	2.0	nd	nd	1.72	38	180	67
4.52	0.100	0.415	9.2	0.170	41	46	36	85	0.092	2.0	nd	nd	1.88	42	-	83
4.54	0.110	0.455	10.0	0.185	41	67	46	87	0.100	2.2	nd	nd	1.93	43	-	85
4.71	0.100	0.430	9.1	0.185	43	60	42	78	0.115	2.4	nd	nd	1.85	39	250	96
5.33	0.095	0.540	10.1	0.200	37	60	46	93	0.110	2.0	5.1	8.0	1.89	35	550	120
6.88	0.130	0.510	7.4	0.200	39	69	50	125	0.120	1.7	5.5	10.0	2.25	33	580	130
7.23	0.130	0.630	8.7	0.220	36	67	60	120	0.140	1.9	6.8	nd	2.61	36	-	150
7.48	0.150	0.650	8.7	0.240	37	82	57	120	0.140	1.9	6.4	nd	2.69	36	-	150
9.63	0.170	0.690	7.1	0.240	35	97	65	130	0.150	1.5	7.8	nd	3.13	33	-	130
10.1	0.180	0.740	7.3	0.260	35	112	67	140	0.160	1.6	10.6	nd	3.64	36	-	160
10.3	0.300	0.900	8.7	0.350	39	130	130	260	0.210	2.1	3.0	3.6	2.77	27		420
11.0	0.220	0.800	7.3	0.250	31	100	75	180	0.130	1.2	10.2	15.9	3.51	32	2100	320
13.0	0.270	0.860	6.6	0.310	36	110	88	220	0.150	1.2	-	-	3.75	29	3870	360
15.0	0.170	0.825	5.5	0.295	36	125	81	160	0.190	1.3	3.2	3.2	3.79	25	3740	350
34.0	0.770	1.93	5.7	0.560	29	265	195	385	0.370	1.1	5.6	10.3	11.60	34	-	-
40.4	0.630	1.99	4.9	0.600	30	160	105	370	0.355	0.88	6.8	9.5	8.63	21	-	-
45.8	0.520	2.25	4.9	0.580	26	210	150	370	0.410	0.89	8.5	9.9	10.8	24	-	1170
57.0	0.720	2.89	5.1	0.780	27	280	190	600	0.410	0.72	10.1	11.0	-	-	-	7220
61.5	0.900	2.58	4.2	0.710	28	260	180	560	0.380	0.62	10.7	16.8	12.6	20	-	5330
62.1	0.880	2.78	4.5	0.730	26	280	220	630	0.420	0.68	11.4	13.8	15.8	25	-	-
68.7	0.800	2.53	3.7	0.720	29	230	200	470	0.420	0.61	9.8	12.2	17.5	25	-	6800
(uterus full of spermatozoa but no eggs - developing oocytes in oviducts)																
81.3	1.09	2.72	3.3	-	-	310	240	580	0.510	0.63	-	-	13.3	16	-	-

* from mammary gland; ** just bifurcating; nd - not discernible

eggs or spermatozoa) was never observed in the sense that a loose cuticular sheath was seen separated from the normal cuticle, although in 3 larvae (males 8.83 and 9.53 mm and female 11.0 mm) a coarsely striated outer cuticular layer was overlying a deeper finely striated layer, indicating the final moult (Fig. 6.8 H & I). Hence, early pre-adults were distinguished from late 4th-stage larvae by the reduction in width of cuticular striae, from 10 - 16 µm to 2 - 5 µm. Striae were broadest in the mid-body region, and narrower anteriorly than in the caudal region, with occasional exceptions.

There being no distinct morphological transformations associated with the few moults observed, the development of T. pteropodis is described here in relation to larval length, from which in most cases the stage may be inferred. Morphology of adult worms is described in Section 6.2.5.

In the cephalic region, detectable changes appeared in larvae beyond 900 µm in length. The ventral cuticular thickening disappeared, the anterior protrusion of the subventral lips (Fig. 6.9 A) receded, although still discernible, a "cervical" thickening demarcated the lips from the body and the labial papillae became more prominent (Fig. 6.9 B). In most young 4th-stage larvae the labial-cervical region appeared as a slightly bulbous swelling (Fig. 6.9 C - E). With growth, the lip region became gradually narrower in relation to the "neck" and the lips angular (Fig. 6.9 G), with barely discernible fine denticular ridges along the inner anterior edges, gradually assuming the adult form (Fig. 6.9 H).

The oesophagus grew slowly in relation to the body, shortening from 15% of total body length in the 1 mm late 3rd-stage larva (Tables 6.2 & 6.3) to about 4% in the late pre-adult. With growth, the anterior oesophagus thickened (Fig. 6.9 D) and the isthmus at the nerve ring disappeared (Fig. 6.9 B, C & E). The secretory follicles of the dorsal oesophageal gland were conspicuous in the anterior dorsal region (Fig. 6.9

FIGURE 6.9

Developmental changes at anterior end of T. pteropodis larvae

A. Ventral view of subventral lip protuberances (arrowed) in 550 µm third-stage larva from bat liver 6 weeks p.i. (bar = 10 µm).

B. Oesophagus anterior to nerve ring (arrowed) of 1.56 mm male larva from intestine of 6-day-old bat. Note dorsal and ventral cervical thickening (t) indicating early demarcation of lips from body, and prominent dorsal and subventral outer labial papillae (bar = 40 µm).

C. Oesophageal region of 1.94 mm late third-stage female larva from 16-day-old experimentally-infected bat. Dorsal edge nerve ring arrowed. Note cervical pre-labial swelling; oesophageal isthmus at nerve ring not constricted; oesophagus and ventriculus axial and junction distinct; ventriculus wider than oesophagus; gut well-developed (scale: bar in E = 100 µm).

D. Anterior oesophagus and lips of 3.16 mm early fourth-stage male larva from 9-day-old bat. Buccal cavity distinct; anterior oesophagus club-shaped with conspicuous secretory follicles (f) of dorsal gland in dorsal sector, lined internally with thick cuticle; lips more distinct with papillae prominent (scale: bar in E = 50 µm).

E. Same larva as in D. Oesophagus narrowest midway between mouth and nerve ring (arrowed); body tapers markedly anteriorly from ventriculus (cf. C); excretory cell nucleus (e) occupies same relative position as in late third-stage larvae (bar = 100 µm).

F. Fourth-stage male larva, 3.59 mm long, from 9-day-old bat, showing cervical post-labial bulge (scale: bar in E = 50 µm).

G. Dorsal lip of 16.2 mm pre-adult male from 6-week-old bat, showing anterior margin with pits representing inner labial papillae (arrowed). Outer labial papillae not in focus. Anterior extensions of labial pulp cells just discernible within lip substance (scale: bar in E = 50 µm).

H. Lips of 16.7 mm male pre-adult from 6-week-old bat, showing early dentigerous ridge (arrowed) along inner edge of dorsal lip (scale as in G).

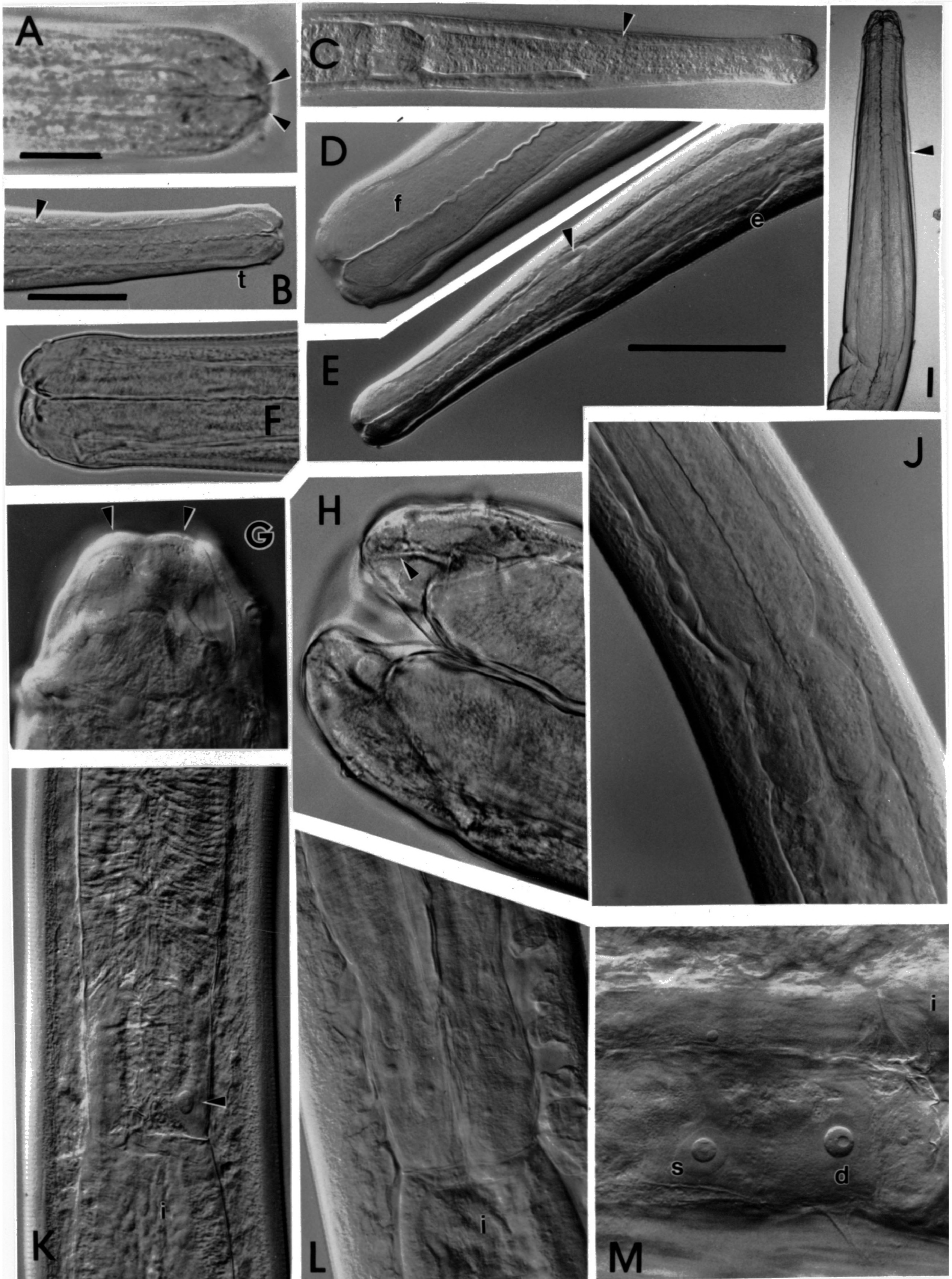
I. Dorsal view of 20.3 mm pre-adult from 6-week-old bat showing cervical alae (arrow) (scale: bar in E = 500 µm).

J. Well-demarcated ventriculus of 3.16 mm larva (same as in D & E), of same width as posterior oesophagus and narrower than anterior intestine. Note lumen in posterior ventriculus, and excretory cell nucleus ventral to posterior oesophagus (scale: bar in E = 50 µm).

K. Poorly-demarcated cylindrical ventriculus of 15.0 mm female pre-adult, showing dorsal oesophageal gland nucleus (arrow) in left subventral segment (i = intestine; scale: bar in E = 100 µm).

L. Ventriculus of 16.7 mm male showing distinct junction with intestine (i) but not with oesophagus (scale as in K).

M. Ventriculus of 41.9 mm male, showing junction with intestine (i), and nuclei of dorsal (d) and left sub-ventral (s) oesophageal gland cells (scale as in K).


A
B
t
C
D
f
E
e
F
G
H
I
J
K
i
L
i
M
i
s
d

D) and the cuticular lining was conspicuous from mouth to ventriculus (Fig. 6.9 E). The nerve ring moved relatively forwards, from about 55% of the oesophageal length from the mouth in the late 3rd-stage to 25 - 30% in advanced pre-adults.

In most larvae, as in many adults, cervical alae were inconspicuous, although in others thin alae extended from just behind the lips for about two-thirds the length of the oesophagus (Fig. 6.9 I). The oesophagus was narrowest midway between lips and nerve ring (Fig. 6.9 E) and from here gradually thickened posteriorly to constrict abruptly at its junction with the ventriculus (Fig. 6.9 E & J). With little growth in the excretory cell, and its gradual relative forward movement, the posterior oesophagus in the late 3rd/early 4th-stage was no longer displaced dorsally, lying axially with the ventriculus (Fig. 6.9 J - L).

In the 3rd-stage larva, the ventriculus was wider than the terminal oesophagus and the proximal intestine (Fig. 6.9 C). With growth, the ventriculus relatively narrowed (Fig. 6.9 J - L). It was cylindrical and almost indistinct from the terminal oesophagus in some larvae (Fig. 6.9 K), but in most maintained its typical barrel shape (Fig. 6.9 L). The nuclei of the dorsal oesophageal gland and subventral oesophageal glands grew little in size in relation to the ventriculus, but maintained their positions in the ventral sector, with the dorsal oesophageal gland nucleus behind the left subventral oesophageal gland nucleus (Fig. 6.9 M). The junctional zone between ventriculus and intestine became increasingly prominent with growth, as did the lumen of the posterior ventriculus (Fig. 6.9 J & L). The excretory nucleus was prominent in late 3rd and early 4th-stage larvae (Fig. 6.9 E & J) but, growing little in size and "tethered" by the excretory canal, moved forwards and became inconspicuous.

The bacillary layer of the intestine, already possessed of a lumen in early 3rd-stage larvae (Fig. 6.3 C, E and F), in the late 3rd/early 4th-stages had become distinct (Fig. 6.9 J).

The genital primordium maintained its size and relative position in 3rd-stage larvae up to 900 µm in length, but in later 3rd- and early 4th-stages, the cells started dividing and its relative postion shifted. In females, the posterior cell bifurcated (Fig. 6.11 B - D), followed by a series of divisions in the anterior cells. This led to increasing length of the undivided segments but not of the 2 branches until larvae had grown beyond 2 mm (Fig. 6.11 E - G & Table 6.3). With the initial bifurcation, the primordium in females commenced moving forwards (Fig. 6.10). In males it did not bifurcate and moved in a relatively posterior direction (Fig. 6.10), growing in length much more rapidly than that in females of the same size. Some larvae designated as males in Figure 6.10 may have been females in which the bifurcation was overlooked, perhaps because of the angle of view. In a 1.78 mm long male, the primordium was 61% of the body length from the mouth; it comprised 4 cells and was 23 µm long. In another of 1.98 mm, it had multiplied to 7 cells, was 37 µm long and 63% of body length from the anterior extremity. Although in males cellular multiplication appeared to proceed at both extremities of the genital primordium (Fig. 6.11 M - O), there was rapid posterior extension so that by 3.40 mm the tract had reached the cloaca (Table 6.2). Following this, the rudimentary spicule appeared (Fig. 6.12 E & F) and shortly afterwards, at a length of 6.23 mm, the caudal papillae and phasmids became discernible. At 9.53 mm, all post-cloacal papillae were visible. In a larva of 8.03 mm, a distinct post-cloacal constriction indicated the formation of the typical digitiform appendage of males. An 8.83 mm larva had a lumen in the primordial ejaculatory duct (Fig. 6.11 P). Beyond this length, it was usually quite simple to differentiate male and female larvae on tail structure alone. Pre-cloacal papillae (Fig. 6.12 G) were more difficult to find. They were not seen in larvae shorter than 17 mm, but in one male 19.6 mm long, 8 pairs were reliably identified. Beyond 20 mm, they were more distinct.

FIGURE 6.10

Position of anterior extremity of genital primordium in relation to body length (log_{10} scale) of T. pteropodis recovered from intestines of juvenile P. poliocephalus. (Based on data from Tables 6.2 and 6.3)

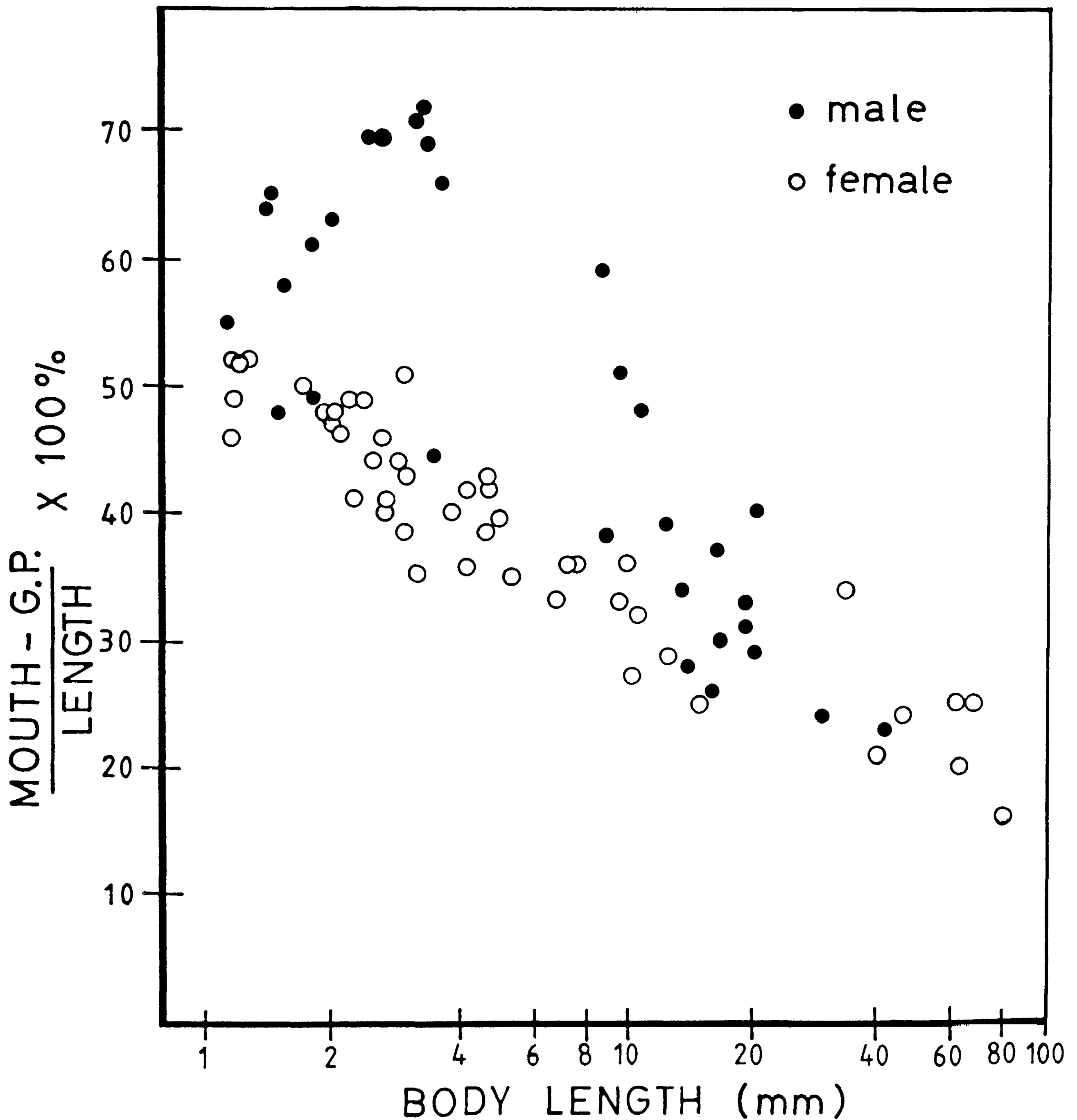


FIGURE 6.11

Developmental stages of genital primordium in T. pteropodis

Note - Orientation in A - I: anterior to top of page (Scale bar in J)

A. Ventral view of 550 μm third-stage larva from bat liver 6 weeks p.i., showing apparently 3 cells (bar = 20 μm).

B. Lateral view of 1.17 mm third-stage larva after first division of posterior cell (arrow) (bar = 50 μm).

C. Lateral view of 1.25 mm third-stage larva with early posterior bifurcation (arrow) (bar = 50 μm).

D. Lateral view of 1.75 mm larva (? late third-stage) with arrow indicating 2 cells in each arm of bifurcation (bar = 50 μm).

E. Oblique view 1.90 mm presumably early fourth-stage larva (bar = 50 μm).

F. Ventral view 2.83 mm fourth-stage larva showing development of anterior rudiment as well as bifurcation (bar = 50 μm).

G. Lateral view 2.94 mm fourth-stage larva with non-patent primordial vulva (arrow) (bar = 50 μm).

H. Ventral view 6.88 mm fourth-stage larva with arrow indicating lumen in bifurcation (bar = 100 μm).

I. Same larva and scale as in H showing cuticular dimple (arrow) but not pore in primordial vulval region.

J. Lateral view 21.6 mm pre-adult showing undifferentiated but well-developed uterine-vaginal segment and bifurcation (bar = 200 μm).

K. Lateral view 15.0 mm pre-adult showing genital tract bifurcation (arrow) (bar = 200 μm).

L. Same as K, showing patent vulva. Intestine (i) has well-defined bacillary layer (bar = 100 μm).

M. Lateral view 2.45 mm male fourth-stage larva showing entire genital primordium (posterior to right) (bar = 50 μm).

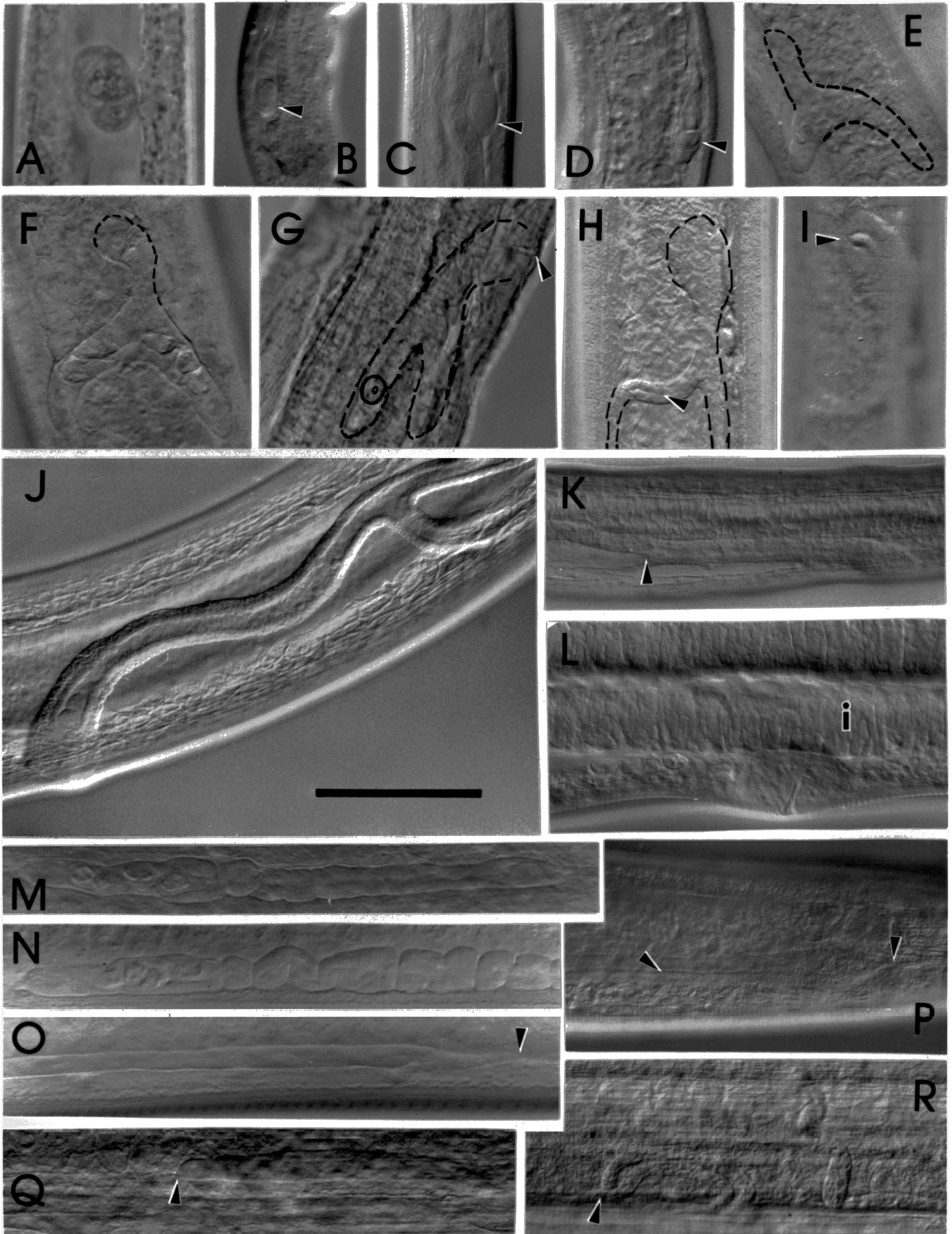
N. Anterior genital primordium 3.30 mm fourth-stage male showing early folding (bar = 50 μm).

O. Same larva and scale as in N. Posterior genital primordium, with terminus arrowed.

P. Lateral view of caudal region 8.83 mm fourth-stage male showing lumen of terminal vas deferens (arrow) and junction with cloaca (arrow) (bar = 100 μm).

Q. Ventral view of 16.2 mm pre-adult showing straight anterior extremity (arrow) of primordial testis (bar = 100 μm).

R. Lateral view 19.6 mm pre-adult showing anterior testis recurved (arrow) (bar = 200 μm).


A
B
C
D
E
F
G
H
I
J
K
L
i
M
N
O
P
Q
R

The ejaculatory duct differentiated with maturation, and was very well-developed in a 41.9 mm long pre-adult (Fig. 6.12 J & K) which also contained spermatocytes in its distal testis.

6.2.5 The adult T. pteropodis

Adults were examined to compare with and extend previous descriptions (Baylis, 1936; Warren, 1971a), which were of specimens from another host, P. geddiei. Specimens included live worms, intact fixed worms cleared in creosote, fixed specimens prepared for SEM and stained sections of fixed worms.

When immersed in hot (70 ^{o}C) 3% formalin, most adult worms ruptured, and this still occurred but to a lesser extent in hot 3% formol-saline. Cold formalin did not damage the live worms, but caused contortion and apparent shortening. Immersion in Berland's fixative followed by storage in alcohol was convenient and produced straighter worms which were easier to examine.

Male worms matured at about 60 mm, and females at 80 mm (Section 4.2.6). Live worms were tapered at both ends, more gradually anteriorly, and were covered with a thin translucent cuticle through which the larger internal structures were clearly visible, including the oesophagus and ventriculus. The intestine, extending almost to the tail tip, was golden-brown, and in both sexes obscured throughout most of its length by the genital tract. Females could be distinguished by their larger size, straight tail, the thick, white uterus (cf. thinner, much-coiled testis and more posteriorly situated seminal vesicle of the male) and the slight body constriction at the vulva, which opened in the midline ventrally in the anterior quarter of the body. The key dimensions of 10 females and 12 males fixed in either Berland's solution or hot formol-saline are presented in Table 6.4.

FIGURE 6.12

Caudal extremity of T. pteropodis larvae from intestine of juvenile P. poliocephalus (Scale bar in E)

A. *Third-stage female, 1.49 mm, showing prominent dorsal gland* cell *(arrow)* and other features typical of earlier third-stage larvae. Tail tapers posterior to anus (bar = 50 µm).

B. Early fourth-stage female, 2.47 mm, showing dorsal rectal gland cell (arrow) (bar = 50 µm).

C. Pre-adult female, 19.7 mm, showing dorsal rectal gland cell (arrow), prominent anterior lip to anus and typical smooth outline of tail (bar = 200 µm).

D. Early fourth-stage male, 2.49 mm, showing no evidence of spicular development. Probable dorsal rectal gland cell (arrow) appears posteriorly displaced (bar = 50 µm).

E. Fourth-stage male, 3.59 mm, with discernible spicule cell (arrow) (bar = 50 µm).

F. Early spicule (arrow) development in 8.83 mm fourth-stage male (bar = 100 µm).

G. Pre-anal papillae (arrow) in oblique view in 18.9 mm early pre-adult male. Note well-developed spicules and moderately differentiated digitiform appendage (bar = 200 µm).

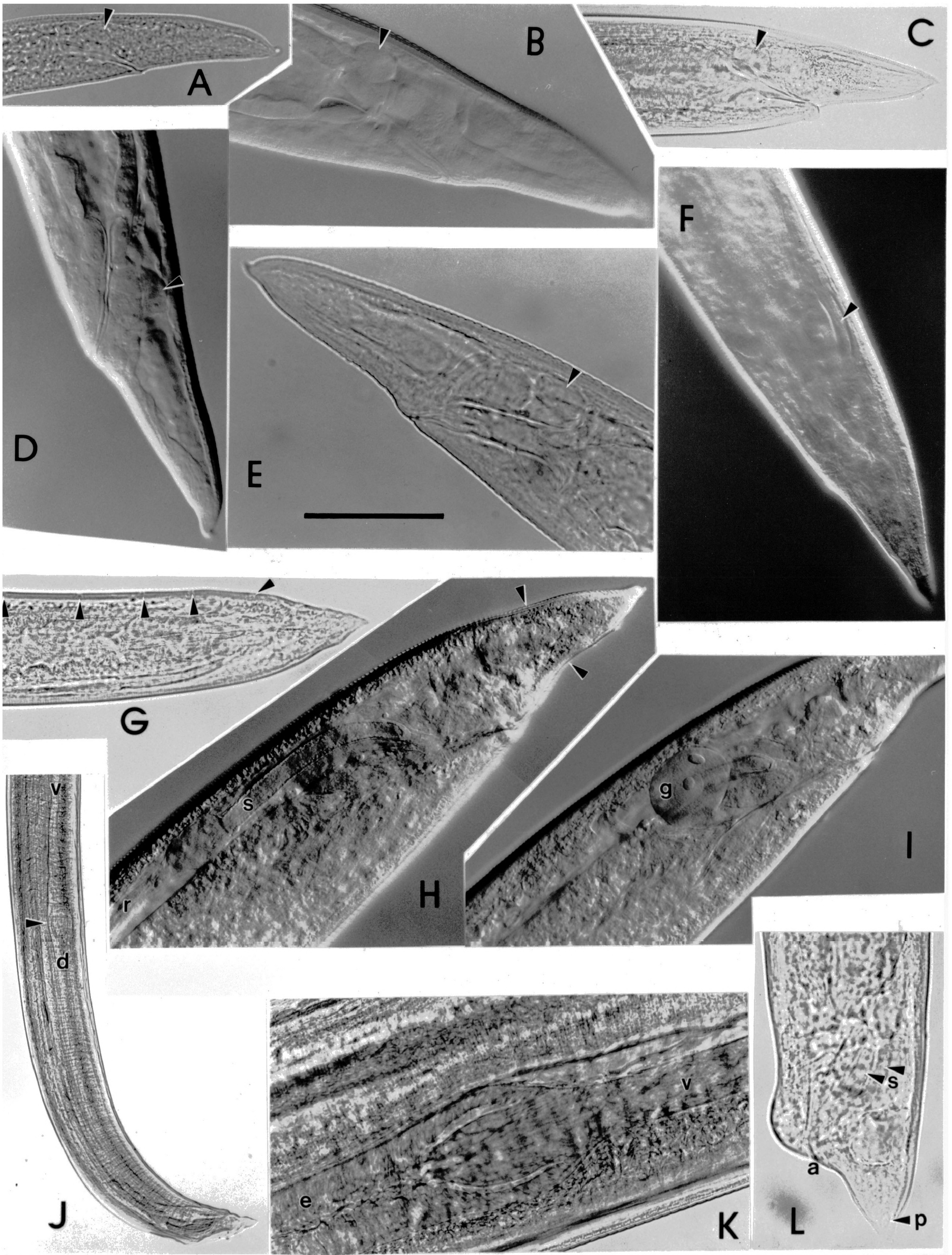
H. Early pre-adult male, 16.7 mm, showing constriction (arrows) separating digitiform appendage, well-developed spicules (s) and spicule-cell continuing to retractor muscle (r) (bar = 100 µm).

I. Same worm and scale as in H, showing well-developed dorsal rectal gland cell (g) between spicules.

J. Almost mature male, 41.9 mm, showing junction (arrow) of vas deferens (v) and muscular ejaculatory duct (d) (bar = 400 µm).

K. *Same worm as in J, showing valve-like junction between vas deferens (v)* and ejaculatory duct (e) (bar = 100 µm).

L. Bulbous malformation of anterior lip of cloacal aperture (a) of 11.3 mm early pre-adult male. Note 2 spicules (arrows at s) and phasmidial opening (p) (bar = 200 µm).



A
B
C
D
E
F
G
H
I
J
K
L
s
r
g
v
d
e
a
p

TABLE 6.4

Dimensions of adult T. pteropodis passed spontaneously by juvenile P. poliocephalus with patent infections

Total length (L) (mm)	Max. width (mm)	Lips length x breadth (µm)	Oesoph. length (LE) (mm)	LE/L %	Mouth-nerve ring (NR) (mm)	NR/LE %	Ventriculus length (mm)	Ventriculus width (mm)	Ventriculus body width (mm)	Cuticular striae Vent. (µm)	Cuticular striae Mid-body (µm)	Cuticular striae Pre-anal (µm)	Anus-tail (T) (mm)	T/L %	Genital tract Spicule (mm)	Genital tract Ejac. duct (ED) (mm)	Genital tract ED/L %		
MALES																			
*68	1.28	115 x 235	3.11	4.6	0.74	24	0.34	0.32	0.82	13	-	24	0.25	0.36	0.70	4.04	5.9		
*72	1.55	140 x 240	3.61	5.0	0.98	27	0.30	0.31	0.98	13	-	23	0.28	0.39	0.63	5.32	7.4		
*74	1.66	125 x 205	2.51	3.4	0.60	18	0.32	0.40	1.06	18	-	25	0.27	0.36	0.58				
*77	1.55	130 x 240	3.44	4.5	0.73	21	0.28	0.28	0.99	15	-	22	0.27	0.35	0.62	4.36	5.7		
78	1.13	105 x 175	3.82	4.9	0.97	26	0.40	0.30	0.76	13	22	-	0.27	0.34	0.53	3.92	5.0		
*80	1.47	110 x 240	3.04	3.8	0.91	30	0.38	0.38	1.00	17	-	25	0.32	0.40	0.71	4.83	6.0		
85	0.92	120 x 240	2.87	3.4	0.87	30	0.34	0.27	0.80	15	-	24	0.33	0.38	0.57	4.10	4.8		
*89	1.23	-	3.55	4.0	0.82	23	0.32	0.36	0.99	17	-	23	0.30	0.34	0.60	3.98	4.5		
92	1.25	150 x 260	3.78	4.1	0.83	22	0.44	0.36	0.97	19	30	-	0.32	0.35	0.70	4.51	4.9		
95	1.37	150 x 305	3.82	4.0	1.01	26	0.44	0.32	0.98	18	33	23	0.35	0.38	0.76	4.96	5.2		
*96	1.32	110 x 270	3.56	3.7	0.91	26	0.44	0.30	0.95	18	-	28	0.26	0.27	0.68				
102	1.38	135 x 230	4.60	4.5	1.00	22	0.45	0.29	0.94	17	31	30	0.31	0.31	0.87	5.56	5.5		
FEMALES															Mouth-vulva (mm)	Vu/L %	Vagina length (mm)	Undiv. uterus (mm)	Uterine branches (mm)
*108	2.15	120 x 225	3.81	3.5	0.94	25	0.42	0.38	0.89	17	-	27	0.30	0.28	13	12			
*114	2.09	130 x 235	3.53	3.1	0.94	27	0.33	0.40	1.23	19	-	42	0.51	0.45	28	25			
*116	2.13	125 x 290	3.15	2.7	0.87	28	0.38	0.47	0.98	15	-	35	0.38	0.33					
*124	1.70	140 x 250	3.87	3.1	1.00	26	0.42	0.38	0.85	17	-	35	0.44	0.35	37	30			
*128	2.26	145 x 280	3.81	3.0	0.98	26	0.42	0.40	1.17	19	-	40	0.64	0.50	33	26			
144	2.29	170 x 340	4.27	3.0	1.01	24	0.48	0.36	1.18	20	46	34	0.73	0.51	33	23			
144	2.12	-	3.57	2.5	0.87	24	0.42	0.36	0.97	20	40	40	0.88	0.61	26	18	6.5	5.5	
145	2.11	170 x 350	3.40	2.3	0.97	29	0.50	0.32	1.05	18	39	35	0.57	0.40	30	16	4.9	15.0	
147	1.94	185 x 305	3.85	2.6	1.01	27	0.48	0.32	1.05	19	43	41	0.62	0.43	[Vagina & uterus run anteriorly]				
150	1.86	185 x 295	4.44	3.0	0.97	22	0.48	0.33	1.15	18	44	45	0.61	0.41	28	19	7.0	5.8	38

* fixed in hot formalin

The mean length of the 7 males fixed in formalin was 79 ± 9.2 mm (range 68 - 96 mm), and of those fixed in Berland's, 90 ± 8.3 mm (range 78 - 102 mm); the difference was not highly significant ($P < 0.10$). The mean length of the 5 females fixed in formalin was 118 ± 7.2 mm (range 108 - 128 mm), which was shorter ($P < 0.001$) than that of the 5 fixed in Berland's, 146 ± 2.3 mm (range 144 - 150 mm).

Overall, the mean length of all 12 males was 84 ± 10 mm (range 68 - 102 mm) and of all females 132 ± 15 mm (range 108 - 150 mm) there being no overlap in the length ranges.

Maximal body thickness, in the midbody region, varied 0.92 - 1.66 mm in males, with an overall mean of 1.34 ± 0.19 mm. The mean width of the formalin-fixed group (1.44 ± 0.15 mm) was greater ($P < 0.05$) than that of the Berland's group (1.21 ± 0.17 mm). Females were thicker than males, with a mean overall width of 2.07 ± 0.17 mm (range 1.70 - 2.29 mm). Mean body widths of the formalin- and Berland's-fixed females were virtually identical.

There was no clear relationship between body length and maximal width in any group. In general, worms fixed in hot formalin tended to be contracted, whereas those from Berland's adopted a crescentic configuration with a gentle ventral curvature of the entire body.

Ten formalin-fixed alcohol-preserved females and 11 males from bats with patent infections were weighed. The mean weight of females was 370 ± 52 mg (range 300 - 450 mg) and of males 110 ± 30 mg (range 70 - 170 mg).

At the cephalic extremity were 3 lips typical of ascaridoids, visible clearly with the stereomicroscope. In some worms, the lips were demarcated from the body by a variable constriction (Fig. 6.13 A & D) but in most were less distinctly separated (Fig. 6.13 B & C). Lips were considered to end at their outer junction, rather than at the fusion of inner margins which were considerably anterior (Fig. 6.13 B & C). In the first 5 worms examined, the dimensions of subventral and dorsal lips in each worm were

FIGURE 6.13

Scanning electron micrographs of cephalic extremity of adult

T. pteropodis (A-F) and *T. canis* (G-I)

A. Low magnification of *T. pteropodis* showing poorly-developed (left) cervical ala (a) and constriction demarcating lips from body.

B. Ventral view of *T. pteropodis* showing excretory pore (arrow), almost indiscernible alae (a) and less marked demarcation of lips. Note inner junction of lips is well anterior to outer junction.

C. Ventral view of another *T. pteropodis*, again with poorly demarcated lips.

D. En face view *T. pteropodis*, showing 2 large double dorso-lateral papillae on dorsal lip (d) and 2 large double ventro-lateral papillae on subventral lips. Note dentigerous ridges along anterior edges of lips, and median bulge (arrow) on buccal surface of each lip.

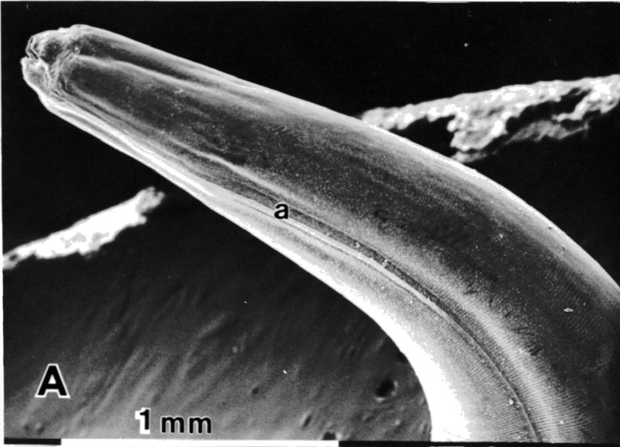
E. Right subventral lip of D. Amphidial pore (am) and single externo-lateral papilla (s) lie anterior to double externo-ventral papillae (dp). Inner labial papillae represented by pits (i). Note second row denticles (arrow), median labial bulge, surface pits between amphid and double papilla and apparent pit in dentigerous ridge at midline of lip (p).

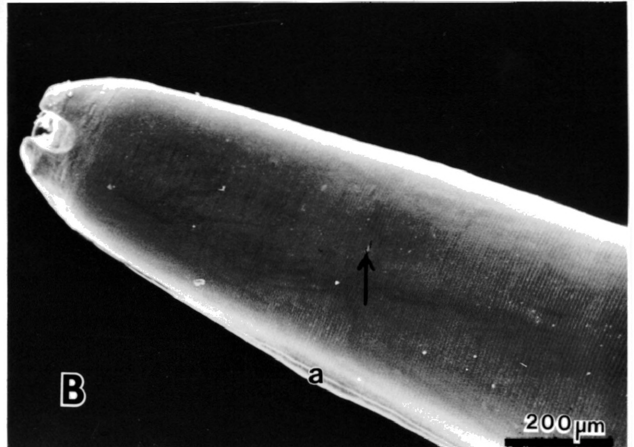
F. Same lip as in E, showing two pits (arrows) on ventral double papilla (dp), amphidial pore (am), indistinct single lateral papilla (s) with single pore (arrow) and apparently random surface pits (arrows).

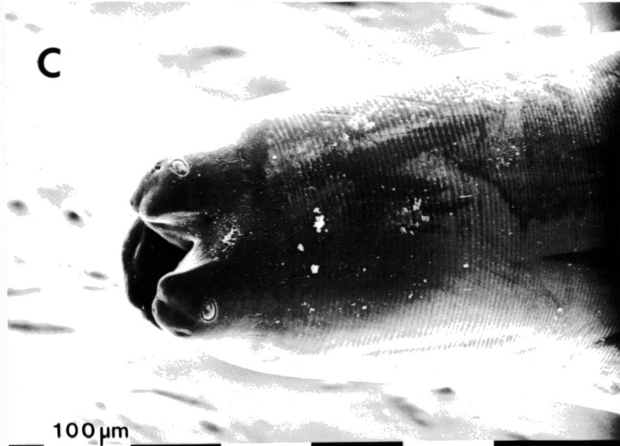
G. En face view *T. canis* showing well-developed cervical alae (a) and general labial and papillary structure as in *T. pteropodis* (d = dorsal lip).

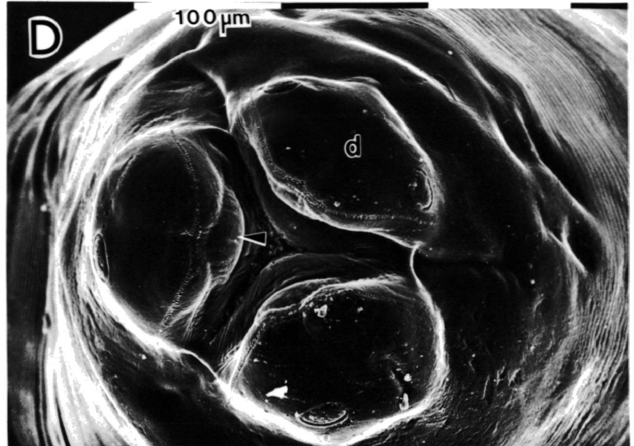
H. *T. canis* lips, showing sharp demarcation from body and median grooves along buccal surface lips (arrow).

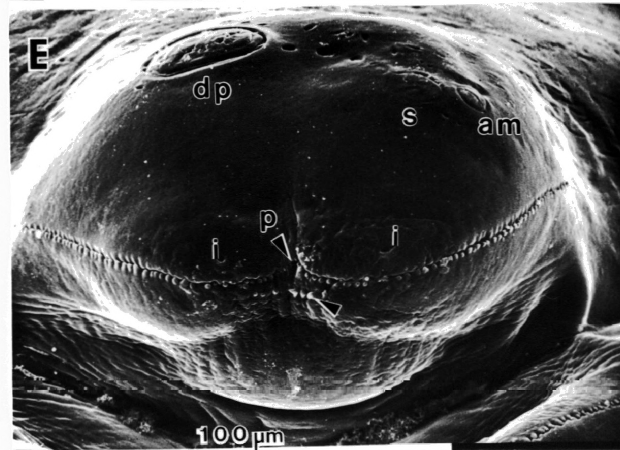
I. Left subventral lip of H, showing single row of evenly spaced denticles along ridge, inner labial papillary pits (arrows), externo-ventral double papilla (dp), slightly recessed externo-lateral single papillae (s) and amphidial pore (am).

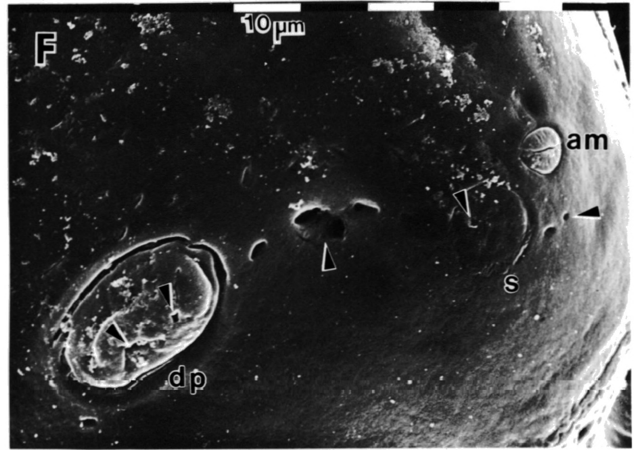

A
a
1 mm

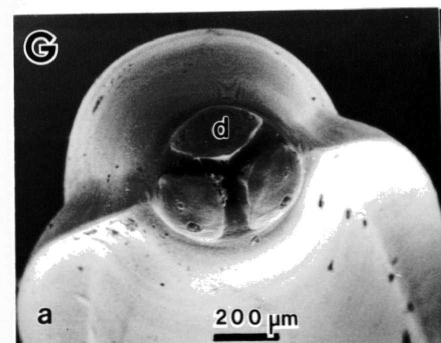

B
a
200 µm

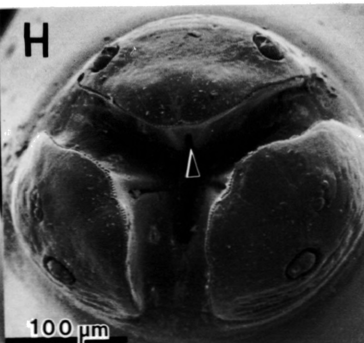

C
100 µm

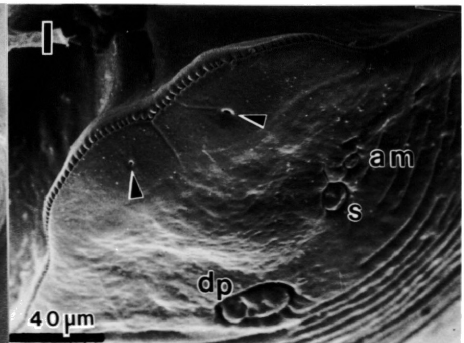

D
100 µm
d


E
dp
s
am
p
i
i
100 µm


F
10 µm
am
s
dp


G
d
a
200 µm


H
100 µm


I
am
s
dp
40 µm

almost identical, so in Table 6.4, only the dimensions of the most readily measurable lip, usually a subventral, are presented for each worm.

The labial papillary pattern was also typical, with 6 outer papillae (Fig. 6.13 D) - 2 double externo-lateral papillae on each side of the dorsal lip, a double ventro-lateral papilla on each subventral lip, and a much less distinct single externo-lateral papilla on each subventral lip (Fig. 6.13 E & F). The single external papilla on each subventral lip was situated slightly anteriorly to the double subventral papilla, and just dorsal to it was the amphidial pore (Fig. 6.13 E & F). The single and double pores in the corresponding papillae were readily discernible in the SEM, and a number of apparently randomly distributed pits were seen between and around these papillae on the subventral lips (Fig. 6.13 E & F). The inner labial papillae were represented by 2 small pits near the outer anterior surface of each lip (Fig. 6.13 E). In some specimens, a fine groove extended from each of these pits to a slight cleft on the anterior lip margin, in which was located an accessory denticle just out of the line of the denticular ridge.

The denticular ridge (Fig. 6.13 D & E), located on the inner margin of the anterior edge of each lip, comprised about 100 - 120 somewhat irregularly-spaced conoid denticles with corresponding denticular grooves along the immediately adjacent outer margin. At the apex of each lip, just inside the dentigerous ridge, was a secondary row of 8 - 10 denticles (Fig. 6.13 D & E). In the apical cleft of each lip, at the centre of each dentigerous ridge, a recessed pit was seen in all 5 worms examined by SEM (Fig. 6.13 E).

The labial pulp, examined in cleared lips removed from 2 adult worms, comprised 2 outer lobes and a larger inner lobe corresponding with the median swelling on the buccal surface of each lip (Fig. 6.13 D & E).

By contrast, in _T. canis_ (2 specimens examined), the lips were sharply

delineated from the body at their bases (Fig. 6.13 G & H), the denticles were more regularly and tightly spaced without a secondary inner row (Fig. 6.13 I), the single externo-lateral papilla on each subventral lip was distinct and slightly recessed (Fig. 6.13 I), there were no obvious irregular pits on these lips, the anterior edges of the lips were sharper and a distinct median groove was seen on the inner buccal surface of each lip (Fig. 6.13 H & I).

The cervical alae of T. pteropodis commenced behind the lips and were poorly-developed, varying in length, width and thickness amongst individual specimens (Figs 6.13 A, B & C; 6.14 A, B & M). They appeared to comprise thickened cuticle and in cleared speimens extended about half the length of the oesophagus. They were markedly distinct from those of T. canis (Fig. 6.13 G). In transverse sections, an alar supporting bar could not be found (Fig. 6.14 A, B & M).

Circumferential cuticular striations covered the entire body except for the lips, being most widely spaced in the mid-body region (range 22 - 44 µm). In male worms, the width of striae at the ventriculus ranged 13 - 19 µm, at the mid-body 22 - 33 µm and in the pre-cloacal region 22 - 28 µm. The corresponding ranges in females were 15 - 20 µm, 39 - 46 µm and 27 - 45 µm. In general, spacing was wider in longer worms, with many individual exceptions.

The oesophagus, 3.4 - 5.0% of the body length in males and 2.3 - 3.5% in females, commenced as a club-shaped swelling under the cuticle of the posterior buccal surface of the lips, and was then cylindrical until the nerve ring, beyond which it gradually widened until constricting at its junction with the ventriculus (Fig. 6.14 C & D). The lumen was lined with cuticle and typically triradiate throughout its length. Elements of the dorsal oesophageal gland were densely scattered amongst muscle anterior to the nerve ring, where the gland communicated with the oesophageal lumen through a longitudinal fissure. Posteriorly, these follicles were found

less densely in patches until reaching the ventriculus, in which they comprised about 50% of the tissue (Fig. 6.14 F). The oesophagus did not constrict on passing through the nerve ring (Fig. 6.14 J), which was situated 0.74 - 1.01 mm from the lips (18 - 30% of oesophageal length, Table 6.4).

As in 4th-stage larvae (6.2.4), the ventriculus was cylindrical to barrel-shaped, in most specimens clearly demarcated from the terminal oesophagus and varying in length 0.28 - 0.50 mm. It occupied about one-third the body width at that level, varying 0.27 - 0.47 mm in diameter, and was wider than long in some worms. The anterior lumen was a continuation of the triradiate oesophageal lumen, but it suddenly expanded in the posterior half into a cavernous triangular space filled with foamy eosinophilic material (Fig. 6.14 C, D, E & F). Large, dense aggregates of glandular elements were symmetrically dispersed throughout the muscle tissue, and the nuclei of the 2 subventral oesophageal glands were seen in the corresponding antero-ventral segments (Fig. 6.14 G). The larger, kidney-shaped nucleus of the dorsal oesophageal gland was located posteriorly in the left subventral segment (Fig. 6.14 H). The ventricular lumen communicated freely with that of the gut (Fig. 6.14 C, D & E). The posterior edge of the ventriculus was lined with an annular, muscular "cushion" structure (Fig. 6.14 C, D & E) in which were found a dorsal and ventral nucleus. The anterior-most intestinal epithelial cells arose from the outer posterior wall of the ventriculus but not from the cushion cells (Fig. 6.14 E). Ventriculi of _T. canis_ and _T. cati_ were examined in creosote-cleared specimens but not in sections. Despite individual variations, that of _T. canis_ was generally barrel-shaped and very similar to _T. pteropodis_, except that the patent lumen in _T. canis_ extended further forwards, almost to the oesophagus, where there was a more distinct valvular structure. The "cushion" cells at the junction with intestine

FIGURE 6.14

Anterior body structures of adult T.pteropodis (scale bar in F)

A. Transverse section (T.S.) left cervical ala at level of nerve ring in 120 mm female (bar = 50 µm).

B. Left cervical ala at nerve ring of 135 mm female (bar = 50 µm).

C. Ventriculus of 144 mm female, showing demarcation from oesophagus (o) and intestine (i), wide lumen in posterior half (l) and "cushion" cells (c) protruding into gut lumen (bar = 500 µm).

D. Longitudinal section (L.S.) of 98 mm male, showing ventriculus (v), nucleus of right subventral oesophageal gland cell (g), nucleus of dorsal cushion cell (arrow) and anterior-most coils of testis (t) around intestine (i) (bar = 500 µm).

E. Same worm as in D, showing dorsal cushion cell (c) at junctional zone between ventriculus (v) and intestine (i) (bar = 50 µm).

F. T.S. posterior ventriculus 130 mm female, showing lumen (l) filled with eosinophilic foamy material. Note extensive darker (basophilic) glandular elements between muscle tissue (bar = 200 µm).

G. L.S. 98 mm male, showing nucleus of left subventral oesophageal gland in antero-ventral portion ventriculus (anterior to left) (bar = 50 µm).

H. Same preparation as G, showing nucleus dorsal oesophageal gland in left postero-ventral ventriculus (arrow at posterior wall ventriculus) (bar = 100 µm).

I. 1 & 2 SEM ventral surface showing excretory pore with apparent accessory openings (I-2) (bar = 100 µm for I-1).

J. L.S. 98 mm male showing excretory canal (ec) just posterior to nerve ring (nr) (bar = 1 mm).

K. T.S. 130 mm female through excretory pore (p), 0.73 mm from lips, showing multi-channelled lumen of excretory canal (ec) and nucleus of canal cell (n) (bar = 50 µm).

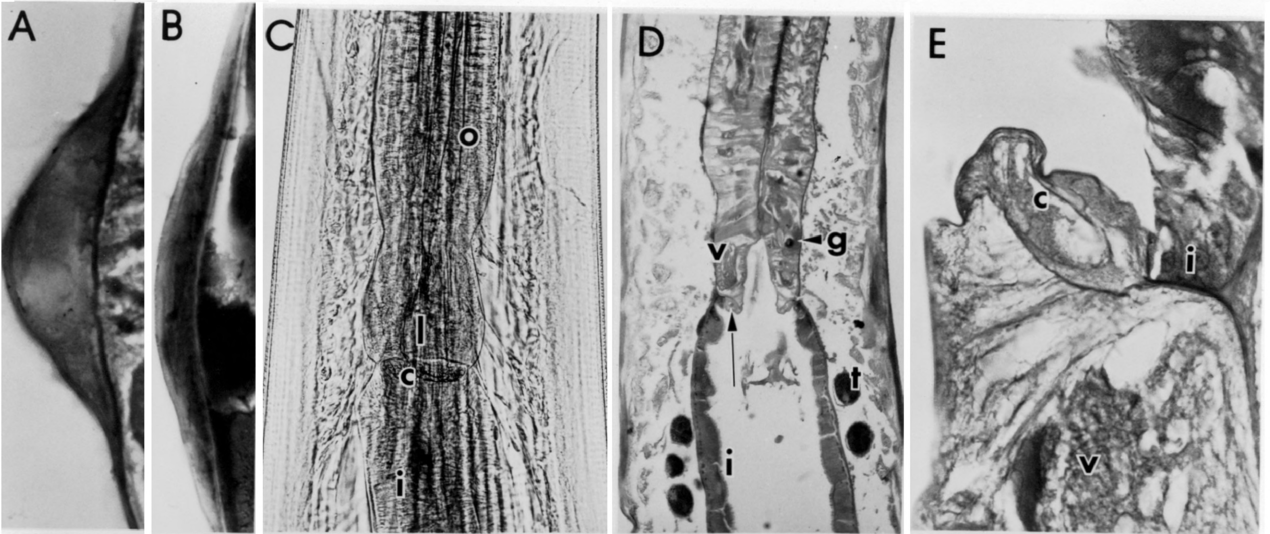
L. Same worm as in K, 0.93 mm from lips, showing left lateral cord (lc) and excretory cell nucleus (en) disrupted in sectioning (bar = 100 µm).

M. Same worm as in K, 0.95 mm from lips showing body of excretory cell (c) (bar = 100 µm).

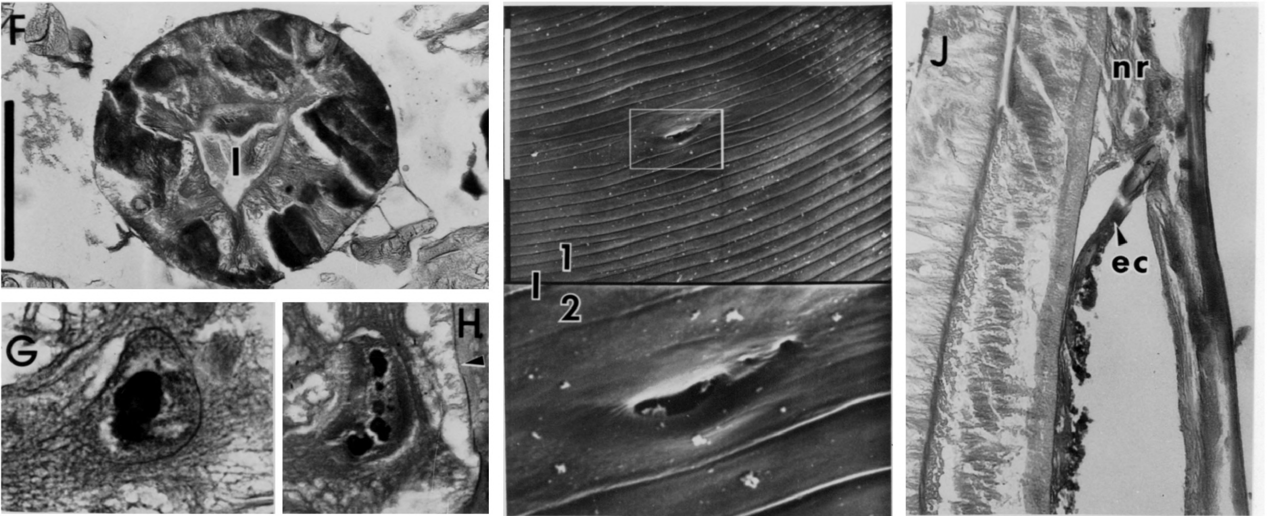
N. Same worm as in K, 1.01 mm from lips, showing left multi-channelled excretory filament (ef) within excretory cell adjacent to lateral cord (lc) just anterior to commissure (bar = 50 µm).

O. Same worm as in K, 1.26 mm from lips, showing excretory commissure (co) damaged in sectioning (o = oesophagus; bar = 200 µm).

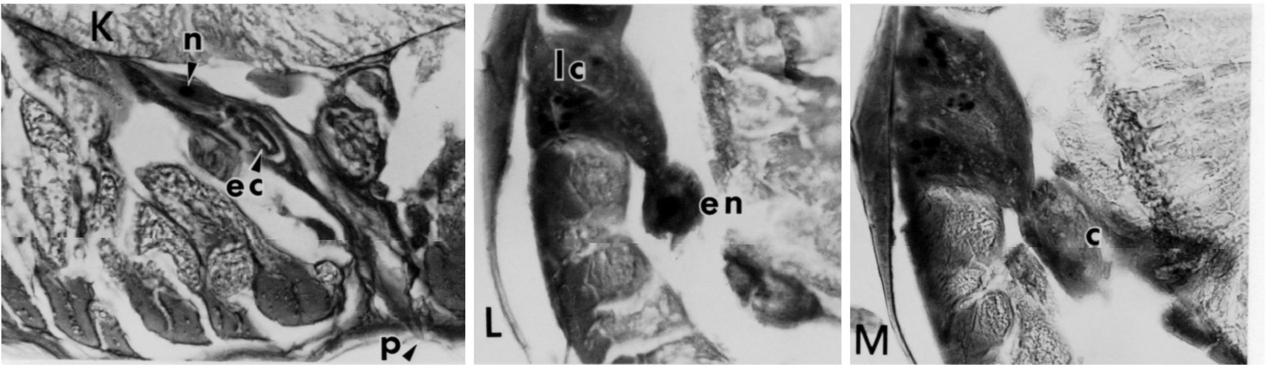
P. Same worm as in K, 1.5 mm from lips, showing hollow excretory filament (arrow) in left lateral cord (bar = 100 µm).



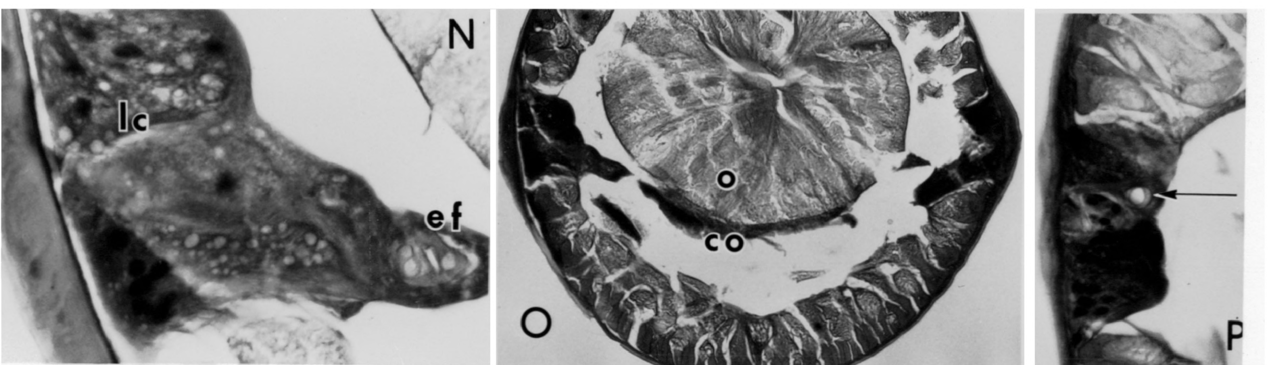
A
B
C
o
l
c
i
D
v
g
t
i
E
c
i
v



F
l
I
1
2
J
nr
ec
G
H



K
n
ec
p
lc
en
L
c
M

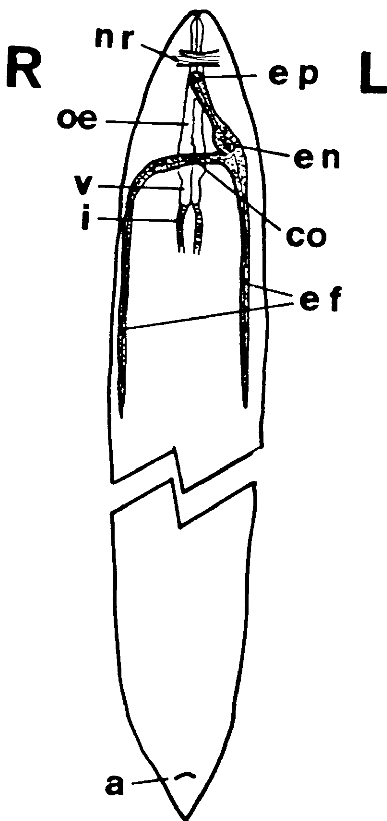


lc
N
ef
o
co
O
P

were smaller than in T. pteropodis. In T. cati, the ventriculus was narrower and generally cylindrical in shape, with the patent lumen occupying about the posterior two-thirds, and cushion cells again smaller than in T. pteropodis.

The intestine of T. pteropodis had no unusual features, comprising innumerable tall, densely-packed columnar epithelial cells with basal nuclei, arising from a basement membrane; it was elliptical in section and tapered abruptly at its junction with the cuticle-lined rectum (cloaca in males). The luminal surface of the epithelial cells was lined with a prominent bacillary layer 6 - 15 µm thick.

A schematic outline of the adult excretory system is shown in the accompanying diagram (legend: a = anus; co = excretory commissure; ef = excretory filament; en = excretory nucleus; ep = excretory pore; i = intestine; nr = nerve ring; oe = eosophagus; v = ventriculus).



The excretory pore was in the ventral midline just posterior to the nerve ring (Figs 6.13 B; 6.14 J), and in some specimens apparently had accessory openings (Fig. 6.14 I). From the pore, the multichannelled excretory canal ran dorsally, posteriorly and to the left (Fig. 6.14 J & K) to pass through the excretory cell, the nucleus of which was located between the left lateral cord and oesophagus (Fig. 6.14 L) just anterior to the excretory commissure (Fig. 6.14 O) almost 1 mm from the mouth. Behind the commissure, an excretory filament with a lumen was seen in both lateral cords (Fig. 6.14 P), but could not be found in sections taken about one-third of the body length further back.

The distal female genital tract terminated at the vulva, a small transverse slit-like opening in the ventral midline 12 - 30% of the body length from the mouth. In most specimens, the vulva could be located by the slight body constriction at that level, and protruded slightly from the body surface (Fig. 6.11 J, K & L). There was no difference in this constriction between worms passing fertile or infertile eggs. The muscular vagina, 4.9 - 7.0 mm long and usually containing a column of eggs and occasionally spermatozoa, was often coiled around the intestine or other genital tract structures. It ran posteriorly to its junction with the undivided uterine segment, which continued backwards for a variable distance before branching. Occasionally, the vagina and undivided uterus ran forwards. In 3 specimens the undivided uterine segment was 5.5 - 15.0 mm long, and in one the uterine branches were 38 mm long. These branches ran side by side and terminated adjacently in a sphincter-like constriction at their junctions with the spermathecae, which also were packed with eggs and often spermatozoa. More proximally, the spermathecae narrowed and continued as the oviducts, which joined the growth zones of the ovaries, seen with a rachis in transverse sections, and terminated in the very short germinal zone of each ovary.

The terminal portion of the male genital tract, the ejaculatory duct (= glandular vas deferens) (Fig. 6.12 J) tapered sharply at its junction with the cloaca, beneath the termination of the intestine. This duct occupied 4.5 - 7.4% of the total body length. At its anterior extremity it connected via a valve-like junction (Fig. 6.12 K) with the narrower vas deferens, which continued anteriorly ventral to the intestine for a length 1 - 1.5 times that of the ejaculatory duct, then gradually widened to form the muscular seminal vesicle, usually packed with spermatozoa, which in some specimens extended to the mid-body. The seminal vesicle was about half the body diameter, and lay ventral or to either side of the intestine.

Just before its anterior termination, the seminal vesicle turned back on itself and narrowed sharply at a sphincter-like junction with the vas efferens, which ran backwards for a variable distance and had sausage-like constrictions along most of its length. This then merged with the growth zone of the testis, which comprised most of the male genital tract structures in the anterior half of the body. Its most anterior loops often approached the ventriculus (Fig. 6.14 D), and sometimes extended beyond it.

The spicules were usually retracted in their sheaths, but protruded in about 30% of fixed and in some live specimens (Fig. 6.15 B, D & K); their length varied 0.45 - 0.87 mm, from almost equal to noticeably subequal (Fig. 6.15 E & F). The spicules were gently curved, had a thick sclerotised ridge running from the muscle insertion at the head almost to the tip of the blade (Fig. 6.15 E & F) and had wide, irregular alae running on each side of their entire lengths. The protoplasmic core was prominent in sections near the head (Fig. 6.15 G) and narrowed distally (Fig. 6.15 H & I). More distally both alae became more blade-like and curved ventromedially (Fig. 6.15 I) so that the rolled edges of the distal spicule blade gave the appearance of an incomplete tube (Fig. 6.15 D & K).

Externally, a distinct constriction of the male tail just behind the anus demarcated the digitiform appendage with its terminal spike (Fig. 6.15 A). This was less marked in some specimens (Fig. 6.15 B) and exaggerated in others (Fig. 6.15 C). The cuticle ventral to the lateral line from the tail to a short distance anterior to the cloaca, was striated longitudinally as well as transversely to give a tessellated appearance (Fig. 6.15 C, D, J & K) and in some males this extended over the caudal appendage (Fig. 6.15 D & K). On each side, posterior to the cloacal aperture, was a double post-cloacal papilla (Fig. 6.15 D, K & L), which occasionally was split into two separate papillae (Fig. 6.15 J). On the caudal appendage were 4 pairs of single papillae, 2 subventral and 2 subdorsal (Fig. 6.15 J, K & M). On each side, lying amidst the 4 single

FIGURE 6.15

Caudal structures of adult male *T. pteropodis* (A-M), male *T. canis* (N,O) and female *T. pteropodis* (P,Q)

A. Typical male *T. pteropodis* tail, with digitiform appendage and terminal spike. (Mature worm 61.2 mm long) (bar in B = 100 µm).

B. Tail of 92 mm male *T. pteropodis* with poorly-developed digitiform appendage and protruded spicules. Arrow indicates junction of intestinal tract (i) and ejaculatory duct (e) (bar = 200 µm).

C. Male *T. pteropodis*, 102 mm, with exaggerated constriction separating digitiform appendage, spicules retracted and spicule cells not discernible (bar in B = 500 µm)

D. SEM of 78 mm male *T. pteropodis*. Right post-cloacal double papilla (d) shown in detail in K & L. First and second left subventral papillae fused into one double papilla (arrow). Alae of spicules curved ventrally, to form tubular-type spicule on left and canoe-shape on right. Note extensive longitudinal and transverse striation of cuticle over tail and around cloaca to give tessellated appearance (bar = 100 µm).

E. & F. Spicules dissected from 95 mm male *T.pteropodis*, about 450 & 620 µm long. Note distinct longitudinal ridge, coarsely sculptured appearance and distinct alae. Spicule retractor muscle (m) still firmly attached to proximal (anterior) extremity and folded in this preparation (scale: bar in B = 200 µm).

G. Transverse section of proximal shaft, near head, of right spicule (rs) encased in spicule sheath (ss), of 90 mm male showing central core and prominent medial ala at this point (scale: bar in B = 50 µm).

H. Transverse section of same worm 40 µm posterior to section in G, showing mid-shafts left (ls) and right (rs) spicules, with narrower cores and irregular thick alae, dorsal and lateral to terminal intestine (i) (scale: bar in B = 100 µm).

I. Same worm, 20 µm posterior to H, showing spicules and alae curving ventrally; g = lateral rectal gland cells; n = rectal nerve plexus; i = terminal intestine just anterior to junction with ejaculatory duct (scale: bar in B = 200 µm).

J. SEM of 80 mm male *T. pteropodis*, showing cloacal opening (C), linearly arranged pre-claocal papillae, two pairs subventral papillae on digitiform appendage and right double post-cloacal papilla separated as two distinct single papillae (arrows) (bar = 100 µm).

K. SEM of male *T. pteropodis* in D, showing protruding spicules, double post-cloacal papilla (d) and two right subventral single papillae on digitiform appendage (arrows) (bar = 100 µm).

L. Enlargement of double papilla (d) in K, showing 2 pores (arrows).

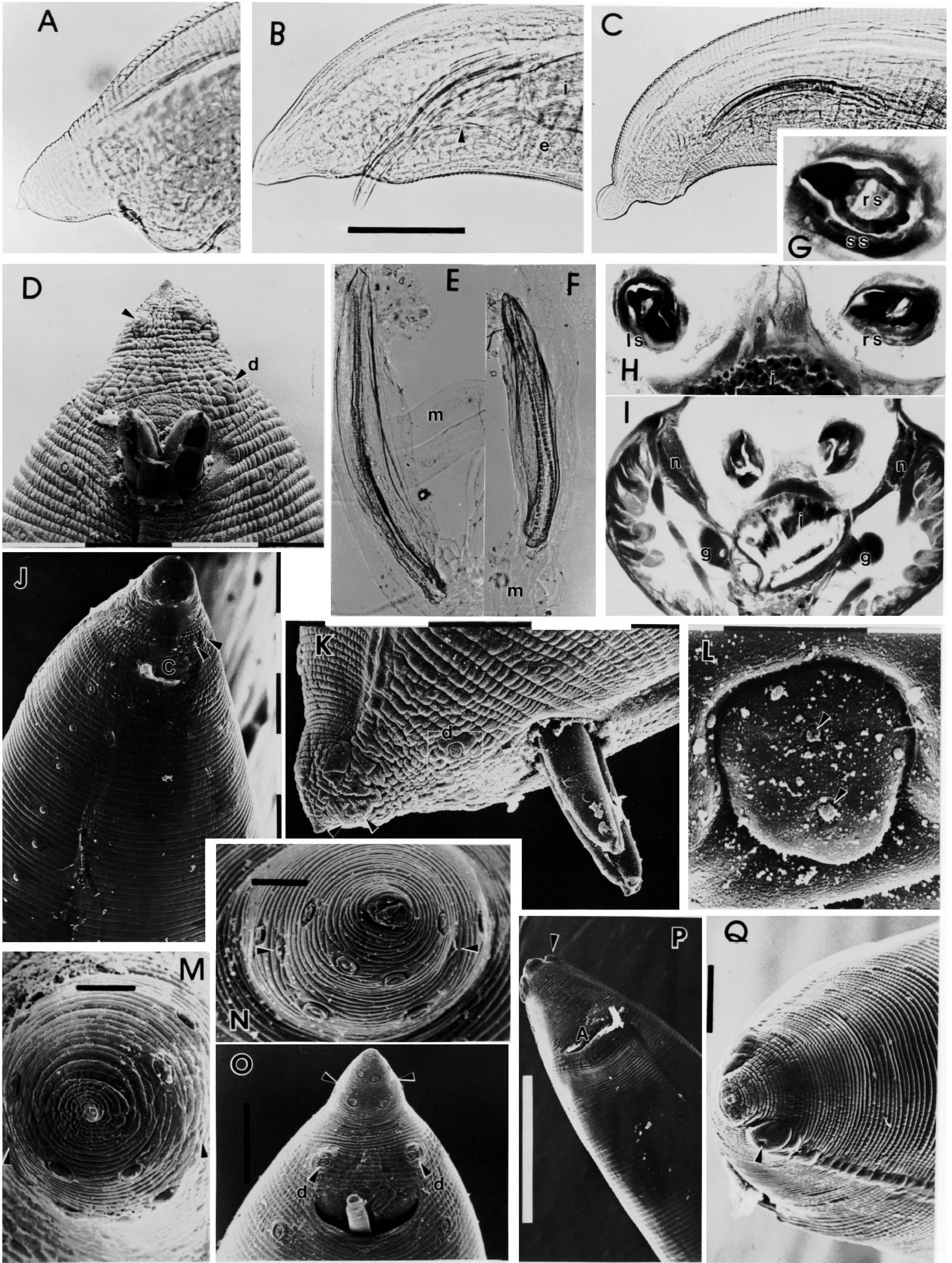
M. SEM of digitiform appendage of 80 mm male *T. pteropodis*, showing 2 pairs single subventral papillae, 2 pairs single subdorsal papillae and phasmidial pores (arrows) (bar = 20 µm).

N. SEM of digitiform appendage of adult *T. canis*, showing button-like single papillae in 2 subventral and 2 lateral pairs, with intermediate distinct phasmidial pores (arrow) (bar = 20 µm).

O. Same *T. canis* as in N, showing distinctly doubled postcloacal papillae (d), button-like first pair pre-cloacal papillae and phasmidial pores (arrows) (bar = 100 µm).

P. Tail of 135 mm female *T. pteropodis* showing relatively large anal opening (A) with transverse cuticular striations except for tessellation limited to anterior lip region, and paired papillae on caudal extremity (arrow), probably phasmidial pores. Note characteristic terminal spike (bar = 500 µm).

Q. Same specimen as in P, showing subterminal papillae (arrow) (bar = 100 µm)


A
B
i
e
C
rs
ss
G
D
d
E
F
m
m
ls
rs
H
i
I
n
n
i
g
g
J
C
K
d
L
M
N
O
d
d
P
A
Q

papillae, was the smaller phasmidial opening (Fig. 6.15 M). Occasionally, either the subdorsal or subventral papillae on one side were partially or totally fused into a double papilla (e.g. Fig. 6.15 D).

Two male T. canis showed a caudal structure distinct from that of T. pteropodis. The cloacal aperture was wider (Fig. 6.15 O), cuticular tessellation was restricted to small areas lateral to the cloaca (Fig. 6.15 O), and papillae were larger, sharper in outline and button-like (Fig. 6.15 N & O). The 2 post-cloacal papillae were double but not fused, and the 4 pairs on the digitiform appendage were lateral and subventral, with the phasmidial openings between and slightly ventral to the lateral papillae. The one protruding spicule appeared to have a solid cylindrical terminal blade (Fig. 6.15 O).

Tails of adult male T. pteropodis ranged in length (anus - tip) 0.25 - 0.35 mm (0.27 - 0.40% body length), and in females 0.30 - 0.88 mm (0.28 - 0.61% body length). The structure of the female tail was relatively simple (Fig. 6.15 P & Q), with a large anus, minimal cuticular tessellation immediately anterior to this, no digitiform appendage and no papillae except for 2 tuberous swellings, one at the termination of each lateral line, which corresponded with phasmidial openings in cleared specimens. Female T. canis and T. cati were not examined by SEM, but in cleared whole mounts comparable phasmidial tuberosities were not found.

6.3 DISCUSSION

Embryonation up to the formation of the cuticularized 1st-stage larva in the egg was as described for other ascaridoid species, except for details of timing. Beyond this stage the findings for this species differ from others. Some internal organization became evident after the 1st moult, at about 7 days. The appearance of a double-layered sheath over the definitive cuticle between days 8 and 11 immediately preceded the onset of infectivity at day 11, and beyond this only larvae with a single loose sheath were seen. The most plausible explanation for these observations is that 2 moults occur in the egg. Maung (1978), after careful observations of larvae of *A. suum* and *A. lumbricoides* expressed from eggs, came to the same conclusion. Schacher's (1957) description of *T. canis* larvae expressed from eggs at 5 and 6 d was virtually identical with that of *T. pteropodis* at the same stage in this study, yet he then commented that "moulting may occur as early as the 9th day". His "2nd-stage" larva fits the description of the 3rd-stage here. Furthermore, Schacher observed "in a very small percentage of larvae, a thin cuticular sheath surrounding the cuticle of the 1st moult may be seen at both ends of the body", but interpreted this as a splitting of the cuticle rather than a 2nd moult. Possibly Schacher missed the true 1st moult, which may commence as early as 6 d in *T. canis* (Prociv, unpublished). His structural details of the 1st-stage larva coincide with those of the 2nd-stage in *T. pteropodis*. He also cited an earlier finding reported by Mozgovoi (1953) of a double moult within the egg of *Ascaris tarbagan*. Araujo (1972) provided convincing evidence of 2 larval moults in the eggs of *A. lumbricoides*, *A. suum*, *T. canis*, *Ophidascaris sprenti*, *Polydelphis quadrangularis* and *Hexametra quadricornis*, concluding that in each species the 3rd-stage was infective. Similar observations were made in *T. cati* by Uhlikova & Hubner (1982), who concluded there must be 5 larval stages in its life-cycle. Such findings

contradict the detailed findings in A. suum of Roberts (1934), who described the 2nd and 3rd moults in the lungs, and Douvres et al. (1969), who were convinced that the 2nd moult occurred in the liver and the 3rd in the intestine. The latter authors did not examine A. suum development in the egg, assuming that the 2nd-stage larva was infective to pigs, and that "artificially-hatched eggs .. released motile larvae that were enveloped in the sheath of the first molt .. or that completed the first ecdysis on hatching". Consequently, they found mixtures of what they considered to be late 2nd and early 3rd stages in pig livers as early as 28 h pi. According to Roberts (1934), the 2nd-stage larvae in A. suum eggs measured 230 - 270 µm, and did not undergo the 2nd moult until reaching 0.8 - 1.0 mm about 6 d pi. Yet, Douvres et al. (1969) reported the 2nd moult to occur in larvae 172 - 252 µm long in the livers of pigs 28 h pi. In view of the individual variations observed in larvae of T. pteropodis in the present study, the precise staging of early A. suum based on such fine morphological details as described by Douvres et al. (1969) is open to question. Furthermore, their photograph of a "larva in 2nd molt from liver, 28 h after infection" appears to show only a small bleb at the mouth, and in fact the larva appears to be less developed than the accompanying photograph of a "larva in first molt pressed from infective egg". With T. pteropodis, much more convincing "moulting" was observed in larvae over a wide length range and at widely variable intervals after infection. These were usually seen in clusters, i.e. most larvae from 1 liver would be affected, yet none from others at a similar period after infection.

By overlooking the 1st moult in the egg of T. canis, Schacher (1957) was obliged to conclude that the 2nd moult in this species occurred soon after eggs hatched in the dog intestine. The few differences between his descriptions of 2nd and 3rd-stage larvae could be explained simply on the basis of 3rd-stage larval growth as seen in T. pteropodis in this study. Nichols (1956) assumed that infective larvae in the eggs of T. canis were

2nd-stage, and found no significant differences between these and larvae he recovered from mouse tissues 24 h to 180 d pi. He also found wide length variations amongst larvae either expressed from eggs or recovered from mice. Sprent (1958) found what he considered to be 2nd-stage *T. canis* larvae in dog tissues and in the intestines of dogs fed eggs or infected mice. His 2nd-stage *T. canis* corresponded morphologically with the 3rd-stage *T. pteropodis* larva of the present study. Sprent concluded that no structural changes occurred throughout the 2nd-stage of *T. canis*, which ranged 0.34 - 0.44 mm, "and some of the larvae showed a loosening of the cuticle indicating the onset of the 2nd moult". The length range of his 3rd-stage larvae was 0.46 - 1.26 mm, and they "were found in the liver, heart, lungs and stomach of puppies in the first week of life, in the liver, lungs and alimentary tract of puppies infected with eggs, and in the alimentary tract of dogs and foxes fed with infected mice". His description of the 3rd-stage was little different from the 2nd. In egg-infected dogs and mouse-infected foxes, Sprent observed the 2nd moult in larvae as short as 0.37 mm from lungs, hearts and livers, yet his smallest 3rd-stage was 0.46 mm long. The size range of 2nd-stage *T. canis* reported by Schacher (1957) was virtually identical with Sprent's, but his shortest 3rd-stage was 0.51 mm, so he concluded "the 2nd molt apparently occurs when the larva approaches 0.5 mm in length". Furthermore, Sprent observed that several 2nd-stage larvae in the lungs of a fox pup had loose cuticles and appeared to be damaged. Greve (1971) found not only 2nd-stage larvae of *T. canis* in various organs of experimentally-infected dogs, but also "an occasional 3rd-stage". This failure by careful workers to distinguish conclusively between the 2nd and 3rd stages of *T. canis* may indicate that they were not observing true moulting and that the 2nd stage simply does not occur in tissues.

In his study of *T. cati*, Sprent (1956) assumed the 2nd moult to occur

at 0.33 - 0.37 mm, although he did not observe this in mouse tissues, and his 3rd stage larvae were 0.35 - 1.24 mm long. He reported the 3rd moult occurring in larvae 0.53 - 1.24 mm long. The surprising absence of shorter 4th stages, and several apparent discrepancies between larval sizes, stages and duration of infection, again could be readily explained on the mistaken interpretation of a damaged 3rd stage larva with lifted cuticle as undergoing "moulting". It is likely that in T. cati the 2nd moult has already occurred before the larva hatches from the egg.

In the present study, loose cuticles were found on T. pteropodis larvae ranging 440 - 750 µm in length from bat livers as early as 3 d, and as late as one year pi. These were likely to have been artifacts, probably resulting from the conditions of incubation or fixation. Because T. pteropodis larvae grew in livers, cuticular lifting over such a wide time period and range of lengths could not be attributed to moulting, which need not be assumed if it is accepted that two moults occur within the egg. Maung (1978), in arriving at her conclusion that the double cuticular sheath in eggs of A. suum indicates 2 moults, had considered a number of other possibilities which are discussed here.

(a) Five moults in the life-cycle. It is clear from the preceding discussion that there is no need to postulate this. Third-stage larvae hatched from eggs may occasionally retain their loose 2nd-moult sheaths, as was shown by Maung (1978) for A. suum, and in the the present study for T. pteropodis, and perhaps may invade the gut wall and reach the liver while still within this sheath. As infective eggs of A. suum aged, Maung (1978) found the 3rd-stage larvae lost their sheaths more readily.

(b) Second loose sheath not a cuticular remnant. Often, vitelline membranes adhere to the cuticular sheaths of larvae expressed from eggs. These are usually quite distinct, and in many larvae with loose double sheaths the vitelline membranes could be identified quite separately.

(c) Loose sheath splits from damage during mechanical expression. It is remarkable that loose sheaths, presumably having been weakened by the action of moulting enzymes, so often do survive the trauma of mechanical expression from eggs, considering the frequent damage to the definitive cuticle of these larvae, with extrusion of internal contents. The further fine splitting of such a sheath into 2 distinct layers is difficult to accept. The finding of a double loose sheath around *T. pteropodis* larvae expressed in water and saline of various concentrations suggests this is not an osmotic phenomenon. Furthermore, the evanescent nature of this 2nd sheath, and its consistent appearance several days before the onset of infectivity, signifies its relationship to larval development. It is likely that, as an energy-conserving measure, the outer (or perhaps inner) loose cuticular sheath is resorbed by the larva within the egg , so that it disappears 1 - 2 two days after the 2nd moult.

(d) Larvae apparently moulting in tissues are damaged. This has been shown for *T. pteropodis* in the present study and was suspected in *T. canis* by Sprent (1958) as discussed above. It is very likely to be common, but apparently unsuspected by most authors, who have assumed a loose sheath indicates moulting. For example, in his study of *Ascaris devosi*, Sprent (1953) noted the timing of the 2nd moult in mouse tissues to be "somewhat variable", from 5 - 12 d pi, and commented that "during the moult the cells of the intestine appeared to become loose from one another and frequently breaks were observed, particularly at the anterior end". In some of the pseudo-moulting hepatic *T. pteropodis* larvae in the present study, not only were gut cells disrupted, but sometimes the larva was torn in 2, or detached portions were still adherent to the lifted cuticle.

(e) Second moult in egg may be the same moult as observed in tissues, early in infection. If the retention of the loose cuticular sheath is interpreted as an ongoing moult, then this may be possible, as

described for T. pteropodis larvae in (a) above. However, the infective larvae are in their 3rd stage.

From the foregoing, it is reasonable to accept that the 3rd-stage larva of T. pteropodis, and also of related ascaridoids, forms within the egg. By overlooking this, careful workers studying other species, in their search for the 2nd moult after larval hatching, have over-interpreted the finding of retained loose cuticles (early in infections) or artificially-induced cuticular lifting in tissue larvae.

The 3rd stage is that of somatic differentiation. Further development of T. pteropodis, to the late-3rd, 4th and pre-adult stages, was entirely consistent with descriptions for T. canis and T. cati. The 4th is a stage of genital tract differentiation and development, and the pre-adult (early 5th stage) of sexual maturation.

With minor differences in detail, the 3rd-stage larva of T. pteropodis is very similar to those of T. canis and T. cati. The intact larvae may be differentiated by detailed measurements, but a confident distinction is unlikely to be made from histological sections.

The 3rd-stage excretory system appears identical with that of T. canis described by Sprent (1958). Nichols (1956), in his illustration of a T. canis "2nd-stage" larva expressed from the egg, depicted the excretory canal as following a tortuous path around the oesophagus on its way to the excretory cell, but this was not Sprent's finding, and certainly different from that described here in T. pteropodis. Furthermore, Nichols described the excretory cell of T. canis as "typical of the ascarid-group, a shortened H-type . . . having 2 lateral columns extending posteriorly on either side of the intestine and anteriorly some distance beyond the oesophageal bulb". This again was different from the Y-system found in the T. pteropodis 3rd-stage larva, which had no anterior lateral prolongations, and appears to be inconsistent with the description of the adult T. canis excretory system by Warren (1971a).

Although several of the larger T. pteropodis larvae from mammary glands had loose sheaths, these may have been damaged late 3rd-stage rather than moulting forms. In only 3 gut larvae, 1.49 - 2.63 mm long, was unequivocal moulting observed. In T. canis, Sprent (1958) observed the 3rd moult in larvae 0.98 - 1.3 mm and Schacher (1957) in larvae of about 1.5 mm. In T. cati, Sprent (1956) observed the 3rd moult at 0.53 - 1.24 mm. If lengths in T. pteropodis are comparable, then it is possible that in some larvae the 3rd moult commences in the mammary gland (Fig. 6.8 B, C & D), although undoubtedly most passing to the offspring are still in the 3rd stage.

As with T. canis and T. cati, the 4th stage essentially is a parasite of the intestine. External features change towards those of the adult, and internally the genital tract develops. Males could not be differentiated from females in early 3rd-stage larvae, although the finding of a larger dorsal rectal gland cell in some larvae perhaps may be significant. In the late 3rd-stage, the genital primordium starts to grow and, in females, bifurcates. In males, there is a relative backward shift of the anterior extremity of the primordium which commences in the late 3rd-stage. The sex difference in relative positions of the primordium is most marked in 3 mm larvae, and is a useful distinguishing feature if the bifurcation of the primordium is not clearly seen. The posterior prolongation of the primordial vas deferens, which reaches the cloaca by 3.40 mm, is an obvious differential feature, as are the subsequent changes in the post-cloacal region. It appeared that in males the secondary characteristics in the tail, i.e. spicule primordia, caudal papillae and digitiform appendage, did not commence development until the vas deferens had reached the cloaca. The posterior shift of the genital primordium, and its more rapid growth than in females, may be viewed as a means of expediting the secondary sexual differentiation of the male. The primordial vulva is not patent in

the 4th-stage, but apparently becomes so after the final moult. All the features of the 4th-stage T. pteropodis larva are comparable with those described for T. canis and T. cati, apart from dimensional details.

The final moult of T. pteropodis occurred about 6.92 - 9.53 mm. Sprent (1958) observed the final moult of T. canis in larvae 5.4 - 7.4 mm, although Schacher (1957) thought this to occur at 17 - 20 mm. All of the developmental changes in the 4th stage described by Schacher appear to occur in longer larvae than in T. pteropodis. However, given the final adult lengths and earlier moults, and assuming comparable development, Schacher's interpretation may be erroneous, particularly as he appears to suggest that the vagina was patent in the advanced 4th stage. In T. cati, the final moult occurs at 4.33 - 5.65 mm (Sprent, 1956), which, in relation to adult lengths, corresponds with Sprent's (1958) observations in T. canis and the present findings in T. pteropodis.

In view of the wide length range of the adult stage, from the final moult to the onset of patency and then until senescence, perhaps a comparison only of the largest worms is meaningful between species.

Another factor to be taken into account is the method of fixation. No nematode has a "true" length, as during movement its length must be altering continually. The method of preservation influences the measured lengths of fixed specimens (Fagerholm & Lovdahl, 1984) and almost certainly accounts for some differences between adult T. pteropodis fixed in hot 3% formol-saline and in Berland's solution. In his description of T. pteropodis, Baylis (1936) reported males as 70 - 80 mm long by 1.5 - 1.8 mm thick, and females 90 - 120 mm by up to 2.5 mm thick. These lengths lie within the lower range for worms examined in this study, but his specimens may have been young or were not fixed optimally. Otherwise, virtually all the other characteristics he described corresponded with those found in the present study, except for minor details which are discussed below. Warren (1971a) measured paratype specimens and recorded males as 58.8 - 80.7 mm

long, and females 91.2 - 131 mm. Nevertheless, general differences in the average sizes of larger worms distinguish *T. pteropodis* from *T. canis* and *T. cati*. The average male *T. pteropodis* was 84 mm long, ranging up to 102 mm, and the average female 132 mm, with a maximum of 150 mm. For *T. canis* Sprent (1958) recorded a maximal length of 28 mm for males and 45 mm for females, although obviously these could not have been fully grown. Warren (1971a), tabulating Mozgovoi's figures, showed the respective length ranges as 99 - 127 mm and 126 - 198 mm. Likewise, Warren recorded lengths for *T. cati* males and females of 30 - 70 mm and 40 - 100 mm, and Sprent's (1956) maximal lengths for this species were 70 and 100 mm. It would appear that *T. canis* generally is a little larger than *T. pteropodis*, which in turn is considerably larger than *T. cati*. Considering the size of the respective hosts, it is not surprising that mechanisms, albeit unknown, limit the worm burdens in infected juvenile bats, whose bodies are not much longer than those of the worms they harbour. Female *T. pteropodis* commence producing eggs on attaining about 80 mm, compared with 55 mm in *T. cati* (Sprent, 1956).

Apart from maximal size, other more specific features distinguished *T. pteropodis* from *T. canis* and *T. cati*, the most obvious being the cervical alae. These occur in all members of the genus *Toxocara* and appear most developed in *T. cati* (Warren, 1971a). In all the species he sectioned, Warren found a longitudinal generally v-shaped alar "supporting" bar. These included *T. vitulorum*, in which cervical alae, as in *T. pteropodis*, were almost absent. He concluded that this bar was a characteristic of the genus *Toxocara*. However, in none of the 4 *T. pteropodis* sectioned was evidence found of such a bar within the ridges of the alae.

Although the general pattern of the lips, labial papillae and dentigerous ridges in *T. pteropodis* conformed with that of other ascaridoid species which have been studied, there were peculiarities. The lips of *T.*

pteropodis, particularly at the bases and on the inner surface, were quite distinct from those in T. canis and described in T. cati by Uni & Takada (1975). The lips of T. cati and T. canis resemble each other more than T. pteropodis. Uni & Takada counted totals of 424 - 542 denticles on the lips of T. cati giving an average per lip of about 140 - 180. Barus et al. (1979) found significant differences between the lips of T. canis and T. cati, and counted 122 - 136 denticles per lip in T. canis and 115 - 129 in T. cati, which is comparable with the 100 - 120 counted in 2 specimens of T. pteropodis in this study. In neither of the other 2 species was a short, secondary dentigerous ridge reported, nor a pit in the centre of the ridge, as found in T. pteropodis, although Kazacos & Turek (1982) have reported such a central pit in B. procyonis. Obviously, the number of lip denticles is not a reliable taxonomic indicator, and Barus et al. (1979) concluded that neither was their shape nor size, although those of T. pteropodis in the present study appear to be quite distinct in their shape and less regular distribution. The shape of the lips of T. pteropodis conformed to the stucture of the labial pulp, which was found to be identical with that described by Baylis (1936).

The external features of the male tail of T. pteropodis, although consistent with the general pattern, were distinct from those of T. canis, and the distribution of papillae showed irregularities even in the small number examined by SEM. Neither by light microscopy nor by SEM were the caudal papillae, particularly the double post-cloacal pair, quite as distinct as depicted by Baylis (1936), although possibly he exaggerated these features in his sketch for illustrative purposes. Baylis described the spicules as subequal and about 0.6 - 0.8 mm long, which is consistent with the 0.45 - 0.87 mm range found here. Their appearance on SEM and light microscopy is also consistent with Baylis' description. Warren (1971a) presented microphotographs of transverse sections of the spicules of T. cati, T. canis, T. vitulorum, T. suricattae and T. mackerrasae, all

quite different from those of T. pteropodis, which has the most irregular features of this group and is of taxonomic significance.

The internal dimensions recorded here correspond with those of Warren (1971a) for T. pteropodis, including lip sizes, absolute and relative oesophageal lengths, nerve ring positions, ventricular sizes, cuticular striations, tail sizes and genital features, although the range of these parameters was wider in the present study owing to the greater number of specimens examined.

The internal structures of T. pteropodis were not found to be remarkably different from those described by others for T. canis and T. cati. The cavernous lumen of the posterior ventriculus illustrated by Baylis' (1936) figure 1 was also found in all specimens. This characteristic could be traced back to the 3rd-stage larva. Baylis did not examine its details or record the presence of the junctional "cushion" zone. Sprent (1956, 1958) did not record this feature either in T. cati or T. canis, and Warren (1971a) did not discuss the ventriculus as a taxonomic indicator within the genus. From the limited observations in this study, it appears that the ventriculus of T. pteropodis differs from that of T. canis and of T. cati.

Baylis' description of the genital tracts agreed with that of the present study, except he reported that "the muscular ejaculatory duct extends forwards to about the middle of the body". In the present study, the term "ejaculatory duct" referred to what is also known as the "glandular vas deferens", and it is most likely that Baylis was describing the more proximal muscular vas deferens and seminal vesicle, which does extend anteriorly to the mid-body. The terminology applied to the male genital system of nematodes is confused and at times inconsistent (Chitwood & Chitwood, 1974).

Other than locating the excretory pore, Baylis did not examine the

excretory system of T. pteropodis. Warren (1971a) also did not describe it, although by examining the excretory systems in serial transverse sections of T. canis, T. vitulorum, T. mackerrasae and T. suricattae he found them all to be identical, except for the positions of the excretory nuclei. Two lateral longitudinal vessels descended anteriorly to fuse midventrally about 600 - 900 μm before reaching the excretory pore. He found no evidence of anterior branches to this system. The excretory system of T. pteropodis appears to be virtually identical with that described by Warren, with the excretory nucleus located beside the left lateral cord just anterior to the commissure.

The huge relative size of the excretory system in 3rd-stage larvae of T. pteropodis, and T. canis and T. cati, in comparison with that in adults, warrants consideration. According to Schmidt & Roberts (1985), "strong evidence exists that most excretion occurs through the intestine" of nematodes, and the excretory system is mainly osmoregulatory. It seems paradoxical that this system is at its highest development in the 3rd-stage larva, which inhabits a relatively stable, homeostatic environment, and yet in the adult worm, which perhaps needs osmotic protection more than any other stage, is almost atrophic. Bone (1982a), in his brief review of the poorly understood role of pheromones in nematode development and behaviour, referred to evidence that the gonads were implicated as the source of sexual attractants, although the precise site remained unknown. That more complex proteinaceous substances are released through the excretory pore of T. canis larvae in culture is confirmed by immunoflorescence studies and by the analysis of excretory-secretory antigens (Arulthilakan & Nicholas, 1986). This had been found also for T. pteropodis (Mak & Prociv, unpublished). As briefly discussed in Section 4.5, it is very likely that nematode larvae influence each other's growth and development. It is possible that this influence may be mediated by peptides and other agents released from the "excretory" system. The intimate relationship of the

excretory filament (or column, in the 3rd-stage larva) with the nerve trunk in the lateral hypodermal cord may be more than coincidence.

In its development and morphology, *T. pteropodis* has all the typical features of the genus *Toxocara*, without any striking irregularities. Perhaps the most "unusual" feature, the 2nd moult within the egg, is also typical of the group but in related species needs to be re-examined more critically.

CHAPTER 7

TOXOCARA PTEROPODIS IN OTHER MEGACHIROPTERA

7.1 INTRODUCTION

Once the prevalence of T. pteropodis in P. poliocephalus had been established, and its life-cycle elucidated, the next step was to determine its distribution in other large Australian Megachiroptera. To gain further insights into its host range, transmission and evolution, specimens from overseas were examined.

7.2 THE BLACK FLYING FOX, PTEROPUS ALECTO

7.2.1 Natural infections

In south-east Queensland, P. alecto were found roosting only in camps occupied by P. poliocephalus, although occasionally winter camps of this species were free of black bats. Smaller numbers of black than of grey-headed flying foxes were shot between October 1981 and January 1986, and smaller numbers of females and young were caught. They were examined for infection as described already.

7.2.1.1 Adult males

The findings from a total of 25 males, including 2 adolescents (2-75, 3-1), collected between November 1981 and January 1986, are shown in Table 7.1. In bat 4-1 only 20% of the sediment was examined; in 4-2 one-third, and in all subsequent specimens 25% of each sediment was examined. In only 7 (28%) were hepatic larvae recovered, varying in number from 3 to an estimated 15. Mean larval lengths ranged 600 - 745 µm, with an overall range of 540 - 765 µm. All 12 animals collected from December onwards were from Eagleby and were free of infection; prior to this time P. alecto

had not been sampled from this site.

A lower proportion of male *P. alecto* was infected than of male *P. poliocephalus* (combining data from Tables 3.1 and 3.2, $P < 0.001$, $Z = 4.153$, $v = 75$). Amongst infected male *P. alecto*, the mean larval recovery, 7.0 ± 3.8, was less than in *P. poliocephalus* (from Table 3.2, $P < 0.01$, Student's *t*-test). The lengths of these larvae were within the range of those from *P. poliocephalus*.

7.2.1.2 Adult females

Between October 1981 and December 1984, 6 adult female *P. alecto*, weighing 500 - 820 g, were examined. One (820 g) was pregnant with a 90 g foetus and 1 (670 g) was nursing a 90 g pup. Larvae were not found in any livers or mammary glands nor in the offspring. One female (675 g) had an approximately 2-month-old juvenile with patent infection which survived.

7.2.1.3 Autopsied young

Over the same period, 13 young *P. alecto* weighing 80 - 143 g were autopsied. Only 1 intestinal *T. pteropodis* female larva, 8.0 x 0.12 mm, was found in a 143 g male bat 3 - 4 weeks old.

7.2.1.4 Suckling juveniles

Between January 1982 and December 1984, of 13 live suckling juveniles examined by rectal swab, only 3 (23%) were positive. One was the pup of the 675 g female; it subsequently passed a male and a female worm. Another harboured an unidentified tapeworm similar to those found in *P. poliocephalus* juveniles. The prevalence of patent infections in *P. alecto* juveniles (3/13) was less than in *P. poliocephalus* (60/119) ($P < 0.05$, $Z = 1.986$, $v = 130$).

TABLE 7.1

Numbers and lengths of P. pteropodis larvae recovered from livers of adult male P. alecto

Autopsy No.	Coll'n Date	Weight (g)	Number of larvae in liver recovered/measured	Larval length (µm) x ± S.D.	Range in length
1-70	13.11.81	500	0	-	-
1-101	26.03.82	750	0	-	-
2-42	14.10.82	850	8/8	610 ± 47	540 - 665
2-43	17.10.82	970	5/5	600 ± 38	540 - 645
2-54	28.10.82	850	0	-	-
2-55	28.10.82	720	9/9	670 ± 41	590 - 720
2-68	14.02.83	575	4/4	625 ± 24	610 - 645
2-71	22.03.83	870	3/3	745 ± 22	720 - 765
2-74	22.03.83	850	0	-	-
2-75	22.03.83	435	0	-	-
3-1	29.09.83	460	0	-	-
4-1	17.07.84	885	5/1	765	765
4-2	17.07.84	800	15/5	730 ± 12	715 - 740
4-4	11.12.84	820	0	-	-
4-5	11.12.84	660	0	-	-
4-6	11.12.84	780	0	-	-
4-7	11.12.84	680	0	-	-
4-8	11.12.84	500	0	-	-
5-1	30.01.86	590	0	-	-
5-2	30.01.86	860	0	-	-
5-3	30.01.86	870	0	-	-
5-4	30.01.86	880	0	-	-
5-5	30.01.86	890	0	-	-
5-6	30.01.86	930	0	-	-
5-7	30.01.86	990	0	-	-

TABLE 7.2

Lengths of T. pteropodis larvae recovered from livers of experimentally-infected P. alecto

	Egg dose	Time p.i.	No. larvae recovered	No. larvae measured	Length (µm) mean ± S.D.	Length (µm) range
juvenile male	5000	10 days	2000*	45	505 ± 29	425 - 565
juvenile male	?	4 months	34	34	620 ± 61	510 - 725
juvenile male	5000	6 months	1000	44	590 ± 30	530 - 660
adult female	?	6 months	41	41	640 ± 49	545 - 730
adult female (10 weeks post-partum)	?	22 months	20	9	695 ± 50	600 - 790

* one larva found in lungs

7.2.2 Experimental infections

7.2.2.1 Recovery and growth of hepatic *T. pteropodis* larvae

A small number of wild-caught adult and juvenile *P. alecto* sharing the flight cages with *P. poliocephalus* were exposed to infection when worm eggs were added to food. A few other juveniles were fed known doses of eggs. The numbers and lengths of larvae recovered from these animals which died from unrelated causes are shown in Table 7.2. All these larval lengths are similar to those from *P. poliocephalus* (Table 4.5 and Fig. 4.1).

One adult male *P. alecto* died of pneumonia 15 weeks pi. Following incubation in saline for 18 h, the liver sediment was divided into 3 portions, 1 examined immediately, 1 fixed in cold formalin and the 3rd fixed in hot formalin. Live larvae from the fresh sediment were mounted in chlorlactophenol under a coverslip and measured when they stopped moving, with the following results:

	Live larvae	Cold formalin	Hot formalin
Number of larvae	13	16	21
Length (μm) mean ± S.D.	680 ± 29	570 ± 37	580 ± 43
Length range	620 - 725	495 - 620	510 - 656

The unfixed larvae mounted in chlorlactophenol were significantly longer than those fixed in formalin ($P < 0.001$).

7.2.2.2 Maternal transmission of infection

In October 1984, young were born to 3 *P. alecto* which had been kept in the same cage as the *P. poliocephalus* described in Section 4.2.6 and hence had been exposed to infections.

The mother of 1 juvenile escaped 42 d pp, and its offspring remained clear of eggs on rectal swabs and did not pass worms after piperazine was given at 70 d of age.

The 2nd juvenile also did not develop patent infection. Its mother was killed at 70 d pp and no larvae were recovered from the liver. The 3rd juvenile commenced passing fertile eggs 45 d pp. Five days later observations were unavoidably interrupted for 1 week following which this bat passed neither eggs, nor worms after piperazine treatment. Its mother was killed at 73 d pp and yielded 20 hepatic larvae (Table 7.2).

7.2.2.3 Haematological response to infection

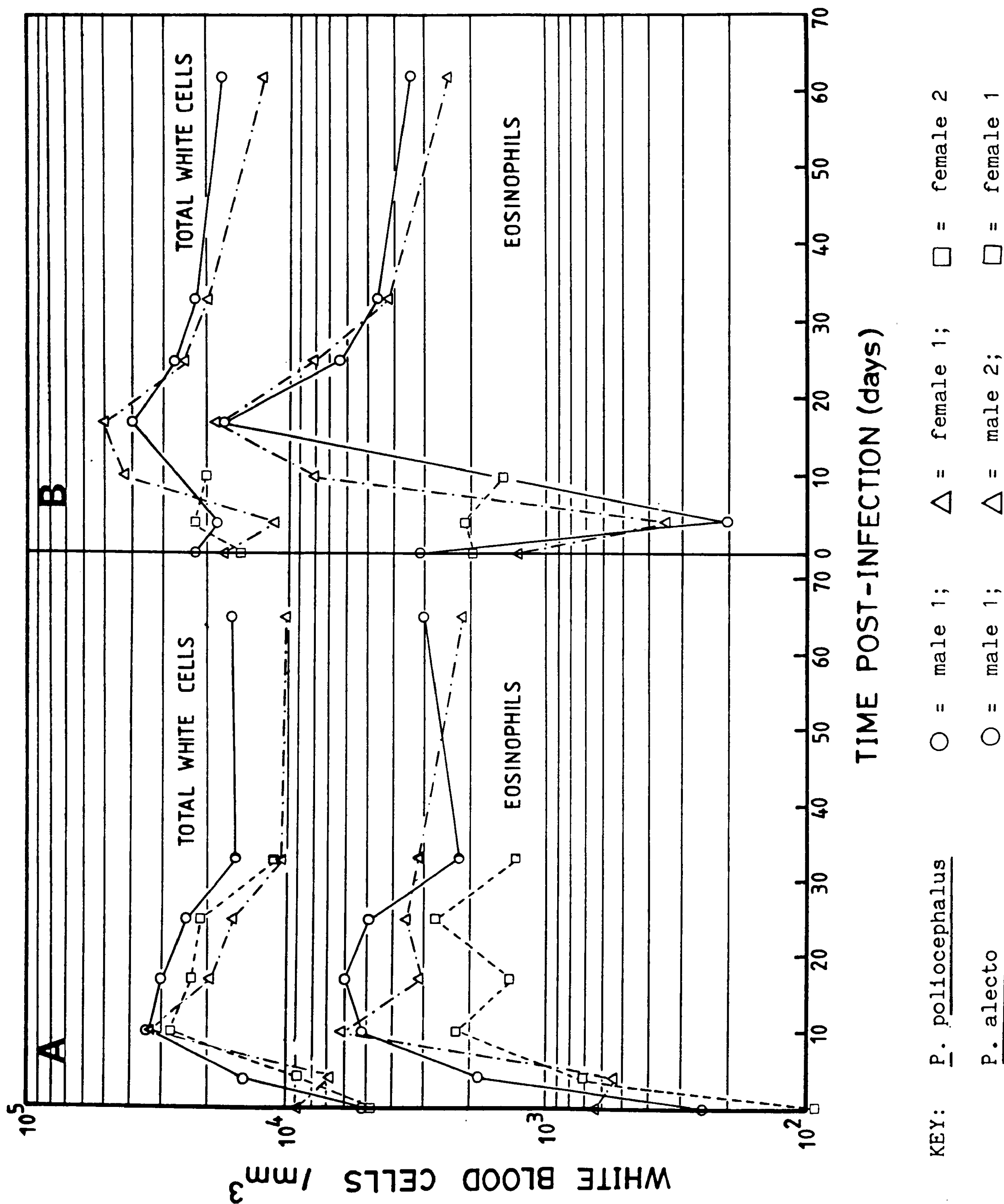
Three juvenile P. alecto aged about 3 months were each fed 5,000 T. pteropodis eggs from the same batch and at the same time as were the juvenile P. poliocephalus in Section 4.3. Blood was collected and examined at times indicated in Figure 7.1, where the results are compared with those from P. poliocephalus. The female died while being anaesthetised, whereas the 2 males were followed for 2 months. In all 3 bats, both total leukocyte and absolute eosinophil counts were high at the outset (15.7 - 24.0 x $10^3/mm^3$ and 1.32 - 3.36 x $10^3/mm^3$ respectively). In 2, both indices were lower at 4 d pi, peaked at 10 - 17 d, then gradually subsided but remained high, even at 62 d pi. Lymphocyte numbers rose in proportion to total white cells, whereas relative numbers of neutrophils fell at the expense of the eosinophil rise. As with P. poliocephalus, the eosinophil levels changed in parallel with total white cell counts.

7.2.2.4 Liver histology

Liver sections from only 4 P. alecto were examined microscopically. One was a 10 d old juvenile, 2 were post-paturient females (of which 1 proved to be uninfected) and the 4th a juvenile, fed 5,000 eggs, which died 6 months pi and from whose liver 1,000 larvae were recovered. The patterns of response appeared similar to those in P. poliocephalus, although very few inflammatory foci or larvae were seen. The last of these bats may have

FIGURE 7.1

Peripheral blood total white cell counts and eosinophil levels following the oral administration of 5,000 infective T. pteropodis eggs to juvenile P. poliocephalus (A) and P. alecto (B).



died as long as 16 h before fixation of the liver. In 110 sections (area of each section approximately 1 cm^2) 12 larvae were found without a single granuloma. There was an increase of plasma cells and eosinophils in the sinusoids, but the only inflammatory foci were clusters of histiocytes and lymphocytes around an extremity of each larva, with the remainder "free" in tissues. The hepatocytes generally were a little swollen and slightly basophilic, but around each larva they were more swollen, with eosinophilic cytoplasm and nuclear features less distinct than in those of other hepatocytes, indicative of early coagulative necrosis.

7.3 THE SPECTACLED FLYING FOX, *PTEROPUS CONSPICILLATUS*

No adult *P. conspicillatus* could be obtained to determine the prevalence of hepatic infection.

In early December 1981, and in late October 1983, a combined total of 8 suckling juveniles were caught alive and another 12 were either shot or found dead after having been apparently abandoned in mangroves 1 - 2 days after the colony had shifted location. Their weights ranged 79 - 155 g, and estimated ages 2 - 6 weeks. Developing gut larvae were found in 6 of the 12 dead bats. Rectal swabs of the 8 live animals over 2 weeks after capture showed *T. pteropodis* infection in 4, and another passed two 3-6 cm long immature adults 6 h after it was fed bananas, terminating an exclusively milk diet. Hence, of 20 juveniles, at least 11 (55%) were infected.

In December 1981, a 135 g female which had previously voided 2 female worms and had stopped passing eggs, was unexpectedly found dead in its cage. At autopsy, a male worm was found coiled and impacted in the gall-bladder and common bile-duct.

A juvenile which was fed 1,000 *T. pteropodis* eggs died 18 months later. From 40% of the liver sediment, 6 larvae (i.e. estimated total 15) were recovered, of mean length 695 $\pm$ 30 µm (range 655 - 745 µm).

7.4 THE SMALL RED FLYING FOX, PTEROPUS SCAPULATUS

7.4.1 Natural infections

On 23 June 1982, 10 adult female, all with suckling young, and 20 adult male P. scapulatus were shot in a nursery camp near Bauhinia Downs in central Queensland (Fig. 1.2). Larvae ranging in number from 1 to 37 were recovered from the livers of 15 (75%) males; lengths ranged 515 - 900 µm (Table 7.3). Mean lengths in bats harbouring more than 1 larva ranged 580 - 775 µm. There was no clear relationship between body weight, numbers of larvae and lengths of larvae. Not a single larva was found in the livers of the females nor in another 2 caught at Indooroopilly Island in March 1984.

The above 10 suckling juveniles plus an additional 13 shot at the same time (weight 145 - 215 g), all capable of flying well, were examined for infection and T. pteropodis were found in the intestines of 2 (8%). One, a female of 147 g, harboured 13 worms ranging in length 25 - 168 mm. Of these, 7 were female (mean length 152 $\pm$ 9.0 mm, range 141 - 168 mm) and 6 male (mean length 60 $\pm$ 21 mm, range 25 - 88 mm). One of these worms was in the distal intestine, with the remainder all in the proximal quarter of intestine. Numerous fertile T. pteropodis eggs were found in the gut contents. In the other juvenile, 2 female (14.7 x 0.34 mm, 18.2 x 0.42 mm) and 3 male worms (13.4 x 0.21 mm, 17.5 x 0.27 mm, 22.3 x 0.35 mm) were found. Larvae were not recovered from the livers of these bats. In 2 others, numerous unidentified hymenolepid tapeworms were found in the upper intestine.

On 14 July 1982, 50 suckling juveniles were caught by hand in the same camp and examined by rectal swab. These bats were then individually fed 300 mg piperazine citrate in milk and kept in a cage overnight prior to release the following day. T. pteropodis eggs were found in only 1 swab taken from a bat not given piperazine. No worms were found in the faeces

TABLE 7.3

Numbers and lengths of T. pteropodis larvae recovered from livers of adult male P. scapulatus in June, 1982

Weight (g)	Number of larvae	Length (µm) mean ± S.D.	Length (µm) range
400	0	-	-
450	0	-	-
450	6	580 ± 39	515 - 625
450	26	595 ± 37	515 - 665
460	0	-	-
460	0	-	-
460	1	515	515
460	4	665 ± 75	610 - 750
460	4	775 ± 13	655 - 900
470	7	605 ± 48	560 - 690
470	27	655 ± 61	540 - 755
470	37	695 ± 45	630 - 805
490	7	605 ± 67	470 - 660
500	0	-	-
500	6	580 ± 30	535 - 610
500	9	605 ± 40	565 - 700
520	12	635 ± 30	605 - 700
520	17	685 ± 32	620 - 745
560	1	605	605
600	15	610 ± 48	525 - 695

TABLE 7.4

Numbers and lengths of T. pteropodis larvae recovered from livers of experimentally-infected adult P. scapulatus

Egg dose	Time p.i.	No. larvae recovered	No. larvae measured	Length (µm) mean ± S.D.	Length (µm) range
2000	18 days	120	27	485 ± 25	440 - 530
2000	53 days [3 days post-partum]	70 0 in mammaries 0 in babe's gut	32	655 ± 39	595 - 750
?	12 weeks	150	25	555 ± 22	515 - 590
?	13 weeks	120	31	555 ± 35	495 - 640
2000	15 weeks [7 weeks post-partum]	220	38	670 ± 42	565 - 760
?	4 months	32	32	625 ± 70	495 - 775
?	5 months [5 weeks post-partum]	3	3	630 ± 27	595 - 650
?	5 months [6 weeks post-partum]	35	35	605 ± 48	495 - 690
?	6 months	165	34	610 ± 38	510 - 680
?	7 months	350	39	640 ± 48	535 - 770

of the juveniles given piperazine. In 4 (8%) of the bats, hymenolepid cestode eggs or segments were detected in rectal swabs.

Hence, the overall prevalence of _T. pteropodis_ gut infections in suckling juvenile _P. scapulatus_ aged about 3 - 4 months was 3/73 (4%). This was considerably lower than the prevalence in juvenile _P. poliocephalus_ ($P < 0.001$, $Z = 6.8$, $v = 190$).

7.4.2 Experimental infections

A number of adult female _P. scapulatus_ caught at the nursery camp in July 1982 were kept in 1 of the large cages with _P. poliocephalus_ and _P. alecto_. In February 1983, infective _T. pteropodis_ eggs were added to their food. In addition, on 7 February 1984, another 13 adult females and 3 males were caught at Pine River and placed in the same cage, also being exposed to infection. On 15 March 1984, another 8 pregnant _P. scapulatus_ were caught at Indooroopilly Island, ear-marked to distinguish them from the others in later studies, fed 2,000 _T. pteropodis_ eggs each and maintained in a 1.5 x 2 x 2 m cage. Some of these small red bats died and the results are shown in Table 7.4. From a bat fed 2,000 eggs, 120 hepatic larvae were recovered 18 d later, of average length 485 ± 25 µm (range 440 - 530). Larvae recovered from a liver 7 months pi were 640 ± 48 µm long (range 535 - 770 µm). In the 3 fed 2,000 eggs, the larval recovery represented 4 - 11% of the egg dose.

Ultimately, 15 of the adult females were found to be pregnant. Three aborted within 6 weeks of capture; in none of the foetuses were larvae found. One of these aborted a 19 g foetus 15 d pi and expelled the placenta the next day. Two days later, i.e. 18 d pi, she was anaesthetised and killed. Larvae were found only in the liver (Table 7.4).

In another 2, the pregnancies proceeded to term but 1 offspring was stillborn and the other rejected by its mother shortly after birth. The

pup (34 g) survived 3 d but apparently had not suckled, and larvae were not found in its intestine. The mother was killed 3 d pp (53 d pi) and yielded 70 hepatic larvae (Table 7.4). Larvae were not recovered from the mammary glands, which were hypertrophied but not lactating.

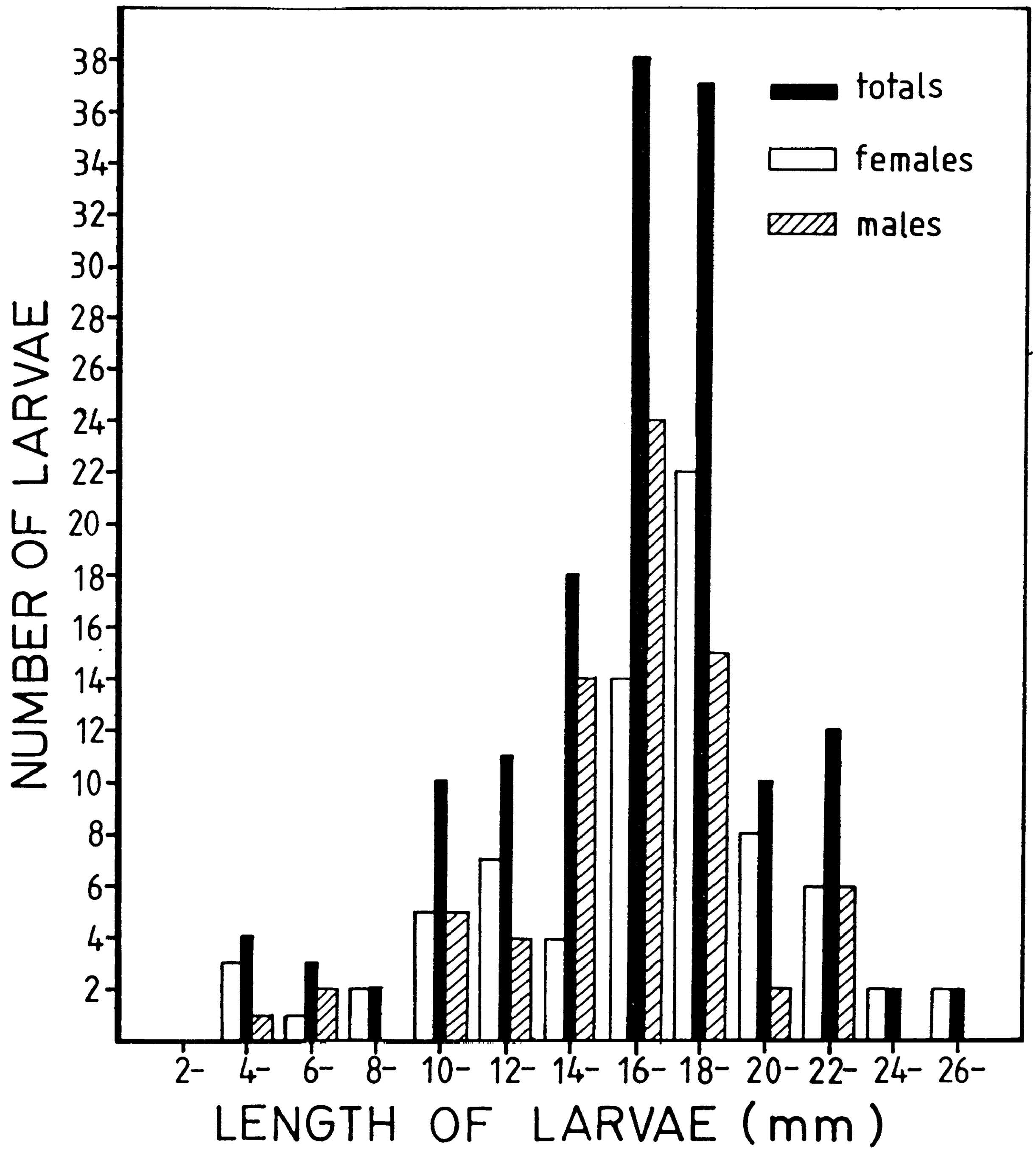
The remaining 10 pregnant females gave birth to healthy pups which suckled and survived. At one stage, 4 of these mothers with their young, all born over a period of 2 weeks, were placed together in a small cage, and the following morning 2 mothers had no pups and the other 2 were suckling 2 each! It was obvious that at least under close conditions the babes transferred readily between mothers, rendering impossible the matching of individual pups from this group with their natural mothers.

One juvenile aged 27 d, from a mother fed 2,000 T. pteropodis eggs 66 d ante-partum, died without obvious cause. On dissection, its intestine was found to be packed with developing T. pteropodis, which were washed in saline then fixed in hot 3% formol-saline. Approximately 50 of these were damaged, but 76 intact females and 73 intact males were measured. The mean length of females was 17.2 $\pm$ 4.75 mm (range 4.23 - 27.6 mm) and of males 16.7 $\pm$ 3.53 mm (range 5.66 - 22.6 mm). These lengths were not significantly different, and their distribution pattern is shown in Figure 7.2. There appeared to be a small "tail" of short larvae, particularly females. The largest 4 worms were females. Another juvenile aged about 30 d, which had been passing larvae in its faeces during the previous week, was killed with parenteral pentobarbitone. Its mother had been infected 3 months ante-partum, with an unknown egg dose. In the intestine approximately 120 worms were found (several damaged), ranging in length from 1.46 to 56.3 mm. In another juvenile aged 45 d, 92 worms were found, ranging 4.96 - 91.6 mm in length. Most of these were in the proximal two-thirds intestine, although some were found to within 10 cm of the rectum, and 2 were in the stomach. The largest were all high in the gut.

One juvenile commenced passing infertile eggs at 40 d of age. On the

FIGURE 7.2

Lengths of 149 intact T. pteropodis larvae recovered from intestine of 27-day-old juvenile P. scapulatus born to mother infected 66 days ante-partum



following day, a 24 hour faecal collection produced no eggs. On the 3rd day, 1,500 infertile eggs were recovered and 500 on the 4th day, when an 83 mm female worm containing several hundred infertile eggs in its uterus was expelled, following which egg output ceased. Another juvenile aged about 5 weeks died and approximately 70 developing T. pteropodis were found in the intestine. However, another 3 bats which subsequently died at ages 7 - 9 weeks were entirely free of worms, and the remaining 3 juveniles (aged about 10 weeks), including that which passed the single female worm, were each fed 300 mg piperazine without expelling any worms. All of these bats had been eating mainly fruit, and larvae had been noticed earlier in some faecal samples. There had been difficulties in maintaining the mother P. scapulatus in small cages, as many of them stopped eating and several died. As mentioned above, in most cases it was impossible to match the mothers with their natural pups. However, 1 mother which died 107 d after eating 2,000 eggs, and 50 d after giving birth to an offspring which subsequently developed a gut infection, still harboured 220 larvae in her liver (see Table 7.4).

Another female which died 125 d after being exposed to eggs in food and 30 d pp, did not yield larvae from her liver, and her pup was uninfected.

7.4.3 Liver histology

In view of the apparently different patterns of behaviour of T. pteropodis in P. scapulatus and P. poliocephalus, the histological changes in the liver are described here in detail.

At 12 d pi, very occasional lymphocytic-eosinophilic cuffing was present around portal tracts. No larvae were found in 50 serial sections, but a large granuloma, about 1.0 x 0.5 mm was present and extended through all sections. The haemorrhagic core of this lesion consisted of necrotic

hepatocytes and was heavily infiltrated with neutrophils, lymphocytes, plasma cells and eosinophils, most of which were disintegrating. Around this was a layer of degenerating hepatocytes and vacuolated hepatocytes infiltrated with epithelioid cells, lymphocytes and eosinophils. Occasional MNGCs appeared to be forming in places.

From the liver fixed at 18 d pi, 60 serial sections examined contained 6 granulomas and 1 larva. The granulomas did not have an outer layer of vacuolated hepatocytes, and were composed mainly of spheroid clusters of well-formed MNGCs infiltrated predominantly with lymphocytes. The cores of these lesions consisted of degenerating hepatocytes and inflammatory cells or amorphous, pink-staining material which appeared to be degenerating MNGC cytoplasm (Fig. 7-3A). The larva was found beside a dense aggregation of epithelioid cells, eosinophils and lymphocytes about 1 mm from the nearest granuloma.

The liver of a bat found dead 52 d pi, 3 weeks after aborting spontaneously, contained larvae which were partly "free" in the tissues but also close to or partly enmeshed within foci of lymphocytes, epithelioid cells and eosinophils from which inflammatory "trails" could be traced to adjacent portal tracts. No granulomas were found. Another bat was killed 3 d pp, at 53 d pi. Many bipolar granulomas (described in Section 4.4.3.1) were found in 60 sections (Fig 7-3B) and a larva was found 100 µm away from the type 1 (cellular core) end of one of these. Several other larvae were found remote from granulomas but closely associated with dense infiltrates of epithelioid cells, eosinophils and lymphocytes. An outer layer of vacuolated hepatocytes was not evident around the granulomas. The eosinophilic necrotic core of type 2 granulomas appeared to consist of degenerating MNGC cytoplasm and eosinophils.

In the liver of an adult female found dead 15 weeks pi (250 larvae recovered), larvae were frequently encountered, in each case being found within a cluster of epithelioid cells, eosinophils and lymphocytes which

FIGURE 7.3

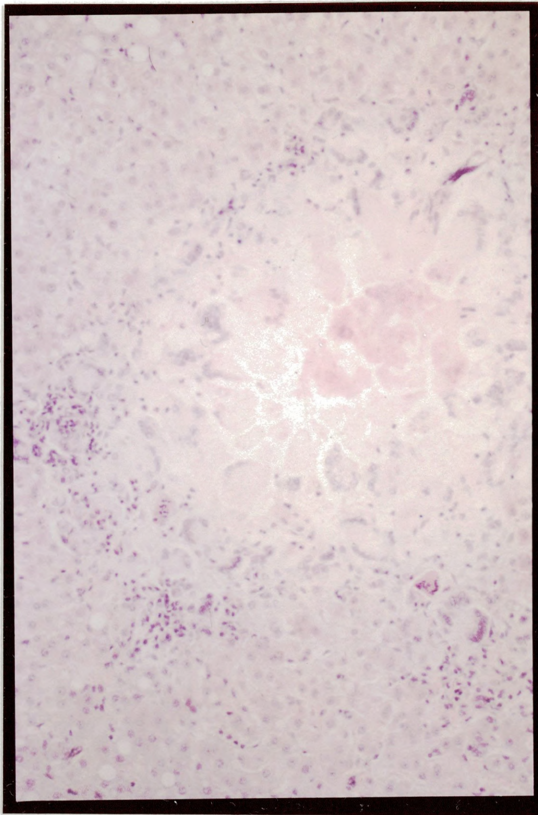
Histological changes in livers of adult *Pteropus scapulatus* experimentally infected with *Toxocara pteropodis*

A. Granuloma 18 days post-infection, comprising necrotic, amorphous eosinophilic core surrounded by dense layer of MNGCs infiltrated with lymphocytes and eosinophils. No larva found in this lesion.
(magnification x 150)

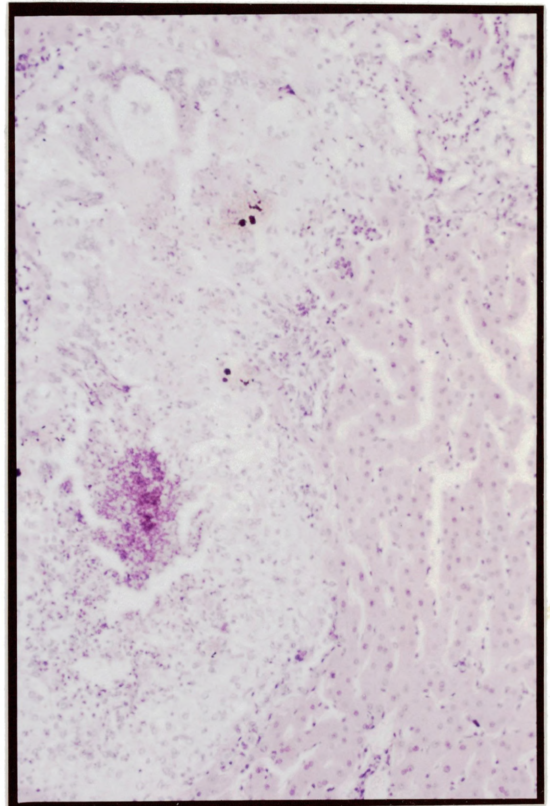
B. Edge of bipolar granuloma from female 3 days post-partum, 53 days post-infection. Lower pole of lesion has heavily-infiltrated, necrotic, cellular core surrounded by lymphocytes, eosinophils and thick layer of epithilioid cells, whereas upper pole core comprises MNGCs. A larva was found 100 µm from lower pole.
(magnification x 150)

C. Larva within type 1 pole of bipolar granuloma, 50 days post-partum and 15 weeks post-infection.
(magnification x 150)

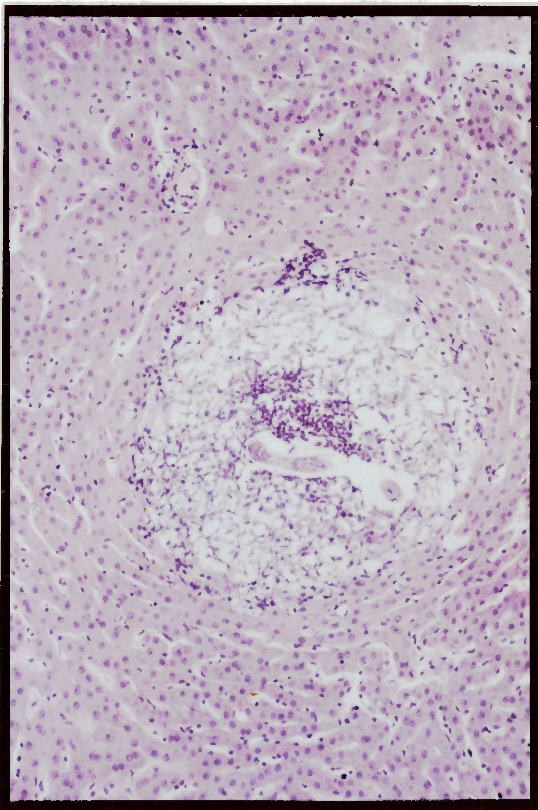
D. Female 5 months post-infection and 6 weeks post-partum. Larva adjacent to granuloma with core densely infiltrated with eosinophils and lymphocytes surrounded by layer of epithelioid cells. No MNGCs found in this lesion.
(magnification x 150)



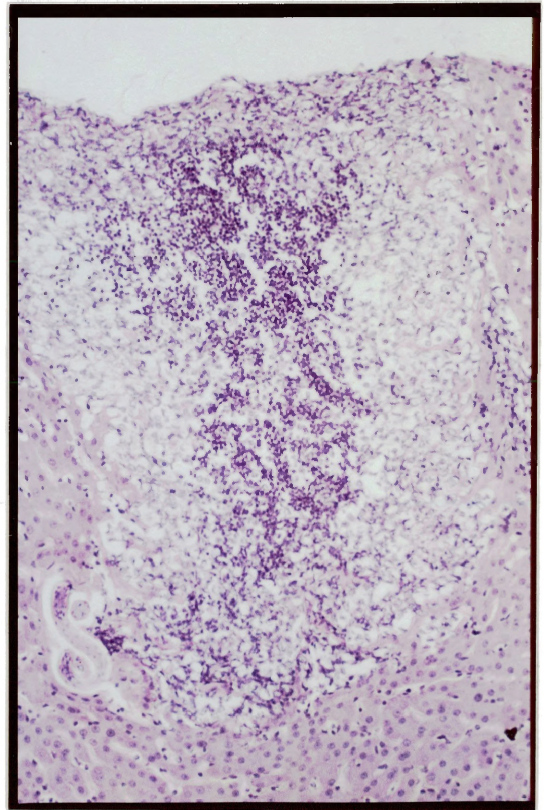
A



B



C



D

could be traced to a nearby granuloma. These granulomas were of the early form (type 1), with cores of live and disintegrating inflammatory cells and necrotic hepatocytes surrounded by a layer of vesiculated epithelioid cells occasionally forming minute "giant cells". Again, there was no outer layer of vacuolated hepatocytes.

Another female died 50 d pp, 15 weeks pi (200 larvae recovered). Its liver appeared grossly normal. In 40 serial sections 5 larvae were found, each located within the type 1 end of a bipolar granuloma, i.e. within the acute cellular region, opposite the pole containing large MNGCs (Fig. 7-3C). As with previous P. scapulatus, there was no layer of swollen, vacuolated hepatocytes in these granulomas.

One female liver, 6 weeks pp and 5 months pi, yielded 35 larvae. Microscopically, numerous scattered small infiltrates of lymphocytes, eosinophils, and histiocytes were seen around portal tracts, and 2 granulomas of similar structure were found in 40 sections. Both had a core of densely packed eosinophils and lymphocytes, and an outer layer of vacuolated cells which had features of epithelioid cells or perhaps small adipocytes. One was subcapsular and elongated (about 500 μm) with a larva immediately adjacent (Fig. 7.3 D). The other was deeper, spheroid (300 μm diam.) with 2 larvae within its core.

In a female which died 9 months pi, small scattered collections of eosinophils, lymphocytes and epithelioid cells were found throughout the sections. One elongated type 1 granuloma was found throughout all the sections, and contained a larva within the inflammatory cell core.

7.5 THE BARE-BACKED FRUIT BAT, DOBSONIA MOLUCCENSIS

In October 1983, 3 adult males of this cave-dwelling species, weighing 425, 450 and 470 g, were shot amongst granite boulders near the mouth of the Pascoe River on Cape York Peninsula (Fig. 1.2). Larvae were not found

in their livers. The liver histology of 1 of these (470 g) appeared entirely normal.

7.6 PTEROPUS TONGANUS TONGANUS

In February 1984, 4 male P. t. tonganus (weights 250, 300, 310 and 550 g) and 2 females (260 g and 1 lactating, 370 g) were shot on Rarotonga and Mangaia in the Cook Islands. Larvae were not recovered from the livers of any of these.

Subsequently 6 more adult carcasses were forwarded from Rarotonga, but these were fixed in formalin so the livers could not be processed to recover larvae. The livers of 2 adult males were examined histologically. Neither larvae nor lesions suggestive of toxocariasis were evident. However, in 1 specimen, numerous minute foci of adipocytes infiltrated with epithelioid cells, lymphocytes, plasma cells and occasional eosinophils were seen, with occasional collections of these cells around portal tracts.

Formalin-fixed carcasses of 3 juvenile P. t. tonganus were forwarded from Niue by Mr Misi Nicholas. These did not harbour intestinal worms.

7.7 PTEROPUS MELANOTUS (NATALIS)

In March 1984, Dr Chris Tidemann collected 20 suckling juveniles, estimated to be 2 - 3 months old, on Christmas Island in the Indian Ocean. On dissecting the alimentary tracts of all these animals, he found no evidence of helminth infection.

7.8 MEGACHIROPTERA FROM MUSEUM COLLECTIONS

Letters were sent to several museums in Europe and North America known to hold large collections of fruit bats. Dr Karl Koopman, of the American Museum of Natural History, forwarded 26 alcohol-preserved specimens of juvenile bats as follows:

Acerodon jubatus jubatus - 1 (Philippines)

Dobsonia moluccensis magna - 4 (New Guinea)

Dobsonia pannietensis - 2 (New Guinea)

Hypsignathus monstrosus - 1 (Belgian Congo)

Pteropus admiralitatus - 2 (New Guinea)

Pteropus alecto gouldii - 1 (Australia)

Pteropus conspicillatus chrysochen - 1 (Irian Jaya)

Pteropus hypomelanus luteus - 3 (New Guinea)

Rousettus amplexicaudatus amplexicaudatus - 11 (Philippines)

Several of these were obviously neonates, still with umbilical cords attached, and several were advanced juveniles whose intestines contained considerable vegetable matter in addition to milk. Nevertheless, nematodes with all the features of T. pteropodis were found as follows:

P. admiralitatus Tabar Island, New Guinea (1935) - 1 male worm 19.4 x 0.32 mm;

P. conspicillatus Irian Jaya (1961) - 1 female worm 9.74 x 0.17 mm;

R. amplexicaudatus Luzon, Philippines (1961) - 3/11 infected: No. 1 - 1 male worm 10.6 x 0.33 mm; No. 2 - 1 male worm (damaged) 20 mm (approx.) x 0.49 mm ; No. 3 - 2 females, 6.15 x 0.22mm and 7.38 x 0.25 mm, and 1 male 6.36 x 0.22 mm.

Detailed measurements of these worms are given in Table 7.5, and photographs of larvae recovered from R. amplexicaudatus in Figure 7.4 D-F).

In May 1987, 1 suckling juvenile of each of the following species was dissected in the Mammals Section of the British Museum (Natural History): Pteropus tonganus, P. seychelliensis, Eidolon helvum, Epomops buttikoferi, Epomophorus gambianus, E. wahlberghei, Rousettus aegyptiacus, R. angolensis and R. leschenaultii. In none of these was T. pteropodis found.

At the same time, in the Parasitic Worms Section of the British Museum (Natural History), 3 worms were examined which had been recovered from the

TABLE 7.5

Key dimensions of nematode larvae recovered from Megachiroptera in the American Museum of Natural History collection

Sex	Total length (L) (mm)	Max. width (mm)	Oesoph. length (LE) (mm)	LE/L %	Mouth -nerve ring (NR) (µm)	NR/LE %	Ventriculus length (µm)	Ventriculus width (µm)	body width (µm)	Mouth - gen. prim. (GP) (mm)	GP/L %	Tail (µm)	Tail/L %	Striae vent. (µm)	Striae pre-anal (µm)	Genital tract
HOST: *P. conspicillatus* Irian Jaya																
F	9.74	0.180	0.730	7.5	275	38	78	74	170	3.22	33	nd	-	-	-	Total length GP 0.91 mm Undivided tract 0.18 mm
HOST: *P. admiralitatus* Tabar Island																
M	19.4	0.320	1.17	6.0	320	27	165	157	285	3.73	19	125	0.65	-		Spicules 150 µm Ejaculatory duct 0.85 mm (4.4%L)
HOST: *Rousettus amplexicaudatus* Philippines																
F	6.15	0.22	0.700	11	240	34	87	73	185	2.43	40	108	1.8	7.6	5.6	Total length GP 330 µm Undivided portion GP 170 µm
M	6.36	0.21	0.680	11	245	36	100	82	190	nd	-	80	1.3	nd	7.7	Spicules 100 µm
F	7.38	0.25	0.815	11	272	33	105	105	210	2.88	39	135	1.8	7.6	13.3	-
M	10.6	0.33	0.920	8.7	310	34	150	140	295	1.42	13	130	1.2	nd	3.9	Spicule 230 µm Caudal papillae & digit-iform appendage evident
M	20*	0.49 (post. half missing)	1.27	6.4*	350	28	190	160	420	5.25	26*	missing	-	12.5		-

* estimated

FIGURE 7.4

Flying fox camps near Brisbane (A, B & C) and
T. pteropodis fourth-stage larvae recovered from *R. amplexicaudatus* from the Philippines (D, E & F)

A. Small section of *Pteropus* summer camp in eucalypts, casuarinas, mangroves and other trees, at Indooroopilly Island, showing vertical and horizontal separation of bats. *P. alecto* selectively roost in higher branches, while *P. poliocephalus* tend to occupy middle and lower levels. Individuals in very low mangroves are predominantly adolescents and young males.

B. Group of adult male *P. alecto* roosting above *P. poliocephalus* at Indooroopilly Island. Bat in centre defaecating.

C. Temporary dense camp of *P. scapulatus* at Eagleby, at periphery of regular *P. poliocephalus* summer camp. Note very dense clusters of small red bats at lower levels, with grey-headed mothers with pups roosting in high denuded branches, individually and in small group (arrows).

D. Late fourth-stage female 6.15 x 0.22 mm. Arrows indicate anterior end vagina (lower) and bifurcation primordial uterus (upper).
(bar = 500 µm)

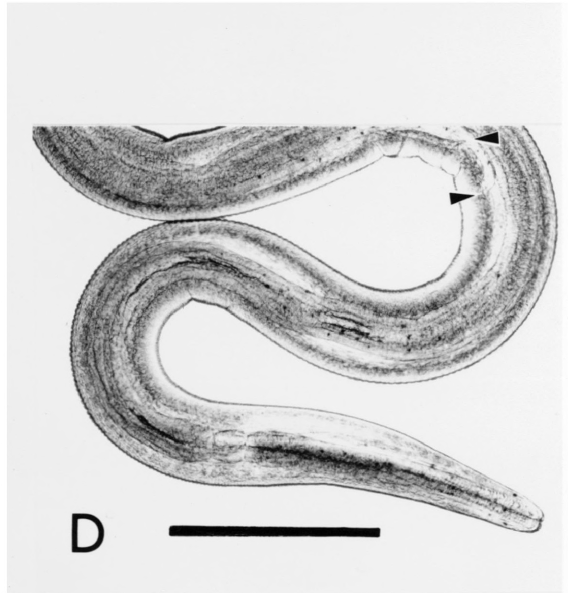
E. Tail of late fourth-stage female 7.38 x 0.25 mm showing typical shape and terminal spike.
(bar in D = 100 µm)

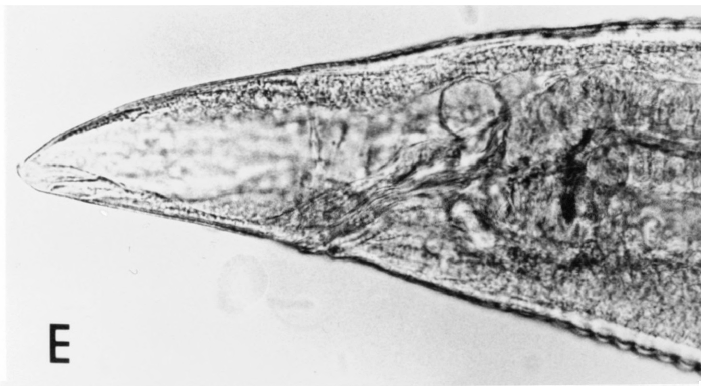
F. Ventriculus of larva in D between oesophagus (o) and intestine (i).
(bar in D = 100 µm)

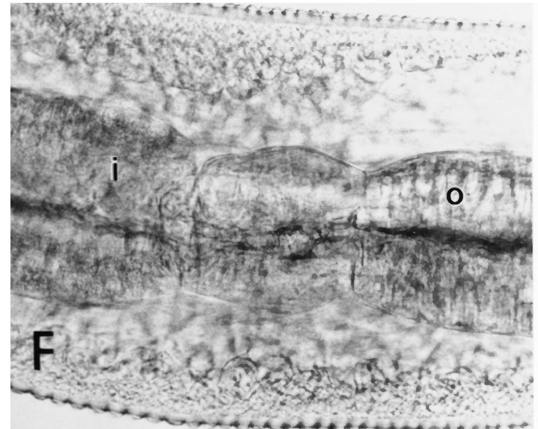
A

B

C


D


E

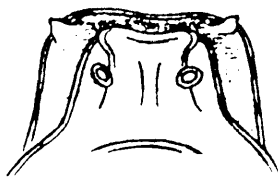

i
o
F

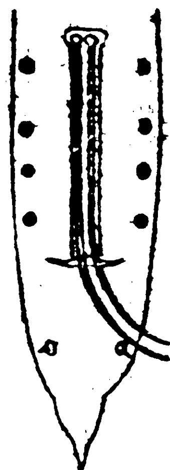
intestine of a flying fox at Calcutta Zoo in 1916 and identified later by Dr Gibson as T. pteropodis. The worms were 2 females, 11.1 cm and 13.2 cm long, and a male, 8.65 cm long; their internal morphology and dimensions were entirely consistent with findings in T. pteropodis from P. poliocephalus (Section 6.2.5).

7.9 CYNONYCTERIS AMPLEXICAUDATA

The only other ascaridoid reported from Megachiroptera is Ascaris cynonycteridis, described from the stomach of Cynonycteris amplexicaudata from Burma by Parona (1889). The taxonomy of this bat is not clear. Anderson (1912) synonymized it with Rousettus amplexicaudatus, although Rookmaaker & Bergmans (1981) indicate it is synonymous with R. leschenaultii leschenaultii, which occurs in Burma but not the Philippines. As R. a. amplexicaudatus also occurs in Burma, as well as the Philippines (Rookmaaker & Bergmans, 1981), either of these species may have been the host for A. cynonycteridis.

Parona (1889) provided a very brief description of A. cynonycteridis, recording the body lengths of 2 females as 60 - 65 mm and 1 male as 50 mm.





The external features he described were entirely consistent with those of T. pteropodis, but he did not elaborate on internal structure, in particular the genital tracts, or whether females contained eggs. Parona's entire sketches of the new species, of a ventral lip and male tail, are reproduced here. The positions of the spicules and 4 pairs of pre-cloacal papillae are not consistent with ascaridoid anatomy, the 2 papillae on the subventral lip are shown as equal and it is not clear what the 2 pointed labial protruberances represent.

Nevertheless, Mozgovoi (1953) was able to re-assign it to the genus *Toxocara*, and described the vulva as being 5 mm (Parona's "a mezzo centimetro"?) from mouth, without indicating whether he had examined the type material. Sprent (1968) included it amongst his *species inquirendae*.

In view of these uncertainties, the 3 type specimens were examined in the Genoa Museum of National History in June 1987. Even though the worms were in poor condition and somewhat damaged, after clearing in creosote their external and internal features were found to resemble closely those of *T. pteropodis*. They were measured, and key dimensions are presented in Table 7.6. Neither a drawing tube nor micrometer were available, so measurements were estimated in relation to the field diameters of the microscope used, and considered accurate to $\pm$ 10%.

The male worm was 78 mm long; its vas deferens was full of spermatocytes, but there were no spermatozoa in its seminal vesicle. The females were 90 and 126 mm long; the larger contained numerous typical *Toxocara* eggs about 70 - 80 μm in diameter.

7.10 DISCUSSION

Patent *T. pteropodis* infection was found in all 4 Australian *Pteropus* species, but the patterns of infection varied.

In *P. alecto* of the Brisbane district, the prevalence of infection appears to be considerably lower than in *P. poliocephalus*, described in detail in Chapter 3. However, on Palm Island (Fig. 1.2) where the flying fox population is exclusively *P. alecto*, the prevalence of *T. pteropodis* is likely to be similar to that in the more southern *P. poliocephalus*, assuming similar population dynamics to maintain natural infection. Moorhouse (1982) found worms in the intestines of 3/7 juvenile *P. alecto* from Townsville, near Palm Island. Data from juvenile and adult bats collected on Palm Island is not presented in this study because it was

TABLE 7.6

Dimensions of type specimens of *Toxocara cynonycteridis* examined in Genoa Museum of Natural History

	Female I	Female 2	Male
Total length (L)	126 mm	90 mm	78 mm
Max. width	1.57 mm	0.93 mm	0.74 mm
Lip length	105 µm	not clear	88 µm
Oesophagus length (LE)	2.31 mm	1.85 mm	? 2.22 mm
LE/L	1.8%	2.1%	2.8%
Mouth - nerve ring (NR)	0.46 mm	0.46 mm	0.49 mm
NR/LE	19.7%	24.6%	22.1%
Ventriculus - length	not clear	0.21 mm	not clear
- width	not clear	0.16 mm	not clear
- body width	0.74 mm	0.53 mm	0.35 mm
Cuticular striae -			
ventriculus	11 µm	9 µm	11 µm
mid-body	28 µm	16 µm	21 µm
pre-anal	22 µm	16 µm	16 µm
Anus - tail (T)	0.21 mm (indented)	0.53 mm	0.18 mm
T/L	0.17%	0.56%	0.22%
Mouth - vulva	24 mm (19%L)	22 mm (24%L)	-
Spicule length	-	-	0.56 mm (equal, divergent)
Ejaculatory duct (ED)	-	-	3.33 mm (4.3%L)
Eggs in uterus	typical	-	-

grossly unreliable as a result of poor field conditions. The finding by Moorhouse of heavy contamination of mangoes with Toxocara eggs supports the assumption that the worm must be common in the bats of Palm Island, and presumably on the northern Queensland mainland as well.

A southward extension of the range of P. alecto by almost 1,000 km over the last 50 years has been well documented (Appendix III), so there is no question of species differences between southern and northern populations. Assuming that T. pteropodis is more prevalent in northern P. alecto, then the explanation for this difference must lie in altered behaviour or ecology. The black flying fox is generally more aggressive than the grey-headed, both in captivity and under natural conditions (pers. obs.) and it may be displacing the latter species from its feeding-grounds and its traditional roosts. In mixed camps, as found in its more southerly distribution, P. alecto invariably roosts in large groups peripherally or in higher branches than P. poliocephalus (Fig. 7.4 A & B). By adopting such roosting sites, the black bat diminishes its risk of exposure to Toxocara infection. In north Queensland, where it aggregates in mono-specific roosts as well as with other species (pers. obs.) a more random stratification would promote the increased acquisition of infection by this species. Were P. alecto to start forming exclusive roosts in its southern range, then the prevalence of infection in this species could increase and approach that in P. poliocephalus.

The prevalence in P. conspicillatus seemed comparable with that in P. poliocephalus. The spectacled flying fox forms large camps along the coast and in the ranges of northern Queensland, and often shares its camps with P. alecto (G. Richards, pers. comm.). Nothing is known of the interaction of these 2 species.

From the limited experimental studies in P. alecto and P. conspicillatus, the migration and development of T. pteropodis appear similar to its behaviour in P. poliocephalus.

The dynamics of infection in the small red flying fox may be quite distinct. This highly migratory bat aggregates in rarely-observed winter nursery camps (Appendix II), although in summer, smaller sub-groups temporarily fly to the coast to share camps with P. poliocephalus, P. alecto and P. conspicillatus (pers. obs.), for periods varying from several days to over 6 weeks. These "tourists" are usually males, at times accompanied by variable numbers of pregnant females which usually stay in the mixed camps for shorter periods. In such situations, their numbers may vary from hundreds to over 10,000 (pers. obs.), and they invariably occupy the outskirts of the camp, densely roosting even on very low and thin branches so thickly that large branches or even trees collapse under their weight. Their roosting territory intrudes in a wedge-like fashion under the sites occupied by the coastal species (Fig. 7.4 C).

The prevalence of natural Toxocara infection in juvenile P. scapulatus (4%) was very low. It is possible that the 50 juveniles examined by rectal swab had already passed their worms, as they appeared to be sufficiently mature to be foraging, but the findings in much younger bats also showed a very low prevalence. It seems unlikely that at such a level the worm could be maintained in this host species. Furthermore, juveniles are born in late autumn in the hinterland where climatic conditions are very dry in winter with night temperatures below freezing for considerable periods of time. Low temperatures would retard the embryonation of T. pteropodis eggs, desiccation would destroy any developing embryos and the absence of rainfall would render transmission exceedingly unlikely. Yet, the prevalence of hepatic infection was found to be very high in adult males (75%), almost at the level found in P. poliocephalus (100%). The most likely explanation is that T. pteropodis infection is acquired when the bats share summer camps with the coastal species, where they increase their risk of infection by roosting beneath the females of other species who

happen to be nursing their infected pups at that time (Fig. 7.4 C). The apparently more nomadic males are more likely to be infected, and also retain their hepatic larvae for at least several years. Perhaps the males sampled were not representative, as they may have been from a group which visited the coast, whereas others had not. The prevalence of hepatic infection in adult females may be lower, either because they are less migratory or because they pass on their larvae to their offspring. The former seems more likely, in view of the low level of infection in pups and the less frequent finding of females in mixed-species summer camps.

The experimental studies in *P. scapulatus* were beset with various difficulties. It was assumed on the basis of findings from naturally-infected animals that the pregnant females used in this study were unlikely to harbour hepatic larvae, but this could not be proved. The small red bats were very difficult to maintain in captivity. Perhaps reflecting their different natural food preferences, they regularly stopped feeding for periods of days, and rejected wide varieties of fruit offered. They did not adjust to handling and human contact as did the other 3 species, and morbidity and mortality were consequently serious problems. Nevertheless, several facts were established from the experiments. Firstly, growth, migration and development of *T. pteropodis* proceeds in this bat species comparably with its behaviour in *P. poliocephalus*. Secondly, it is not essential for larvae to occupy the maternal liver for the duration of gestation in order then to undergo transmammary migration. One female was fed eggs only 66 d ante-partum, and still produced an infected offspring. Another had larvae remaining in her liver 15 weeks after giving birth to an infected pup, indicating that not all larvae were transmitted. Thirdly, a much greater proportion of larvae entered the juvenile intestine than with infections in *P. poliocephalus*, yet patent infection rarely developed. In fact, in only one of these small red pups were eggs ever found in the faeces, and these in very small numbers and for

only a few days before the solitary, barely-mature female worm was voided.

Perhaps, by invading the maternal liver late in gestation, the larvae did not have sufficient time to "bed in" before they were stimulated to migrate by the hormonal changes of parturition. Alternatively, physiological cues which may inhibit most larvae from leaving the liver were absent. Such reasoning contradicts the earlier suggestion (Section 4.5) that all hepatic larvae are mobilized around parturition. With T. canis in dogs, infection of the bitch nearer to term causes proportionately more larvae to pass through the milk than through the placenta, and infection at, or shortly after, parturition results in high numbers of larvae passing through the milk for up to 3 weeks (Burke & Roberson, 1985b; Stoye, 1976). In post-partum T. canis-infected bitches, presumably the larvae are circulating and hence have greater opportunities for transmammary passage. In pregnant P. scapulatus fed T. pteropodis eggs 2 months ante-partum, by parturition all the larvae should be well-established in the liver, so the heavier migration cannot be explained on a similar basis to that of T. canis. This migration does not occur before parturition, as evidenced by the failure to find larvae in aborted foetuses and in the mammary glands of those females which aborted and that which rejected its 3-day-old pup.

Whatever the explanation, much greater numbers of larvae were consistently found in the intestines of the offspring from these experimentally-infected females. The larvae were measured only from the pup, aged 27 d, which had the heaviest infection, and their lengths did not show any remarkable patterns, although they were all below the length when a wide dispersal could be expected to appear (Fig. 4.2). The finding of almost equal numbers of male and female larvae confirms the earlier conclusion, from P. poliocephalus (Chapter 4), that there is an equal sex distribution in early gut infection. Although not statistically

significant, the females were slightly longer than the males, and comprised the 4 longest larvae.

The total worm burdens were not analysed from the other experimental juveniles, but the larval length ranges were much greater in the slightly older pups, ranging 1.46 - 56.3 mm (total 120) in a 30-d-old pup, and 4.96 - 91.6 mm (total 92) in a 45-d-old pup. Clearly, there was a great difference in growth rates between the shortest and longest larvae. Possible mechanisms were discussed previously (Section 4.5). The final moult of T. pteropodis occurs at about 10 mm; most of the larvae in Figure 7.2 were well past this stage. Hence, retardation of development by immunologically-mediated suppression of moulting, suggested for A. suum by Soulsby (1961), does not appear to be the case with T. pteropodis. It is also unlikely that antigens released at moulting play a significant role in this phenomenon. Despite the much heavier infections, larval lengths were within the range of those from lightly-infected P. poliocephalus of comparable ages (Table 4.6). The high intensity of infection therefore did not suppress development of the larger larvae. The normal distribution of larval lengths from the 27-d-old P. scapulatus, and the wider ranges from slightly older juveniles, is consistent with the earlier conclusions from P. poliocephalus (Fig. 4.2) that when older larvae attain a critical length, probably 50 - 60 mm, the shorter ones are arrested in their development, perhaps through the release of inhibitory factors through the excretory system.

Owing to the author's absence, close observations of these infected juveniles was interrupted for two weeks, over which time they were given access to fruit. Subsequently, the 3 which died aged 7 - 9 weeks and the 3 10-week-old survivors were totally free of infection. Probably most of these, if not all, had been infected earlier, but had 'self-cured'. Most of their mothers had been infected individually, and the other mothers exposed to several batches of eggs in food. Furthermore, some of these

juveniles had earlier passed larvae in their faeces. Worm loss may have resulted from fruit ingestion, or may have been comparable with massive A. suum infections in pigs which fail to result in patent infections (Schwarz, 1959; Douvres et al, 1969).

Of all these juveniles, only 1, aged about 40 d, developed patent infection with a solitary female worm. Its very low and irregular egg output over the first 3 days, compared with the rising output in P. poliocephalus (Fig. 5.1), indicates that the female worm was abnormal, although in length (83 mm) it was similar to those found at the onset of patency in the latter host. The factors suppressing egg output may have been the same as those causing earlier worm expulsion, and similar to those acting in heavily-infected P. poliocephalus. However, in the latter species, 1 or 2 normal female worms survived to produce eggs for considerable periods. Although 3 juvenile P. scapulatus were found to be naturally-infected (Section 7.4.1), only 1 actually had a patent infection, and harboured the unusually high number of 7 adult females and 6 males.

Therefore, not only is P. scapulatus unsuitable ecologically for the transmission of T. pteropodis, but it may be physiologically incompatible as well. With individual variations, however, an occasional patent infection under natural conditions could be expected.

Histologically, the hepatic responses of P. scapulatus to T. pteropodis larvae were similar to those of P. poliocephalus. Most of the tissue sections examined, from post-parturient females, demonstrated bipolar granulomas which may suggest recent larval mobilization. Again, post-mortem larval migration may have occurred; in some livers fixed immediately after the death of the bat, larvae were consistently within inflammatory foci, whereas in bats dead for some time prior to autopsy, larvae were usually "free" in tissues. Occasional exceptions to this pattern are difficult to explain.

Anatomically, physiologically and behaviourally, the 3 coastal Pteropus species have more in common with each other than with P. scapulatus. In captivity, P. alecto and P. poliocephalus may interbreed (Helen Luckhoff, pers. comm.) and bats which appeared to be hybrids of these species have been seen occasionally (pers. obs.).

The only other large megachiropteran found in Australia, D. moluccensis, is restricted to Cape York. It may be that only males venture south from New Guinea, where this bat is very common (Moorhouse, pers. comm.). The sighting of females or juveniles has never been reported in Australia. Furthermore, as Dobsonia roosts in well-ventilated rocky crevices and caverns, hanging from walls and ceilings, it is unlikely to harbour T. pteropodis. The absence of hepatic infection from the 3 males examined was not surprising, although this was hardly a significant sample.

The finding of T. pteropodis in Pteropus species north of Australia, including P. admiralitatus and P. conspicillatus from New Guinea, also is not surprising; their distribution suggests a radiation of the genus from the Indonesian region (Andersen, 1912), so possibly T. pteropodis originated here also.

The specimens of T. pteropodis collected from the Calcutta Zoo in 1916 almost certainly were from P. giganteus, which is found throughout India and is the only flying fox likely to have been kept in a zoo there. Confirmation of this is awaited from the Zoo's director. This finding indicates that T. pteropodis has radiated westward as well as eastward.

The occurrence of T. pteropodis in remote island bat populations warrants further consideration. Flights of several hundred kilometres are well within the capability of most Pteropus species, but large numbers would be unlikely to undertake transoceanic migrations and establish colonies on remote islands. Individual P. scapulatus have been found in New Zealand (Daniel, 1975), presumably having been blown there by high winds. Were a small number to settle on a remote island with the eventual

establishment of a viable population, *T. pteropodis* is unlikely to be found there, as for many years the bat population would be too sparse for successful infection transmission. This may explain the absence of the worm from *P. melanotus* of Christmas Island, 400 km south of Java. In Vanuatu (New Hebrides), where *T. pteropodis* was first found (Baylis, 1936), it is likely that *P. t. geddiei* started from a small initial population, although infected individuals arriving later to join the established camps may have re-introduced the worm.

In the more remote Cook Islands, *T. pteropodis* is even less likely to have survived. Furthermore, local folklore (pers. obs.) has it that *P tonganus*, a much-desired delicacy, was introduced to Mangaia, the most south-eastern island of the group, from Tonga by canoe about 400 years ago. Should *T. pteropodis* occur in Tonga, it is unlikely to have survived a sea passage in captive bats fattened almost from birth on a diet of papayas, mangoes, coconuts and bananas. The small dispersed wild populations on Mangaia and Rarotonga (pers. obs.) do not form large roosts and are highly mobile, neither factors being conducive to the establishment or survival of the worm.

The morphology and dimensions of worms from *P. conspicillatus* of Irian Jaya and *P. admiralitatus* of Tabar Island (Table 7.5) were virtually identical to those of *T. pteropodis* of similar lengths (Tables 6.2, 6.3). The worms from *R. amplexicaudatus* from the Philippines also closely resembled *T. pteropodis*, but the internal dimensions (Table 7.5) in comparably sized worms differed; generally, they were wider, their oesophagi were longer, nerve rings further back, ventriculi larger and spicules longer than in *T. pteropodis*. The type specimens of *T. cynonycteridis* from the Burmese *Rousettus* (Table 7.6) were found to be much longer than reported by Parona (1889), and were of the same size as mature *T. pteropodis* (Table 6.4), which they closely resembled. However, measuring showed them to be generally narrower, with oesophagi shorter,

nerve rings further forwards, ventriculi smaller (clearly discernible only in Female 2), tails shorter and eggs smaller than in *T. pteropodis* (the ejaculatory duct and spicules were similar in length). It is interesting that the adult worms were equal in size to *T. pteropodis*, yet *Rousettus* sp. are much smaller than *Pteropus* (Anderson, 1912; Discussion, Appendix IX). It is very likely that *Rousettus* from Burma is the same as or very closely related to the species from the Philippines, so the *Toxocara* from each also are likely to be identical species. However, if on the basis of internal dimensions either the Burmese or Philippine *Toxocara* is assumed not to be *T. pteropodis*, then they must represent two distinct species. Neither groups of specimens were optimally fixed, particularly those of Parona (1889) which were shrivelled and contained air bubbles, so it is likely that some internal features were distorted. Furthermore, in comparing such small samples individual variations may be misleading. It would seem reasonable, therefore, to assume that the specimens examined represent *T. pteropodis*, with the proviso that *T. cynonycteridis* remain a *species inquirenda* pending a detailed examination of optimally preserved specimens from *Rousettus* in Burma and the Philippines. Should they not be identical species of *Toxocara*, their life-cycles are likely to be very similar.

The genus *Rousettus* extends from the African continent to southern Asia and New Guinea, but does not occur in Australia (Andersen, 1912). The Egyptian tomb-bat, *R. aegyptiacus*, roosts in the twilight zone of large caverns and in dark rocky crevices, and other members of the genus, including *R. amplexicaudatus*, probably behave likewise (Dr Karl Koopman, pers. comm.). It is almost certain that the transmission of *T. pteropodis* in *Pteropus* populations occurs in arboreal roosts (Section 5.11). As little is known of the behaviour of *Rousettus*, the transmission of the worm in this genus is open to speculation. If mother-young pairs roost on cliff-faces, perhaps sufficient surface contamination occurs for bats to be

infected by licking during rain. Infected juveniles may venture from their caves at night while the adults are foraging, and await the return of their mothers in nearby trees. Branches and foliage could then become contaminated with Toxocara eggs, and the adults may rest in these trees prior to departure on their foraging flights or on returning. None of the worms found in R. amplexicaudatus were mature adults, but it is unlikely to be an accidental host, in view of the apparently high prevalence of infection and low worm burdens (cf. P. scapulatus). The worms from the Burmese Roussetus sp. were adults.

It appears that R. amplexicaudatus is an adequate maintenance host of T. pteropodis. If so, then the worm is likely to be found in other Rousettus species, and perhaps extends into Africa, beyond the range of Pteropus (Fig. 1.1). Many other megachiropteran genera occur in Africa, including the Straw-coloured Fruit Bat, Eidolon helvum, which is about the same size as P. scapulatus and roosts very densely in trees (Funmilayo, 1979). A nematode which may have been T. pteropodis was found in the stomach of a suckling juvenile E. helvum (Professor Festo Mutere, pers. comm.). The negative findings in the African bats examined in the British Museum (Natural History) are inconclusive as only one of each species was available for dissection.

It is clear that T. pteropodis, and perhaps very closely-related species, occur in a range of megachiropteran genera. The extent of the parasite's penetration into the suborder will become more fully appreciated as more juvenile bat specimens are examined, and may provide insights into aspects of ascaridoid and chiropteran evolution and dispersal.

CHAPTER 8

T. pteropodis INFECTIONS IN NON-CHIROPTERAN HOSTS

8.1 INTRODUCTION

To gain an indication of the fate and potential pathogenicity of *T. pteropodis* infections in humans, the behaviour of this nematode was studied in a variety of experimental hosts. For obvious reasons, the most detailed work was undertaken in mice, but smaller-scale comparative studies were also carried out in other mammals. To compare its host range with that of *T. canis*, limited studies were undertaken in chicks and ducklings.

8.2 EXPERIMENTAL PROCEDURES AND RESULTS

8.2.1 Mice

8.2.1.1 Summary of published findings

Details of procedures and full results of mouse infections are presented in published papers included here as Appendices IV and V, and a summary of findings follows.

a) Following light oral infections (less than 1,000 eggs), larvae hatching from eggs penetrated the mucosa mainly of the distal intestine to reach the liver via the portal venous circulation. Virtually all larvae were established in the liver by 3 d pi and remained there indefinitely. Hepatic larval recoveries in the first 3 months represented 7.9 - 22.5% of administered egg doses. Although in 1 mouse killed 14 months pi 11.0% of the dose was recovered, in most mice kept for longer than 1 year the number of hepatic larvae recovered was considerably less.

b) In heavy oral infections (7,500 eggs), occasional larvae migrated through lymphatics and lungs to appear transiently in distal sites, such as brains and kidneys, whereas the bulk of larvae settled in livers, representing 13-16% of the egg dose.

c) Eggs injected parenterally hatched over 1 - 2 weeks, and larvae ultimately reached the liver. Following sc inoculation, larvae travelled via the circulation, through the lungs, where perhaps a small proportion underwent tracheal migration. With ip inoculation, many larvae seemed to penetrate the liver directly although a considerable proportion travelled via the circulation to arrive eventually in the liver.

d) Larvae commenced growth following hatching, attaining a plateau length of about 600 µm at 50 d pi, although there were marked differences between individual mice and different strains of mice (which were not analysed further owing to small numbers).

e) Pregnancy and suckling did not induce larvae to leave the livers of mice.

f) Mice ingesting larvae in infected mouse livers or bat lungs subsequently developed hepatic infections.

g) Blood white cell responses, including eosinophilia, were comparable with those seen in T. canis-infected mice (unpublished).

h) Age-resistance to T. pteropodis did not develop (unpublished).

i) Histological responses in mouse livers included generalized lymphocytic-eosinophilic perivascular infiltration and localised granulomatous infiltration, with MNGCs, but typical foci as seen in bat livers did not develop (unpublished).

8.2.1.2 Post-mortem larval migration

To test the hypothesis that the larvae of T. pteropodis were capable of migrating through tissues following the death of the host and that this tendency underlies the success of larval recovery techniques, a variety of studies were carried out in mice.

a) Comparison of "digestion" techniques

Six adult male Quackenbush mice which had been fed 4,000 T. pteropodis

eggs 4 months previously were killed by concussion and cervical dislocation. Their livers were immediately removed, minced and mixed well, then divided into 6 equal portions, which were suspended over gauze in 0.85% saline, 2% trypsin or 1% pepsin solutions and incubated at 37 ^{0}C. At the intervals indicated in Table 8-1, each minced liver sample in its gauze pocket was transferred to a fresh container of corresponding solution, and the sediments fixed in hot formalin and examined for larvae. One week later this experiment was repeated in identically-infected adult female mice, so that 4 tissue samples were incubated in each "digestion" fluid, and the combined results are presented in Table 8-1.

More larvae were recovered from the saline sediments than from trypsin, which appeared to be more effective than pepsin. However, by analysis of variance, the differences amongst the 3 groups were not found significant at the 0.05 level. The patterns of larval recovery with time varied. In saline, about one-third had left the tissue in the first 4 hours then, after a "quiet period" of 4 hours, more than half were recovered between 8 and 20 hours of incubation. In trypsin, peak recovery was at 4 - 8 hours, with very few recovered beyond 20 hours. In pepsin, almost half of recovered larvae had egressed within the first 4 hours, and later recoveries declined rapidly. There was insignificant variation of the mean lengths of larvae from different solutions. However, most of those from pepsin were damaged, having disrupted ventriculo-intestinal junctions. The internal features of most larvae from trypsin were not clear.

Earlier smaller scale studies comparing recoveries from mouse livers, infected 7 d to 14 months, in saline, trypsin and pepsin generally showed a similar pattern, with considerable variations which did not appear to depend on the duration of infection.

The carcasses of 2 mice killed 12 weeks after ingesting 5,000 _T. cati_

TABLE 8.1

Recovery of T. pteropodis larvae from mouse livers (4,000 eggs, 4 months p.i.) incubated at 37 C over varying intervals in different media

"Digesting" medium	Total no. larvae recovered mean ± SD (range)	% total recovered mean ± SD (range) 0-4 hrs	4-8 hrs	8-12 hrs	12-20 hrs	20-32 hrs
0.85% saline	780 ± 40 (720 - 840)	32 ± 12 (12 - 47)	7 ± 2 (3 - 9)	23 ± 4 (18 - 28)	32 ± 7 (25 - 42)	6 ± 2 (3 - 8)
2% trypsin	620 ± 50 (560 - 740)	16 ± 3 (12 - 19)	35 ± 4 (24 - 40)	25 ± 5 (18 - 33)	22 ± 5 (15 - 27)	2 ± 1 (1 - 3)
1% pepsin	540 ± 80 (450 - 670)	47 ± 10 (32 - 59)	37 ± 9 (25 - 48	11 ± 5 (2 - 19)	4 ± 3 (1 - 8)	1 ± 1 (0 - 3)

TABLE 8.2

Recovery of T. pteropodis and T. canis larvae from peritoneal cavities of mice immediately and at 4 hours post-mortem (p.m.)

Time p.i.	Time p.m.	No. of mice	No. larvae in peritoneal cavity mean ± S.D. (range)	No. larvae from intact GIT incubated 4 hours mean ± S.D. (range)
T. pteropodis - 2,000 eggs				
16 hrs	0 hrs	4	2 ± 2 (0 - 4)	6 ± 2 (4 - 9)
	4 hrs	4	5 ± 2 (3 - 8)	-
T. canis - 5,000 eggs				
16 hrs	0 hrs	4	8 ± 2 (5 - 11)	305 ± 57 (220 - 360)
	4 hrs	4	485 ± 105 (370 - 640)	-
21 hrs	0 hrs	2	14 ± 3 (11 - 19)	485 ± 75 (410 - 560)

eggs were ground in a Waring blender at high speed for 30 sec, then divided into 2 equal portions which were incubated for 18 h in 600 ml saline or in 600 ml pepsin, with almost identical recoveries from both solutions.

b) Recovery of non-viable larvae from livers

In Appendix IV, it was shown that prior freezing of infected livers resulted in a significant reduction in larval recovery. In this experiment, 4 mice, each fed 4,000 *T. pteropodis* eggs, were killed 4 weeks pi. The livers were minced together, then divided into 4 equal portions. Two samples were immersed for 30 sec in liquid nitrogen, with the remaining 2 left at room temperature (26 ^{o}C). One from each group (frozen or unfrozen) was then incubated over gauze in saline for 18 h at 37 ^{o}C. The remaining 2 were simply incubated in saline at 37 ^{o}C for 18 h then filtered through a single 8-ply gauze swab prior to sedimentation and fixation.

Larvae in a drop of sediment from non-frozen liver were motile, whereas those from frozen liver appeared dead. Likewise, non-viability of larvae from frozen livers was confirmed by examining small portions pressed between glass slides. Larvae were recovered as follows:

Frozen, incubated over gauze	-	76
Frozen, filtered through gauze	-	240
Non-frozen, incubated over gauze	-	940
Non-frozen, filtered through gauze	-	590.

c) Larval migration through peritoneal cavity

In this study, 8 adult male Quackenbush mice were each fed 2,000 *T. pteropodis* eggs and another 10 were each fed 5,000 *T. canis* eggs. They were killed by concussion and cervical dislocation at the times pi indicated in Table 8-2. Immediately after death, 10 ml 0.85% saline was injected via syringe and 23G needle into the peritoneal cavity of 4 mice from each group killed 16 h pi, agitated gently for 30 sec then drained through a longitudinal midline incision. The peritoneal cavity and

contents were then rinsed with an additional 20 ml saline and the washings from each mouse left to stand for 1 h prior to fixation of the sediment. The lower oesophagus and rectum in each of these mice were tied off, and the entire abdominal contents removed *en bloc* and immersed in saline for 4 h (room temperature 28 ^{o}C) after the peritoneal lavage described above. An additional 2 *T. canis*-infected mice were killed at 21 h pi.

The results are shown in Table 8.2. Immediately post-mortem, very small numbers of larvae were rinsed from peritoneal cavities of mice in both infection groups. In the *T. pteropodis* group, again only small numbers (3 - 8) were found in the peritoneal washings of mice left dead at room temperature, and also from the intact alimentary tracts incubated in saline.

In the *T. canis*-infected mice left dead for 4 h, large numbers of larvae (370 - 640) were found in the peritoneal cavities, and also large numbers emerged from the incubated intact alimentary tracts. In those killed 21 h pi, again small numbers (11 - 19) were found in peritoneal cavities, but many larvae emerged from the gut during incubation in saline.

d) Histological findings

Three female Quackenbush mice fed 2,000 *T. pteropodis* eggs were killed 6 weeks, 3 months and 8 months pi. The liver from each was immediately removed and cut into 3 approximately equal portions. One piece was immersed in liquid nitrogen for 30 sec to kill larvae prior to fixing in formalin, another into 10% buffered formalin and the 3rd kept in saline for 2 h prior to fixing in formalin. These fragments were then sectioned and stained for microscopic examination. Inflammatory reactions and larvae could be found in all sections. Although the impression was gained that some larvae were more remote from their associated lesions in the late-fixed specimens, in all livers, including those frozen in nitrogen, larvae were found both in granulomatous regions and in normal tissue.

8.2.2 Rats

Investigations in rats are presented in detail in Appendix VI. Salient findings are listed below.

a) Following the oral administration of eggs to suckling rats, larvae migrated to livers but in much lower numbers than in mice.

b) Infection was associated with blood leukocytosis and eosinophilia.

c) Older rats were refractory to infection. However, in an adult rat killed 8 h pi, larvae were recovered from the intestinal wall, and in 1 of 4 adults bled 3 weeks after a dose of 2,000 eggs, moderate eosinophilia was detected (unpublished).

d) Hepatic larvae in orally-infected suckling rats were short-lived.

e) Following the ip or sc inoculation of eggs in suckling rats, larvae migrated to livers, but again were short-lived.

8.2.3 Guinea-pigs

In guinea-pigs, oral infections with _T. pteropodis_ were compared with those of _T. canis_ and _T. cati_, and details are presented in Appendix VII. The essential findings are listed below.

a) _T. pteropodis_ larvae penetrated the mucosa mainly of the caecum, distal small intestine and proximal colon.

b) Larvae accumulated in the liver, but in smaller numbers than seen with comparable mouse infections (maximal recovery 6% at 7 d pi), and perished quickly, with none being recovered beyond 25 d pi.

c) Larvae in livers did not grow appreciably in length.

d) _T. canis_ larvae passed through livers and lungs to accumulate in skeletal muscle and brains, where they persisted indefinitely (at least 7

months).

e) *T. cati* larvae settled almost exclusively and survived indefinitely in skeletal muscle, with small numbers being recovered from the brain and lungs.

f) Peripheral blood white cell and eosinophil responses to the 3 *Toxocara* species were virtually identical except that in *T. pteropodis* infections eosinophilia gradually approached normal whereas in the other 2, although levels fell, they remained above the baseline at 5 months pi.

g) The hepatic tissue response to *T. pteropodis* comprised classical MNGC granuloma-formation around larvae, which were present and intact at least 70 d pi, with extensive eosinophil-lymphocyte periportal infiltration (unpublished).

8.2.4 Rabbits

Two 6-week-old rabbits were fed 5,000 eggs each then killed 2 weeks later. Livers and lungs were subjected to saline separation, but larvae were not recovered.

Two 6-month-old rabbits were fed 10,000 eggs each, and bled and killed 2 weeks later. Neither blood leukocytosis nor eosinophilia developed, nor were larvae recovered from livers or lungs.

Two 2-year-old rabbits were fed 20,000 eggs each, again without a white blood cell response or recoverable larvae at 2 weeks.

8.2.5 Dogs

Two 6-week-old labrador pups, both with patent *T. canis* infection, were each fed 2,000 *T. pteropodis* eggs mixed into their food, and killed 12 d pi. Of each liver 25% was subjected to saline separation. No *Toxocara* larvae were found in 1 liver, but from the sediment of the other 18 were recovered, giving an estimated total of 72 larvae for the entire liver. Most of these larvae had very loose cuticular sheaths and many were

macerated so that internal features were not clear. Their mean length was 430 ± 17 µm (range 400 -460). In 10 with discernible internal structures, 3 (lengths 415-445 µm) had tails 42 - 43 µm long (9.5 - 10.3% total body length), whereas in the remaining 7 the tails were 33 ± 1.5 µm long (range 30 - 34 µm), representing 7.4 - 7.9% total body length. Oesophageal lengths ranged 108 - 121 µm (25 - 30% body length).

8.2.6 Cats

Two 6-week-old kittens were each fed 2,000 T. pteropodis eggs mixed with their food, and killed 13 d pi. Four Toxocara larvae were recovered from 25% of the liver of 1, giving an estimated total of 16. Salient features of these larvae were: mean length 415 ± 13 µm (range 405 - 435 µm); oesophageal lengths 102 - 117 µm (22 - 28% body length); nerve ring 64 µm from mouth in each (55 - 60% oesophageal length); tails 30 - 32 µm (7.3 - 7.6% body length).

8.2.7 Brush-tailed possums (Trichosurus vulpecula)

A female brush-tailed possum with a babe in its pouch was trapped in a suburban backyard. The babe, a 170 g male, was fed 1,500 T. pteropodis eggs and killed 6 d later. From its liver 140 Toxocara larvae (9% total dose) were recovered, of mean length 465 ± 12 µm (range 440 - 475 µm). The mother possum was fed 5,000 eggs, bled at weekly intervals under ether anaesthesia, then killed 6 weeks pi. From its liver, 3 Toxocara larvae were recovered, 440 - 670 µm long (mean 575 ± 123 µm). At the time of infection, the haemoglobin was 13.0 g/100 ml, and remained fairly steady throughout the study. White cell count was 8,400/mm^3, rising to 10,500 at one week and returning to 7,800 at the end of the study. Eosinophil levels were 0 on day 1, 950/mm^3 after 1 week, 1,200/mm^3 at 2 weeks, 960/mm^3 at 3 weeks and 230/mm^3 on the last day.

In April 1983, the livers of 2 adult possums found freshly-killed on a road 10 km from the Indooroopilly Island camp were examined for larvae and found to be free of infection. In February 1984, another possum found killed 4 km from the same camp was likewise uninfected.

8.2.8 Chickens and ducklings

Three 2-d-old chicks were each fed 3,000 T. pteropodis eggs, and another 3 were given 3,000 eggs in 0.3 ml saline intraperitoneally.

One from each group was killed at 3 d, and the remainder killed at 7 d. Larvae were not found in the livers or lungs of any.

Two 6-d-old ducklings hatched in 1 of the large bat cages were fed 6,000 T. pteropodis eggs each and killed 5 d later. Larvae were recovered from neither livers nor lungs.

8.2.9 Pigs

Two 6-week-old pigs from the specified-parasite-free piggery at the University Veterinary School Farm were fed 20,000 T. pteropodis eggs administered in sweetened milk intra-orally via syringe; another 2 were fed 10,000 eggs and the same dose repeated 1 week later. Eggs from the same batch were infective to mice. The pigs were observed for clinical signs regularly, and were bled initially, then weekly, for 8 weeks pi. Haemoglobin and white cell studies, carried out at the diagnostic laboratory of the Veterinary School, showed no significant changes over the 8 weeks, with total white cell counts remaining fairly steady and eosinophils fluctuating between 0 and $400/mm^3$ in each pig. Biochemical investigations were performed by the Chemical Pathology Laboratory of the Royal Brisbane Hospital, and included serum electrolytes, urea, creatinine, bicarbonate, bilirubin, albumin, globulins, alkaline phosphatase, lactic dehydrogenase and several "hepatic" transaminases. There were no significant changes in any of these indices throughout the study.

When the pigs were killed at the end of the study, their livers were found to be normal macroscopically and histologically. Approximately 5% of each liver was examined for larvae, with negative findings.

8.2.10 Cynomolgus monkeys (*Macaca fascicularis*)

8.2.10.1 Methodology

Nine wild-caught *M. fascicularis*, purchased from a monkey-dealer in Kuala Lumpur, were housed in the primate facilities of the Malaysian Institute for Medical Research, and fed and maintained by experienced staff under the supervision of Dr Mak Joon Wah. The monkeys were divided into 3 groups, A, B and C, each comprising 2 infected animals and 1 sham-infected control (see Table 8-3).

They were anaesthetised with ketamine hydrochloride and the egg doses administered in 5 ml saline via naso-gastric tube according to the protocol in Table 8-3. To assess the infectivity of these eggs, 2,000 were administered via stomach tube to 2 Quackenbush mice on the first day of the experiment.

Ten ml of blood was collected into EDTA tubes from the femoral vein of each monkey on the first day then at weekly intervals until autopsy. Thin smears were stained with Giemsa for microscopic examination. The following values were determined from whole blood: haemoglobin, total and differential white blood cell counts and absolute eosinophil count. Serum was analysed using routine methods in an Abbot ABA-100 automatic analyser for albumin, globulins, bilirubin, alkaline phosphatase and transaminases (aspartate aminotransferase, alanine aminotransferase and γ-glutamyl-transferase). The animals were observed daily for diarrhoea, and examined weekly for body temperature, jaundice, pallor, hepato-splenomegaly, lymphadenopathy and abnormal respiratory signs. Formol-ether concentrates of faeces were examined microscopically for parasites at these times.

At the times indicated in Table 8-3, the monkeys were anaesthetised with ketamine hydrochloride and killed with an overdose of pentobarbitone. At autopsy, abdominal, thoracic and cranial contents were examined *in situ* then removed for larval recovery. Small portions from each liver were fixed in 10% formol-saline for routine histological examination. From each animal the alimentary tract was freed of its mesenteries and divided into stomach, duodenum, proximal, middle and distal thirds of small intestine, caecum, and proximal and distal colon. Each segment was opened longitudinally, rinsed vigorously in 3 changes of tapwater, examined for ulcers and haemorrhages, minced finely with scissors and then incubated over gauze in 0.85% saline at 37°C for 18 hours. The following organs were minced and incubated likewise: mesenteric lymph-nodes, mesentery, liver, right and left lungs, kidneys, spleen, trachea and main bronchi (opened longitudinally but not minced), heart, right and left cerebral hemispheres and cerebellum. The sediments were fixed in hot formalin as described in Chapter 2, and examined in Petri dishes by stereomicroscopy. All larvae found were mounted in chlorlactophenol and examined at higher power for identification, drawing and measuring. The 2 mice were killed 3 d pi and their livers examined for larvae.

8.2.10.2 Results

Clinical response:

None of the infected or control animals developed fever, diarrhoea, jaundice, hepatosplenomegaly, lymphadenopathy or other clinical abnormalities which could have been attributed to the infection, although monkey no. 1 developed pneumonia and was killed prematurely on d 26.

Biochemistry:

The serum biochemical analyses showed no significant changes

TABLE 8.3

Infection schedules, timing of autopsy and recovery of larvae from tissues of monkeys fed infective Toxocara pteropodis eggs

(Group A: 1, 2, 3; Group B: 4, 5, 6; Group C: 7, 8, 9)

Monkey ID No.	Description	T. pteropodis egg dose	Autopsy (Days post infection)	Toxocara larvae recovered from tissues
1	Juvenile male, 0.5 kg	20,000 day 0	26	Stomach: 2 Lungs: 8 Duodenum: 1 Heart: 6 Liver: 150 Brain: 3
2	Juvenile male, 1kg	20,000 day 0	38	Liver: 1
3	Young adult male, 1.5kg	CONTROL	35	0
4	Juvenile male, 1kg	5,000 day 0 15,000 day 14	38	0
5	Young adult male, 2kg	5,000 day 0 15,000 day 14	40	Liver: 11
6	Young adult male, 2kg	CONTROL	35	0
7	Young adult female, 2 kg	2,000/day day 0 - day 9	10	0
8	Adult male 4kg	2,000/day day 0 - day 9	10	Mesenteric lymph nodes: 2 Liver: 650 Brain: 1
9	Adult female 3kg	CONTROL	40	0

throughout the experiment and no differences between infected and control animals, except for elevated transaminase levels on d 14 in monkey 1 and on d 21 and d 28 in monkey 5.

Haematology:

Haemoglobin levels did not vary significantly with time or between groups. White cell counts rose in all infected animals (except nos 7 and 8, which were killed at 10 d pi) but not controls, and peaked between the 2nd and 4th weeks. Eosinophils accounted largely for this leukocytosis. Blood eosinophil responses of individual animals are shown in Figure 8.1. In each group eosinophil levels rose higher in infected than control animals, but 2 controls (6 and 9) demonstrated abnormal peak levels. The highest levels were seen in monkeys given a priming dose followed 2 weeks later with a 2nd dose (4 and 5).

Autopsy findings:

In all control animals the livers and other organs appeared normal. Pale, punctate lesions varying in number and in diameter from about 1 mm (monkey 4) to 4 mm (monkey 5) were observed in all infected animals. In monkey 7, killed 10 d after the first egg dose, several petechial haemorrhages were evident on the lung surfaces. Monkey 8, in the same group, exhibited moderate numbers of petechiae over the lungs and liver and throughout the gastrointestinal mucosa.

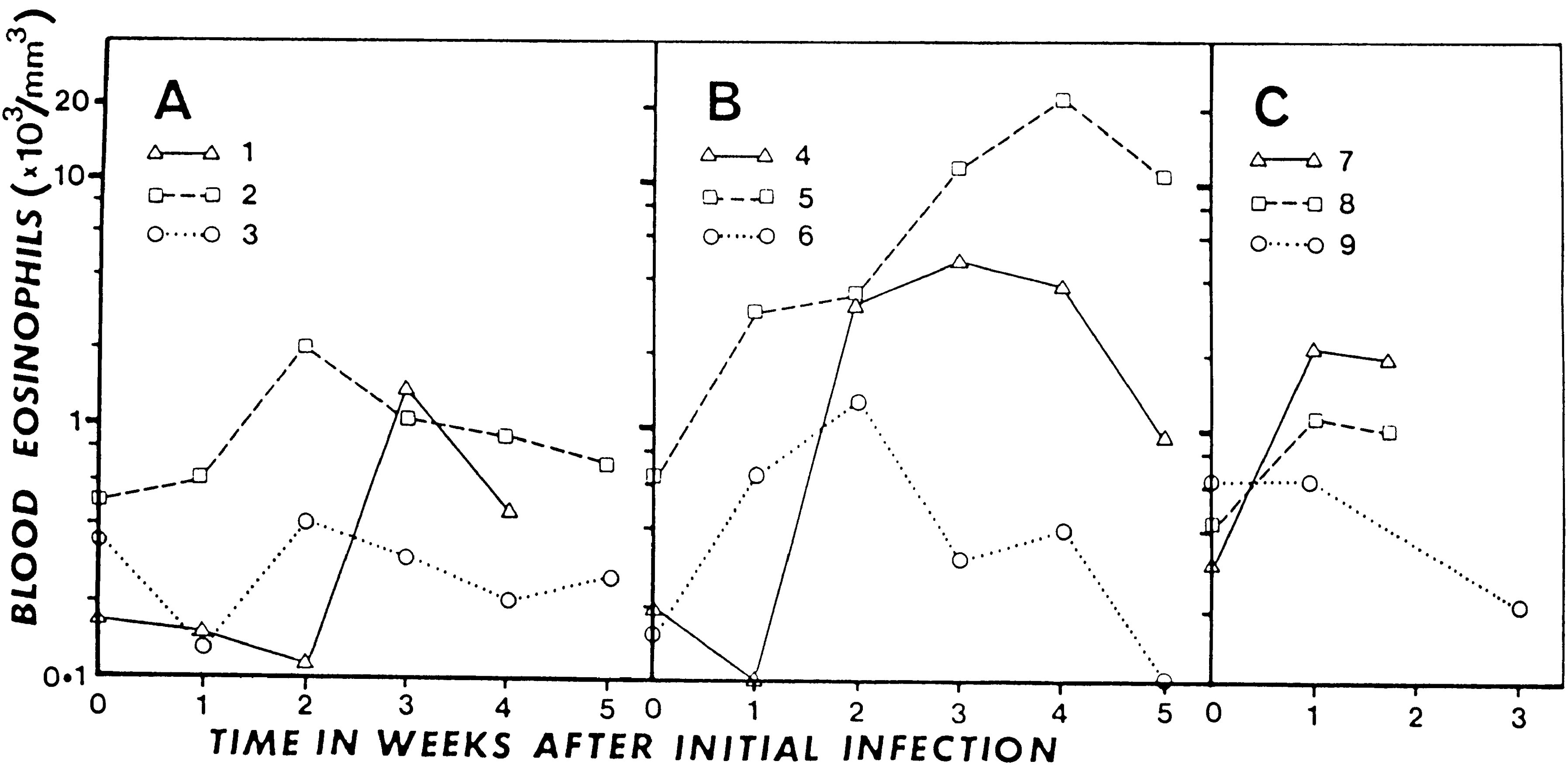
Liver histology:

In monkeys 7 and 8, patchy periportal lymphocytic infiltration was associated with large numbers of focal lesions, ranging from microabscesses consisting predominantly of neutrophils with necrotic hepatocytes, through intermediate stages to young classical foreign-body granulomata comprising central eosinophilic necrotic cores of degenerate cells, intermediate zones of epithelioid cells and MNGCs and outer layers of lymphocytes, plasma

FIGURE 8.1

Absolute blood eosinophil counts (log_{10} scale) in monkeys given T. pteropodis eggs as single dose of 20,000 (group A), as priming dose of 5,000 followed at two weeks with 15,000 (group B) or as 10 daily doses of 2,000 eggs (group C)

Numbers correspond with animals described in Table 8.1. Note 3, 6 and 9 were non-infected controls



cells and eosinophils. Small numbers of T. pteropodis larvae were found. Those associated with microabscesses were usually beside the lesion, from the periphery (Fig. 8.2 A) to 230 µm away (Fig. 8.2 B). Larvae associated with evolving granulomas were enmeshed within the lesions. Fewer larvae were seen in the sections from monkey 7, and all were found within granulomata. In monkey 8, most larvae were associated with "younger" lesions, but were beyond the periphery (Fig. 8.2 B).

The liver sections of monkey 4, killed 38 d after the initial egg dose, contained the greatest density of larvae. Without exception, these were enmeshed in giant-cell systems within the cores of classical foreign-body granulomata, heavily infiltrated with eosinophils (Fig. 8.2 C). Monkey 5, from the same infection group, demonstrated a distinct tissue response (Fig. 8.2 D) of dense eosinophil and lymphocytic infiltrates extending from portal tracts to larger vessels, with no classical granulomata. Numerous eosinophil "microabscesses" were found throughout the sections, but larvae were scarce and most were located near the necrotic centres of such lesions.

The histology of monkeys 1 and 2 was similar to that of number 4. In monkey 1, the larvae were as numerous as in number 4, but most appeared clearer in cellular detail and many gave the impression of attempting to push out of the granuloma (Fig. 8.2 E). One was found free in the tissues, without associated inflammation. In monkeys 1, 2 and 4, dense tracts of chronic inflammatory cells could be followed from granulomata to aggregations around portal tracts (Fig. 8.2 F) or hepatic veins, with very little inflammatory infiltration away from these foci.

In all 3 control animals, the liver tissues were normal with no larvae or focal or perivascular inflammatory collections.

Larval recovery:

The numbers of Toxocara larvae recovered from various tissues are shown

FIGURE 8.2

Histological sections from livers of monkeys infected with *T. pteropodis* (arrows indicate larvae)

A. Monkey 8. Eosinophilic microabscess with necrotic core and larva within peripheral inflammation (bar = 200 μm)

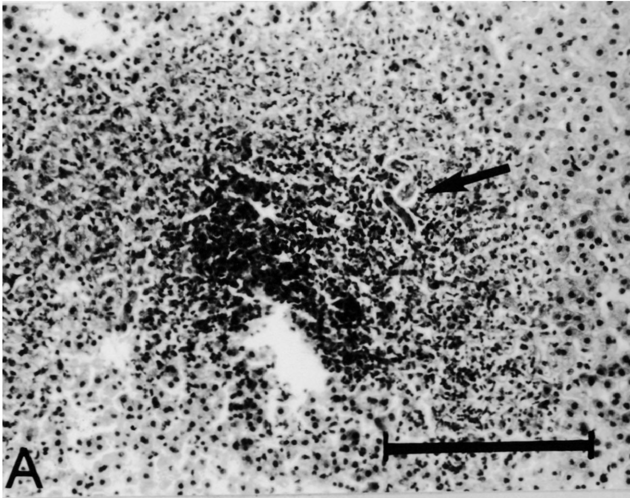
B. Monkey 8. Acute inflammatory focus, chiefly of neutrophils and lymphocytes, with larva 230 um distant (bar = 200 μm)

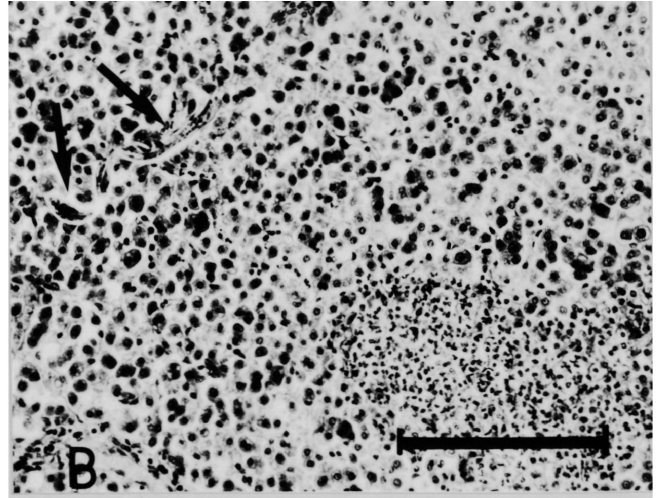
C. Monkey 4. Typical granuloma with presumably dead larva enmeshed within giant-cell "shell". Eosinophilic material within necrotic core represents degenerate giant-cell cytoplasm and eosinophils (bar = 100 μm)

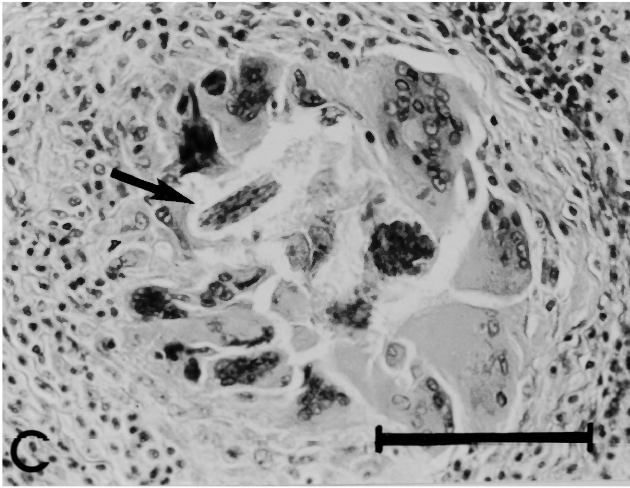
D. Monkey 5. Typical inflammatory focus of eosinophils, lymphocytes and plasma cells, with larva nearby (bar = 200 μm)

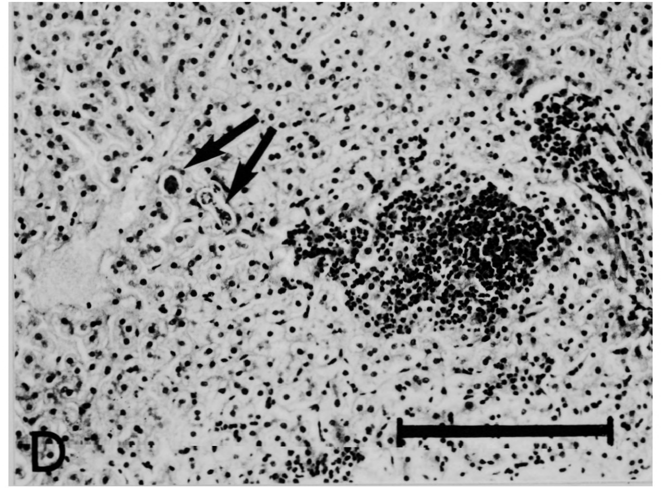
E. Monkey 1. Section towards edge of typical granuloma, with larva apparently attempting to break out. Cells around larva are all eosinophils, whereas peripheral infiltrate comprises eosinophils, plasma cells and lymphocytes (bar = 100 μm)

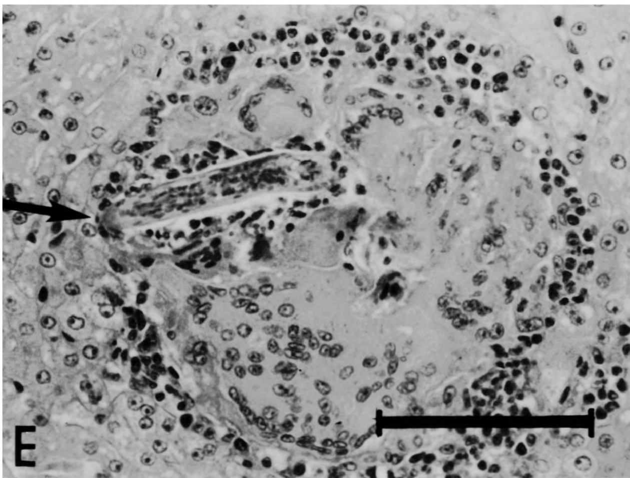
F. Monkey 2. Classical granuloma with core of eosinophils, many of which are disintegrating both inside and outside giant-cell cytoplasm. Outer layer of eosinophils, lymphocytes, plasma cells and fibroblasts contiguous with asymmetric inflammatory cuff extending along portal tract (bar = 200 μm)

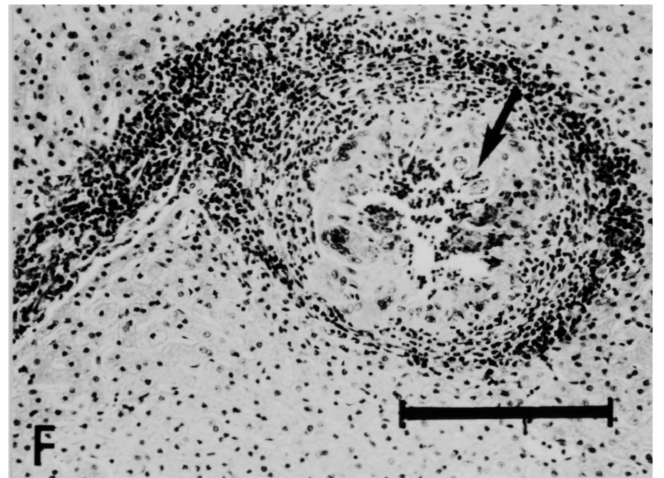

A


B


C


D


E


F

in Table 8-3. Larvae were not recovered from any of the control animals, nor from numbers 4 and 7 of the infected animals. Most larvae were recovered from livers, the highest yield being 650 from the liver of monkey 8, killed after 10 daily doses of 2,000 eggs. Small numbers were found in other organs, including the cerebella of monkeys 1 and 8, and the cerebral hemispheres, lungs, heart and upper gastrointestinal tract of monkey 1. The lengths of these larvae are shown in Table 8.4. Larvae at 26 d pi (monkey 1), were longer than at 10 d pi (monkey 8) ($P < 0.001$, Student's t-test). Larvae at 40 d pi (monkey 5) were longer than at 26 d pi (monkey 1) ($P < 0.10$). From the 2 mouse livers, 354 larvae were recovered (9% of total egg dose).

8.3 DISCUSSION

In mice, T. pteropodis larvae hatched from eggs in the intestine and penetrated the mucosa, mainly in the distal small gut and colon, to reach the liver via the portal circulation. Larval growth in mice (Table II, Appendix IV) was slower, with a shorter final length, than in bats (Fig. 4.1). Larval recovery from mice as a fraction of egg dose was similar to that in bats, and they survived extended periods, albeit in diminishing numbers. Their distinct hepatotropism was confirmed by the parenteral inoculation of eggs. They demonstrated a paratenic tendency in mice (Appendix V), as in bats, but did not undergo transmammary passage or migration from livers in response to gestation and parturition. In a limited study (Prociv & Brindley, unpublished), larvae did not leave the livers of mice immunosuppressed with prednisone and cyclophosphomide. The histological responses (unpublished) were also quite distinct from those in bats. In mice, T. pteropodis either mimics its behaviour in the definitive host, or exhibits a relict paratenic tendency.

The findings from the studies of post-mortem larval migration in mice

TABLE 8.4

Numbers and lengths of T. pteropodis larvae recovered from tissues of experimentally-infected monkeys

Monkey	No. days post-infection	Tissue	No. larvae recovered	No. larvae measured	Length (μm) mean ±SD	Length (μm) range
8	10	mesenteric nodes	2	2	405 ± 7	400 - 410
		liver	650	50	410 ± 17	375 - 445
		brain	1		460	460
1	26	stomach/ duodenum	3	3	430 ± 24	415 - 465
		lungs	8	8	435 ± 30	405 - 510
		heart	6	6	470 ± 38	405 - 510
		brain	3	3	430 ± 23	415 - 460
		liver	150	50	465 ± 25	410 - 505
2	38	liver	1	1	470	470
5	40	liver	11	11	495 ± 49	410 - 565
Two mice*	3	livers	354	30	410 ± 17	380 - 455

* infectivity controls

are consistent with the conclusion drawn from liver histology, particularly in flying foxes (Section 4.4.1.4) and in monkeys (see below), that viable larvae may move considerable distances prior to fixation of the tissues. It appears that this migration, coupled with tissue autolysis, is more important in larval recovery techniques than is digestion by extraneous enzymes. Saline extraction of larvae was consistently shown to be at least as effective as tryptic or peptic digestion. The transfer of minced liver into fresh solution at the end of each collection interval (Table 8-1), led to repeated dilution and loss of the enzymes released by autolysis. This may explain the protracted recovery of larvae from the saline samples, which normally would have been undisturbed for 18 h. It is likely that autolytic enzymes play an important role in the tryptic and peptic digests as well. Although the recovery peak with pepsin occurred early, the total numbers recovered were less than with trypsin or saline, and the larvae were damaged. Perhaps its high acidity (pH 1) irritates larvae, inducing excessive motility which results in early egress from tissues, disruption of alimentary tracts and diminished later recoveries owing to larval morbidity. In most other published studies, tissues were first blended before digesting, for much shorter periods, in trypsin or pepsin. Under such circumstances, enzymatic damage of larvae may be lessened, but addition of enzymes seems unnecessary. In the blended mouse carcass, equal numbers of _T. cati_ larvae were recovered from the saline- and pepsin-incubated samples. The protracted recovery of larvae in all solutions, but especially saline, probably reflects the size of tissue fragments incubated, as larvae would take longer to reach the surface of larger pieces (up to 3 mm in length). Blending the tissue would minimise this delay, but must be balanced against the inconvenience and greatly increased workload, particularly when processing larger numbers of specimens. Sediments from peptic digests were much clearer than those in saline or

trypsin, but this advantage may be lost with blended specimens.

Although other workers have occasionally used saline extraction (modified Baermann's technique), few systematic studies comparing the efficiency of different techniques in recovering tissue larvae have been published. Johnstone et al. (1978) compared their "spin method" for recovering A. suum larvae from mouse lungs and livers with Baermann separation and a digestion method. They found the digestion technique considerably inferior to the other two methods, and presented data which indicated the Baermann technique was almost as effective as the spin method. Jackson et al. (1984) found that saline incubation was as effective as pepsin digestion in recovering Ostertagia circumcincta larvae from the abomasum of sheep.

Freezing the liver in liquid nitrogen killed T. pteropodis larvae. Nethertheless, simple incubation of this liver in saline still resulted in sufficient autolysis to passively free many of these larvae, allowing their recovery by filtration through gauze followed by sedimentation. When the frozen liver was suspended in a gauze pocket ("saline extraction"), the tissue fragments were held tightly together, so that in the absence of movement, few larvae "fell" through the multi-layered gauze. This contrasted with recoveries from non-frozen liver, in which larvae were viable. In those simply incubated and then filtered, presumably many were retained in the filtered material, perhaps because they had died during incubation. In those incubated over gauze, a higher proportion of larvae were recovered because each motile larva, once traversing the gauze, was effectively trapped in the sediment, and prolonged viability therefore became less critical. Successful recovery of larvae by "digestion" techniques, particularly those based upon modifications of Baermann's method, requires live larvae; small numbers of dead larvae may be recovered, by falling passively through the gauze.

The question of transperitoneal migration by larval ascaridoids has

concerned workers for many years, and was shown by Bhowmick (1964) to be a major pathway in the life-cycle of T. canis and A. lumbricoides. However, Bhowmick's photograph of serosal haemorrhages in acutely infected mouse intestines (his figure 24) shows distinct features of an animal which has been dead for many hours - the gut is flaccid, the serosa dull and the haemorrhagic areas becoming diffuse. He fed A. lumbricoides eggs to mice which were killed at half-hourly intervals for 24 h. It is likely that the dead mice were put aside for more convenient examination in batches. All of his findings, including massive numbers of larvae in the peritoneal cavity, histological evidence of larvae penetrating the serosal surfaces of the gut, liver, kidneys and diaphragm, and recovery of larvae from the pleural cavity, can be explained on the basis of post-mortem larval migration. From the findings in Table 8-2, it seems that T. canis larvae are much more active than T. pteropodis in this type of migration. Perhaps this reflects the greater propensity of T. canis to undergo paratenic transmission. Other species undoubtedly undergo post-mortem migration, including larval cestodes and trematodes. Mason et al. (1976) recovered A. cantonensis from the central nervous systems of naturally-infected dogs by incubating in saline. Third-stage larvae of this nematode are readily obtained in large numbers from infected snails by incubating in saline (pers. obs.). Tetrathyridia of Mesocestoides corti have been recovered from mouse livers by saline incubation (Dr P.J. Brindley, pers. comm.) and metacercariae of Opisthorchis viverrini have been recovered from infected fish by incubating over gauze in saline (S. Kaewekes, pers. comm.). The larvae of Anisakis sp. migrate from viscera to the flesh of fish post-mortem (Smith & Wootten, 1975), so that the potential infectivity of fish to humans increases with the time elapsed between capture and gutting.

This propensity of helminthic larvae to undergo post-mortem migration must be considered in all studies of larvae in tissues, including

histological investigations. Mason et al. (1976) found that degenerate A. cantonensis larvae in dogs' spinal cords were found within granulomas, whereas healthy-looking larvae occurred in normal tissue, without surrounding inflammation. Such considerations may explain many of the histological findings in bats' livers, in which delays between death and tissue fixation seemed to be associated with greater separations of larvae from inflammatory lesions. However, there were exceptions, and the finding of larvae "free" in tissues, although close to zones of inflammation, in the frozen mouse livers indicates that these larvae may move out of inflammatory zones during life. This is in keeping with the slow "step-wise" larval migrations which probably occur in the bat liver infected with T. pteropodis.

In rats, larvae again were hepatotropic, but the much lower yields, and only from young animals, indicates an innate refractoriness which rapidly consolidates with age. The limited findings in orally-infected adult rats of larvae in the gut wall and of blood eosinophilia suggest that larvae may penetrate the gut mucosa but perish there.

The fate of T. pteropodis in guinea-pigs was different from that in either rats or mice. Larvae reached the livers where they survived longer than in rats, but not as long as in mice, and did not grow significantly. The focal histological response differed from the more diffuse granulomatous changes in mice. The last viable larvae were recovered from guinea-pig livers 25 d pi, although histologically they were detectable in apparently "good" condition as late as 70 d (unpublished). Larvae of T. canis and T. cati seem to survive indefinitely in guinea-pig tissues, without growing; this may explain the prolonged elevation of blood eosinophilia in these infections compared with the gradual decline to normal in T. pteropodis infections.

From the limited studies, rabbits appeared to be entirely refractory to infection with T. pteropodis.

The infection in the 2 dogs was complicated by the presence of *T. canis*. By comparing the features of larvae recovered with those in Table 6.1, it is clear that most dimensions overlapped with those of *T. canis* larvae and of *T. pteropodis* from mice at 7 d pi. On the basis of tail length, which seems the most reliable differential index, the larvae from the dog's liver fell into two distinct groups, those with short tails (< 7.9% body length) and those with long tails (> 9.5% body length), corresponding to *T. pteropodis* and *T. canis* larvae (Table 6.1). Therefore, the dog harboured larvae of both species in its liver. The *T. pteropodis* larvae were shorter than those from mice 12 d pi (Table 6.1).

The even smaller number of larvae recovered from one of 2 infected cats had features consistent with *T. pteropodis* rather than *T. cati*. Again, there was no evidence of growth in length. It appears that cats and dogs may be similar to rats in their refractoriness to *T. pteropodis* and similar to guinea-pigs in that larvae do not grow significantly.

The brush-tailed possum was studied as a possible sentinel host to indicate the degree of environmental contamination with *T. pteropodis* eggs, as these animals probably eat the same type of fruit as flying foxes, with whom they often engage in territorial conflict (pers. obs.). From such a small study, conclusions must be drawn cautiously, but there is no doubt that possums are susceptible to hepatic infection with *T. pteropodis*. With 9% of administered eggs recovered as larvae, the suckling possum was as susceptible as mice, but its mother was more resistant; her blood eosinophil response suggests that she had harboured larger numbers of larvae, most of which had perished by the time of autopsy. This limited viability of larvae in possum livers renders them useless as indicators of fruit contamination with *T. pteropodis* eggs. Therefore, the negative findings in the road-killed possums were to be expected, regardless of their possible exposure to infection.

Chicks and ducklings were totally refractory to infection, even via the ip route in chicks. This contrasts with the susceptibility of chickens to T. canis, whose larvae settle in the liver and persist indefinitely (Galvin, 1964).

In view of the varying susceptibilities of small animals to infection with T. pteropodis, it was impossible to predict its potential to infect humans. Pigs were chosen for the closer monitoring of pathogenicity for several reasons. Firstly, in view of the susceptibility of both pigs and humans to the very closely-related A. suum and A. lumbricoides (Beaver et al., 1984), it was considered that another ascaridoid might also behave comparably in these hosts. Secondly, considerable anatomical and physiological similarities to humans underlie the use of pigs as experimental models of human metabolic diseases, including atherosclerosis (Cevallos et al., 1979; Vesselinovitch, 1979). Thirdly, in pigs fed T. canis eggs, the organ distribution of larvae and pathological response was similar to that found in humans (Done et al., 1960). The dose of 20,000 T. pteropodis eggs, representing approximately the total daily output of a female worm, was considered to be beyond the upper limit of what a human was likely to ingest under appropriate circumstances. The administration of two separate doses to 2 pigs was to allow for pre-sensitization. Nevertheless, the pigs did not manifest clinical, haematological, biochemical or histological evidence of infection.

In view of the negative findings in pigs, it was decided to undertake studies in monkeys, as non-human primates are considered to be a more realistic model of human parasites than are standard laboratory animals (Orihel, 1970). The 3 different dosage regimens were given in an attempt to simulate possible natural exposure patterns.

Counting and identification of Toxocara larvae was complicated by the presence of Strongyloides larvae in the intestines of all of the monkeys. However, not a single Toxocara larva was found in the controls, and all

infected monkeys harboured T. pteropodis. These findings show that M. fascicularis is susceptible to infection with T. pteropodis, but the proportional recovery of larvae was considerably less than in mice. Furthermore, it appeared that in monkeys the larvae rapidly died.

Histologically, Toxocara larvae were found in the livers of all experimentally-infected monkeys, yet the numbers recovered were considerably lower than indicated in sections. In monkey 4, the finding of larvae in every liver section meant that several thousand were in the liver, yet none was recovered from this or from monkey 7. Every larva in monkey 4 was enmeshed in the core of a granuloma, whereas in monkey 8, which yielded the highest number of larvae, each one was free of its inflammatory focus. The liver of monkey 1, from which 150 larvae were recovered, was as densely infected as that of 4, but many larvae seemed to be attempting to escape from their granulomata, the one found free in tissues apparently having succeeded. This supports the premise that larval recovery from host tissues correlates with their viability. Larvae remain viable for variable periods within classical granulomata, which seem to be a mechanism for larval disposal. Larvae remain alive for variable periods. In early infection, more larvae would be viable, whereas in older granulomata, few would be mobile perhaps because of damage by tissue responses. In mouse livers, where T. pteropodis larvae may remain viable for 2 years or more, classical granulomata do not develop with this infection (unpublished). In guinea-pigs, granulomata do develop and the fate of larvae is similar to that in monkeys. The gross discrepancy between numbers of larvae recovered and those found histologically indicates that most larvae died early but retained their morphology. Unless grossly degenerate, the viability of a larva in tissues is impossible to assess histologically.

In every granuloma examined completely through serial sections, dense

trails of inflammatory cells could be traced to adjacent portal tracts (Fig. 8.2 F) or hepatic venules, or both. The periportal infiltrates extended to larger vessels, which they ensheathed asymmetrically, indicating perhaps a localised response to larval antigens passing through veins and lymphatics draining the granuloma.

The finding of larvae in mesenteric lymph-nodes and small numbers in the brain early in infection (monkey 8) was not surprising, but the findings in monkey 1, killed 26 d pi, were unexpected. In mice, bats and guinea-pigs, even after parenteral infection, larvae quickly accumulated in livers and did not persist in other sites. In monkey 1, perhaps a small number underwent somatic and even tracheal migration to arrive and persist in other tissues.

Larvae of T. pteropodis grew in monkeys but at a slower rate than in mice, attaining a shorter final length owing to limited viability. The aggressive tissue reaction which kills larvae may initially retard their development.

Interpretation of blood eosinophil responses was complicated in the monkeys by their universal infection with Strongyloides, which may account for the high levels in some of the controls. Nevertheless, in each group, eosinophil levels rose faster in the Toxocara-infected animals and reached much higher peaks. This experiment was organised at short notice; the limited viability of T. pteropodis eggs (Chapter 5) precluded attempts to clear the monkeys of natural infections, which in any case, may have been unsuccessful (Dr J. W. Mak, pers. comm.). Furthermore, these animals may have been pre-sensitized by previous helminthic infections.

The absence of a consistent rise in biochemical liver function indices indicated that hepatocellular function was not disturbed significantly. Although serum electrolytes, urea and creatinine were not measured, at no time was there clinical evidence of such fluid and electrolyte disturbances as seen in the Palm Island epidemic (Byth, 1980).

In the first reported studies of toxocariasis in primates (Beaver, 1962), massive doses of *T. canis* eggs (300/g body weight) were given to a male and female *Macaca mulatta*. Larvae distributed to liver, lungs, muscle and brain and survived up to 10 years (Beaver, 1966). Wiseman & Woodruff (1967) fed 500 and 1,000 eggs of *T. canis* to 2 rhesus monkeys to produce a peak blood eosinophilia of 3-4,000/mm^3 at 3 weeks pi and peak leukocytosis at 3 - 4 weeks, both of which had returned to normal by 12 weeks. These monkeys were naturally-infected with *Enterobius vermicularis*, hookworms and *Trichuris*. In one *Macaca irus* fed 2,500 *T. canis* eggs, blood eosinophilia peaked at 2 weeks pi and was still elevated 4 months later (Bisseru, 1969). In baboons, blood leukocytosis and eosinophilia rose in proportion to the *T. canis* egg dose, peaked at 2 - 4 weeks pi and persisted for up to 6 months (Aljeboori & Ivey, 1970). Larvae distributed amongst livers, lungs, brains, kidneys and hearts (skeletal muscle was not examined) with considerable individual variation (Aljeboori et al., 1970). Experimental infections with up to 400,000 *T. canis* eggs, in 4 *Macaca* species which apparently harboured unspecified intestinal parasites, produced essentially similar results (Tomimura et al., 1976). A high proportion of larvae was found in skeletal muscle. The authors concluded that the rapid fall in eosinophil levels after the early infection peak differed from the response seen in humans, although their published results in fact showed most infected monkeys exhibited a chronic hypereosinophilia. In a more detailed study of long-term infection in a larger group of *M. fascicularis*, also harbouring unspecified endoparasites, blood eosinophilia appeared at 14 d pi, peaked at 30 - 50 d and persisted throughout the study, i.e. to 220 d (Glickmann & Summers, 1983). Haemoglobin, urea, glucose and serum alkaline phosphatase did not change, serum globulins rose by 3 - 4 weeks and albumin levels fell slightly, and serum alanine aminotransferase rose by day 4 and declined to normal over 6 months. Liver histopathology was

essentially identical with that found here in T. pteropodis infections.

Another ascaridoid recently implicated in human disease, B. procyonis, produces clinical and pathological responses in monkeys which are comparable with those reported in humans (Kazacos et al., 1981; Kazacos, 1986).

Despite the paucity of detailed human studies, it is clear that experimental T. canis infections in monkeys closely resemble observed responses in humans (described in Section 1.3.5). Therefore monkeys should be a reliable model of human T. pteropodis infection as well. Under normal circumstances, people are unlikely to ingest large numbers of infective eggs (Section 5.10), but even high doses probably would not produce clinical disease. However, blood eosinophilia, hepatic granulomata and serological responses, perhaps cross-reacting with T. canis or other helminths, could be expected, in which case T. pteropodis should be considered in the differential diagnosis of unusual cases of hepatic larva migrans or blood hypereosinophilia with an appropriate background. It fails to explain the clinical presentation of cases in the Palm Island epidemic.

CHAPTER 9

CONCLUSIONS

9.1 HOST RANGE AND GEOGRAPHICAL DISTRIBUTION OF T. PTEROPODIS

At least 2 genera of Megachiroptera, Pteropus and Rousettus, harbour Toxocara. As they extend over the entire range of the large bats, from Africa to Polynesia (Fig. 1.1), potentially many other species may also be infected. T. pteropodis occur in all 4 Australian pteropids, although P. scapulatus may be an accidental host incapable of maintaining the parasite under natural conditions. The other 3 species, which amongst them range along the entire tropical and eastern coastline of Australia, seem to be natural and adequate hosts, capable of maintaining T. pteropodis within their own populations. The gradual range extension of P. alecto, and its dominance over P. poliocephalus, have resulted in the parasite's decline in southern P. alecto, presumably as a consequence of selective roosting behaviour.

The worm occurs in Pteropus in New Guinea, and Baylis found it in P. tonganus geddiei in Vanuatu. Closely-related flying fox species in Fiji, Tonga, Samoa, Nuie and the Cook Islands may also be infected, although T. pteropodis was not found in the last 2 locations, where the bats may have been artificially introduced under conditions unfavourable to the worm's survival and transmission. Larger Pteropus populations, on islands such as Vanuatu which were colonised naturally, may be infected, while other groups with behaviour unfavourable to the parasite's survival, or which may have invaded remote islands only in small numbers, such as P. melanotus of Christmas Island in the Indian Ocean, may be free of the parasite.

On gross morphology, nematodes from Rousettus sp. from Burma and the Philippines appeared identical with T. pteropodis, but on key internal

dimensions T. pteropodis fitted between those of the other two groups of specimens. It seems likely that these were all T. pteropodis, or perhaps very closely related species with similar life-cycles. The finding of Toxocara in the genus Rousettus means that perhaps the worm extends into Africa and other megachiropteran genera.

9.2 LIFE-CYCLE OF T. PTEROPODIS

All the evidence indicates that the life-cycle is direct, in keeping with the dietary habits of the host, although eggs need a minimum of 10 d after passing in the faeces of juveniles to become infective. Adult bats acquire infection almost entirely within the roost and its immediate surroundings, as contamination of fruit remote from the camps is minimal. In effect, T. pteropodis is a soil-transmitted nematode.

Two moults occur in the egg, so that the 3rd-stage larva is infective. Larvae hatch from the eggs throughout the gastrointestinal tract and reach the liver mainly via the portal venous circulation, with perhaps a small number entering lymphatics and the systemic blood circulation. Larvae remain in the liver not through anatomical constraints, but because they are hepatotropic. Eggs injected parenterally hatch readily and larvae eventually reach the liver, to grow from an initial length of about 420 µm to about 600 µm at 3 months; beyond this time there is minimal growth over perhaps another 1 - 2 years. Hepatic larvae may survive indefinitely.

In adult female bats, it is likely that rapid hormonal changes at the commencement of parturition induce some, or perhaps all, of the larvae to leave the liver. The precise timing and limited duration of this migration suggests that immunological changes are not directly responsible. Some of these larvae reach the mammary glands, undoubtedly via the circulation, while those which remain in, or return to, the liver may infect the offspring of subsequent pregnancies. Larvae may be ready for hepato-

mammary migration as early as 2 months after infection.

Arrival in the mammary glands induces a resurgence of larval growth, with many larvae exceeding 1 mm in length. Perhaps some moult before reaching the pup's intestine, although most arriving there are still in the 3rd-stage. There is no tissue phase in the juvenile, and the infection becomes patent from as early as 35 d after birth.

The liver-mammary complex in bats is equivalent to the canine foetal liver-lung in the *T. canis* life-cycle. In *T. canis*, hormonal changes at mid-pregnancy induce larval migration and a burst of rapid growth, which then ceases until parturition; in *T. pteropodis*, growth commences after larval hatching from eggs, then effectively ceases within 3 months, to be reactivated with larval migration at parturition.

Large numbers of *T. pteropodis* larvae may occupy maternal livers, and pass through the mammaries to the neonatal gut, but small numbers of adult worms mature to produce patent infections. Even though *P. poliocephalus*, *P. alecto* and *P. conspicillatus* are amongst the largest bats in the world, *T. pteropodis* is big in relation to their juveniles, which are protected effectively from excessive burdens by unknown mechanisms. These may be mediated immunologically or through chemical transmitters released from within the worm population. The more rapid growth of female larvae, with a greater susceptibility to spontaneous expulsion of smaller larvae, leads to a change in the sex ratio from 1:1 in late 3rd- and 4th-stage larvae to about 2:1 in adults. Small numbers of adult worms in the host lead to a greater frequency of single-sex infections, but this is a small trade-off against the threat to host-survival of larger worm burdens.

Peak egg production coincides with juvenile confinement to the camp, ensuring maximal contamination of the roost environment and successful infection transmission. Worm loss during weaning results from "senescence", perhaps accelerated by the change in host diet. The limit of egg infectivity corresponds with the duration of the camp, for most summer

camps are deserted soon after the juveniles are weaned. Adult bats are infected by the unintentional ingestion of faeces while grooming and licking water from leaves and branches in wet weather. The 3rd-stage larvae in livers of females then have more than adequate time to develop prior to the birth of the next offspring, whereas in males they simply accumulate.

9.3 TAXONOMIC AFFINITIES

T. pteropodis has all the features typical of the genus Toxocara, except for the absence of cervical alar bars. As a relatively large ascaridoid of herbivorous, terrestrial, mammalian hosts, with a direct life-cycle, it may be considered a highly developed member of this group. The genotype, T. canis, is unusual in that it is the only member of the genus known to undergo transplacental migration, although its ability also to pass through the milk suggests that the latter route is a relict and therefore more "primitive". Certainly, transmammary transmission is more typical of the genus, occurring naturally in at least T. cati, T. vitulorum and T. pteropodis. Even so, T. pteropodis exhibits a relict paratenic tendency which is of no advantage under natural conditions and may suggest its recent ancestors were carnivores. However, Sprent (1983) cautioned that "it is unlikely that life-history patterns have taxonomic significance even at the generic level".

It is highly improbable that T.pteropodis evolved from parasites of microchiropterans. Firstly, no ascaridoids have yet been found in this group, and secondly, accumulating evidence from many different areas indicates that Microchiroptera and Megachiroptera are descended from different groups, and simply represent evolutionary convergence (Pettigrew, 1985). Microchiroptera have affinities with rodents, whereas Megachiroptera have more in common with primitive primates. A systematic

search for ascaridoids in the lower primates of the Indonesian archipelago, which harbours the greatest number of Pteropus species and presumably represents their origins (Andersen, 1912), may shed light on the ancestry of Pteropus and Toxocara.

9.4 T. PTEROPODIS AS A MODEL OF ASCARIDOID INFECTIONS

Experimental work was hindered by difficulties in maintaining breeding Pteropus populations and the seasonality of the worm, although the first could be overcome with adequate facilities and staffing. Attempts to produce 2 annual "worm harvests" by experimentally infecting P. scapulatus, whose breeding is 6 months out of phase with the other 3 Pteropus species, were unsuccessful. Perhaps with optimal conditions and the careful titration of worm egg doses, it may be possible to establish a breeding colony of P. scapulatus with patently infected juveniles.

Studies of larval migration through tissues are easier in flying foxes than in the larger dogs and cats, but the production of only 1 juvenile annually and reluctance to breed in captivity are major drawbacks. A more reliable source of infective eggs than the wild bat population used in this study would facilitate further investigation of experimental infections in bats, particularly if a colony of Toxocara-free Pteropus were also established. Studies in a variety of areas could provide valuable insights into the host-parasite relationships of ascaridoids, eg transplantations of worms to non-infected bats, immunosuppression of bats with hepatic or gut infections, induction of pseudopregnancy in infected male and female bats (as more information becomes available on Pteropus reproductive endocrinology) and a search for possible chemical inhibitors of intestinal larval growth, perhaps based on in vitro studies.

In non-chiropteran hosts, the influence of immunity on hepatic larval growth, and the evaluation of hepatic larvae for systemic anthelminthic pharmacological trials, are worthy of investigation as the other models, T.

canis and *T. cati*, disperse more widely in body tissues, confounding the interpretation of findings.

9.5 POST-MORTEM LARVAL MIGRATION

It is clear that many viable larval helminths, including *T. pteropodis*, may migrate from their original sites following, or perhaps in response to, the death of their host and in the early stages of tissue fixation. This mobility is essential for the success of larval recovery techniques based on modifications of Baermann's method, and explains the effectiveness of saline extraction in comparison with tryptic and peptic digestion. It also necessitates caution in the interpretation of the spatial relationships in histological sections of larvae and associated tissue changes.

9.6 PATHOGENICITY OF *T. PTEROPODIS*

9.6.1 Bats

Although the larval stages, in early infections and during migration, stimulated peripheral blood eosinophilia, there was no overt indication of morbidity in experimental bats. Most juveniles with intestinal infections developed normally, and limited studies (unpublished) indicated that weight gain was not retarded in infected bats. In only 2 captive naturally-infected juveniles, a *P. conspicillatus* and a *P. poliocephalus*, was unexpected death subsequently attributed to adult worm migration, to the gall-bladder and to the pharynx, respectively. No information is available regarding similar complications under natural conditions. Several of the juvenile *P. scapulatus* born to experimentally-infected mothers may have died from gut obstruction or other consequences of their very heavy gut larval burdens, as on dissection their intestines were found literally

packed with worms.

It is unlikely that under natural conditions T. pteropodis is a major cause of morbidity or mortality in bat populations.

9.6.2 Other experimental animals

Mice were susceptible to infection at all ages, with larvae not travelling beyond the livers, even during and after pregnancy. Hepatic numbers slowly declined with time, but some larvae were still viable more than 2 years after infection. There was no indication of morbidity resulting from these infections. In fact, infected young mice gained weight faster than non-infected controls, but this was attributed to their enlarged livers (unpublished). Rats demonstrated a lower susceptibility than mice, and became almost totally refractory to oral infection with increasing age. Guinea-pigs were susceptible, but larvae perished in livers within 45 days of infection. Rabbits and pigs were totally refractory to oral infection and results in other animals were variable.

The susceptibility of Macaca fascicularis to infection was confirmed by haematological and histological findings in the absence of clinical abnormalities.

9.6.3 Humans

In view of the susceptibility of macaques, it is likely that humans ingesting T. pteropodis eggs will also develop hepatic larval infection, i.e. visceral larva migrans. However, exposure is likely to occur only under unusual circumstances such as those of the Palm Island epidemic in November, 1979. Even so, negative clinical findings and the absence of metabolic disturbances in the monkeys fed large egg doses indicate that humans also would remain clinically unaffected. Larvae found in the liver sections from an infected individual probably would be indistinguishable from those of T. canis, unless specific morphological features including

larval length could be measured. Therefore, *T. pteropodis* does not represent a human public health risk.

9.7 THE PALM ISLAND EPIDEMIC

The failure of toxocariasis pteropodis to account for the clinical and biochemical features of the Palm Island outbreak necessitates a re-appraisal of other possible aetiologies. The algal toxicity hypothesis is untenable on epidemiological considerations. An alternative possibility, not previously considered, is that of copper sulphate toxicity, which is reviewed in Appendix XI. Acute copper toxicity, well-described in humans, does not closely resemble the features of the epidemic. However, chronic copper poisoning, studied extensively in experimental animals but not well-documented in humans, could produce a syndrome virtually identical with the clinical and laboratory manifestations of the Palm Island cases. A likely scenario is proposed in Appendix XI.

CHAPTER 10

REFERENCES

Abraham PS, 1896. Remarks on cutaneous myiasis, due to oestridean larvae. Trans Derm Soc GB & Ireland, 3: 62-74.

Aljeboori TI & Ivey MH, 1970. Toxocara canis infections in baboons. I. Antibody, white cell and serum protein responses following infection. Am J Trop Med Hyg, 19: 249-254.

Aljeboori TI, Stout C & Ivey MH, 1970. Toxocara canis infections in baboons. II. Distribution of larvae and histopathologic responses. Am J Trop Med Hyg, 19: 815-20.

Andersen K, 1912. Catalogue of the Chiroptera in the collection of the British Museum. I. Megachiroptera: i-ci, 1-854. Trustees Brit Mus (Nat Hist) London.

Andersen S, Jorgensen RJ, Nansen P & Nielsen K, 1973. Experimental Ascaris suum infection in piglets. Acta Path Microbiol Scand, Sect B 81: 650-6.

Anderson RM, 1986. The population dynamics and epidemiology of intestinal nematode infections. Trans R Soc Trop Med Hyg, 80: 686-96.

Anya AO, 1976. Physiological aspects of reproduction in nematodes. Advances in Parasitology, 14: 267-351.

Araujo P, 1972. Observacoes pertinentes as primeiras ecdises de larvas de Ascaris lumbricoides, A. suum & Toxocara canis. Rev Inst Med Trop Sao Paulo, 14: 83-90.

Arean VM & Crandall CA, 1971. Toxocariasis. In: Pathology of protozoal and helminthic diseases. (Marcial-Rojas RA, ed.) Williams & Wilkins Co. Baltimore: 808-42.

Arulthilakan T & Nicholas WL, 1986. The specificity of antigens used for immunodiagnosis of human larva migrans. In: Parasitology - Quo Vadit? Proc 6th Int Cong Parasitol (Howell MJ, ed). Aust Acad Sci, Canberra: Abstract 36.

Augustine DL, 1927. Development in prenatal infestation of Belascaris. J Parasitol, 13: 256-9

Baer GM, 1975. Rabies in nonhematophagus bats. In: The natural history of rabies. (Baer GM, ed.) Vol II. Academic Press, NY: 79-97.

Baer JG, 1952. Ecology of animal parasites. University of Illinois Press, Urbana: 12.

Barron CN & Saunders LZ, 1966. Visceral larva migrans in the dog. Path Vet, 3: 315-30.

Barus V, Wiger R, Tenora F & Stanek M, 1979. Scanning electron microscopy of the lip denticles of Toxascaris leonina, Toxocara canis and T. cati (Nematoda). Vest Cesko Spol Zool, 43: 3-6.

Baylis HA, 1936. A new ascarid from a bat. Ann Mag Nat Hist, Ser 10, 17: 360-5.

Beautyman W, Beaver PC, Buckley JJC & Woolf AL, 1966. Review of a case previously reported as showing an ascarid larva in the brain. J Path Bact, 91: 271-3.

Beautyman W & Woolf AL, 1951. An Ascaris larva in the brain in association with acute anterior poliomyelitis. J Path Bact, 63: 635-47.

Beaver PC, 1952. Observations on the epidemiology of ascariasis in a region of high hookworm endemicity. J Parasitol,38: 445-53.

Beaver PC, 1956. Larva migrans. Exp Parasitol, 5: 587-621.

Beaver PC, 1962. Toxocarosis (Visceral Larva Migrans) in relation to Tropical Eosinophilia. Bulletin de la Societe de Pathologie Exotique, 55: 555-76.

Beaver PC, 1966. Zoonoses, with particular reference to parasites of veterinary importance. In: Biology of parasites (Soulsby EJL, ed). Academic Press, NY: 215-27.

Beaver PC, 1969. The nature of visceral larva migrans. J Parasitol, 55: 3-12.

Beaver PC, Jung RC & Cupp EW, 1984. Clinical parasitology, 9th ed. Lea & Febiger, Philadelphia: 325-9.

Beaver PC, Snyder CH, Carrera GM, Dent JH & Lafferty JW, 1952. Chronic eosinophilia due to visceral larva migrans. Pediatrics, 9: 7-18.

Beeson PB, 1980. The clinical significance of eosinophilia. In: The eosinophil in health and disease (Mahmoud AAF & Austen KF, eds). Grune & Stratton Inc, NY: 313-21.

Beeson PB, 1983. Eosinophilia and the hypereosinophilic syndromes. In: Oxford textbook of medicine (Weatherall DJ, Ledingham JGG & Warrell DA, eds). Oxford University Press, Oxford: 23.6-23.8.

Behrer MR, 1951. Hyperoesinophilia with eosinophilic granuloma of the liver associated with Ascaris infestation. J Pediat, 38: 635-40.

Bentley CA, 1902. On the causal relationship between "ground itch", or "pani-ghao", and the presence of the larvae of Ankylostoma duodenale in the soil. Brit Med J, 1: 190-3.

Bhowmick DK, 1964. Beitragezudem Problem der Wanderwege der Ascariden-larven (Ascaris lumbricoides Linnee 1758 und Toxocara canis Werner 1782) im experimentellen und naturlichen Wirt. Z Parasitenkde, 24: 121-68.

Bird AF, 1971. The egg. In: The structure of nematodes. Academic Press, NY: 279-300.

Bisseru B, 1969. Studies of the liver, lung, brain and blood of experimental animals infected with Toxocara canis. J Helminthol, 43: 267-72.

Bone LW, 1982a. Pheromone communication in parasitic helminths. In: Parasites - their world and ours (Mettrick DF & Desser SS, eds). Elsevier Biomedical Press: 138-40.

Bone LW, 1982b. Chemotaxis of parasitic nematodes. In: Cues that influence behaviour of internal parasites. Agricultural Research Service, US Dept Agr, New Orleans: 52-66.

Borg OA & Woodruff AW, 1973. Prevalence of infective ova of Toxocara species in public places. Brit Med J, 2: 470-472.

Boulard Y, Landau I, Miltgen F, Baccam D & Humphery-Smith I, 1985. Observations ultrastructurales sur la sporogonie d'Hepatocystis levinei, Haemoproteide, parasite de chiropteres australiens chez Culicoides nubeculosus. Protistologica, 21: 93-105.

Bourke ATC, Hawes RB, Neilson A & Stallman ND, 1983. An outbreak of hepato-enteritis (the Palm Island Mystery Disease) possibly caused by algal intoxication. Toxicon, Supp 3: 45-48.

Bremner KC, 1956. The parasitic life-cycle of Haemonchus placei (Place) Ransome (Nematoda: Trichostrongylidae). Aust J Zool, 4: 146-51.

Brindley PJ, Prociv P, Creevey CA & East IJ, 1985. Regulation of toxocariasis in mice selectively reared for high and low immune responses to Nematospiroides dubius. J Helminthol, 59: 157-66.

Brown HW & Cort WW, 1927. The egg production of Ascaris lumbricoides. J Parasitol, 14: 88-90.

Brown RC & Girardeau MHF, 1977. Transmammary passage of Strongyloides sp. larvae in the human host. Am J Trop Med Hyg, 26: 215-9.

Brygoo ER, Simond J-P & Mayoux AM, 1971. Les enterobacteries pathogenes chez Pteropus rufus (Megachiroptere) a Madagascar. Compte rendus des Seances de la Societe de Biologie et de ses filiales (Paris), 165: 1793-5.

Buckland-Wright JC & Pye JD, 1973. Dietary deficiency in fruit bats. Int Zoo Year Bk, 13: 271-7.

Bunch C, 1983. Leucocytes in health and disease. In: Oxford textbook of medicine (Weatherall DJ, Ledingham JGG & Warrell DA, eds). Oxford University Press: 19.84-19.89.

Burke TM & Roberson EL, 1985a. Prenatal and lactational transmission of Toxocara canis and Ancylostoma caninum: experimental infection of the bitch before pregnancy. Intl J Parasitol, 15: 71-5.

Burke MT & Roberson EL, 1985b. Prenatal and lactational transmission of Toxocara canis and Ancylostoma caninum: experimental infection of the bitch at midpregnancy and at parturition. Intl J Parasitol, 15: 485-90.

Burren CH, 1968. Experimental toxocariasis. I. Some observations on the histopathology of the migration of Toxocara canis larvae in the mouse. Z Parasitenkde, 30: 152-61.

Done JT, Richardson MD & Gibson TE, 1960. Experimental visceral larva migrans in the pig. Res Vet Sci, 1: 133-51.

Douglas JR & Baker NF, 1959. The chronology of experimental intrauterine infections with Toxocara canis (Werner, 1782) in the dog. J Parasitol, 45 (Suppl): 43-4.

Douglas JR & Baker NF, 1965. Some host-parasite relationships of canine helminths. In: McCauley JE (ed), Proc 26th Biol Colloquium, Corvallis, Oregon State University Press: 97-115.

Douvres FW, Tromba FG & Malakatis GM, 1969. Morphogenesis and migration of Ascaris suum larvae developing to the fourth stage in swine. J Parasitol, 55: 689-712.

Dubey JP, 1967. Egg production of Toxocara cati. Vet Rec, 81: 671-2.

Dubey JP, 1978. Patent Toxocara canis infection in ascarid-naive dogs. J Parasitol, 64: 1021-3.

Elleman TC, Falconer IR, Jackson ARB & Runnegar MT, 1978. Isolation, characterization and pathology of the toxin from a Microcystis aeruginosa (= Anacystis cyanea) bloom. Aust J Biol Sci, 31: 209-18.

Fagerholm HP & Lovdahl M, 1984. Induced morphometric variation in the preparation of nematode parasites for the LM and SEM. Syst Parasitol, 6: 241-55.

Falconer IR, Jackson ARB, Langley J & Runnegar MT, 1981. Liver pathology in mice in poisoning by the blue-green alga Microcystis aeruginosa. Aust J Biol Sci, 34: 179-87.

Falconer IR, Beresford AM & Runnegar MTC, 1983. Evidence of liver damage by toxin from a blue-green alga, Microcystis aeruginosa. Med J Aust, 1: 511-4.

Foor WE, 1967. Ultrastructural aspects of oocyte development and shell formation in Ascaris lumbricoides. J Parasitol, 53: 1245-61.

Foor WE, 1983. Nematoda. In: Reproductive biology of invertebrates. Vol I: Oogenesis, oviposition and oosorption. (Adiyodi KG, Adiyodi RG eds). John Wiley & Sons Ltd, NY: 223-56.

Foor WE & McMahon JT, 1973. Role of the glandular vas deferens in the development of Ascaris spermatozoa. J Parasitol, 59: 753-8.

Fox AS, Kazacos KR, Gould NS, Heydemann PT, Thomas C & Boyer KM, 1985. Fatal eosinophilic meningoencephalitis and visceral larva migrans caused by the raccoon ascarid Bayliscaris procyonis. N Eng J Med, 312: 1619-23.

Fulleborn F, 1921. Askarisinfektion durch Verzehren eingekapselter Larven und uber gelungene intrauterine Askarisinfektion. Arch fur Schiffs und Tropenhyg, 25: 367-75.

Fulleborn F, 1923. Biological considerations about the "wandering" of the larvae of parasitic nematodes in the body of the host. Arch fur Schiffs und Tropenhyg, 27: 444-52.

Gupta SC, 1986. Pattern and control of Neoascaris vitulorum infection in calves. Indian Vet J, 63: 71. (Cited: Helm Abs, 1986, Series A, 55: abs 3769).

Hall LS, 1987. Identification, distribution and taxonomy of Australian flying foxes (Chiroptera: Pteropodidae). Aust Mammal 10: 75-9.

Harris AH, Harkema R & Miller GC, 1967. Maternal transmission of Pharyngostomoides procyonis Harkema, 1942 (Trematoda: Diplostomatidae). J Parasitol 53: 1114-5.

Hartwich G, 1974. Keys to genera of the Ascaridoidea: CIH keys to the nematode parasites of vertebrates. No. 2 (RC Anderson, AG Chabaud, SM Willmott, eds). Commonwealth Agricultural Bureaux, Farnham Royal: 1-15.

Hawkins PR, Runnegar MTC, Jackson ARB & Falconer IR, 1985. Severe hepatotoxicity caused by the tropical cyanobacterium (blue-green alga) Cylindrospermopsis raciborskii (Woloszynska) Seenaya and Subba Raju isolated from a domestic water supply reservoir. Applied & Environmental Microbiology, 50: 1292-5.

Heydon GM, 1929. Creeping eruption or larva migrans in north Queensland and a note on the worm Gnathostoma spinigerum (Owen). Med J Aust, 1: 583-91.

Hill JE & Smith JD, 1984. Bats: a natural history. Rigby Publishers, Adelaide.

Humphery-Smith I, 1982. Biology of Trichostrongyloidea (Nematoda) and their Miniopterus (Chiroptera) hosts in eastern Australia. Ph D Thesis, Department of Parasitology, University of Queensland, Australia.

Irfan M & Sarwar M, 1954. Studies on the development of Ascaris vitulorum. Proc 6th Pakistan Sci Conf, Part III: 228.

Jackson E, Jackson F & Smith WD, 1984. Comparison of saline incubation and pepsin digestion as methods for recovering Ostertagia circumcincta larvae from the abomasum of sheep. Res Vet Sc, 36: 380-1.

Johnstone C, Leventhal R & Soulsby EJL, 1978. The spin method for recovering tissue larvae and its use in evaluating C57Bl/6 mice as a model for the study of resistance to infection with Ascaris suum. J Parasitol, 64: 1015-20.

Jung RC, 1954. The predominance of single-brood infections in human ascariasis. J Parasitol, 40: 405-7.

Kayes SG & Oaks JA, 1978. Development of the granulomatous response in murine toxocariasis. Am J Pathol, 93: 277-85.

Kazacos KR, 1986. Raccoon ascarids as a cause of larva migrans. Parasitol Today, 2: 253-5.

Kazacos KR & Turek JJ, 1982. Scanning electron microscopy of the labia of Baylisascaris procyonis (Nematoda). J Parasitol, 68: 634-41.

Kazacos KR & Turek JJ, 1983. Scanning electron microscopy of the eggs of Baylisascaris procyonis, B. transfuga, and Parascaris equorum, and their comparison with Toxocara canis and Ascaris suum. Proc Helminthol Soc Wash, 50: 36-42.

Kazacos KR, Wirtz WL, Burger PP & Christmas CS, 1981. Raccoon ascarid larvae as a cause of fatal central nervous system disease in subhuman primates. J Am Vet Med Assoc, 179: 1089-94.

Kean BH, Mott KE & Russell AJ (eds), 1978. Tropical medicine and parasitology: Classic investigations. Vol II: Helminths - Helminthic infections. Cornell University Press, Ithaca & London.

Kelley GW & Nayak DP, 1964. Acquired immunity to migrating larvae of Ascaris suum induced in pigs by repeated oral inoculations of infective eggs. J Parasitol, 50: 499-503.

Kelley GW, Olsen LS & Hoerlein AB, 1958. The influence of diet on the development of Ascaris suum in the small intestines of pigs. Am J Vet Res, 19: 401-4.

Kirby-Smith JL, Dove WE & White GF, 1926. Creeping eruption. Arch Derm & Syph, 13: 137-75.

Koopman KF, 1970. Zoogeography of bats. In: About bats: a chiropteran biology symposium (Slaughter BH & Walton DW, eds). Southern Methodist University Press, Dallas.

Krishnasamy M, Singh M & Iyamperumal R, 1981. Makifilaria inderi gen. et sp. nov. (Filarioidea: Onchocercidae) from the Island Flying Fox, Pteropus hypomelanus Temminck in Malyasia. SE Asian J Trop Med Pub Hlth, 12: 185-8.

Kuberski T, 1979. Eosinophils in the cerebrospinal fluid. Ann Int Med, 91: 70-5.

Larsh JE, 1950. Relationship in mice of intestinal emptying time and natural resistance to pig ascaris infection. Science, 111: 62-3.

Lee DL, 1982. Ecological physiology of parasites. In: Parasites - their world and ours (Mettrick DF & Desser SS, eds). Elsevier Biomedical Press: 125-34.

Lewis JH, 1977. Comparative hematology: Studies on Chiroptera, Pteropus giganteus. Comp Biochem Physiol, 58A: 103-7.

Looss A, 1901. Uber des eindringen der Ankylostomalarven in die menschliche Haut. Centralblatt fur Bakteriologie und Parasitenkunde, 29: 733-9.

Looss A, 1911. The anatomy and life-history of Agchylostoma duodenale Dub. A monograph. Records of the School of Medicine, Ministry of Education, Egypt, 3 parts: 159-613.

Lyons ET, Drudge JH & Tolliver SC, 1969. Parasites from mare's milk. The Blood-Horse, July 19, 1969: 2270-1.

Lyons ET, Drudge JH & Tolliver SC, 1970. Strongyloides larvae in milk of sheep and cattle. Mod Vet Pract, 51: 65-8.

Mackerras MJ, 1959. The haematozoa of Australian mammals. Aust J Zool, 7: 105-35.

Martin J, Keymer A, Isherwood RD & Wainwright SM, 1983. The prevalence and intensity of Ascaris lumbricoides infections in Moslem children from northern Bangladesh. Trans R Soc Trop Med Hyg, 77: 702-6.

Mason KV, Prescott CW, Kelly WR & Waddell AH, 1976. Granulomatous encephalomyelitis of puppies due to Angiostrongylus cantonensis. Aust Vet J, 52: 295.

Maung M, 1975. An ascaridoid nematode from a hippopotamus. Intl J Parasitol, 5: 431-39.

Maung M, 1978. The occurrence of the second moult of Ascaris lumbricoides and Ascaris suum. Intl J Parasitol, 8: 371-8.

Mercer RD, Lund HZ, Bloomfield RA & Caldwell FE, 1950. Larval ascariasis as a cause of chronic eosinophilia with visceral manifestations. Am J Dis Child, 80: 46-56.

Mettrick DF, 1986. Interactions between parasites and their hosts: metabolic aspects. In: Parasitology - Quo Vadit? Proc 6th Int Cong Parasitol (Howell MJ, ed.). Aust Acad Sci, Canberra: 111-7.

Mia S, Dewan ML, Uddin M & Chowdhury MUA, 1975. The route of infection of buffalo calves by Toxocara (Neoascaris) vitulorum. Trop Anim Hlth Prod, 7: 153-6.

Michel JF, 1974. Arrested development of nematodes and some related phenomena. Adv Parasitol, 12: 279-366.

Miller GC, 1981. Helminths and the transmammary route of infection. Parasitol, 82: 335-42.

Miller HRP, 1986. Vaccination against intestinal parasites. In: Parasitology - Quo Vadit? Proc 6th Int Cong Parasitol (Howell MJ, ed.). Aust Acad Sci, Canberra: 43-51.

Moncol DJ & Batte EG, 1966. Transcolostral infection in newborn pigs with Strongyloides ransomi. Vet Med, 61: 583-6.

Moorhouse DE, 1982. Toxocariasis: a possible cause of the Palm Island mystery disease. Med J Aust, 1: 172-3.

Mozgovoi AA, 1953. Essentials of nematology. Vol II, pt 2, Ascaridata of animals and man and the diseases caused by them. Monogr Ser Helm Lab, Acad Sci, Moscow Acad Sci Publ.

Muhleisen JP, 1953. Demonstration of pulmonary migration of the causative organism of creeping eruption. Ann Int Med, 38: 595-9.

Naidu TSV, 1981. Two new ascarid nematodes from vertebrate hosts from India. Folia Parasitol (Praha), 28: 327-34.

Nelson JE, 1965a. Behaviour of Australian Pteropodidae (Megachiroptera). Animal Behaviour, 13: 544-57.

Nelson JE, 1965b. Movements of Australian flying foxes (Pteropidae: Megachiroptera). Aust J Zool, 13: 53-73.

Nichols RL, 1956. The etiology of visceral larva migrans. I. Diagnostic morphology of infective second-stage Toxocara larvae. J Parasitol, 42: 349-62.

Noda R, 1956. Survey of the incidence of ascarid, Toxocara canis in dogs, with special reference to the postnatal infection as compared with prenatal infected. Bull Univ Osaka Prefec, Ser B, 6: 65-72.

Noda R, 1958. Some observations on the experimental Toxocara canis infections in young and adult dogs. Bull Univ Osaka Pref, Ser B, 8: 73-85.

Okoshi S & Usui M, 1968. Experimental studies on Toxascaris leonina. IV Development of eggs of three ascarids, T. leonina, Toxocara canis and Toxocara cati, in dogs and cats. Jap J Vet Sci, 30: 29-38.

Olsen OW & Lyons ET, 1965. Life cycle of Uncinaria lucasi Stiles, 1901 (Nematoda: Ancylostomatidae) of Fur Seals, Callorhinus ursinus Linn., on the Pribilof Islands, Alaska. J Parasitol, 51: 689-700.

Orihel TC, 1970. Primates as models for parasitological research. In: Medical primatology. Proc 2nd Conf Exp Med Surg Primates, NY, 1969, Karger, Basel: 772-82.

Oshima T, 1976. Observations of the age resistance, eosinophilia and larval behaviour in the helminth-fee beagles infected with Toxocara canis. Jap J Parasitol, 25: 27-35.

Otto GF, 1929. A study of the moisture requirements of the eggs of the horse, the dog, human and pig ascarids. Am J Hyg, 10: 497-520.

Otto GF, 1930. Ascaris lumbricoides: treatment, loss of worms and reinfestation. J Am Med Assoc, 95: 194-6.

Pal SR, Arora B, Chhuttani PN, Broor S, Choudhury S, Joshi RM & Ray SD, 1980. Rabies virus infection of a flying fox bat, Pteropus poliocephalus (sic) in Chadigarh, Northern India. Trop Geogr Med, 32: 265-7.

Parona C, 1889. Sopra alcuni elminti di vertebrati birmani raccolti da Leonardo Fea. Ann Mus Civ Stor Nat Genova, Ser 2, 7: 763-80.

Pawlowski ZS, 1978. Ascariasis. Clinics in Gastroenterology, 7: 157-78.

Perlingiero JG & Gyorgy P, 1947. Chronic eosinophilia: report of a case with necrosis of the liver, pulmonary infiltrations, anemia and Ascaris infestation. Am J Dis Child, 73: 34-43.

Pettigrew JD. 1985. Flying primates? Megabats have the advanced pathway from eye to midbrain. Science, 231: 1304-6

Phills JA, Harrold AJ, Whiteman GV & Perelmutter L, 1972. Pulmonary infiltrates, asthma and eosinophilia due to Ascaris suum infestation in man. New Eng J Med, 286: 965-9.

Postlethwaite AE, Jackson BK, Beachey EH & Kang AH, 1982. Formation of multinucleated giant cells from human monocyte precursors. J Exp Med, 155: 168-78.

Radcliffe-Crocker H, 1907. Diseases of the skin. 3rd ed, Vol II. P Blakiston's Son & Co, Philadelphia.

Ransom BH & Foster WD, 1917. Life history of Ascaris lumbricoides and related forms. J Agr Res, 11: 395-8.

Ratcliffe FN, 1931. The flying fox (Pteropus) in Australia. Bulletin of the Council for Scientific and Industrial Research of Australia, No. 53: 1-80.

Refuerzo PG & Albis-Jimenez FS, 1954. Studies on Neoascaris vitulorum. III. Further observations on inoculation of calves with notes on prenatal infection. Am J Vet Res (Oct): 532-4.

Refuerzo PG, Albis-Jimenez FS & De La Cerna G, 1954. Studies on Neoascaris vitulorum (Goeze, 1782) I. Observations on the morphology of the ova and on their development to the infective stage with notes on feeding experiments. Philippine J Anim Ind, 13: 15-23.

Reid LA, 1985. Studies on Hepatocystis pteropi and Trypanosoma pteropi. BSc (Hons) Thesis, Department of Parasitology, University of Queensland.

Richards GC, 1983. Bats. In: The Australian Museum Complete Book of Australian Mammals (R Strahan, ed). Angus & Robertson, Sydney: 271-3.

Robbins SL, Cotran RS & Kumar V, 1984a. Inflammation and repair. In: Pathologic basis of disease (3rd edn). WB Saunders Co, Philadelphia: 40-84.

Robbins SL, Cotran RS & Kumar V, 1984b. The liver and biliary tract. In: Pathologic basis of disease (3rd edn). WB Saunders Co, Philadelphia: 884-959.

Roberts FHS, 1934. The large roundworm of pigs, Ascaris lumbricoides L., 1758. Its life history in Queensland, economic importance and control. Yeerongpilly Animal Health Station Bulletin No. 1, Qld Dept Agr & Stock.

Roneus O, 1971. Studies on the inter-relationship between the number of orally administered A. suum eggs, blood eosinophilia and the number of adult intestinal ascarids. In: Pathology of parasitic diseases (Proc 4th Int Conf WAAVP, Glasgow, 1969) Purdue University Studies, Lafayette: 339-43.

Rookmaaker LC & Bergmans W, 1981. Taxonomy and geography of Rousettus amplexicaudatus (Geoffroy, 1810) with comparative notes on sympatric congeners (Mammalia, Megachiroptera). Beaufortia, 31: 1-29.

Rosen L, Chappell R, Laqueur CL, Wallace GD & Weinstein PP, 1962. Eosinophilic meningoencephalitis caused by a metastrongylid lungworm of the rat. J Am Med Assoc, 179: 620-4.

Schacher JF, 1957. A contribution to the life history and larval morphology of Toxocara canis. J Parasitol, 43: 599-612.

Schmidt GD & Roberts LS, 1985. Phylum Nematoda: Form, function and classification. In: Foundations of parasitology. Ch 22. Times Mirror/Mosby College Publishing, St Louis: 409-49.

Schwartz B, 1959. Experimental infection of pigs with Ascaris suum. Am J Vet Res, 20: 7-13.

Schwimmer M & Schwimmer D, 1968. Medical aspects of phycology. In: Algae, man and the environment. (Jackson DF, ed.). Syracuse University Press, Syracuse: 279-358.

Setasuban P, Punsri W & Meunnoo C, 1980. Transmammary transmission of Necator americanus larva in the human host. SE Asian J Trop Med Publ Hlth, 11: 535-8.

Shillinger JE & Cram EB, 1923. Parasitic infestation of dogs before birth. J Am Vet Med Assoc, 63: 200-3.

Shoho C, 1970. Finding of the nematode larvae in the fresh cow milk during the post-parturitional period. In: Proc 2nd Int Cong Parasitol, 1970. J Parasitol, 56(4) (Suppl), Sect II, Part I: 318.

Sinniah B, 1982. Daily egg production of Ascaris lumbricoides: the distribution of eggs in the faeces and the variability of egg counts. Parasitology, 84: 167-75.

Smith MHD & Beaver PC, 1953. Persistence and distribution of Toxocara larvae in the tissues of children and mice. Pediat, 12: 491-7.

Smith JW & Wootten R. 1975. Experimental studies on the migration of Anisakis sp. larvae (Nematoda: Ascarida) into the flesh of the herring, Clupea harengus L. Intl J Parasitol, 5: 133-6.

Soulsby EJL, 1957. Immunization against Ascaris lumbricoides in the guinea pig. Nature, 179: 783-4.

Soulsby EJL, 1961. Some aspects of the mechanism of immunity to helminths. J Am Vet Med Assoc, 138: 355-61.

Spillmann RK, 1975. Pulmonary ascariasis in tropical communities. Am J Trop Med Hyg, 24: 791-800.

Sprent JFA, 1953. On the life history of Ascaris devosi and its development in the white mouse and domestic ferret. Parasitol, 42: 244-58.

Sprent JFA, 1954. The life-cycles of nematodes in the family Ascarididae Blanchard 1896. J Parasitol, 40: 608-17.

Sprent JFA, 1955. On the invasion of the central nervous system by nematodes. I. The incidence and pathological significance of nematodes in the central nervous system. Parasitol, 45: 31-40.

Sprent JFA, 1956. The life-history and development of Toxocara cati (Schrank 1788) in the domestic cat. Parasitol, 46: 54-78.

Sprent JFA, 1957. The development of Toxocara canis (Werner, 1782) in the dog. J Parasitol, 43: Suppl p45.

Sprent JFA, 1958. Observations on the development of Toxocara canis (Werner, 1782) in the dog. Parasitol, 48: 184-209.

Sprent JFA, 1959. The life history and development of Toxascaris leonina (von Linstow 1902) in the dog and cat. Parasitol, 49: 330-71.

Sprent JFA, 1961. Post-parturient infection of the bitch with Toxocara canis. J Parasitol, 47: 284.

Sprent JFA, 1962. The evolution of the ascaridoidea. J Parasitol, 48: 818-24.

Sprent JFA, 1963. Visceral larva migrans. Aust J Sci, 25: 344-54.

Sprent JFA, 1968. Notes on Ascaris and Toxascaris with a definition of Baylisascaris gen.nov. Parasitology, 58: 185-98.

Sprent JFA, 1972. Toxocara vajrasthirae sp. nov. from the hog-badger (Arctonyx collaris) of Thailand. Parasitol, 65: 491-8.

Sprent JFA, 1982. Host-parasite relationsips of ascaridoid nematodes and their vertebrate hosts in time and space. Mem Mus Nat d'Hist Nat Paris Ser A, Zool, Tome 123.

Sprent JFA, 1983. Observations on the systematics of ascaridoid nematodes. Pp 303-19. In: Systematics Association Special Vol 22, Concepts in nematode systematics (Stone AR, Platt HM & Khalil, eds), Academic Press, London & NY.

Sprent JFA & English PB, 1958. The large roundworms of dogs and cats - a public health problem. Aust Vet J, 34: 161-71.

Stevenson P, 1979. Toxocara and ascaris infection in British pigs: a serological survey. Vet Rec, June 9: 526-8.

Stewart FH, 1916. On the life history of Ascaris lumbricoides. Brit Med J, 2: 5-7.

Stone WM & Girardeau MH, 1967. Transmammary passage of infective-stage nematode larvae. Veterinary Medicine/Small Animal Clinician, 62: 252-3.

Stone WM & Girardeau MH, 1968. Transmammary passage of Ancylostoma caninum larvae in dogs. J Parasitol, 54: 426-9.

Stone WM & Smith FN, 1973. Infection of mammalian hosts by milk-borne nematode larvae: A review. Exp Parasitol, 34: 306-12.

Stoye M, 1976. Galaktogene und pranatale Infektionen mit Toxocara canis beim Hund (Beagle). Deutsche Tierarztliche Wochenschrift, 83: 107-8.

Sulkin SE & Allen R, 1970. Bats: carriers of human disease-producing agents. In: About bats: a chiropteran biology symposium (Slaughter BH & Walton DW, eds). Southern Methodist University Press, Dallas: 284-302.

Swerczek TW, Nielsen SW & Helmboldt CF, 1971. Transmmary passage of Toxocara cati in the cat. Am J Vet Res,

Tamura H, 1921. On creeping disease. Brit J Dermatol & Syphilis, 33: 81-102, 138-51.

Tedman RA & Hall LS, 1985. The morphology of the gastrointestinal tract and food transit time in the fruit bats Pteropus alecto and P. poliocephalus (Megachiroptera). Aust J Zool, 33: 625-40.

Thayer WS, 1897. On the increase of the eosiniphilic cells in the circulating blood in trichinosis. Lancet, 2: 787-8.

Thienpont D & De Keyser H, 1981. Toxocariasis of cattle in Belgium. In: Current topics in veterinary medicine and animal science. Epidemiology and control of nematodiasis in cattle (Nansen P, Jorgensen RJ & Soulsby EJL, eds). Martinus Nijhoff Publishers, The Hague. 9:569-581.

Tiner JD. 1953. The migration, distribution in the brain, and growth of ascarid larvae in rodents. J Inf Dis, 92: 105-13.

Tomimura T, Yokota M & Takiguchi H, 1976. Experimental visceral larva migrans in monkeys. I. Clinical, haematological, biochemical and gross pathological observations on monkeys inoculated with embryonated eggs of the dog ascarid Toxocara canis. Jap J Vet Sci, 38: 533-48.

Ubelaker JE, 1970. Some observations on ecto- and endo-parasites of Chiroptera. In: About bats: A Chiropteran Biology Symposium (Slaughter PH & Walton DW, eds) Southern Methodist University Press, Dallas: 247-61.

Ubelaker JE & Allison VF, 1975. Scanning electron microscopy of the eggs of Ascaris lumbricoides, A. suum, Toxocara canis, and T. mystax. J Parasitol, 61: 802-7.

Uhlikova M & Hubner J, 1982. A study on the morphology of early larval stages of Toxocara cati (Schrank, 1788). Folia Parasitologica, 29: 165-6.

Unanue ER, 1978. The immune granulomas. In: Immunological diseases, 3rd Edn, Vol 1 (Samter M, ed.). Little, Brown & Co: 297-306.

Uni S & Takada S, 1975. The surface structure of sense organs of Toxocara cati (Schrank, 1788) viewed with the scanning electron microscope. Jap J Parasitol, 24: 365-74.

Van Thiel PH, 1966. The final hosts of the herring worm Anisakis marina. Trop Geogr Med, 18: 310-28.

Van Thiel PH, Kuipers FC & Roskam TH, 1960. A nematode parasitic to herring, causing acute abdominal syndromes in man. Trop Geogr Med, 12: 97-113.

Vesselinovitch D, 1979. Animal models of atherosclerosis, their contributions and pitfalls. Artery, 5: 193-206.

Wakelin D, 1978. Immunity to intestinal parasites. Nature 273(Suppl): 617-20.

Wakelin D, 1984. Immunity to parasites: how animals control parasitic infections. Edward Arnold Ltd, London.

Wakelin D & Donachie AM, 1983. Genetic control of eosinophilia. Mouse strain variation in response to antigens of parasite origin. Clin Exp Immunol, 51: 239-46.

Warren EG, 1969. Nematode larvae in milk. Aust Vet J, 45: 388.

Warren EG, 1971a. Studies on the morphology and taxonomy of the genera *Toxocara* Stiles, 1905 and *Neoascaris* Travassos, 1927. Zool Anz, 185: 393-442.

Warren EG, 1971b. Observations on the migration and development of *Toxocara vitulorum* in natural and experimental hosts. Intl J Parasitol, 1: 85-99.

Warren EG, 1972. Two new species of *Toxocara* from viverrid hosts. Parasitol, 65: 179-87.

Webster GA, 1958. On prenatal infection and the migration of *Toxocara canis* Werner, 1782, in dogs. Canadian J Zool, 36: 435-40.

Weller PF & Goetzl EJ, 1979. The regulatory and effector roles of eosinophils. Adv Immunol, 27: 339-71.

Wharton DA, 1979. *Ascaris* sp.: water loss during desiccation of embryonating eggs. Exp Parasitol, 48: 398-406.

Wharton D, 1980. Nematode egg-shells. Parasitology, 81: 447-63.

White GF & Dove WE, 1928. The causation of creeping eruption. J Am Med Assoc, 90: 1701-4.

Wilder HC, 1950. Nematode endophthalmitis. Trans Am Acad Ophthalmol Otolaryngol, 55 (Nov-Dec 1950): 99-109.

Wiseman RA & Woodruff AW, 1967. *Toxocara* skin sensitivity tests and other observations in animals experimentally infected with *Toxocara canis*. Trans R Soc Trop Med Hyg, 61: 827-33.

Yutuc LP, 1949. Prenatal infection of dogs with ascarids, *Toxocara canis* and hookworms, *Ancylostoma caninum*. J Parasitol, 35: 358-60.

Zamirdin M & Wilson PAG, 1974. *Strongyloides ratti*: relative importance of maternal sources of infection. Parasitology, 69: 445-53.

Zervos S, 1986. How and why parasitic hind gut thelastomid nematodes regulate their own populations. In: Parasitology - *Quo Vadit*? Proc 6th Int Cong Parasitol (Howell MJ, ed.) Aust Acad Sci, Canberra: Abstract 740.

APPENDIX I

Z Parasitenkd (1983) 69:773–781

Zeitschrift für
Parasitenkunde
Parasitology Research

© Springer-Verlag 1983

Observations on the Transmission and Development of *Toxocara pteropodis* (Ascaridoidea: Nematoda) in the Australian Grey-Headed Flying-Fox, *Pteropus poliocephalus* (Pteropodidae: Megachiroptera)

P. Prociv

Department of Parasitology, University of Queensland, St. Lucia, Queensland 4067, Australia

Abstract. Findings in the Australian Grey-Headed Flying-Fox, *Pteropus poliocephalus*, have elucidated the life-cycle of *Toxocara pteropodis*. In adult bats, other than parturient females, larvae were found only in the livers. Following parturition, larvae were recovered only from mammary glands up to 2 weeks post-partum. Developing larvae were found only in the intestine of young bats from the age of two days onwards; there was no evidence of pulmonary migration.

The evidence indicates that juvenile bats commence passing *Toxocara* eggs in their faeces at about 2 months of age and expel the worms spontaneously following weaning at about 5 months. The eggs passed in the faeces of the young bat and its mother are disseminated throughout their environment and embryonate rapidly, being infective to mice after 10 days. Under natural conditions the eggs remain viable for 6 weeks or less and are infective to bats by the oral route.

Introduction

Baylis (1936) described *Toxocara pteropodis* from the intestine of a suckling fruit-bat, *Pteropus geddiei*, in the New Hebrides (now Vanuatu). Since then the only recorded work on this species was by Warren (1971a) who reviewed the genus *Toxocara* and added measurements from seven paratype specimens to Baylis' original description. The life-cycle of this nematode has remained unknown.

The present work results from the suggestion of Moorhouse (1982) that an outbreak of acute hepatitis/gastroenteritis amongst the children of Palm Island in November 1979 (Byth 1980) may have been a manifestation of epidemic visceral *larva migrans* resulting from the ingestion of *T. pteropodis* eggs, which he identified from contaminated mangoes.

In a number of *Toxocara* life-cycles, adult worms occur in the intestines of juvenile hosts. In *T. canis*, transmission of infection to the pup occurs transplacentally (Sprent 1958) while in *T. vitulorum* of cattle it occurs shortly

after birth, through milk (Warren 1971b). The transmission of *T. pteropodis* is now described.

Materials and Methods

Host Biology

The distribution and seasonal behaviour of the four Australian *Pteropus* species have been studied by Ratcliffe (1931) and Nelson (1965a, b). The young of the Grey-Headed Flying-Fox (*P. poliocephalus*) are born in October when this species is aggregating in large "summer camps". At first, the young bat suckles almost continuously, but from about 4 weeks of age it is left behind in the camp at night as the mother flies off to feed. At about 3 months of age the juvenile begins to accompany its mother on feeding flights and is totally independent by 5 months. Adults copulate in March–April, and shortly thereafter the summer camps disperse.

Sources of Material

This study commenced in August 1981 when a winter camp of several thousand *P. poliocephalus* was located in rainforest 50 km south-east of Brisbane. Being a protected species, only a limited number could be obtained by shooting. Specimens were also received from the local animal shelter and from linesmen who removed numerous dead bats from power lines, often with live offspring still attached. Between January and April 1982 live juveniles, some with mothers, were caught in a mangrove swamp.

Determination of Age

Because of a great variation in birth weight (Nelson 1965b) the weight and forearm length of the young bat does not provide a useful indication of its age. Hence the ages of these animals, which are approximations only, were determined by considering the maturation of the umbilical stump. development of body hair, general appearance and behaviour as well as weight and forearm length.

Examination of Tissues

Freshly dead adult animals were weighed, measured and dissected. The intestine was opened in saline and examined macroscopically for parasites. It was then cut into small pieces with scissors and placed into trypsin solution (trypsin 0.4 mg, NaCl 0.8 g, $NaHCO_3$ 1.0 g, water to 100 ml). The liver, lungs, kidneys, spleen, heart, brain and samples of pectoral muscle and diaphragm were minced separately with scissors into pieces roughly 2–3 mm in diameter, placed into 50 ml trypsin solution and incubated overnight at 37° C. Each sample was then centrifuged and the supernatant discarded. To the sediment was added an equal volume of 10% formalin solution. Sediments were then scanned under a stereo-microscope for larvae.

In pregnant females the mammary glands, uteri and placentae were treated similarly, as were the foetal lungs, hearts, livers and intestines.

The intestines of young bats were first syringed out with saline, and then the gut mucosa was scraped with the back of a scalpel blade into saline, these washings then being examined for larvae. The gut wall and other organs were digested in trypsin as described above.

Examination of Faeces

Juveniles were kept in small cages and maintained on a diet of double strength skimmed cow's milk, supplemented with sugar and vitamins, until they had developed sufficiently to eat fruit. Faeces were examined microscopically for the presence of eggs. Animals passing *Toxocara* eggs were placed in a separate cage. Their faeces were collected daily, shaken up with tap water and washed through a nest of sieves, the mesh in the final sieve being 50 µm. To obtain the eggs, this last sieve was backwashed with saline and the eggs and residual fruit pulp allowed to settle for about 1 h before the supernatant was discarded. Daily egg

output was calculated by counting the eggs in a small sample of sediment. The sediment was mixed into charcoal on Petri dishes and stored, or used for egg embryonation studies.

Adult worms were identified as *T. pteropodis* by Dr. D.E. Moorhouse and Professor J.F.A. Sprent.

Results

Findings in Pteropus poliocephalus

Adult males. Between August and December 1981 26 males, weighing 445–875 g, were examined. No worms were found in the intestines. Larvae (1–455), subsequently identified as *T. pteropodis,* were recovered from the livers of 14 (54%). The larvae from five male livers were measured and no relationships were found between host body weight and mean length of larvae nor between larval lengths and numbers recovered. There was a positive correlation ($r = 0.45$) between the number of larvae recovered and the body weight.

Non-pregnant adult females. Only five specimens were obtained between August and November. Four were nulliparous weighing 420–560 g. The heaviest had only two larvae in its liver, the others being negative. One parous 580 g female, which was neither lactating nor pregnant, was also free of larvae.

Pregnant adult females. Eleven were examined between August and November at stages of pregnancy estimated between 1 and 6 months (i.e. full-term). In five no larvae were found in any tissue. The remainder yielded from 2 to 31 larvae from their livers. Larvae were not found in the mammary glands, uteri, placentae nor in any foetal organs. In addition, five aborted foetuses were examined without larvae being detected in any tissues.

Mothers with neonates (less than 4 weeks old.). In October and November 1981, twelve females and their young were examined. Larvae were found in the mammary glands of two mothers, but not in any other organs. One of these mothers had a single third-stage larva (706 × 32 µm) in its mammae and its baby (approximately 2 days old) harboured one third-stage larva (870 × 37 µm) in its intestine. The other infected female, which died at capture, yielded six larvae (length 746–955 µm) from its mammae. Her baby died 2 weeks later and two larvae (approximately 5 mm long, presumably fourth stage) were recovered from its intestine. Of the ten uninfected mother bats, four had juveniles aged 2–4 weeks that harboured between four and six developing larvae in their small intestines. These are included in Table 2.

Orphaned juveniles (October–December 1981). Twelve orphaned juvenile bats aged between 2 days and 10 weeks were obtained. Some of these had been hand-reared for varying periods before dying, predominantly from pulmonary infections. Two, three and eleven developing adult worms were

recovered from the intestines of three of these bats (two are included in Table 2). Larval worms were not found in any organs.

Mothers with juveniles (January–March 1982). Rectal swabbing of 46 juvenile bats aged between 2 and 4 months revealed that 16 (34%) were passing eggs of *T. pteropodis* (diam. 102 ± 8 mm). Eight of these infected young were with their mothers, and in each case eggs were also detected in the mother's faeces, although in much smaller numbers than in the respective baby's faeces. One young bat was passing infertile eggs and similar infertile eggs were found in its mother's faeces. Administration of piperazine citrate (600 mg in milk with sugar) to each of three mother bats which had infected young did not clear the eggs from their faeces. Piperazine citrate (300 mg) was then administered to the offspring resulting in the expulsion of adult worms with the subsequent disappearance of eggs from the faeces of both offspring and mother. Separation of babe from mother resulted in the clearance of eggs from the mother's faeces, the time before clearance depending on the mother's diet. When adult or juvenile flying-foxes were fed juicy fruit (e.g. peaches, mangoes, rockmelons, papayas) identifiable fruit pulp was passed *per rectum* within 20 min. A mother bat on such a diet ceased passing eggs within 30 min of being separated from its infected young.

Diet and infection. Many young bats given free access to fruit (including those on mothers given fruit) expelled live adult worms rapidly, often within 12 h of capture. Those maintained on a milk and sugar diet usually retained their worms, sometimes for several weeks. One juvenile female, about 4 months old (wt 260 g), passed eggs for 14 days while suckling, then expelled one female worm the day after its mother escaped. On a milk diet it continued passing eggs for a further 11 days then expelled another live female and live male worm after apples were introduced to the diet.

Development of Eggs of *T. pteropodis*

By collecting all faeces passed and subsequently recovering all worms from three juvenile bats it was estimated that one adult female worm released 20,000–30,000 eggs per 24 h, intermittently rather than continuously. Eggs were at the one-cell stage when released in the faeces and cell division commenced within several hours of being passed. Motile larvae were present in some eggs by day 5 and by 10 days virtually all eggs were fully embryonated. Eggs embryonated at ambient room temperature (average daily range 20–26° C) on moist charcoal, as well as on fruit (green and ripe), on plant leaves, on filter paper and even on glass slides left exposed to the air, without any significant difference in rates of development on these substrates.

Bat faecal "sludge" (eggs, concentrated by sieving, with a large residual volume of fruit pulp) was painted onto leaves of palm, eucalypt, avocado and mango trees. By day 10, eggs were infective to mice as shown by the recovery of larvae from the liver of a grey mouse killed 6 days after being

Table 1. Embryonation and growth of larvae of *Toxocara pteropodis*

Origin and age of larvae	Number of larvae measured	Length $\bar{x} \pm$ S.D. (μm)	Range in length (μm)
Eggs embryonated in charcoal 10 days	51	350 ± 50	220–450
Eggs embryonated in charcoal 30 days	43	400 ± 40	320–490
Gut wall experimental adult bat infected over 10 days (see text)	12	425 ± 25	380–460
Liver of same bat at 10 days	43	445 ± 40	365–525
Liver 3 weeks after infection[a]	48	460 ± 40	380–525
Liver 6 weeks after infection[a]	69	560 ± 30	520–665
Liver 11 weeks after infection[a]	39	640 ± 45	555–730
Liver 6 months after infection[a]	41	640 ± 50	550–730
Mammary glands	5	815 ± 105	705–955

[a] Juveniles infected experimentally

fed these eggs. Studies on infection in mice will be reported separately. The period of viability on trees varied; those surviving longest were eggs coated onto the upper surface of mango leaves and found infective to mice 6 weeks later. Throughout this period the leaves had been exposed to an estimated 8 h of dappled sunlight daily and a total rainfall of 250 mm.

Experimental Infection in *Pteropus poliocephalus*

An adult female (wt 720 g) was fed 500 infective eggs each day for 10 days. The total white cell count on the day prior to feeding eggs was 3.9×10^9/litre (eosinophils 10%) and on day 11 was 4.7×10^9/litre (eosinophils 36%). On day 11 this bat was killed with intraperitoneal pentobarbitone and its organs were examined for larvae. No larvae were recovered from the lumen of the gastrointestinal tract or from the stomach, duodenum or the upper one-third of the intestine. One larva was digested from the middle third of the intestine, and 12 from the lower third. None was found in the mesentery or mesenteric lymph nodes. From the liver 194 larvae were recovered, similar to larvae of naturally infected bats but shorter in length (see Table 1).

Fifteen captive juveniles were fed infective eggs in milk. Some of these bats died after varying time intervals from unrelated causes and were dissected and examined as described above. Larvae were found only in their livers, and the results are shown in Table 1.

Discussion

Infection of Neonates

These findings indicate that *T. pteropodis* undergoes transmammary transmission which occurs within 2 weeks of the birth of the young. The evidence suggests that all the larvae in the mother bat's liver migrate to the mammary glands at about the time of parturition. These larvae then pass in the milk

Table 2. Growth of *Toxocara pteropodis* in juvenile *P. poliocephalus* intestine (natural infection)

Estimated age of host bat	Weight (grams)	No. of larvae recovered	Length (mm) mean ± S.D.	Range in length	Range in body width
2 days	75	1	0.81	0.81	0.037
3 weeks	100[a]	2	approx. 5	nm	nm
3–4 weeks	85[a]	4	7.22 ± 0.53	6.69– 7.75	0.16–0.18
3–4 weeks	100	6	8.58 ± 1.32	7.34– 10.30	0.13–0.18
4–5 weeks	75[a]	6	9.40 ± 3.35	5.33– 13.00	0.09–0.23
4–5 weeks	75[a]	6	9.52 ± 2.31	6.23– 13.09	0.12–0.29
7–9 weeks	120[a]	2[b]	64.9 ± 6.3	58.6 – 71.2	0.88–1.21
7–9 weeks	150[a]	6 F	51.0 ± 21.0	11.0 – 68.7	0.22–0.90
		5 M	41.0 ± 22.7	8.36– 62.3	0.15–0.91
3 months	180–210	5 F[c]	117 ± 8	107 –128	1.70–2.19
(total of 5 bats)		7 M[c]	78.5 ± 10.6	67.8 – 96.0	1.13–1.66

nm = not measured; F = female; M = male

[a] Hand reared for varying periods before dying, so weight often much lower than normal
[b] Both females, the longer (71.2 mm) containing fully developed but unfertilized eggs in undivided segment uterus
[c] All fully mature with females containing developed fertile eggs

to the neonate in the first few days after birth, and perhaps over a period as long as 2 weeks. Mother bats with offspring older than 2 weeks did not have larvae in their mammary glands. Once in the young bats' intestine the larvae grow rapidly (Table 2). Developing worms in one animal sometimes vary greatly in their lengths. This may reflect the prolonged period of transmission of larvae in the milk, or individual variations in the worms. Larvae were never found in the lungs of young bats indicating that tracheal migration does not occur. Developing worms attained maturity and commenced producing eggs at about 2 months. Individual worm burdens were small, with many juveniles harbouring only one or two worms. Eleven was the largest number found in one bat.

Infection of Adults

The mode of acquisition of infection by the adult bat in nature has not been confirmed. At 2 months of age, when the first eggs are appearing in their faeces, the young bats are being left behind at night in the camp by their mothers. They avoid self contamination by inverting to defaecate or urinate, and groom themselves frequently. At night the juveniles also indulge in mutual grooming while the parents are away feeding. When the mothers return before dawn the young climb onto their chests and commence suckling. The juveniles then appear to defaecate indiscriminately, so that the mothers frequently groom their offspring and ingest most of the faeces produced. Hence the trees, foliage and ground within the camp and its surroundings become heavily contaminated with *Toxocara* eggs deposited in the faeces of infected juveniles and their mothers. The mother

bats would disperse the eggs more widely when they fly off to feed at night. However, this does not explain how the mothers acquire larvae, as the eggs take at least 10 days to become infective. For a worm which occurs in relatively small numbers in its host and produces relatively small numbers of eggs, survival could not depend on widespread dispersal in the environment by a host which rarely, if ever, alights on the ground. Although the mother bats defaecate while feeding, only a small proportion of their faeces actually drops onto the fruit in the tree. Some contaminates foliage, but most falls on the ground. As the fruit is usually ripe, it is unlikely that there will be any left to eat 10 days later when the eggs are fully embryonated, unless green fruit is also heavily contaminated, which would seem unusual but which obviously occurred at Palm Island (Moorhouse 1982). Blossom forms a much greater component of the flying-fox's diet (Ratcliffe 1931) and the chance of this being contaminated is even more remote. Furthermore, it appears that the mother bat passes eggs in her faeces only for a short time after leaving her offspring at dusk. This suggests that the infection of adults occurs in the camps, where the environment is much more heavily saturated with eggs. The intervention of an intermediate host is unlikely, as flying-foxes eat only blossom or fruit (Nelson 1965b), and adult and juvenile bats are readily infected by eggs which have developed in the faeces.

The most likely explanation is that flying-foxes acquire infection via accidental coprophagy. For many herbivores faeces is the major source of vitamin B12 and other B-group vitamins, and coprophagy in animals has been recognized for many years (Mickelsen 1956). Green et al. (1975) produced neurological changes due to vitamin B12 deficiency in the Egyptian fruit-bat *Rousettus aegyptiacus* by feeding the animals clean, peeled, pest-free fruit. Coprophagy was not observed in their study.

Nelson (1965a) observed that *P. poliocephalus* chewed leaves of trees in their camps, and in the present study bats were seen chewing and eating leaves of trees growing in their cages. During rainstorms wild bats were seen to lick water from leaves and captive animals licked the wet netting of their cages.

Caged animals lost interest in fruit contaminated with fresh faeces, but showed less aversion to stale faeces which were less offensive and presumably contained more vitamins from microbial sources.

Viability and Fate of Eggs

Eggs were shown to be relatively resistant to sunlight and not readily washed off leaves by rain. Nevertheless, their infectivity in the natural environment is very short-lived compared with eggs of other ascaridoid species.

After infective *T. pteropodis* eggs are eaten by the bat, larvae hatch in the intestine, penetrate the wall mainly of the lower third as shown in the experimental animal, and pass presumably via the portal venous system into the liver where they remain and grow in size. In males this is a dead-end, and with the passage of years large numbers of larvae may accumulate.

Period of Infection

The evidence indicates that intestinal worms and the associated dissemination of viable eggs leading to infection occur seasonally. Worms have never been found in the gut of fully weaned or adult bats. In this study, the prevalence of infection amongst juvenile bats was found to fall noticeably in autumn (March–April) which is the time of weaning. Live adult worms have been found on the forest floor in bat camps at about this time (Rhys Puddicombe, personal communication). Larvae, presumably third-stage, recovered from the livers of flying-foxes in winter are fully-developed and represent long-term infection.

As occurs with *T. canis* in dogs (Dubey 1978) intestinal worms are expelled spontaneously by the growing host, a phenomenon which has not been explained. The findings of the present study suggest that the rapid change in the captured juvenile bat's diet from predominantly milk to mainly fruit leads consistently to the expulsion of worms, perhaps through a cathartic effect. Under natural conditions a similar end result is achieved but more gradually.

Infection of Related Species and Humans

Baylis (1936) described *T. pteropodis* from *P. geddiei* in Espiritu Santo, New Hebrides. Moorhouse (1982) identified its eggs from mangoes contaminated by *P. alecto* on Palm Island, northern Queensland, and recovered adult worms from juveniles of that species. It is possible that other megachiropterans in the region are also infected.

The ranges of all four Australian species overlap and they often share summer camps (Nelson 1965b; Hall and Richards 1979), so that they could all be expected to harbour *T. pteropodis*. The highly nomadic Little Red Flying-Fox, *P. scapulatus,* has a breeding cycle 6 months out of phase with the other species, so the finding of *T. pteropodis* in this species would be of interest.

Undoubtedly people ingest eggs deposited on fruit by mother bats while feeding and perhaps by juveniles, but more work needs to be done before *T. pteropodis* can be included in the list of nematodes causing visceral *larva migrans* in humans.

Acknowledgements. The author is grateful to Dr. D.E. Moorhouse for encouraging this study and for much helpful advice and criticism, to Professor J.F.A. Sprent for useful advice and to Miss C.A. Creevey for assistance in the field and laboratory. The work was supported by the Mayne Bequest Foundation of the University of Queensland.

References

Baylis HA (1936) A new ascarid from a bat. Ann Mag Nat Hist Ser 10, 17:360–365
Byth S (1980) Palm Island mystery disease. Med J Aust 2:40
Dubey JP (1978) Patent *Toxocara canis* infection in ascarid-naive dogs. J Parasitol 64:1021–1023
Green R, Tonder SV van, Oettle GJ, Cole G, Metz J (1975) Neurological changes in fruit-bats deficient in vitamin B12. Nature, 254:148–150

Hall LS, Richards GC (1979) Bats of Eastern Australia. Queensland Museum booklet No. 12
Mickelsen O (1956) Intestinal synthesis of vitamins in the non-ruminant. Vits and Hormones 14:1–95
Moorhouse DE (1982) Toxocariasis – a possible cause of the Palm Island mystery disease. Med J Aust 1:172–173
Nelson JE (1965a) Behaviour of Australian Pteropidae (Megachiroptera). Animal Behaviour 13:544–557
Nelson JE (1965b) Movements of Australian flying foxes (Pteropodidae: Megachiroptera). Aust J Zool 13:53–73
Ratcliffe FN (1931) The flying-fox (*Pteropus*) in Australia. Bull Coun Sci Ind Res Aust 53:1–80
Sprent JFA (1958) Observations on the development of *Toxocara canis* (Werner 1782) in the dog. Parasitology 48:184–209
Warren EG (1971a) Studies on the morphology and taxonomy of the genus *Toxocara* Stiles, 1905 and *Neoascaris* Travassos, 1927. Zool Anz 185:393–442
Warren EG (1971b) Observations on the migration and development of *Toxocara vitulorum* in natural and experimental hosts. Int J Parasitol 1:85–99

Accepted June 1, 1983

306

APPENDIX II

SEASONAL BEHAVIOUR OF *PTEROPUS SCAPULATUS* (CHIROPTERA: PTEROPODIDAE)

SINCE the pioneering work of Ratcliffe (1931) the only studies on migration and breeding of Australian flying foxes have been by Nelson (1965a, 1965b).

Ratcliffe described the distribution of the Grey-headed Flying Fox, *Pteropus poliocephalus,* and the formation by this species of summer camps, which dispersed in winter because, he presumed, these animals migrated northwards.

Nelson showed that *P. poliocephalus* did not undertake extensive migrations and concluded that the dispersal of summer camps resulted from a more localised reorganisation of flying fox populations. Copulation occurs in March-April, just before these camps disperse, and young are born at about the time these camps re-establish in October-November. Both Nelson and Ratcliffe found that the breeding cycle of the Little Red Flying Fox, *P. scapulatus,* was about six months out of phase with that of *P. poliocephalus* and *P. alecto,* the Black Flying Fox.

As neither author was able to find evidence of *P. scapulatus* forming camps between April and September, Nelson concluded that the young of this species are born when the individuals are widely dispersed. This seems a surprising finding, as it would appear from his own extensive work on *P. poliocephalus* behaviour (Nelson 1965) that juvenile interactions in camps play an important role in the development of the relatively complex social behaviour of flying foxes.

The finding for the first time of a *P. scapulatus* winter camp in which the young are born and reared indicates that the seasonal behaviour of this species is comparable, although six months out of phase, with that of other Australian *Pteropus* species.

The presence of the camp was brought to the author's attention by property owners who responded to a letter in the 'Queensland Country Life' newspaper. It was located in a thickly wooded area of about four hectares, on a slightly elevated area of gently undulating land. Although severely defoliated, the predominant trees appeared to be Bauhinia (*Lisiphyllum carronii*), Brigalow (*Acacia harpophylla*) and other acacias (*A. pendula* and *A. cambagei*), Native Cascarilla Bark (*Croton insularis*) and bottle-trees (*Brachychiton australe* and *B. rupestre*). At a conservative estimate, there were about 100 large trees with an average of 300 adult bats per tree, giving an approximate total of 30,000 animals.

The bats appeared suddenly in large numbers in March, 1982, and by April most of the females were carrying young. In June, when the author first visited the site, the young could fly well and, although left at night by their mothers, flew around very actively with large numbers being found in bottle-trees several hundred metres from the camp.

In daytime the camp seemed to comprise predominantly females with young. Adult males were difficult to locate perhaps because they were more wary and readily took to flight on the arrival of intruders. Males when located were clustered in taller trees on the periphery of the camp but on being disturbed soon mingled with the females and young. Mothers actively abandoned their young and rejoined them later when the disturbance settled. At dusk (around 5.30 p.m.) the adults commenced their departure from camp and by 7 p.m. only juveniles remained. At 8 p.m., hundreds of juveniles were seen in large bottle-trees several hundred metres from the camp. Some of these possibly spent the night there, as several mothers with young were seen in these trees the following morning but were gone later in the day.

The diet of the adults at the time probably comprised blossom of many *Eucalyptus, Acacia* and *Melaluca* species which were in flower in the surrounding districts. Brigalow trees in the vicinity of the homestead, 3 km from the camp, had been visited by bats at night. The ground in the morning was covered with small broken branches and blossom much of which smelt very strongly of male bats.

By mid-August, the colony was noticeably smaller and at night juveniles were observed around the homestead. Bats were last seen in the camp on 1 September. Two days later it was completely deserted. However, for two weeks after this, bats were still observed at night flying around the homestead. In late September a report was received from Fanning River Station, about 80 km west of Townsville and 500 km NNE of the camp, that *P. scapulatus* had appeared suddenly in large numbers. It is possible that these were the same bats.

A similar winter camp had formed at the same site in the preceding year and another on a different part of the property (which has since been cleared) two years prior to that.

The finding of such a large group of *P. scapulatus* which stayed in the one place, from March to Setpember, during which time babies were born and reared (no attempt had been made to observe copulation), indicates that this species does indeed form camps equivalent to the summer camps of the more coastal *Pteropus* species. Presumably these have not been recorded previously because they form in scattered forest areas remote from human settlements.

I am deeply indebted to Kit and Tom Rodda, of "Kurrajong Park" via Moura, for bringing the flying fox colony to my attention and for their encouragement and substantial support during my field studies.

REFERENCES

NELSON, J. E., 1965a. Movements of Australian flying foxes (Pteropodidae: Megachiroptera). *Aust. J. Zool.* **13:** 53-73.

NELSON, J. E., 1965b. Behaviour of Australian Pteropodidae (Megachiroptera). *Anim. Behav.* **13:** 544-57.

RATCLIFFE, F. N., 1932. Notes on the fruit bats (*Pteropus* spp.) of Australia. *J. Anim. Ecol.* **1:** 32-57.

Postscript: In early December, 1982, a large camp comprising perhaps 100,000 *Pteropus scapulatus* was observed near Grantham, about 100 km west of Brisbane, in woodland consisting mainly of *Casuarina, Eucalyptus* and *Acacia.* Large numbers of juveniles were dispersed in packs mainly around the periphery of the camp. Most of the adult females were associated with males in "harem" groups and exhibited distinctive territorial behaviour. Smaller numbers of adult males formed exclusively male groupings scattered apparently at random. Many male-female pairs were actively copulating with perhaps a hundred such couples being observed over a three-hour perod. If this represents the peak of the mating season, and most young are born in April, then the gestation period in *P. scapulatus* may be as short as four months, compared with six months for *P. poliocephalus* and *P. alecto* (Nelson 1965b).

Paul Prociv, *Department of Parasitology, University of Queensland, St Lucia, Queensland, Australia 4087.* *Received 3 November, 1982. Aust. Mammal.* **6:** *45-46, 1983.*

APPENDIX III

OBSERVATIONS ON THE PREVALENCE AND POSSIBLE INFECTION SOURCE OF *TOXOCARA PTEROPODIS* (NEMATODA: ASCARIDOIDEA) IN QUEENSLAND FLYING-FOXES

PAUL PROCIV

Prociv, P., 1985. Observations on the prevalence and possible infection source of *Toxocara pteropodis* (Nematoda: Ascaridoidea) in Queensland flying-foxes. *Aust. Mammal.* **8**: 319-21.

The ascaridoid nematode, *Toxocara pteropodis*, was found in all four Australian *Pteropus* species. Its eggs were recovered in large numbers from foliage in a coastal flying fox camp, and presumably this is where adult bats acquire infection.

In *Pteropus poliocephalus*, northern *P. alecto* and *P. conspicillatus*, the nematode is maintained by a seasonally recurring life cycle. Adult *P. scapulatus* are exposed to infection when they share camps with these coastal species in late summer, but this species is probably unable to maintain the parasite in its own populations. The prevalence in southern *P. alecto* may be lower than in other coastal groups.

P. Prociv, Department of Parasitology, University of Queensland, St. Lucia, Queensland, Australia 4067.

THIS work was prompted by the hypothesis (Moorhouse 1982) that the unexplained Palm Island epidemic (Byth 1980) was a manifestation of visceral *larva migrans* caused by *Toxocara pteropodis.*

Baylis (1936) originally found *T. pteropodis* in *Pteropus geddiei* from the New Hebrides (now Vanuatu). Moorhouse (1982) found this worm in *P. alecto* from Townsville, northern Queensland, and it is highly prevalent in *P. poliocephalus* of southeastern Queensland (Prociv 1983a).

Adult *T. pteropodis* are found only in the intestines of suckling bats. Eggs are passed in the faeces from five weeks of age, embryonate rapidly and, under optimal conditions, are infective after 10 days but lose their viability within six weeks (Prociv 1983a). Adult bats become infected probably by ingesting stale faeces from foliage in the camps rather than from fruit or blossom while foraging (Prociv 1983a). Eggs hatch in the intestine and third-stage larvae travel to the liver where they remain, perhaps in male bats for the rest of the host's life. In females, at the end of pregnancy, larvae pass through the mammary glands to the neonate in its first two weeks of life (Prociv 1983a). Additional data from *P. poliocephalus* and *P. alecto,* evidence of natural infection in the other two species found in Australia (*P. conspicillatus* and *P. scapulatus*) and leaf and fruit contamination with *Toxocara* eggs are presented here.

MATERIALS AND METHODS

EXAMINATION OF FOLIAGE

Leaves were examined from a mixed camp of *P. poliocephalus, P. alecto* and *P. scapulatus* situated in mangroves along the South Pine River, 30 km north of Brisbane. In January, 1983, 200 faecally-contaminated leaves between 1 and 2 m above ground level were picked at random in an area known to have been occupied by nursing mother bats. These leaves were soaked overnight in weak detergent solution then carefully washed. The sediment was then passed through a bank of sieves with the mesh opening in the final sieve being 63 μm. This sieve was then backwashed with tapwater, and *Toxocara* eggs were concentrated by gravitational sedimentation. The

total egg count was calculated by counting the eggs in five 1 ml aliquots from a total sediment volume of 500 ml. In addition, 50 faecally contaminated leaves were examined individually in January and another 50 in February, 1983. Each leaf was soaked for two hours in 10 ml tapwater then faeces brushed off. The suspension was strained through gauze then centrifuged. Sediments were examined microscopically for eggs.

Examination of Fruit

In February, 1982, twenty green mangoes and three ripe pawpaws presumably contaminated with bat faeces were collected from trees growing within 1 km of a large *Pteropus* camp at Indooroopilly, Brisbane. The pooled washings from the mangoes were strained and sedimented, and eggs were counted in a 1 ml aliquot from a total sample of 6 ml. Each pawpaw was examined individually in a similar manner.

Examination of Bats

Specimens of *P. poliocephalus* were obtained as described previously (Prociv 1983a) and the animals from that earlier study are included here. Likewise, *P. alecto* were collected from camps around Brisbane as well as an animal refuge, interested individuals and from power lines. Only juveniles of *P. conspicillatus* were examined, these being recovered from mangroves near Mossman, northern Queensland, where they had been abandoned by their mothers. Juveniles of *P. scapulatus* were caught by hand in a patch of brigalow scrub in central Queensland (see Prociv 1983b) and adult males were shot in the same camp.

Patent infections in live young were detected by rectal swab, whereas the intestines of dead young were dissected and examined for worms. Livers of adult bats were "digested" in normal saline and examined as previously described (Prociv 1983a).

RESULTS

Most of the mangrove leaves at the camp site were contaminated with bat faeces, many covered by several separate droppings. The 200 leaves yielded an estimated 48,000 eggs, i.e. an average of 240 eggs per leaf. Only about 10% of these eggs contained fully developed larvae. Of the 100 contaminated leaves examined individually, 12% carried worm eggs (6/50 in January and 6/50 in February). Hence, the calculated average egg burden per infected leaf was 2,000.

Two degenerate and one embryonated *T. pteropodis* eggs were found in 1 ml of the mango sediment, giving a total recovery of 18 eggs from 20 mangoes. Eggs were not recovered from the pawpaws. Moorhouse (pers. comm.) recovered a very small number of eggs from mangoes collected in January, 1980 at Yepoon, near Rockhampton.

The rate of infection in various *Pteropus* species is shown in Table 1. Adults of *P. alecto* and *P. conspicillatus* from northern Queensland have not been studied. Likewise, the prevalence of infection in northern *P. alecto* juveniles is known only from Moorhouse's limited study (1982).

DISCUSSION

Egg Dispersal

Moorhouse (1982) proposed that adult bats become infected by eating eggs from fruit, but subsequent work (Prociv 1983a) suggests that most adult infection is acquired in the camps. The finding of such heavy contamination of leaves with *Toxocara* eggs in a typical camp supports this conclusion, as an adult bat need only consume one infected faecal dropping to acquire a sizeable larval burden in its liver. Because bats evacuate their intestine prior to taking flight (pers. obs.), and because their faeces rapidly become diluted when they commence eating fruit (pers. obs.), it is unlikely that egg contamination of foods that are distant from camp sites

Table 1. Infection rates of *Toxocara pteropodis* in Australian *Pteropus* species (no. infected/no. examined).

Species	Adult Males with third-stage larvae in livers	Juveniles—autopsy findings in intestine (worms from third-stage larvae to adult)	Juveniles—patent infections (eggs in rectal swab)
P. poliocephalus	23/35 (66%) (1-460 larvae)	19/24 (79%) (1-30 worms)	31/69 (45%) Infertile eggs in 8 (26%)
P. alecto northern	?	3/7 (43%) (Moorhouse 1982)	?
P. alecto southern	9/17 (53%) (3-9 larvae)	2/11 (18%) (1 & 2 larvae)	1/9 (11%)
P. conspicillatus	?	6/12 (50%) (1-5 larvae)	4/8 (50%)
P. scapulatus	15/20 (75%) (1-27 larvae)	2/23 (9%) (5-12 larvae)	1/50 (2%)

would be sufficiently concentrated to maintain *Toxocara* infection in a bat population. Furthermore, the highest concentration of eggs occurs in the faeces of suckling young which do not fly far beyond the camp perimeters.

Many mangoes and pawpaws were collected to find the small numbers contaminated with bat faeces examined in this study. Even so, very small numbers of eggs were recovered from these fruits, which is in keeping with earlier conclusions (Prociv 1983a).

The camp of *P. alecto* on Palm Island is located only about 1 km from the human settlement, so mother bats could still retain moderate numbers of *Toxocara* eggs in their intestines on arriving at the mango trees, and suckling juveniles could reach the settlement during nocturnal practise flights.

Infection in Bats

The distribution of the four Australian *Pteropus* species is shown in Fig. 1.

Pteropus poliocephalus

This species is a satisfactory maintaining host for *T. pteropodis*. Because larvae probably remain viable for several years in adult male bats, the prevalence of liver infections in this sex was considered a suitable index of exposure of this species to infection. In females, because most larvae are passed on to the young post-partum (Prociv 1983a), larval recovery from livers provides less information unless specimens are obtained during early pregnancy. Refinement of the modified Baermann technique used indicates that the true prevalence of infection in males is higher than the cumulative total of 23/35 (66%).

The infection of adult females is reflected by the prevalence of larvae in the intestines of young bats (79%). It appears that at the most three or four of these larvae attain maturity, and in some cases (8/31) only infertile eggs are passed because adult male worms have not developed. This failure of many larvae to develop, or perhaps the spontaneous expulsion of developing larvae (pers. obs.), could explain the lower detection rate of infection by rectal swab than by intestinal dissection. Approximately equal numbers of male and female worms were recovered, so it could be assumed that because 8/69 bats examined were passing infertile eggs, i.e., harboured only female worms, then a similar number harboured only male worms and hence were not detected on faecal examination. This would raise the proportion infected with adult worms to 8+31/69, i.e. 57%.

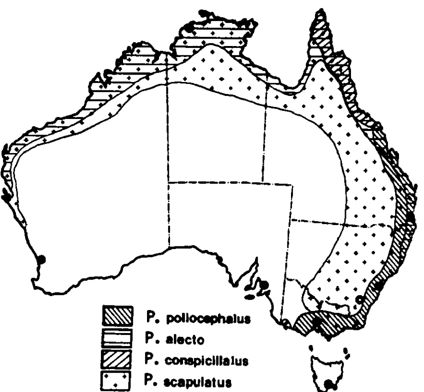


Fig. 1. Geographic distribution of Australian *Pteropus* species (after Strahan 1983 and Hall 1984). •, capital cities.

The prevalence of infection among juveniles diminished later in the season, and they spontaneously passed worms more readily, so examination of young bats early in the season would yield even higher infection rates. The finding of adult *T. pteropodis* on the floor of a *P. poliocephalus* camp in Sydney (R. Puddicombe pers. comm.) indicates that the nematode probably occurs throughout the entire range of this host species.

Pteropus alecto

Insufficient data is available for valid conclusions to be drawn for this species. Moorhouse (1982) showed that in the Townsville district this species successfully maintains the parasite and hence the prevalence figures may be similar to those of *P. poliocephalus* further south. According to Ratcliffe (1931), in 1930 *P. alecto* extended only as far south as Rockhampton, but by 1960 this species had reached Brisbane (Nelson 1965). Its range now extends to northern New South Wales (see Fig. 1). In specimens obtained around Brisbane, larvae were found in 53% of male livers, but in comparatively low numbers (3-9). In only two of eleven young dissected were intestinal worms found, and only one of nine live young were passing eggs. This indicates a lower prevalence of infection in this species, perhaps insufficient to maintain the parasite.

Maybe the behaviour of southern *P. alecto* has been modified; for example, adults may prefer to drink water from streams and estuaries rather than licking it from leaves during rainstorms. More extensive studies are required to confirm the findings. Because *P. alecto* in southern districts invariably share summer camps with *P. poliocephalus* (pers. obs.), there is adequate opportunity for "cross-infection".

Pteropus conspicillatus

The finding of intestinal worms or patent infection in 10/20 young of this species indicates it is an adequate maintaining host. Although the range of *P. conspicillatus* falls within that of northern *P. alecto*, in many areas the camps of the two species are quite separate (pers. obs.). It is probable that most adult males are infected and the worm occurs in this species, as with *P. alecto*, throughout its range into New Guinea.

Pteropus scapulatus

A high prevalence of infection was found among adult males, with up to 27 larvae recovered from one liver, indicating a high exposure of this species to infection. However, only two out of 23 dissected young yielded intestinal larvae and only one of 50 suckling young, about 3 months old, was passing eggs. This suggests either that adult females are not exposed to infection as are males, or that they are not physiologically suitable for larval development and migration, or that the gut of the young bat is not a suitable environment for normal maturation of worms. Whatever the explanation, *P. scapulatus* probably is incapable of maintaining the nematode among its own population. This species is highly migratory and frequently shares summer camps with all the coastal species with which it mingles freely. This is where infection may be acquired by the adults. Assuming that these bats mate in November-December (Prociv 1983b), and that the heaviest contamination of foliage in *P. poliocephalus* camps with infective *Toxocara* eggs occurs in February, it is possible that larvae in *P. scapulatus* do not have sufficient time to develop by term and hence are incapable of migrating to the mammary glands at the end of gestation. If this is the case, larvae would not be capable of such migration in subsequent pregnancies, otherwise the prevalence of infection in young bats would be higher than found. Hence behavioural, physiological or environmental factors, or a combination of these, preclude *P. scapulatus* from maintaining *Toxocara* among its own populations.

More studies on these four species and an extension of this work to other megachiropterans, including those which roost in caves, will delineate the geographical and host range of the parasite and could provide insights into pteropodid behaviour, physiology and taxonomy.

These findings do not explain the Palm Island outbreak, but show that toxocariasis may be common in fruit bats elsewhere, so that people may be inadvertently exposed to infection when eating fruit contaminated by pteropodid droppings. Whether such exposure leads to clinical manifestations of *larva migrans* in humans remains to be determined.

ACKNOWLEDGEMENTS

I am deeply indebted to Dr Douglas Moorhouse for encouraging this work; to Catherine Creevey, for technical assistance; and to Jim and Kay Davidson of Beenleigh, Charlie and Val McCracken of Port Douglas, and Tom and Kit Rodda of "Kurrajong Park" for their kind hospitality and invaluable help with procuring specimens.

The study was funded by the University of Queensland.

REFERENCES

BAYLIS, H. A., 1936. A new ascarid from a bat. *Ann. Mag. Nat. Hist., Ser. 10* **17**: 360-65.

BYTH, S., 1980. Palm Island mystery disease. *Med. J. Aust.* **2**: 40-42.

HALL, L. S., 1984. And then there were ba... Pp. 837-52 *in* "Vertebrate zoogeography and evolution in Australia" ed by M. Archer and G. Clayton. Hesperian Press: Perth.

MOORHOUSE, D. E., 1982. Toxocariasis — a possible cause of the Palm Island mystery disease. *Med. J. Aust.* **1**: 172-73.

NELSON, J. E., 1965. Movements of Australian flying-foxes (Pteropodidae: Megachiroptera). *Aust. J. Zool.* **13**: 53-73.

PROCIV, P., 1983a. Observations on the transmission and development of *Toxocara pteropodis* (Ascaridoidea: Nematoda) in the Australian Grey-headed Flying-fox, *Pteropus poliocephalus* (Pteropodidae: Megachiroptera). *Z. Parasitenkd. (1983)* **69**: 773-81.

PROCIV, P., 1983b. Seasonal behaviour of *Pteropus scapulatus* (Chiroptera: Pteropodidae). *Aust. Mammal.* **6**: 45-46.

STRAHAN, R., (ed.), 1983. "The Australian Museum complete book of Australian mammals." Angus and Robertson: Sydney.

APPENDIX IV

Journal of Helminthology (1985) **59**, 267–275

Observations on *Toxocara pteropodis* infections in mice

P. PROCIV

Department of Parasitology, University of Queensland, St. Lucia, Queensland, 4070, Australia

ABSTRACT

Infection in mice with *Toxocara pteropodis* was investigated. In mice fed infective eggs, third-stage larvae hatched out and penetrated the mucosa, predominantly that of the lower intestine. They travelled via the portal vein to the liver, where they remained at least 14 months. They grew in length from $430 \pm 15\,\mu m$, at three days post infection (p.i.), to $600 \pm 50\,\mu m$, at six to nine weeks p.i., after which time growth ceased. Blood eosinophilia appeared at 28 days p.i., and esosinophil levels continued to rise gradually beyond this time. In female mice the larvae did not migrate from the liver in response to pregnancy or lactation. When infective eggs were inoculated subcutaneously or intra-peritoneally, larvae hatched out and ultimately appeared in the liver in larger numbers than seen with oral infections.

INTRODUCTION

The role of *Toxocara canis* as the prime aetiological agent of visceral *larva migrans* (VLM) in man has been established convincingly (see Beaver, 1969). Larvae of many ascaridoid nematodes, including *T. canis*, undergo tissue migration in various laboratory animals, but only *T. canis* and *Ascaris lumbricoides* have been definitely implicated in human disease under natural conditions. Recently, however, Moorhouse (1982) suggested that *T. pteropodis*, an intestinal parasite of suckling fruit bats (*Pteropus* spp.) in northern Australia and elsewhere, may have been responsible for an outbreak of an illness with some features of VLM in an aboriginal community at Palm Island in north Queensland, Australia (Byth, 1980).

The manifestations of murine toxocariasis with *T. canis* have been studied in detail by many workers (see Dunsmore *et al.*, 1983) and to some degree parallel infections in man. This present study was undertaken to investigate *T. pteropodis* infections in mice, in order to gain an indication of its potential for pathogenicity in man.

MATERIALS AND METHODS

Collection of eggs

Eggs of *T. pteropodis* were collected from the faeces of naturally infected captive juvenile *Pteropus poliocephalus*, as previously described (Prociv, 1983), and cultivated on moist activated charcoal at room temperature. They were used after a minimum of three weeks' embryonation. Eggs of *T. canis* were recovered from the uteri of female *T. canis* obtained from dogs necropsied at the University of Queensland Veterinary School, and were embryonated on moist activated charcoal for two months before use.

Infection of mice

Most work was done on six-week-old female Quackenbush (QB) mice, an allogeneic strain, obtained from the Central Animal Breeding House of the University of Queensland. Limited studies were also carried out using syngeneic C3H, DBA/2, CBA, BALB/C and C57BL 6J strains of mice. *Toxocara* eggs were washed from the charcoal cultures with tap water and concentrated by sedimentation. An egg was considered infective if the larva moved either inside the egg or after being expressed from it. Doses of infective eggs, in 0·3 to 0·5 ml water, were given to mice via a stomach tube.

Migration of larvae in tissues

Four female C3H mice were each inoculated with 100 eggs and killed by cervical dislocation under ether anaesthesia 6, 12, 24 and 48 hours after infection. Ten female QB mice were each given 1000 eggs and killed in pairs at 4, 8, 12, 24 and 48 hours. All peritoneal surfaces in each opened abdomen were lavaged with 50 ml 0·9% saline and the washings left to stand for one hour. The sediments were examined for larvae under a stereo microscope. The alimentary tract, from stomach to rectum, was removed, stripped of its mesenteries and divided into stomach, proximal and distal halves of small intestine, and large intestine. These segments were opened longitudinally, scraped out and rinsed vigorously. The ingesta and washings were fixed in 3% formalin. The mesenteries, gut wall segments, liver, kidneys, entire thoracic contents and brain were minced finely with scissors and each incubated in 40 ml trypsin (trypsin 4 g, NaCl 8 g and $NaHCO_3$ 10 g in 1·0 litre of water) for six hours at 37°C. After removal of the pelt, tail and paws, the remaining carcass was ground in a Waring blender at high speed for 30 sec then digested in 600 ml trypsin solution, as above. All digests were subjected to a modified Baermann procedure (Prociv, unpublished) to separate larvae from tissues. To each sediment was added an equal volume of boiling water followed immediately by 10% formalin to give a final concentration of 3% formalin in the sediment. Specimens were scanned under a stereo microscope for *T. pteropodis* larvae.

Growth of larvae

26 female QB mice were each infected with 200 eggs, and another 16 were given 1000 eggs each. They were anaesthetized with ether at the times shown in Table II, exsanguinated by cardiac puncture then killed by cervical dislocation. The intestines were discarded and the remaining tissues digested and examined as described above. In mice killed beyond 14 days p.i., only the livers were digested separately, whilst other organs were ground and digested with the carcasses. Larvae recovered from livers were drawn, using a camera lucida attached to a compound microscope, then measured.

Blood white cell response

The blood collected from mice was dispensed into EDTA-coated tubes and the total white cell counts determined with a Coulter counter. At the time of killing (always between 0900 and 1000 hours) thin blood smears were prepared and stained with Giemsa and differential white cell counts determined. Four female QB mice, each inoculated with 700 *T. canis* eggs, were bled two weeks and four weeks after infection as positive controls for murine eosinophilia.

Comparison of inbred mouse strains

15 six-week-old male mice of five different strains were each infected with 500 eggs of *T. pteropodis* and one of each strain was killed 16, 35 and 64 days later. In this, and subsequent experiments, tryptic digestion was replaced with overnight incubation (18 hours) in 0·85% saline.

Effects of pregnancy on larval distribution

Four six-week-old female QB mice were fed 1000 eggs each. Three weeks later they were separated and mated with individual male QB mice. One pair produced a litter of five offspring eight weeks p.i. One suckling mouse was killed at each 24-hour interval from birth and its liver and intestines were digested. The dam was killed with the last baby, five days after parturition, and its liver, mammary glands and remaining carcass 'digested' in saline.

Another female produced one litter 10 weeks after infection and a second litter eight weeks later, following which this dam and its second litter were killed and examined.

Paratenic tendencies

Four male mice were each inoculated with 1000 *T. pteropodis* eggs and killed 5, 15, 30 and 60 days later. The liver from each mouse was fed immediately to another mouse which had been starved for the preceding 24 hours. Each recipient mouse was killed one week after eating infected liver and the liver examined for larvae by digestion.

Parenteral routes of inoculation

A dose of 650 infective *T. pteropodis* eggs in 0·25 ml saline was injected into four anaesthetized male C57Bl 6J mice, two intraperitoneally (i.p. group) and two subcutaneously in the interscapular region (s.c. group). One mouse from each group was killed 10 days and the other 21 days later. The intestines were discarded, and the livers and carcasses were examined for larvae.

Tolerance of larvae to refrigeration

Three male QB mice were fed 1000 eggs of *T. pteropodis* and killed after 30 days. The liver of one was divided into ten similar portions which were stored in a plastic container at 5°C. Each day one portion was squashed between two glass slides and examined at room temperature (25°C) for motile larvae.

The livers from the two other mice were minced separately and each divided into equal portions. One half of each liver was incubated overnight in saline at 37°C and subjected to Baermann separation. The two remaining halves were frozen at −20°C for three days then thawed to room temperature. One was subjected to Baermann separation overnight in saline at 37°C, while the other was simply incubated overnight in saline at 37°C. The sediments of both were examined for larvae.

Comparison with T. canis *larvae*

One female QB mouse was inoculated with 200 eggs of *T. pteropodis* and another with 200 eggs of *T. canis*. Both mice were killed seven days later and their livers digested. Ten larvae from each were drawn and measured.

RESULTS

Larval migration

In the alimentary tract of QB mice, larval penetration of the mucosa occurred as early as four hours after inoculation of eggs and throughout all sections of the gut, with most larvae penetrating the distal small intestine and the colon (see Table I). Total recovery of larvae was greatest at 12 hours (46% of inoculum) and fell rapidly to 11% at 24 hours. By 48 hours, larvae were found only in the the liver. The results from C3H mice reflect the findings in QB mice except that larvae were slower to penetrate the gut wall, being found there as late as 48 hours in one mouse. Larvae were already in the liver by six hours in the C3H mouse and at 12 hours one larva was found in the liver of a QB female. Two larvae recovered from livers at 24 hours and examined alive before fixing were enclosed in loose sheaths. No larvae were found free in the peritoneal cavity, or in any other organs.

Growth of larvae

In the two groups of female QB mice fed 200 and 1000 eggs, larvae were found only in the livers. Larval growth had ceased in the 200 egg group by day 50, whereas in the 1000 egg group length did not increase beyond 65 days p.i. (Table II). Larval recoveries

TABLE I. Numbers of third-stage larvae recovered from mice killed within 48 hours of oral inoculation with infective *T. pteropodis* eggs

	*Female C3H mice fed 100 eggs				**Female Quackenbush mice fed 1000 eggs				
	6 h	12 h	24 h	48 h	4 h	8 h	12 h	24 h	48 h
Stomach									
Contents	ne	0	ne	0	3	1	16	0	0
Wall	1	0	0	0	5	0	5	0	0
Proximal small intestine									
Contents	ne	3	ne	0	3	10	21	2	0
Wall	3	3	0	0	16	34	49	3	0
Distal small intestine									
Contents	ne	7	ne	2	48	25	37	3	0
Wall	1	12	0	4	214	98	110	11	0
Large intestine									
Contents	ne	17	ne	7	24	25	39	29	0
Wall	2	24	0	3	23	44	177	53	0
Mesentery	0	1	0	1	0	0	3	1	0
Liver	2	11	12	8	0	0	1	1	115
Total	≥9	78	≥12	25	336	237	458	103	115

ne—not examined.
*C3H mice were single experiments. Total larvae in C3H mice at 6hrs and 24hrs appear low because alimentary tract contents were not examined.
**Female Quackenbush results are average of pairs.

TABLE II. Hepatic larval recovery and blood leucocyte responses in pairs of female Quackenbush mice infected orally with *T. pteropodis* eggs

Days after infection	Larvae recovered from liver No.	% egg dose		Larval length* (μm) Mean ± SD	Range	WBC count $\times 10^3$/mm^3	Eosinophils %
1	1	0·5	Mice fed 200 infective eggs	410±20	385–430	6·0	0
2	24	12·0		—	—	—	—
3	18	9·0		430±15	395–455	8·4	0
5	19	9·5		460±18	425–505	6·5	0
7	28	14·0		465±22	415–500	5·4	0
10	20	10·0		480±18	435–510	10·0	0
14	23	11·5		535±54	435–635	7·8	0
21	26	13·0		550±45	435–670	12·6	0
28	17	8·5		550±38	445–610	6·5	0
40	27	13·5		—	—	6·7	1
50	29	14·5		610±35	520–660	9·5	2
65	45	22·5		590±52	430–670	7·7	1
85	41	20·5		625±50	485–700	4·2	7
2	83	8·3	Mice fed 1000 infective eggs	—	—	5·5	0
5	79	7·9		435±22	380–490	6·0	0
10	109	10·9		465±27	420–510	7·1	1
21	220	22·0		470±40	420–540	7·4	0
28	195	19·5		490±25	445–550	12·0	2
50	115	11·5		535±30	445–580	13·5	8
65	134	13·4		595±40	440–645	6·0	6
420**	110	11·0		595±40	545–670	—	—

* 25–40 were measured, unless numbers recovered were lower, then all measured.
** single mouse.

did not decline with time after infection and one mouse, given 1000 eggs, harboured 110 hepatic larvae at 14 months.

Leucocyte responses

In the group fed 200 *T. pteropodis* eggs, eosinophils were not detected in the peripheral blood until day 40, when they comprised 1% of a total white cell count of 6,700 mm^3. At day 50 the eosinophil count was 2% of 9,500 white cells and at day 85 this was 7% of 4,200 white cells, or 294/mm^3. In the group fed 1000 *T. pteropodis* eggs, significant eosinophilia was not detected until day 28 when it comprised 2% of 12,000 white cells/mm^3. This rose to 8% of 13,500 cells by day 50. The mice fed *T. canis* eggs had absolute eosinophil counts of 180/mm^3 at two weeks, and 550 mm^3 at four weeks.

Syngeneic strains

The numbers and lengths of larvae recovered from individual male mice of five different strains appear in Table III. It was evident that the numbers and the rate of growth were similar in all genotypes, except that DBA 2 mice harboured fewer larvae, and their growth was retarded in CBA mice.

Effects of pregnancy

The pregnant mouse killed after its first litter, five days post-partum, yielded 193 third-stage larvae (length 580±36 μm, range 510–650 μm) only from the liver. No larvae were found in any of the suckling mice.

The second female mouse, killed after its second litter, harboured 126 larvae in its liver, and its offspring were free of infection.

Paratenic tendencies

The recipient mouse which ate the liver of the mouse killed five days after infection was found dead 24 hours later. Motile larvae were recovered only from its gut contents. The other three cannibal mice, which were killed seven days after eating infected liver, were all free of larvae.

Parenteral infection

At 10 days, three larvae were recovered from the carcass of the i.p. mouse and five from its liver. The corresponding numbers from the s.c. mouse were eight and five.

At 21 days, no larvae were found in the carcass of the i.p. mouse, whereas 227 were recovered from its liver. In the s.c. mouse, 10 and 320 were recovered from carcass and liver respectively.

TABLE III. Numbers and lengths of third-stage *T. pteropodis* larvae recovered from livers of inbred single male mice killed at intervals after oral inoculation of 500 infective eggs

Time post-infection	16 days		35 days		64 days	
Strain	No. of larvae	Length (μm) ±SD	No. of larvae	Length (μm) ±SD	No. of larvae	Length (μm) ±SD
B ALB C	36	519±26	84	595±34	25	591±42
CBA	48	518±26	53	548±34	41	560±31
C3H	39	540±32	47	589±36	59	602±35
C57 B16J	46	538±44	40	600±32	25	592±38
DBA 2	6	519±35	15	635±28	7	595±55

Tolerance to low temperatures

In the liver segments stored at 5°C, larval motility was detected until the fifth day, after which all larvae appeared degenerate.

The two fresh half-livers yielded 59 and 97 motile larvae. Their corresponding portions which had been frozen at −20°C for three days yielded only two (Baermann portion) and 19 (simple incubation) non-motile larvae respectively.

Comparison with T. canis *larvae*

The dimensions of third-stage larvae of both species recovered seven days p.i. are shown in Table IV. Although *T. pteropodis* were larger than *T. canis*, there was some overlap in lengths. Other parameters were similar in both species, except that the tail in proportion to body length was longer in *T. canis*.

DISCUSSION

The results of these experiments need to be interpreted with caution because of the small numbers of mice used and the small numbers of infective eggs administered. These restrictions could not be avoided because the eggs were difficult to collect, they were contaminated with much residual faecal matter, few embryonated and those that did were viable for only a short time. Nevertheless, the findings indicate several unique features of *T. pteropodis* larval behaviour. In *Pteropus* spp., the natural host, ingested eggs of *T, pteropodis* hatch in the intestine and the third-stage larvae penetrate the mucosa of the lower gut to travel to the liver via the portal vein (PROCIV, 1983). Larval penetration occurs higher in the intestine of mice, presumably because their gut transit time is longer than the 30 to 60 min in *P. poliocephalus* eating fruit. Many free larvae and apparently viable eggs were present in the faeces of mice 24 hours after infection indicating relatively slow hatching of eggs. This would explain the high total recovery of larvae from alimentary contents (see Table II) and wall digests up to 12 hours p.i., compared with the lower subsequent recoveries from the livers. The absence of larvae from the peritoneal cavity probably excluded the direct migration route from gut wall to liver capsule and the failure to detect larvae in the thorax indicated that the central lymphatic route was not a significant pathway. Hence, larvae recovered from the mesenteries at 6 and 12 hours were more likely to be in veins than lymphatics.

Upon reaching the mouse liver, the larvae stopped within the sinusoids and elicited a strong inflammatory response (descriptions of the histological response to this infection will be published separately). No larvae were found in any tissue beyond the liver, and viable larvae were still present at 14 months after infection. Nevertheless, it is possible that in heavier infections small numbers of larvae may enter lymphatics and travel to sites beyond the liver.

TABLE IV. Dimensions of third-stage larvae of *T. pteropodis* and *T. canis* recovered from livers of female QB mice seven days after oral infection (all dimensions in μm)

Dimension	*T. pteropodis*	*T. canis*
Total length $\bar{X} \pm SD$	470±22	425±14
Range in length	435–505	410–455
Body width at ventriculus	19±1·2	19±1·6
Mouth—nerve ring	72±5	70±3
Mouth—ventriculus	126±8	127±5
Ventriculus length	21±1·8	19±1·6
Genital primordium—tail tip	199±12	180±11
Genital primordium—tail tip (as fraction of total length)	42±1·8%	42±1·8%
Anus—tail tip (tail length)	34±2·7	37±1·5
Tail length (as fraction of total length)	7·2±0·3% (range 6·8–7·8)	8·6±0·5% (range 7·9–9·4)

This hepatic arrest of larvae can be attributed either to mechanical obstruction dependent on the relative dimensions of hepatic sinusoids, or to a positive hepatotropism by the larvae. The latter alternative is strongly favoured by the results of the parenteral infection experiment in which larvae accumulated in the liver after being inoculated into remote sites. Hepatic arrest of *T. pteropodis* larvae also occurs in other mammals after experimental infection and, furthermore, the larvae of *T. canis*, which are of similar dimensions to those of newly hatched *T. pteropodis*, passed through the liver of orally infected *Pteropus poliocephalus* and rapidly dispersed to the central nervous system and other tissues (Prociv, unpublished).

The results in Table II indicate that *T. pteropodis* larvae remained viable in the liver for at least 14 months. They grew from about 430 μm at three days after infection to a maximum length of about 610 μm (range 485–700 μm). It appears from Table II that larvae in lighter infections grew faster and reached maximal lenghts by 50 days after infection, although the numbers examined were insufficient for statistical validation. In *P. poliocephalus*, a natural host, larvae had grown to 640 ± 45 μm by 11 weeks (PROCIV, 1983).

Infective eggs hatched after parenteral inoculation into mice and the larvae ultimately found their way to the liver. At 21 days, almost 50% of the inoculum was recovered there as larvae. After oral inoculation, many viable eggs and hatched larvae were lost with the faeces, whereas larvae from parenteral sites had no such means of egress. Presumably they reached the liver via the blood, and accordingly passed through considerable capillary beds. Some may have undergone tracheal migration, but the results of the cannibalism experiment suggested they subsequently would have been unable to reach the liver by penetrating gut mucosa. It is difficult to explain the low yield from the two mice killed 10 days after parenteral infection. Perhaps many eggs or larvae were still present at the sites of inoculation and so were excluded from the digestion procedures. Alternatively, larvae may have accumulated in the gastrointestinal vasculature before migration to the liver; these tissues were not examined.

In male *P. poliocephalus* the larvae probably remain viable in the liver for many years, whereas in females these larvae are mobilized at the end of pregnancy and pass to the neonate with the milk (PROCIV, 1983). Neither pregnancy nor laction stimulated further larval migration in QB mice, even by 18 weeks after infection when the larvae had long ceased growing. The third-stage larvae of *T. canis* and *T cati* do not grow in size in mice (NICHOLS, 1956) and migrate rapidly beyond the liver. *T. canis* larvae undergo transmammary transmission in mice soon after infection (BAUM & STOYE, 1981).

The absence of a peripheral eosinophilic response in the first six weeks of infection in the female QB mice, some of which harboured more than 200 larvae, was surprising, particularly in view of the response seen with the *T. canis*-infected controls. KAYES & OAKS (1980) demonstrated a peak eosinophilic response at two weeks in their mice which were fed 1000 eggs of *T. canis*. The onset of blood eosinophilia in *T. pteropodis*-infected mice may be retarded by the failure of larvae to reach the pulmonary capillaries. The eosinophilic response of rats to *Trichinells spiralis* was most effective when particulate antigens passed through the lung circulation (BASTEN *et al.*, 1970), and eosinophilia in rats has been induced by pulmonary microembolism of inert particles (WALLS & BEESON, 1972; SCHRIBER & ZUCKER-FRANKLIN, 1975).

Inability of larvae to undergo paratenic transmission could be expected of *T. pteropodis*, a parasite of herbivores. However, owing to the growth in size of larvae in mice, it is possible that paratenic transmission may still occur in very early infections. The premature death of the one mouse which ate a liver infected five days prevents further speculation at this stage.

Low tolerance to freezing is not surprising in a parasite of mammals living in tropical regions, in contrast the cold-tolerance exhibited by the larvae of ascaridoids from colder climates (Sprent, 1953). The freezing experiment confirmed that, for the successful recovery of larvae by the Baermann technique, the larvae must be motile. In fact, the successful replacement of tryptic digestion with incubation in saline indicates that the chief criterion for effective Baermann recovery of tissue larvae is that they are motile. This has been shown for *T. canis* (Brindley *et al.*, 1985).

There appeared to be little variation in *T. pteropodis* migration and growth among different inbred strains of mice. The restriction of larvae to the liver could make this a useful model for systemic pharmacological studies, as the use of *T. canis* in this role (e.g. Burren, 1968; Abdel-Hameed, 1984) is complicated by the dispersal of its larvae throughout many tissues.

On microscopic examination, the only significant distinguishing feature between the third-stage larvae of *T. pteropodis* and *T. canis* was the slightly longer tail of the latter. In late infection, *T. pteropodis* larvae are considerably longer and, in mice, are recovered only from the livers, but early in the infection the considerable overlap in dimensional parameters (see Table IV) could make it impossible to identify individual larvae.

Hepatitis was a major component of the clinical presentation of the affected children in the Palm Island outbreak of 1979 (Byth, 1980). In mice, larvae of *T. pteropodis* remained in the liver and caused an inflammatory response. These results are consistent with the hypothesis of Moorhouse (1982) that this epidemic may have been a manifestation of visceral *larva migrans* caused by this nematode. In addition, *T. pteropodis* may prove to be a useful tool in analysing further aspects of systemic anthelminthic chemotherapy and host-parasite inter-relationships.

ACKNOWLEDGEMENTS

I am indebted to Dr D. E. Moorhouse for inspiring this study, to Catherine A. Creevey for assistance in the field and laboratory and to the University of Queensland for financial support.

REFERENCES

ABDEL-HAMEED, A. A. (1984) Effects of benzimidazole anthelminthics on the survival and migratory behaviour of *Toxocara canis* larvae in the mouse. *American Journal of Veterinary Research*, **45**, 1430–1433.

BASTEN, A., BOYER, M. H. & BEESON, P. B. (1970) Mechanism of eosinophilia. I Factors affecting the eosinophil response of rats to *Trichinella spiralis*. *Journal of Experimental Medicine*, **131**, 1271–1287.

BAUM, J. & STOYE, M. (1981) Pranatale und galaxtogene Infektionen mit *Toxocara canis* Werner 1782 (Anisakidae) bei der Maus. *Zentralblatt für Veterinarmedizin*, **28**, 319–328.

BEAVER, P. C. (1969) The nature of visceral larva migrans. *Journal of Parasitology*, **55**, 3–12.

BRINDLEY, P. J., PROCIV, P., CREEVEY, C. A. & EAST, I. J. (1985) Regulation of toxocariasis in mice selectively reared for high and low immune responses to *Nematospiroides dubius*. *Journal of Helminthology*, **59**, 157–166.

BURREN, C. H. (1968) Experimental toxocariasis. II. *Toxocara canis* in the mouse as a system for testing compounds of potential anthelmintic activity. *Zeitschrift für Parasitenkunde*, **30**, 162–170.

BYTH, S. (1980) Palm Island Mystery Disease. *Medical Journal of Australia*, **2**, 40–42.

DUNSMORE, J. D., THOMPSON, R. C. A. & BATES, I. A. (1983) The accumulation of *Toxocara canis* larvae in the brains of mice. *International Journal for Parasitology*, **13**, 517–521.

KAYES, S. G. & OAKS, J. A. (1980) *Toxocara canis*: I. Lymphocyte function in murine visceral larva migrans and eosinophilia onset. *Experimental Parasitology*, **49**, 47–55.

MOORHOUSE, D. E. (1982) Toxocariasis: a possible cause of the Palm Island Mystery Disease. *Medical Journal of Australia*, **1**, 172–173.

NICHOLS, R. L. (1956) The etiology of visceral larval migrans. I. Diagnostic morphology of infective second-stage *Toxocara* larvae. *Journal of Parasitology*, **42**, 349–362.

PROCIV, P. (1983) Observations on the transmission and development of *Toxocara pteropodis* (Ascaridoidea: Nematoda) in the Australian Grey-Headed Flying Fox, *Pteropus poliocephalus* (Pteropodidae: Megachiroptera). *Zeitschrift für Parasitenkunde*, **69**, 773–781.

SCHRIBER, R. A. & ZUCKER-FRANKLIN, D. (1975) Induction of blood eosinophilia by pulmonary embolization of antigen-coated particles: the relationship to cell-mediated immunity. *Journal of Immunology*, **114**, 1348–1353.

SPRENT, J. F. A. (1953) On the migratory behaviour of the larvae of various *Ascaris* species in white mice. (ii) Longevity of encapsulated larvae and their resistance to freezing and putrefaction. *Journal of Infectious Diseases*, **92**, 114–117.

WALLS, R. S. & BEESON, P. B. (1972) Mechanism of eosinophilia. XI. Induction of eosinophilia in rats by certain forms of dextran (36532). *Proceedings of the Society of Experimental Biology and Medicine*, **140**, 689–693.

Accepted 13th February, 1985

APPENDIX V

International Journal for Parasitology Vol. 16, No. 5, pp. 471–474, 1986.
Printed in Great Britain.

0020–7519/86 $3.00 + 0.00
Pergamon Journals Ltd.
© *1986 Australian Society for Parasitology.*

ORAL, PARENTERAL AND PARATENIC INFECTIONS OF MICE WITH *TOXOCARA PTEROPODIS*

PAUL PROCIV and PAUL J. BRINDLEY

Department of Parasitology, University of Queensland, St. Lucia, Q 4067, Australia

(*Received* 19 *June* 1985; *in revised form* 12 *December 1985*)

Abstract—PROCIV P. and BRINDLEY P. J. 1986.Oral, parenteral and paratenic infections of mice with *Toxocara pteropodis. International Jóurnal for Parasitology* **16**: 471–474. Groups of adult male CBA mice were inoculated with 7500 eggs of *Toxocara pteropodis* intragastrically, 2000 eggs subcutaneously or 2000 eggs intraperitoneally. Regardless of the infection route, larvae accumulated in the livers of these mice in comparable numbers. Following peroral infection, most larvae rapidly appeared in the liver with very small numbers passing through mesenteric lymph-nodes to the lungs. Intraperitoneal inoculation of eggs was followed also by a rapid accumulation of larvae in the liver, with larvae being recoverd also from lungs, intestines and other organs. Larvae from subcutaneous sites passed through the lungs en route to the liver, where they accumulated more slowly.

Larvae within acutely-infected mouse lung and chronically infected flying fox liver which were fed to non-infected mice were capable of invading the livers of the latter, indicating a relict paratenic tendency.

This marked hepatotropism of *T. pteropodis* indicates that larval accumulation in host livers is a goal-directed phenomenon independent of host capillary dimensions.

INDEX KEY WORDS: *Toxocara pteropodis*; mice; visceral *larva migrans*; *Pteropus poliocephalus*; paratenic infection.

INTRODUCTION

SINCE Beaver (1956) confirmed the role of *Toxocara canis* in human visceral *larva migrans*, the migration of several *Toxocara* species has been studied in natural and experimental hosts. Following oral infection of mice with *T. canis* eggs, larvae are distributed widely, passing through liver and lungs and then many other tissues to accumulate ultimately in the central nervous system (see Dunsmore, Thompson & Bates, 1983). Third stage larvae of *T. cati*, which are smaller than those of *T. canis*, also pass through the liver and lungs of mice but accumulate in skeletal muscle (Dubey, 1968). The larvae of both *T. canis* and *T. cati* are long-lived in mice, which are considered to be natural reservoirs of infection for dogs and cats. Larvae of *T. vitulorum* of cattle also distribute to diverse sites in mice but survive only for several weeks (Warren, 1971).

Investigation of the behaviour of *T. pteropodis*, a parasite of *Pteropus poliocephalus* and other flying foxes (Prociv, 1983), in experimental hosts was prompted by its possible implication in human visceral *larva migrans* (Moorhouse, 1982). The first study of *T. pteropodis* in mice (Prociv, 1985) showed that following intragastric administration of infective eggs, third-stage larvae accumulated and remained at least 14 months in the liver where they grew in length from about 400 μm to about 600 μm over 5–7 weeks. Eggs inoculated subcutaneously or intraperitoneally hatched and larvae again travelled to the liver. Egg doses of 1000 or less were used in that study. Improvements in the maintenance of juvenile flying foxes and in egg-recovery techniques have enabled higher dose infections to be investigated in greater detail, and the findings are now presented.

MATERIALS AND METHODS

Source of T. pteropodis *eggs*. Five suckling 2-month-old Grey Headed Flying Fox (*P. poliocephalus*) naturally infected with *T. pteropodis* were caught by hand in mangroves 50 km south of Brisbane. They were kept in a 45 × 45 × 60 cm wire cage and maintained on normal strength reconstituted skimmed cows' milk fortified with an equal quantity of Digestelact (Sharpe Laboratories, Artarmon, NSW, Australia) and supplemented with sucrose (10 g 100 ml^{-1}) and 0.3 ml 100 ml^{-1} Avidrops [Medical Research (Marketing), North Ryde, NSW, Australia] rationed at 100 ml bat^{-1} day^{-1}. All faeces were collected daily from a metal tray on the floor of the cage, emulsified in household detergent solution then washed through a bank of sieves with the final mesh opening at 63 μm. Eggs were backwashed from the final sieve with tapwater then left to settle by gravitation. Four hours later the supernatant was discarded and the sediment was mixed into activated charcoal and left to stand in a covered 14 cm Petri dish at room temperature (25–30°C). After at least 2 weeks embryonation, eggs were washed from the charcoal with normal (0.15 M) saline, passed through a 175 μm sieve and again concentrated gravitationally. They were then left to stand in 10% sodium hypochlorite solution for 2 h to destroy contaminant microorganisms, and total egg numbers were extrapolated from the average count in four 0.1 ml aliquots.

The final volume was adjusted with saline to give the required egg dose in 0.4 ml.

Infection of mice. Six-week-old male CBA mice, provided by the Central Animal Breeding House of the University of Queensland, were used exclusively in this study. Twenty mice (oral group) were each given 7500 infective eggs by stomach tube. Under ether anaesthesia, another 12 were given 2000 eggs intraperitoneally via a 22 gauge needle (intraperitoneal, or i.p., group) and a third group of 12 were given 2000 eggs subcutaneously in the interscapular region (subcutaneous, or s.c., group).

Examination of mice. Mice were killed in pairs by cervical dislocation under ether anaesthesia at the times indicated in Tables 1 and 2. From each carcass the pelt, paws, snout and tail were removed and discarded. The alimentary tract was removed *en bloc* from the abdomen, stripped of its mesenteries, opened longitudinally and rinsed vigorously in three changes of normal saline. The brain, lungs, kidneys, liver, mesenteric lymph-nodes, mesentery and intestinal wall were then minced separately with scissors. Each minced organ was suspended in a 24-layer gauze pocket (three 8-ply 7.5 × 7.5 cm gauze swabs, supported by nylon insect mesh invaginated into 0.15 M saline in a 70 ml plastic container and incubated for 18 h at 37°C ("saline digestion"). The remaining carcass of each mouse was ground in a Waring blender at high speed for 30 s then digested as above in a 600 ml container. At the end of incubation the sediment in each container was fixed with an equal volume of boiling 6% formalin. A half of each liver sediment, one-eighth from each carcass sediment and the entire sample of each remaining sediment were then examined for larvae in a grid-marked glass tray by stereomicroscopy.

Paratenic transmission. Ten mice were each inoculated subcutaneously with 10,000 *T. pteropodis* eggs in 0.5 ml saline and killed 3 days later. Their lungs were removed and minced then fed to five mice which had been starved for 24 h previously. Seven days later the cannibal mice were killed, and their livers removed and subjected to saline digestion. One laboratory-reared juvenile male *P. poliocephalus* (under ether anaesthesia) was given a subcutaneous injection of 20,000 *T. pteropodis* eggs and 5 days later was killed with intraperitoneal pentobarbitone. One lung was digested in saline for larval recovery and the other minced and fed to three mice, which were killed and examined 1 week later. Another young *P. poliocephalus* was given 20,000 eggs by mouth and killed 40 days later. Three-quarters of its liver was fed to two mice, and the remaining quarter was examined for larvae. The mice were killed 1 week later and their livers examined for larvae.

RESULTS

Oral infections (Table 1)

The total number of larvae recovered was maximal at 18 h post-infection (p.i.), when a large proportion was still in the gut contents and wall, only 35 had reached the liver, and one had already entered the lungs. On the fourth day p.i. 99% of larvae recovered were in the liver, although very small numbers were still found in other organs up to day 7. From day 4 onwards hepatic recoveries represented 13–16% of the total egg dose. Small numbers of larvae were found in the mesentery and mesenteric nodes up to day 4, and in the lungs to day 7, when larvae were found also in brain and kidneys. On day 10 larvae were found only in the liver. On days 4 and 7, one or two fine petechial haemorrhages were noticed on the surfaces of the lungs. On days 7 and 10, numerous fine white dots were discernible in the livers.

Parenteral infections (Table 2)

At 2 days p.i. 205 hepatic larvae were recovered from the i.p. group, but none from the s.c. group. Hepatic larval recovery remained fairly constant throughout in the i.p. mice, but gradually increased with time in the s.c. group. Conversely, early lung recoveries were higher in the s.c. than in the i.p. mice, and in both groups diminished gradually with time, except for the peak at day 4 in the i.p. group. A considerable number of larvae (39) was still found in the s.c. lungs at 15 days p.i. Extensive fibrinous exudates containing viable eggs and free motile larvae were found in the peritoneal cavity of the i.p. mice killed on days 2 and 4, so that their intestines were not examined for larval distribution. Small numbers of larvae were recovered from the other organs examined, but the numbers declined with time after a general peak in both groups at day 4. Very small numbers (0–2) of larvae were recovered from the carcass sediments and were not included in Table 2. Two of the s.c. mice died, so none of this group were left to examine at 21 days.

The larvae recovered from the livers and lungs of the two s.c. mice killed on day 15 were drawn and measured, with results shown in Table 3.

Table 1—Recovery of larvae from mice given 7500 eggs *T. pteropodis* intragastrically

Time after infection	No. mice examined	Average no. larvae recovered									
		Gut contents	Gut wall	Mesenteric nodes	Mesentery	Liver	Lungs	Brain	Kidneys	Carcass	Total
18 h	2	1000	555	5	2	35	1	0	0	0	1600
24 h	4	450	175	6	3	385	2	0	0	0	1020
48 h	6	235	88	4	2	625	5	0	0	0	960
4 days	2	1	2	3	0	1060	4	0	0	0	1070
7 days	4	0	1	0	0	1430	4	1	1	0	1440
10 days	2	0	0	0	0	1270	0	0	0	0	1270

Table 2—Average recovery of larvae from pairs of mice inoculated subcutaneously (s.c.) or intraperitoneally (i.p.) with 2000 eggs of *T. pteropodis*

Days after infection	Lungs	Gut contents	Gut wall	Brain	Kidneys	Liver	Total
		s.c. Group					
2	130	0	4	4	5	0	139
4	68	7	9	14	18	127	243
7	29	8	6	11	10	130	194
10	19	4	2	2	1	235	263
15	39	1	2	1	2	341	386
		i.p. Group					
2	38	NE	NE	4	3	205	251
4	74	NE	NE	11	8	356	458
7	45	2	6	6	7	202	268
10	26	1	2	2	3	225	259
15	11	1	1	3	0	208	224
21	2	0	0	1	2	214	219

NE = Not examined.

Table 3—Lengths of larvae recovered from two mice 15 days following subcutaneous inoculation of 2000 *T. pteropodis* eggs

	Lungs			Liver		
	No. measured	Length $\bar{x} \pm$ S.D. (μm)	Range (μm)	No. measured	Length $\bar{x} \pm$ S.D. (μm)	Range (μm)
Mouse 1	18	425 ± 27	370–460	28	445 ± 30	385–490
Mouse 2	56	415 ± 23	370–465	34	455 ± 32	390–530

In both mice, larvae recovered from livers were significantly larger than those from lungs (Student's *t*-test: mouse 1 — $P < 0.05$; mouse 2 — $P < 0.001$). Early in the infection numerous petechial haemorrhages in the lungs correlated with larval recoveries, but beyond day 10 haemorrhagic areas were confluent and showing signs of resolution. Numerous granulomata were visible in the livers from day 7 onwards.

Paratenic transmission

A total of 252 larvae were recovered from the livers of those mice which had eaten the lungs of the 10 mice inoculated subcutaneously with 10,000 eggs each.

One lung of the subcutaneously infected flying fox yielded 170 larvae, and 23 larvae were recovered from the livers of the three mice which had eaten the other lung.

The young flying fox killed 40 days after oral infection harboured 187 larvae in 25% of its liver (i.e. estimated total hepatic burden 750) and the two mice which ate the remaining 75% yielded 11 larvae from their livers.

DISCUSSION

These findings necessitate a modification of earlier conclusions (Prociv, 1985) based on studies of lighter infections in mice.

Following oral inoculation, most infective larvae of *T. pteropodis* travel to the liver via the portal venous system and lodge there perhaps for the natural duration of the mouse's life. However, a small number enter the lymphatics, and presumably these are the ones which then reach the lungs. The subsequent fate of this latter group is not certain, but they may perish there, or move to the liver via the circulation, or undergo tracheal migration to re-enter the gut and perhaps reach the liver after mucosal re-penetration. The small numbers preclude definite conclusions.

Studies of larval distribution in the intestine are unreliable. Because the gut was rinsed and not scraped, larvae remaining in the crypts may have been counted with those recovered from the gut wall. However, following scraping, larvae which had penetrated the mucosa could be included in "intestinal contents". After vigorous rinsing of the intestine in saline

numerous villi were seen in the washings. The one larva recovered from the gut wall 7 days after oral infection (Table 1) may have been a straggler or may have undergone tracheal migration.

In view of the propensity for larvae of *T. pteropodis* to accumulate in the liver as demonstrated in the parenteral infections, it is probable that the larvae found in the lungs after oral infection had travelled via lymphatics and therefore had not passed through the liver. Likewise, the larvae recovered from brain and kidney on day 7 (Table 1) probably reached the central circulation via lymphatics and then the pulmonary and systemic circulations.

The proportion of larvae recovered from livers in this study (13–16% of total intragastric egg dose) is similar to that of the earlier study (Prociv, 1985). However, following parenteral inoculation, larval recovery (10–15%) was inexplicably lower than predicted from the previous study.

The parenteral infections demonstrated the ability of eggs to hatch in tissues and the remarkable hepatotropism of *T. pteropodis* larvae. Following subcutaneous inoculation, many eggs hatch slowly so larval release is protracted. These larvae presumably reach the liver via the circulation, so that large numbers should appear in the lungs prior to their recovery from the liver. Upon arriving in the lungs, some may undergo tracheal migration to reach the gut, but most probably continue in the circulation until ultimately lodging in the liver. The protracted presence of larvae in the lungs may reflect either their slow release from inoculation sites, or repeated circulation of larvae destined ultimately for the liver, which they may reach either via the hepatic artery or via the intestinal-portal circulation. The size difference between hepatic and pulmonary larvae in the s.c. group indicates the latter were less mature and hence represented later hatchlings, i.e. the continuing recovery of larvae from lungs reflected prolonged hatching of eggs at the inoculation site of the s.c. mice.

In the i.p. group, hepatic recoveries were relatively high at the outset and subsequently remained fairly constant, suggesting that most of these larvae had reached the liver after penetrating portal tributaries in serosal surfaces of the gut and perhaps the liver capsule directly. Those found in the lungs probably had penetrated the parietal peritoneum to enter the somatic circulation or lymphatics. The failure of hepatic larval numbers in the i.P. group to increase with time despite the protracted pulmonary recoveries suggests that most larvae which reached the lungs in these mice did not continue to the liver but may have undergone tracheal migration, and were subsequently lost (see below).

Larvae recovered from the intestinal lumen in both i.p. and s.c. groups most likely had undergone tracheal migration. The paratenic study indicates that these larvae retained an ability to penetrate the gut mucosa even after 40 days in the liver of a flying fox, when they would have grown to 560 μm in length (Prociv, 1983), and pass to the liver again, although only a small proportion actually did reach the liver and most were lost. That larvae of *T. pteropodis*, a parasite of strict herbivores, undergo paratenic transmission in mice demonstrates an affinity with closely related ascaridoids of carnivores, notably *T. canis* and *T. cati*, with which it must share a common ancestry.

The marked hepatotropism demonstrated by *T. pteropodis* shows that larval migration is governed by factors other than their dimensions in relation to blood vessels (see Sprent, 1955). It is likely that the poorly understood sensory receptors of these organisms play a critical role in their migration, growth and development throughout a maze of host tissue compartments.

Although further work needs to be done on its potential pathogenicity to humans, *T. pteropodis* exhibits unique larval behaviour which may asist in our understanding of host-parasite interactions.

Acknowledgements—We are grateful to Catherine A. Creevey for laboratory and field assistance, and to the University of Queensland for funding.

REFERENCES

Beaver P. C. 1956. Larva migrans. *Experimental Parasitology* **5**: 587–621.

Dunsmore J. D., Thompson R. C. A. & Bates I. A. 1983. The accumulation of *Toxocara canis* larvae in the brains of mice. *International Journal for Parasitology* **13**: 517–521.

Dubey J. P. 1968. Migration of *Toxocara cati* larvae in mice. *Tropical & Geographical Medicine* **20**: 172–176.

Moorhouse D. E. 1982. Toxocariasis. A possible cause of the Palm Island mystery disease. *Medical Journal of Australia* **1**: 172–173.

Prociv P. 1983. Observations on the transmission and development of *Toxocara pteropodis* (Ascaridoidea: Nematoda) in the Australian Grey-headed Flying-Fox, *Pteropus poliocephalus* (Pteropodidae:Megachiroptera). *Zeitschrift für Parasitenkunde* **69**: 773–781.

Prociv P. 1985. Observations on *Toxocara pteropodis* infections in mice. *Journal of Helminthology* **59**: 267–275.

Sprent J. F. A. 1955. On the invasion of the central nervous system by nematodes: II. Invasion of the nervous sysem in ascariasis. *Parasitology* **45**: 41–55.

Warren E. G. 1971. Observations on the migration and development of *Toxocara vitulorum* in natural and experimental hosts. *International Journal for Parasitology* **1**: 85–99.

APPENDIX VI

Z Parasitenkd (1986) 72:487–491

Zeitschrift für
Parasitenkunde
Parasitology Research

© Springer-Verlag 1986

Susceptibility of rats to infection with *Toxocara pteropodis*

Paul Prociv and Paul J. Brindley *

Department of Parasitology, University of Queensland, St. Lucia, Queensland, Australia 4067

Abstract. Infective eggs of *Toxocara pteropodis* were administered to Wistar rats via oral and parenteral routes. Third-stage larvae were recovered from the livers of suckling young 8 days after oral infection, and from livers and lungs after intraperitoneal or subcutaneous inoculation of eggs. These larvae were short-lived as none were found in suckling mice killed 2 weeks post-infection. Larvae were not recovered from tissues of rats aged 22 days or more when inoculated orally, indicating that refractoriness to infection develops rapidly with growth. Small numbers of larvae were recovered from the lungs of older rats 4 days after subcutaneous but not after oral inoculation. Adult male Buffalo and Fisher rats were also totally resistant to oral infection. Hence, rats differ from mice in their susceptibility to *T. pteropodis*.

Introduction

Toxocara canis, the prototypal agent of human visceral *larva migrans*, has been found to be infective to all mammals so far studied (Beaver et al. 1984). Its migration in mice parallels its behaviour in humans (Smith and Beaver 1983). Although little work has been reported on its behaviour in rats, as in mice it has been shown to invade the central nervous system (Olson and Rose 1966).

An intestinal parasite of suckling flying-foxes, *Toxocara pteropodis*, may have been involved in an outbreak of hepatitis in humans (Moorhouse 1982). Following the oral administration of infective eggs of *T. pteropodis* to mice, third-stage larvae settle in the livers where they elicit a chronic granulomatous response (Prociv 1985). In a natural host, *Pteropus polioce-*

Reprint requests to: P. Prociv

* *Present address:* Laboratory of Parasitic Diseases, National Institute of Allergy & Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA

phalus larvae also accumulate in the liver but at the end of pregnancy migrate to the mammary glands and infect the neonate via the milk (Prociv 1983).

To predict its behaviour in humans, *T. pteropodis* has been studied in a variety of experimental hosts and the findings in rats are presented here.

Materials and methods

Eggs of *T. pteropodis* were collected and doses calculated as previously described (Prociv 1985). Rats were supplied by the Central Animal Breeding House of the University of Queensland and kept under standard conditions with free access to food and water. With one exception, outbred Wistar rats were used in all experiments.

Infection of adult rats. Five males aged 4 months, of each of Wistar, Fisher and Buffalo strains, were lightly anaesthetised with ether and given 1000 infective eggs of *T. pteropodis* in 0.4 ml saline via stomach tubes. Eight days later they were killed with ether and their livers and lungs examined for larvae by incubating at 37° C in saline as previously described (Brindley et al. 1985).

Age susceptibility to oral infection. Eighteen male rats, in six groups of three, aged 12, 22, 30, 40, 60 and 100 days respectively were each inoculated intragastrically with 2,000 infective eggs and killed 8 days later when their livers were examined for larvae. Another two 100-day-old rats were given 5,000 eggs and killed at 3 days for recovery of hepatic larvae. A further two were given 1,000 eggs and killed 8 h later, and their intestines examined as described previously (Prociv 1985).

Age susceptibility to subcutaneous infection. Two 50-day old and two 100-day old rats, under ether anaesthesia, were inoculated subcutaneously by a 22-g needle with 5,000 eggs in 0.5 ml saline. They were killed 4 days later and lungs and livers were examined for larvae.

Infection of suckling rats. Two 8-day-old litters, totalling 15 rats, were divided into three equal groups. Under ether anaesthesia, those in one group were each given 2,000 eggs *T. pteropodis* in 0.4 ml saline intragastrically (oral group), those in the second group the same dose intraperitoneally (i.p. group) and the third subcutaneously (s.c. group) in the interscapular region. They remained with their dams and suckled until all were killed with ether 8 days later. Blood was collected at necropsy for total and differential white blood counts as described previously (Brindley et al. 1985) and the livers and lungs were examined for larvae.

Duration of infection. Twenty-four 12-day-old suckling rats were each inoculated subcutaneously with 2,000 eggs of *T. pteropodis* and three were killed at weekly intervals. From the first group, only livers were examined, but subsequently livers, lungs, kidneys, brains and skeletal muscle samples were processed for larval recovery.

Control mice. In conjunction with each rat experiment, two adult male C3H mice were inoculated intragastrically with 2,000 eggs from the same batch as given to the rats. These mice were killed 1 week later and their livers examined for larvae.

Results

Adult male rats. Larvae were not found in the livers of any of the rats of the three different strains.

Age susceptibility to oral infection. Larvae (mean number 60, ±S.D. 12) were recovered only from the livers of the three rats infected at 12 days

of age. All older groups were free of infection, including the two 100-day-old rats killed at 3 days. The two rats killed at 8 h yielded the following average larval numbers from their gastrointestinal tracts: stomach wall 0; proximal small gut wall 19; distal small gut wall 20; total small gut contents 43; colon wall 0; colon contents 0; liver 0 (total larval recovery: 82).

Age susceptibility to subcutaneous infection. The two 50-day-old rats harboured 31 and 71 larvae in their lungs and none in their livers. The two 100-day-old rats yielded 9 and 16 larvae only from their lungs.

Suckling rats. In the oral group, between 3 and 45 (mean 20±S.D.17) larvae were recovered from livers and none from lungs. The i.p. group yielded 58–110 (82±21) hepatic larvae, which was significantly higher than the oral group ($P<0.002$, Students' "t" test), and 2–39 (19±14) larvae from the lungs. The s.c. group harboured 3–17 (11±5) hepatic larvae (less than the i.p. group, $P<0.001$) and 10–15 (13±2) pulmonary larvae.

Most rats showed a peripheral leucocytosis (total white cell count range 7,000–29,000/mm^3) with no consistent difference amongst the three groups. Owing to technical problems eosinophil counts were not obtained from the oral group. The absolute eosinophil count from the i.p. group was 860/mm^3 (S.D. 200) and from the s.c. group 250/mm^3 (S.D. 200). This was a significant difference (Students' "t" test, $P<0.05$).

Duration of infection. The three 12-day-old rats killed at 1 week yielded seven, four and four larvae (average 0.25% of egg dose) from their livers. Larvae were not found in any organs of rats killed subsequently.

Mouse controls. All the mice harboured larvae in their livers, the numbers recovered ranging from 9% to 25% of the administered egg dose.

Discussion

In a previous study in mice, the oral administration of 1,000 *T. pteropodis* eggs was followed by the recovery of 110–220 larvae from livers between 10 days and 14 months post-infection (Prociv 1985). Larvae were not found in any other tissues. Following parenteral inoculation of eggs, larvae travelled through lungs and other organs, but eventually accumulated in livers (Prociv and Brindley, in press) where they presumably remained indefinitely.

The fate of larvae in rats is different. Eggs administered orally perhaps do not hatch as effectively as in mice. The two rats fed 1,000 eggs and killed at 8 h yielded only 8% of the egg dose as larvae, yet 24% of larvae were recovered from mice fed 1,000 eggs and killed 8 h later. (Prociv 1985). At parenteral inoculation sites in rats, eggs also either hatch less efficiently than in mice, and/or the freed larvae encounter difficulty in penetrating tissues and blood vessels.

Suckling rats are partially susceptible to infection from orally administered eggs, but less larvae reach the liver than in comparable infections

in mice. Furthermore, these larvae are very short-lived, as none were found in suckling rats killed 2 weeks after infection. Larvae must be viable for their successful recovery from tissues by dissection and modified Baermann separation techniques (Prociv 1985). As they grow young rats rapidly become refractory. Perhaps if they were killed earlier than 1 week after infection, the older rats may have yielded live larvae from their livers, but even in adult rats examined only 3 days after a 5,000 egg dose larvae were not found.

In suckling rats, the highest larval recoveries were from the livers (up to 5.5% of total dose) and lungs (up to 2% egg dose) of the i.p. group. Yields from livers of the oral and s.c. groups were significantly lower. Following i.p. inoculation of eggs, larvae may reach the liver by penetrating the capsule directly or via the portal system after entering capillaries of the visceral peritoneal surfaces. Those reaching the lungs probably do so after penetrating gut lymphatics or perhaps capillaries in the parietal peritoneum. Subcutaneous sites are less vascularised so larvae there would have greater difficulty reaching the circulation. They then would need to negotiate the pulmonary capillaries before proceeding to the liver, and attrition of larvae here could explain the lower recovery from livers of the s.c. group.

An uninfected control group of suckling rats was not included in this study, so the blood eosinophilia is difficult to interpret. The higher levels in the i.p. group may simply reflect the larger number of larvae entering the circulation and tissues than in the s.c. group.

In older rats inoculated subcutaneously with eggs, a small number of larvae managed to enter the circulation and reach the lungs, but presumably they perished quickly as none were found in livers. Oral administration of eggs in older rats of three different strains produced uniformly negative results. This refractoriness of rats to infection with *T. pteropodis* must result not from acquired humoral or cellular immunity, but from unfavourable tissue factors which develop with age.

Rats are more resistant than mice to a variety of nematodes. Olsen and Kelley (1960) recovered less larvae from young rats than from young mice given identical numbers of *Ascaris suum* eggs. In a limited study, Sprent (1955) found that 7 days after oral infection less larvae of *T. canis* had reached the brains of juvenile rats than of mice examined and even less of adult rats, indicating a relative resistance to this nematode which increases with age in rats. Olson and Rose (1966) recovered 1–4% of an egg dose of *T. canis* as larvae from the brains of rats killed 4 weeks after oral infection, which is less than found in published studies on mice. For example, Brindley et al. (1985) recovered 6.3–12.9% of a *T. canis* egg dose as larvae from brains of mice killed 25 days after infection.

Suckling rats are susceptible, although less so than adult mice, to infection with *Namatospiroides dubius* (Cross and Duffy 1963) but rapidly develop resistance with age. This resistance is associated with an acute inflammatory and fibroblastic response in the gut submucosa which can be partially suppressed with corticosteroids (Cross 1960). Larvae of *N. dubius* are capable of penetrating the gut mucosa of normal adult rats, but their development is suppressed and they are rapidly destroyed. It is likely that similar aggressive tissue responses destroy larvae of *T. pteropodis* in the tissues of rats. In this study, histological examination was not undertaken nor were our animals treated with steroids. Nevertheless, it is obvious that the response of rats differs markedly from that of mice and therefore at present neither of these animals can be used as a predictive model of the fate of *T. pteropodis* in humans.

Acknowledgements. We are grateful to Catherine A. Creevey for technical assistance. This study was funded by the University of Queensland.

References

Beaver PC, Jung RC, Cupp EW (1984) Clinical parasitology, 9th edition. Lea and Febiger, Philadelphia, p 321

Brindley PJ, Prociv P, Creevey CA, East IJ (1985) Regulation of toxocariasis in mice selectively reared for high and low immune responses to *Nematospiroides dubius*. J Helminthol 59:157–166

Cross JH (1960) The natural resistance of the white rat to *Nematospiroides dubius* and the effect of cortisone on this resistance. J Parasitol 46:175–185

Cross JH, Duffy CE (1963) *Nematospiroides dubius* in the abnormal host. Ann NY Acad Science 113:88–99

Moorhouse DE (1982) Toxocariasis – a possible cause of the Palm Island mystery disease. Med J Aus 1:172–173

Olsen JS, Kelley GW (1960) Some characteristics of the early phase of migration of larvae of *Ascaris suum*. Proc Helm Soc Washington 27:115–118

Olsen LJ, Rose JE (1966) Effect of *Toxocara canis* infection on the ability of white rats to solve maze problems. Exp Parasitol 19:77–84

Prociv P (1983) Observations on the transmission and development of *Toxocara pteropodis* (Ascaridoidea: Nematoda) in the Australian Grey-Headed Flying Fox, *Pteropus poliocephalus* (Pteropodidae: Megachiroptera). Z Parasitenkd 69:773–781

Prociv P (1985) Observations on *Toxocara pteropodis* infections in mice. J Helminthol 59:267–275

Prociv P, Brindley PJ (1986) Oral, parenteral and paratenic infections of mice with *Toxocara pteropodis*. Int J Parasitol (in press)

Smith MH, Beaver PC (1983) Persistence and distribution of *Toxocara* larvae in the tissues of children and mice. Pediatrics 12:491–497

Sprent JFA (1955) On the invasion of the central nervous system by nematodes (ii) invasion of the nervous system in ascariasis. Parasitology 45:41–55

Accepted January 10, 1986

APPENDIX VII

Tropical Biomedicine 3: 97–106 (1986)

Toxocara pteropodis, T. canis and *T. cati* infections in guinea pigs

Paul Prociv
Department of Parasitology, University of Queensland, St. Lucia, Queensland 4067, Australia

Abstract. In guinea-pigs fed infective eggs of *Toxocara pteropodis*, larvae penetrated mainly the distal regions of the intestine and reached the liver via the portal system. Smaller numbers appeared in the lungs, having travelled presumably through lymphatics, but none were found in other tissues. These larvae did not grow in size, and none were recovered beyond the 25th day post-infection. Viable *T. pteropodis* larvae recovered from mouse livers failed to develop in the intestines of suckling guinea-pigs. Larvae of *T. canis* gradually accumulated in the brains of guinea-pigs where they persisted at least seven months, whereas those of *T. cati* showed a marked predilection for skeletal muscle where they persisted in high numbers for at least ten months. Infection with all three species stimulated comparable blood eosinophil responses. After five weeks post-infection, eosinophilia in *T. pteropodis*-infected animals declined more rapidly presumably as a result of the earlier destruction of larvae in tissues.

INTRODUCTION

Consequent to the suggestion that *Toxocara pteropodis* may have been responsible for a human epidemic of hepatitis/gastroenteritis (Moorhouse, 1982), work was commenced on the life-cycle of this nematode (Prociv, 1983) and its behaviour in experimental hosts.

Infective eggs ingested by flying foxes of the genus *Pteropus* hatch in the intestine and larvae accumulate in the liver. In pregnant females these larvae then migrate to the mammary glands at term and pass via the milk to the neonate's intestine, where they develop to maturity (Prociv, 1983). In adult mice infected orally, larvae likewise travel to the liver and remain there for at least 14 months (Prociv, 1985*a*). In rats, however, only suckling juveniles are susceptible to enteral infection. A lower proportion of larvae appear in the livers (Prociv & Brindley, 1986) where they survive less than two weeks. Older rats are totally refractory to oral infection. Such behaviour of *T. pteropodis* in mice and rats contrasts with that of T. *canis,* the classical cause of visceral *larva migrans* in humans which disperses similarly through the tissues of mice, rats, guinea-pigs and rabbits (Beaver, 1956).

In view of their differing susceptibility to *T. pteropodis,* neither mice nor rats could be viewed reliably as a model of human infection. Furthermore, as very little work has been published on the fate of *T. canis* and *T. cati* larvae in the guinea-pig, infections with the three *Toxocara* species were studied in this host.

MATERIALS AND METHODS

Toxocara eggs

Eggs of *T. pteropodis* were collected from the faeces of naturally-infected young *P. poliocephalus* as previously described (Prociv 1983) and embryonated for four weeks on moist charcoal. Eggs of *T. canis* and *T. cati* were dissected from adult worms obtained from dogs and cats autopsied at the University of Queensland Veterinary School and embryonated at room temperature for two months prior to use. Eggs were washed from the charcoal with tapwater and concentrated by gravitation. Egg doses were calculated from the numbers of infective eggs counted in 0.1 ml aliquots of the re-suspended sediments.

Experimental Animals

Male and female outbred English short-

haired guinea pigs aged 6 weeks and weighing 250–350 gm were kept in large cages with free access to commercial food pellets and water in an airconditioned room. They were infected by the slow administration of calculated egg doses in 0.5–1.0 ml sweetened water deep into the buccal cavity by syringe with a blunt 18 g needle. Adult male C3H mice were inoculated via stomach tube to test the infectivity of the eggs used.

Migration of *T. pteropodis*

Nine male guinea-pigs were each fed 10,000 infective eggs and killed with intraperitoneal injections of pentobarbitone sodium (325 mg in 1 ml) at times post-infection (p.i.) indicated in Table 1. The alimentary tract was removed from the abdominal cavity and divided into stomach, proximal and distal halves of small intestine, caecum and proximal and distal halves of large intestine. These organs were opened longitudinally, washed out under a running tap then rinsed vigorously in three changes of 0.9% saline. Each portion was then minced with scissors and incubated over gauze in normal saline at 37°C for 18 hours. Mesenteric lymph nodes, lungs, kidneys, brains, livers and approximately 20% of skeletal musculature were likewise separately minced and subjected to "saline digestion" for 18 hours. The sediments were fixed with an equal volume of boiling 6% formalin and examined for larvae in gridmarked glass trays under a stereomicroscope. Some larvae were mounted in chlorlactophenol and drawn and measured using a compound microscope with camera lucida.

Two mice were given 3,000 eggs from the same batch, killed one week later and their livers examined for larvae.

Oral administration of *T. pteropodis* larvae to suckling guinea-pigs.

Three six-day old suckling guinea-pigs were each fed 30 live third-stage larvae of *T. pteropodis* recovered from the liver of a male mouse three months p.i.. They were killed 5, 7 and 9 days later and their livers and intestines examined for larvae.

Tissue dispersal of *T. canis* and *T. cati* larvae

Nine male guinea-pigs were each fed 2,500 *T. canis* eggs and another nine given the same dose of *T. cati* eggs. These were killed at the intervals indicated in Figure 1, and their livers, lungs, brains and skeletal musculature examined for larvae as described above. Two mice were each fed 2,000 eggs of *T. canis* and two fed 2,000 eggs of *T. cati* as infectivity controls. These mice were killed at one month p.i. and their entire carcasses examined for larvae as described previously.

Secondary infection with *T. canis*

Two male guinea-pigs were fed 2,500 eggs of *T. canis* and after five months were fed another 3,000 eggs. Three months later they were killed and their tissues examined for larvae.

Haematological response

Guinea-pigs in seven groups of six were fed infective eggs as follows: *T. pteropodis* 100, 500 or 2,500; *T. canis* 100, 500 or 2,500; *T. cati* 2,500; At the times p.i. indicated in Figures 2 and 3, between 0900 and 1200 hours, they were lightly anaesthetised with ether and 1.0 ml blood taken from each by cardiac puncture. Three sham-infected animals served as controls. A thin smear was made immediately from the fresh blood and the remainder placed into EDTA tubes for determination of red cell and total white cell counts by Coulter counter. The differential white blood cell counts were determined by examining 200 white cells in Giemsa-stained smears under the oil-immersion objective (X100).

Table 1. Numbers of third-stage larvae recovered from tissues of guinea-pigs fed 10,000 eggs of *Toxocara pteropodis* and killed at intervals indicated.

Days after infection	Stomach Wall	Small Gut Wall		Caecum Wall	Large Gut Wall		Mesenteric Lymph Nodes	Lungs	Liver	Brain, Kidneys, Muscles
		Proximal	Distal		Proximal	Distal				
1	34	18	19	240	40	0	1	0	15	0
2	7	30	69	230	21	0	13	0	112	0
4	7	4	1	55	11	0	11	0	87	0
7	1	0	0	43	2	0	45	19	152	0
10	0	0	0	1	0	0	5	15	56	0
14	0	0	0	0	0	0	4	25	111	0
17	0	0	0	0	0	0	0	0	85	0
25	0	0	0	0	0	0	0	0	8	0
45	0	0	0	0	0	0	0	0	0	0

RESULTS

Migration of *T. pteropodis*

The tissue distribution of *T. pteropodis* larvae from 24 hours to 45 days p.i. is summarised in Table 1. Most larvae were recovered from the caecal wall, although many also invaded the mucosa of the small and proximal large intestines. That only a small fraction of these succeeded in migrating further is indicated by recoveries from the liver and lymph nodes. None were found in the wall of the distal colon, a region where the faecal contents were solid and pelletised. By

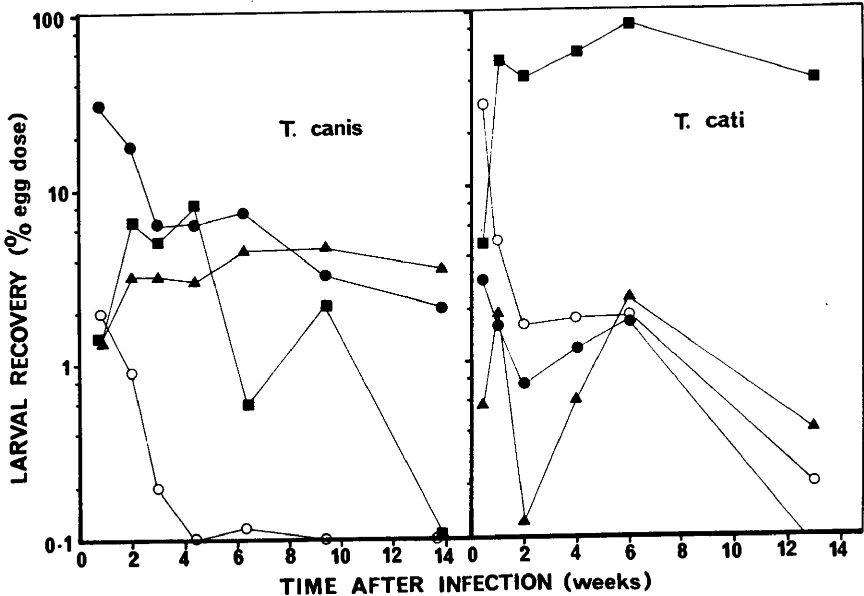


Figure 1. Numbers of larvae, expressed as percentage ($\log_{10}$ scale) of total egg dose, recovered with time from lungs (●), livers (○), skeletal muscles (■) and brains (▲) of individual guinea-pigs fed 2,500 eggs of either *T. canis* or *T. cati*.

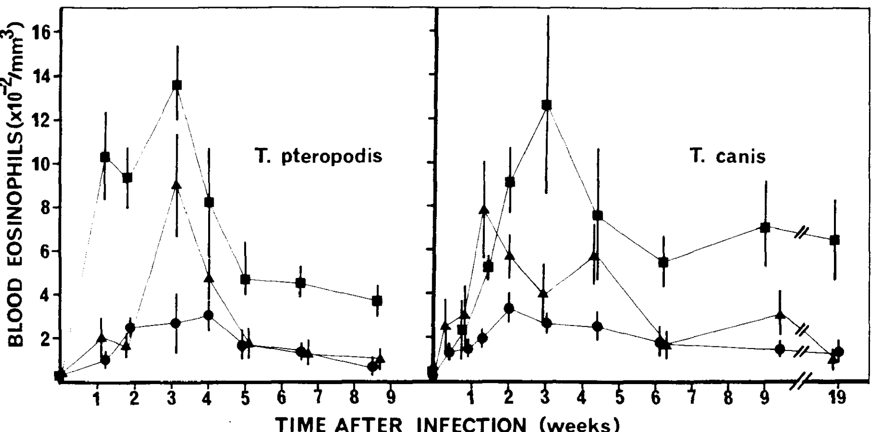


Figure 2. Mean peripheral blood eosinophil levels in groups of guinea-pigs fed 100 (●), 500 (▲) and 2,500 (■) infective eggs of *T. pteropodis* or *T. canis*. Vertical bars represent ±S.E.M.

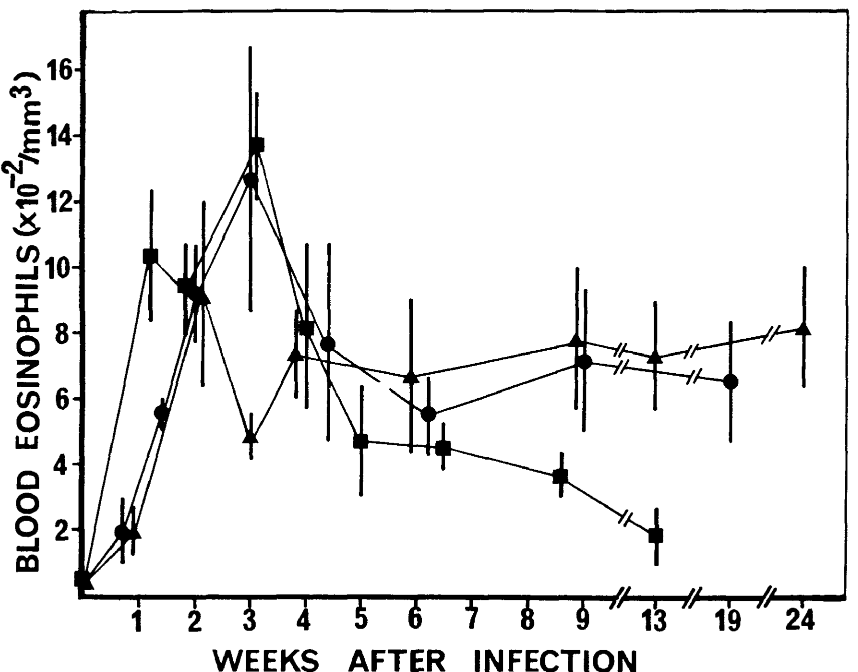


Figure 3. Mean peripheral blood eosinophil levels of groups of guinea-pigs fed 2,500 eggs of *T. pteropodis* (■). *T. canis* (●) or *T. cati* (▲). Vertical bars represent ±S.E.M.

the end of the first week, virtually all live larvae had left the intestines. Small numbers of larvae had reached the liver by 24 hours, and maximal numbers were recovered here at one week p.i.. Maximal numbers in lymph nodes were also found at this time. Larvae were found in the lungs from 7 to 14 days p.i. From the 14th day they were recovered only from the liver, in diminishing numbers, until day 25. At no stage were larvae found in the brain, kidneys or skeletal muscles. The lengths of these larvae are shown in Table 2. The average recovery from the livers of the two mice was 18% of the total egg dose. Developing larvae were not found in the intestines of the three suckling guinea-pigs.

Dispersal of *T. canis* larvae

The recovery of *T. canis* larvae from various tissues of individual guinea-pigs is shown in Figure 1. At 6 days p.i., 31% of the infective egg dose was recovered from the liver, and this fraction dimished with time to 2% at 14 weeks. From the lungs, 47 larvae (1.9% total egg dose) were recovered at day 6, with a rapid subsequent fall-off. Three larvae were found in the lungs at 6 weeks, one at 9 weeks and none later. At 6 days p.i., 36 larvae (1.4% egg dose) were found in the brain and the same number in skeletal muscle. Skeletal muscle recovery peaked at 4 weeks when 205 (8.2%) larvae were recovered, then declined with time so that none were found at 14 weeks. Brain larvae rose gradually to a plateau of 113 (4.5%) at 6 weeks. One guinea-pig was killed 7 months p.i., with following results: brain 99 (3.3% egg dose), liver 44 and lungs 2 larvae. The two animals given a second dose of 3,000 eggs and killed three months later yielded the following averages: liver 590 (10.7% total egg dose), brain 390 (7.1%), muscles 60 (0.1%) and lungs 0. The two mice killed 2 weeks p.i. yielded 34% of their total egg dose as larvae.

Dispersal of *T. cati* larvae

Recovery of *T. cati* larvae is shown in Figure 1. At 3 days p.i., most larvae (29% of egg dose) were in the lungs, with 4.8% (120) already in skeletal musculature. Muscle recoveries rose rapidly to a peak at day 7 and subsequently remained high, varying between 42% and 84% of the total egg dose. Numbers recovered from lungs fell rapidly but even at 13 weeks p.i. 5 larvae were still found here. Liver recoveries were also maximal at day 3 and declined slowly, so that no hepatic larvae were found at 13 weeks p.i.. One guinea-pig was killed 10 months after infection, and yielded 1,500 larvae (60% egg dose) from skeletal muscle, 9 (0.36%) from the brain, 7 (0.28%) from the lungs and none from the liver. An adult female which had been fed 2,500 eggs of *T. cati* and found to be pregnant was killed about mid-gestation, 27 days p.i., and larvae were recovered as follows: muscles 1,400 (56% egg dose), liver 28 (1.1%), lungs 44 (1.8%), brain 16 (0.6%), kidneys 9, heart 2, placentae 4, foetuses 0. From the two mouse carcasses 69% of the administered *T. cati* egg dose was recovered as larvae.

Table 2. Lengths of *Toxocara pteropodis* larvae recovered from guinea-pig tissue.

Days after Infection	Tissue	Number larvae measured	Length (µm) Mean ± S.D.	Range in length (µm)
1	Caecum wall	27	445 ± 24	400 – 490
2	Liver	23	440 ± 23	395 – 490
9	Liver	28	445 ± 18	410 – 475
14	Liver	43	435 ± 18	395 – 465
	Lungs	25	430 ± 17	400 – 455
17	Liver	27	455 ± 16	410 – 485
25	Liver	4	445 ± 18	425 – 465

Larval dimensions

As shown in Table 2, larvae of *T. pteropodis* did not grow in length after penetrating the gut wall and reaching the liver or lungs. Larvae of *T. canis* and *T. cati* also did not grow, and there was no difference between sizes of larvae in mice and guinea-pigs. Dimensions of the three larval species are shown in Table 3. All parameters overlap considerably, except the oesophageal length (mouth-ventriculus) of *T. pteropodis* which is shorter than in the other two species, both in absolute terms and in proportion to overall length. Microscopically, the oesophagus between the nerve-ring and ventriculus in *T. pteropodis* appeared markedly buckled, both in live and fixed larvae, and the ventriculus was more clearly discernible. In *T. canis*, the ventriculus was displaced dorsally in all larvae, presumably by the excretory columns, whereas in *T. cati* it was axial. In *T. canis* and *T. cati* the tail was longer than in *T. pteropodis*. The tail-tip of *T. canis* was bent sharply dorsally in most larvae, whereas in *T. cati* it was generally in line with the axis and tapered more gradually.

Haematological responses

Total red blood cell counts remained constant throughout this study in all infected groups. Total white cell counts did not vary markedly, except for mild elevations which paralleled increases in circulating eosinophil numbers in the groups given 2,500 eggs. Circulating eosinophils, but not neutrophils, did vary between groups. Eosinophils in the control guinea-pigs remained at a constant low level (mean 36 ± S.E. 17, range 0–86 eosinophils/mm^3).

In the *T. pteropodis* groups (Figure 2), eosinophilia was highest in the 2,500 egg group. Following a secondary peak of 1,400 ± 150/mm^3 at 3 weeks p.i. levels declined, but even at 8 weeks p.i. were higher than those of control animals, particularly in the high-dose group.

In the *T. canis* groups (Figure 2), again maximal blood eosinophilia was attained in the 2,500 egg group, with a single peak at 3 weeks p.i.. In the first two weeks there was considerable overlap in levels between the 500 and 2,500 egg groups, and in fact the former peaked earlier at 9 days with a lower secondary peak at 4 weeks. In all *T. canis* groups eosinophilia persisted for the duration of the study, to 19 weeks p.i., and was most marked in the high dose groups.

Figure 3 compares eosinophil responses of the three high dose groups. Absence of readings from *T. pteropodis*-infected animals in the first week makes comparisons of early responses difficult, but it appears that this species stimulated a more rapid rise in blood eosinophilia. Both *T. canis* and *T. pteropodis* showed comparable peaks at three weeks, when there was a temporary fall in *T. cati* eosinophil levels. Subsequently, eosinophilia in *T. pteropodis* infection declined while in the *T. canis* and *T. cati* groups it persisted for at least 19 and 24 weeks respectively. The last two *T. pteropodis* readings were from only two animals, and the last two *T. canis* and last three *T. cati* readings were from three animals each.

Small numbers of basophils, rarely higher than 1% of total white cell count, were detected in some animals, including controls. However, a peak was discerned in the high-dose *T. canis* group at 2 weeks p.i. (mean 117 ± S.E. 37 basophils/mm^3, range 0–270) and in the corresponding *T. cati* group (51 ± 22/mm^3, range 0–120).

DISCUSSION

The susceptibility of guinea-pigs to *T. pteropodis* infection is shown to lie between that of adult rats, which are totally refractory to infection (Prociv & Brindley, 1986), and mice. Larval recovery from mouse livers (18% of the administered egg dose) is comparable with the 9–22% recovery in an earlier study (Prociv, 1985*a*).

In guinea-pigs, the larvae also demonstrated marked hepatotropism, but the maximal number recovered from the liver, 152 on day 7, represented only 6% of the administered egg dose. These larvae perished rapidly, as none were found beyond 25 days p.i.. Viable, and hence motile, larvae are most reliably recovered from tissues using digestion techniques. Dead larvae do not migrate from minced tissues during incubation (Prociv,

Table 3. Dimensions (mean ± standard deviation) in µm of larvae recovered from mouse and guinea-pig tissues at times indicated. Ranges shown in brackets

SPECIES	*T. pteropodis*	*T. pteropodis*	*T. canis*	*T. cati*
SOURCE	Guinea-pig liver	Mouse liver	Guinea-pig brain	Guinea-pig muscle
TIME p.i.	2 days	7 days	14 weeks	13 weeks
NUMBER MEASURED	23	27	33	31
LENGTH (L)	440 ± 23 (395 – 490)	465 ± 16 (420 – 495)	435 ± 14 (415 – 465)	455 ± 17 (430 – 505)
DIAMETER (at ventriculus)	17 ± 0.8 (15 – 18)	20 ± 1.7 (16 – 23)	22 ± 1.3 (19 – 24)	19 ± 0.7 (18 – 21)
VENTRICULUS LENGTH	17 ± 1.8 (15 – 23)	20 ± 1.3 (18 – 23)	19 ± 1.0 (17 – 21)	19 ± 1.0 (18 – 21)
MOUTH-VENTRICULUS (M–V)	111 ± 6.2 (99 – 120)	108 ± 6.0 (90 – 113)	125 ± 3.5 (119 – 135)	135 ± 4.6 (128 – 145)
M–V/L	25 ± 1.2% (23 – 27)	23 ± 1.2% (20 – 25)	29 ± 0.8% (27 – 30)	30 ± 1.2% (28 – 32)
MOUTH-NERVE RING (M–N.R.)	64 ± 2.4 (60 – 69)	64 ± 3.9 (51 – 71)	69 ± 2.1 (66 – 73)	70 ± 3.5 (64 – 78)
M–N.R./M–V	58 ± 2.8% (54 – 63)	60 ± 3.2% (50 – 65)	55 ± 1.2% (53 – 57)	52 ± 2.3% (47 – 56)
GENITAL PRIMORDIUM-TAIL TIP (GP–T)	187 ± 11 (170 – 205)	197 ± 10 (177 – 209)	179 ± 12 (160 – 213)	187 ± 11 (170 – 212)
GP–T/L	43 ± 1.4% (41 – 46)	42 ± 1.7% (39 – 45)	41 ± 2.0% (38 – 46)	41 ± 1.4% (38 – 43)
ANUS-TAIL TIP (T)	34 ± 1.2 (32 – 37)	34 ± 1.4 (31 – 37)	37 ± 2.0 (34 – 43)	40 ± 2.5 (35 – 44)
T/L	7.8 ± 0.2% (7.4 – 8.2)	7.2 ± 0.3% (6.4 – 7.8)	8.5 ± 0.3% (7.9 – 9.2)	8.8 ± 0.5% (8.0 – 9.5)

1985*a*). Intact *T. pteropodis* larvae were found in tissue sections as late as 70 days p.i. (histological findings will be published separately). In mice, viable larvae have been recovered from livers as late as 22 months p.i. (Prociv, unpublished). As in mice (Prociv, 1985*a*) and flying foxes (Prociv 1983), in guinea-pigs larval penetration of the gastro-intestinal mucosa is more pronounced distally. The guinea-pig caecum is a zone of relative stasis where larvae have time to hatch and penetrate the mucosa. Already at 24 hours p.i. 240 larvae were in the caecal wall and 40 in the proximal colonic wall. This may indicate that *T. pteropodis* eggs are relatively slow to hatch, but little work has been published on the precise sites of larval penetration in other ascaridoid infections. For example, Sprent (1952) in his studies on various *Ascaris* and *Toxocara* species, infected mice by feeding them eggs mixed with food, then examined the entire alimentary tract for larvae. Olson (1962) studied organ distribution of *T. canis* larvae in mice but did not examine the large

intestine. However, Larsh (1950) did show that mice considered resistant to infection with *Ascaris suum* were rendered more susceptible by the concurrent administration of eggs with morphine which presumably slowed gut transit and gave larvae more time to hatch and invade the mucosa.

Nevertheless, most of the *T. pteropodis* larvae which penetrated the gastrointestinal wall failed to proceed further. The rapid disappearance of live larvae from the liver reflects an aggressive tissue response which is probably also active in the guinea-pig intestine. The accumulation of larvae in mesenteric lymph-nodes paralleled that in the liver, on a reduced scale, indicating that most larvae travelled via the portal system. The late appearance of larvae in the lungs suggests a retardation in their passage through either the liver or the lymphatics. The latter course is more likely in view of their marked hepatotropism. No larvae managed to complete somatic migration beyond the lungs. The possibility of a limited tracheal migration cannot be excluded, as tracheae were not examined for larvae. The one larva found in the stomach wall on day 7 may have undergone such migration.

In mice, *T. pteropodis* larvae commence growing on arrival in the liver (Prociv, 1985*a*) and by two weeks in light infections are longer than 500 μm. In guinea-pigs, failure of larvae to grow (Table 2) may indicate that the tissues are an unsuitable environment for *T. pteropodis*. The intestine of suckling guinea-pigs did not support the further development of *T. pteropodis*. However, it is possible that larvae from mouse livers may not develop further in the natural definitive hosts, suckling flying foxes. Although larvae were not recovered from any of the young guinea-pigs' livers, limited paratenic transmission has been demonstrated in mice (Prociv & Brindley, in press).

The distribution of *T. canis* larvae in guinea-pigs is shown to be comparable with that in mice. Although total numbers gradually declined, levels in the brain remained steady later in this study and viable larvae were present in the one animal killed at seven months. This agrees with the statement of Beaver (1956), proposed without supporting data or references, that larvae persist in guinea-pig tissues for at least two years, and contradicts the conclusions of Olson & Schulz (1963) who did not recover *T. canis* larvae from their guinea-pigs beyond 12 days p.i..

It appears that *T. canis* larvae gradually disappeared from peripheral tissues, either perishing or recirculating, accounting for the sporadic small numbers recovered from the lungs beyond the first few days. Those reaching the brain survived for much longer periods. Following secondary infection, enhanced immune responsiveness retarded larval dispersal, so that many were still enmeshed in the liver 3 months after the second infection, whereas after a single infection few larvae were found in this organ (See Fig. 1). Although these results were from two animals only, they agree with those of similar studies in mice, such as those of Olson (1962) and Brindley *et al.* 1985). Absence of larvae from the lungs suggests they do not retard larval passage, even in sensitised hosts.

Actual doses of *T. cati* eggs were underestimated. Most of the eggs were clumped, and carbon particles obstructed the detection of many viable larvae. This could explain the apparent higher proportional recovery of *T. cati* larvae. The rapid accumulation and prolonged survival of these in skeletal muscle, without any detectable attrition, is similar to infections in mice. Dubey (1968) recovered 63% of 2,000 eggs as larvae from muscle in mice 28 days p.i., but failed to detect larvae in other tissues at this time. In guinea-pigs, larvae persisted elsewhere so that small numbers were found in the brain and lungs of the animal killed 10 months p.i.. Invasion of the placentae of the pregnant guinea-pig probably reflected the random circulation of small numbers of larvae rather than incipient congenital infection.

Because the larvae of *T. pteropodis* did not grow in size in guinea-pig tissues, their lengths were similar to those of *T. canis* and *T. cati*. In the first three days after infection in mice, *T. cati* larvae grow about 8% in length (Sprent, 1956) but after that neither *T. cati* nor *T. canis* show any increase in size (Sprent, 1955). Nichols (1956) studied in detail the morphology of *T. canis* and *T. cati* larvae in mice, concluding that they could be differentiated only by the greater equatorial width of *T. canis*. In this study, the maximal diameter, at the ventriculus, was measured, showing *T. canis* to be wider but with some overlap. However, other dimensions which, when considered together, could help in distinguishing *T. canis* from *T. cati* larvae, included oesophageal length and the relative position of the nerve ring. In *T. pteropodis* larvae, oesophageal and tail lengths were distinctly shorter, showing little overlap with the other two species.

In the original work on *T. pteropodis* in mice (Prociv, 1985*a*) blood eosinophilia was delayed, but in repeated studies using larger numbers of mice (Prociv & Brindley, unpublished) little difference was found between mouse responses to *T. pteropodis* and *T. canis*. Guinea-pig eosinophil responses to the two species are comparable and proportional to the infective dose (Fig. 2). However, beyond the fifth week, eosinophilia in the *T. pteropodis* groups declined, perhaps as a result of early larval destruction, whereas the *T. canis* groups' levels seemed to plateau. This was most obvious in high-dose infections with *T. canis* and *T. cati* (Fig. 3), and presumably reflected the prolonged survival of larvae. Olson & Schulz (1963) showed a very similar dose-related eosinophilia in guinea-pigs infected with *T. canis*. There were marked individual differences within the infected groups, with some animals showing very early and others delayed high peaks, partly explaining the apparent bimodal response to high-dose infection with *T. cati* (Fig. 3). The apparent rapid rise in high-dose *T. pteropodis* animals is inexplicable and may reflect the absence of data from the first 8 days.

The virtually identical eosinophil responses to three *Toxocara* species whose larvae follow different migration routes, and in particular to *T. pteropodis*, whose larvae remain in the liver, indicates that the stimulus for systemic eosinophilia is not as localised as previously suggested. Basten *et al.* (1970) showed that in rats, *Trichinella* larvae administered parenterally (with the exception of intra-aortic injection) produced eosinophilia only if they could reach the pulmonary circulation. Walls & Beeson (1972) induced eosinophilia in rats by injecting dextran particles which embolised in the lungs, and Schriber & Zucker-Franklin (1975) achieved the same by injecting latex particles coated with human – globulin. In guinea-pigs the hepatic embolisation of *T. pteropodis* larvae is capable of inducing a major blood eosinophilia.

Of all the small laboratory animals, guinea-pigs most closely resemble humans with regard to endocrinology, haematology, immunology and susceptibility to infection (Altman & Katz 1979). It is reasonable to conclude therefore that humans would be susceptible to infection with *T. pteropodis* but a very large egg dose would be required to produce a haematological, let alone a clinical, response. Such an occurrence is unlikely in view of the environmental distribution of *T. pteropodis* eggs (Prociv, 1985*b*), but was possible under the conditions prevailing at the time of the Palm Island epidemic (Moorhouse, 1982).

Regardless of its potential pathogenicity to humans, *T. pteropodis* may prove a useful tool in analysing further the mechanisms of eosinophilia and other host parasite interactions.

Acknowledgements. I am grateful to Catherine A. Creevey for assistance in the field and laboratory, and to the University of Queensland for financial support.

REFERENCES

Altman, P.L. & Katz, D.D. (1979). Inbred and genetically defined stains of laboratory animals. Part 2 Hamster, guinea-pig, rabbit and chicken, *Federation of American Societies for Experimental Biology, Bethesda, Maryland.* pp. 505–510.

Basten A., Boyer M.H. & Beeson P.B. (1970). Mechanism of eosiniphilia. 1. Factors affecting the eosinophil response of rats to *Trichinella spiralis*. *Journal of Experimental Medicine* **131**: 1271–1287.

Beaver P.C. (1956). Larva migrans. *Experimental Parasitology* **5**: 587–621.

Brindley P.J., Prociv P., Creevey C.A. & East D.J. (1985). Regulation of toxocariasis in mice selectively reared for high and low immune responses to *Nematospiroides dubius*. *Journal of Helminthology* **59**: 157–166.

Dubey J.P. (1968). Migration of *Toxocara cati* in mice. *Tropical & Geographical Medicine* **20**: 172–176.

Larsh J.E. (1950). Relationship in mice of intestinal emptying time and natural resistance to pig *Ascaris* infection. *Science* **111**: 62–63.

Moorhouse D.E. (1982). Toxocariasis – a possible cause of the Palm Island mystery disease. *Medical Journal of Australia* **1**: 172–173.

Nichols R.L. (1956). The etiology of visceral larva migrans. 1. Diagnostic morphology of infective second-stage *Toxocara* larvae. *Journal of Parasitology* **42**: 349–362.

Olson L.J. (1962). Organ distribution of *Toxocara canis* larvae in normal mice and in mice previously infected with *Toxocara, Ascaris* or *Trichinella. Texas Reports on Biology & Medicine* **20**: 651–657.

Olson L.J. & Schulz C.W. (1963). Nematode induced hypersensitivity reactions in guinea pigs: onset of eosinophilia and positive Schultz-Dale reactions following graded infections with *Toxocara canis. Annals of the New York Academy of Science* **113**: 440–455.

Prociv, P. (1983). Observations on the transmission and development of *Taxocara pteropodis* (Ascaridoidea: Nematoda) in the Australian grey-headed flying-fox, *Pteropus poliocephalus* (Pteropodidae: Megachiroptera). *Zeitschrift fur Parasitenkunde* 69: 773–781.

Prociv, P. (1985*a*). Observations on *Toxocara pteropodis* infections in mice. *Journal of Helminthology* 59: 267–275.

Prociv, P. (1985*b*). Observations on the prevalence and possible infection source of *Toxocara pteropodis* (Nematoda: Ascaridata) in flying-foxes of Queensland. *Australian Mammology* **8**: 319–323.

Prociv, P. & Brindley, P.J. (1986). Susceptibility of rats to infection with *Toxocara pteropodis. Zeitschrift fur Parasitenkunde* **72**: 487–491.

Prociv, P. & Brindley, P.J. (in press), Oral, parenteral and paratenic infections of mice with *Toxocara pteropodis. International Journal for Parasitology.*

Schriber, R.A. & Zucker-Franklin, D. (1975). Induction of blood eosinophilia by pulmonary embolization of antigen-coated particles: the relationship to cell-mediated immunity. *Journal of Immunology* 114: 1348–1353.

Sprent, J.F.A. (1952). On the migratory behaviour of the larvae of various *Ascaris* species in white mice. I. Distribution of larvae in tissues. *Journal of Infectious Diseases* 90: 165–176.

Sprent, J.F.A. (1955). On the invasion of the central nervous system by nematodes. II. Invasion of the nervous system in ascariasis. *Parasitology* 45: 41–55.

Sprent, J.F.A. (1956). The life history and development of *Toxocara cati* (Schrank 1788) in the domestic cat. *Parasitology* 46: 54–78.

Walls, R.S. & Beeson, P.B. (1972). Mechanism of eosinophilia. IX. Induction of eosinophilia in rats by certain forms of dextran (36532). *Proceedings of the Society of Experimental & Biological Medicine* 140: 689–693.

APPENDIX VIII

PARASITES OF AUSTRALIAN FLYING-FOXES (CHIROPTERA: PTEROPODIDAE)

IT appears that the Microchiroptera harbour a greater number and more diverse range of parasite species than do the Megachiroptera (Ubelaker 1970). Perhaps this is because most of the latter group prefer a herbivorous diet, practise hygienic waste disposal and select well-ventilated, high roosting sites where they space themselves widely. Alternatively, as Megachiroptera are dispersed almost exclusively through underdeveloped countries, they have not been as well studied as the Microchiroptera which range widely throughout Europe and North America. Australia is the only economically-developed country with a significant megachiropteran population. In a study of *Toxocara pteropodis* conducted over the last 5 years in the four Australian *Pteropus* species, several parasites, including new species, have been encountered. These are reviewed here along with other infections of Megachiroptera.

Potentially serious zoonotic **virus** infections in Megachiroptera are rare. Presumed rabies virus has been found in a *Pteropus* species in India (Pal *et al.* 1980) and related viruses in Africa may have been transmitted from fruit bats to humans (Crick *et al.* 1982). In Australia, serological evidence of Ross River Virus and Murray Valley Encephalitis infections has been reported in three *Pteropus* species (Doherty *et al.* 1971, Gard *et al.* 1973). Antibodies to leptospirosis have been found in *P. conspicillatus* (Emanuel *et al.* 1964).

The pathogenic **bacteria** *Salmonella typhi, S. typhimurium, Shigella flexneri* and *Sh. sonnei* were isolated from *P. rufus* in Madagascar, but the source was not determined (Mayoux *et al.* 1971). It is possible that other viral and bacterial (including chlamydial and rickettsial) as well as fungal infections occur in flying-foxes but have yet to be reported.

Two blood **protozoa**, the malaria-like *Hepatocystis pteropi* and the haemoflagellate *Trypanosoma pteropi,* have been reported from Australia (Mackerras 1959) and probably occur in all four *Pteropus* species. The proliferating merocysts of *Hepatocystis* account for some of the white spots frequently encountered in flying-fox livers, and gametocytes develop and circulate within red blood cells. The vector probably is the midge *Culicoides* (Boulard *et al.* 1985) which breeds below flying-fox roosts. Limited observations (pers. obs. and Reid 1985) indicate that parasitaemia fluctuates seasonally and that most adult *P. poliocephalus* are infected (13/22 examined in April 1982). Hepatic merocysts have been found in suckling juveniles as young as 5 months. Similar species have been found in Megachiroptera from New Guinea (Ewers 1973), Asia (Garnham 1966) and Africa (Ayala *et al.* 1980).

Much less is known of the trypanosome, but its likely vectors are nycteribiid flies (Reid 1985). Related species have been reported from the Indian *P. medius* and *P. tonganus* of Tonga (Marinkelle 1979). A tissue cyst, presumably of *Sarcocystis,* has been found in the heart muscle of a *P. poliocephalus* in Brisbane (Reid 1985).

Only one intestinal **nematode,** *Toxocara pteropodis,* is known in Megachiroptera, being first described from *P. geddiei* in Vanuatu (Baylis 1936). It occurs in all Australian *Pteropus* species (Prociv 1985) and in others from New Guinea and Indonesia, as well as in *Rousettus amplexicaudatus* from the Philippines (pers. obs.). Patent infections develop only in suckling juveniles, with a prevalence exceeding 50% in the three coastal species. Contamination of the roost environment with *Toxocara* eggs over summer leads to the infection of most adult bats, which harbour third-stage larvae in their livers. In females, larvae move to mammary glands after parturition to infect offspring via the milk. Infected juveniles rarely harbour more than 3-4 of these large worms (up to 15 cm long), which are expelled spontaneously prior to weaning at about 5 months of age. It is almost certain that the *Ascaris cynonycteridis* described from *Xantharpyia* (= *Rousettus*) *amplexicaudatus* from Burma by Parona (1889) were in fact immature *T. pteropodis* (Prociv, unpublished).

All other nematodes found in *Pteropus* spp. have been filarioids. Johnston (1916) reported a '*Filaria* species' from the body cavity of *P. poliocephalus* in Brisbane. In Fiji, *Chiropterifilaria brevicaudata* from the peritoneal cavity plus a blood microfilaria have been described in *P. hawaiiensis* (Yeh *et al.* 1958, Symes and Mataika 1959). In Malaysia, a *Litomosa* species (Ramachandran *et al.* 1966) and *Makifilaria inderi* (Krishnasamy *et al.* 1981) have been described from the peritoneal cavities of *P. vampyrus* and *P. hypomelanus,* respectively.

In the present study, unidentified filarioids were found under the liver capsules of *P. alecto* on Palm

Island and *P. poliocephalus* in Brisbane. Moorhouse (pers. comm.) found a *Dirofilaria*-like nematode in the right ventricle of *Dobsonia moluccensis* in Papua New Guinea. The vectors of these filarial nematodes are blood-sucking insects, but no life cycles in bats have yet been elucidated.

Johnston (1916) reported the **cestode**, *Hymenolepis*, in *P. poliocephalus* from Caloundra. In the present study hymenolepid cestodes, eggs and segments, have been found in the intestines and faeces of juvenile and adult *P. scapulatus* and juvenile *P. poliocephalus* and *P. alecto*.

Similar cestodes, common in Microchiroptera, have been reported from *Pteropus* species elsewhere. Johri (1934) described *Pseudoligorchis magnireceptacula* from 'a bat' from Lucknow, India and later *Hymenolepis minimedius* was described from a 'vampire bat', *P. medius* (Johri 1960). Prudhoe and Manger (1969) recovered *Ps. magnireceptacula* from the intestine of *P. vampyrus* in Malaysia. Hymenolepids of other mammals utilise arthropod intermediate hosts so it is likely that larval stages develop in mites found on these bats which are ingested during grooming.

Flying-foxes are well endowed with an ectoparasitic fauna of **arthropods.** From an early age, virtually every animal acquires its own colony of nycteribiid bat flies (see Allison, this volume), which probably are the intermediate hosts for trypanosomes and perhaps other parasites. Their numbers gradually decline on captive bats (pers. obs.).

A variety of poorly understood mites inhabit different locations on the skin, wing membranes and fur of flying-foxes. Blood-sucking spinturnicid mites of the genus *Meristaspis* were found on virtually every adult and juvenile examined in the present study and readily transferred to human skin. Laelapid mites were found on the skin and wing membranes and frequently in fresh faecal samples in large numbers, sometimes still alive. Readily transferring between individuals, the haematophagous mites are likely vectors of other parasitic infections. Some mites may be transferred between hosts on nycteribiid flies (pers. obs.). At least three sarcoptid mite species occur on Australian flying-foxes. The sessile adults live buried in various skin sites, and the mobile larval and nymphal stages transfer to other sites and hosts. Other families of mites could be expected on our *Pteropus* species but have not yet been reported. Unidentified mites were found in the lungs of a 5-month old juvenile *P. poliocephalus* captive for 3 months. Halarachnid mites have been reported from the respiratory tracts of seals, primates and dogs (Schmidt and Roberts 1985) but not yet from bats. Ticks also occur on Megachiroptera. Reid (1985) recovered one *Haemaphysalis humerosa* and one *Ixodes holocyclus* from two *P. poliocephalus* in Brisbane, and Moorhouse (pers. comm.) found *Ornithodoros papuensis* on *Dobsonia moluccensis* in Papua New Guinea.

Representatives of a broad range of species parasitise the flying-foxes. Undoubtedly a systematic study would reveal a higher prevalence of these than indicated by the few published reports and would bring to light new species. Microorganisms were not sought in my study, so they will not be discussed. The successful parasites depend on bat behaviour favourable to their transmission and establishment. The conspicious ectoparasitic arthropods take advantage of the frequent individual contact fundamental to the social life of bats. The haemoflagellates utilise blood-sucking ectoparasites or flying vectors, such as mosquitoes and midges, which abound in bat camps and undoubtedly transmit the filarial nematodes. The hymenolepid cestodes probably use ectoparasitic intermediate hosts.

Only one endoparasite of flying-foxes, *T. pteropodis*, is independent of intermediate hosts. It is a 'soil transmitted' nematode, the 'soil' in this case deposited on leaves and branches of the roost. Its eggs are thick shelled to resist desiccation. The absence of other intestinal nematodes and faecally transmitted protozoa reflects the unsuitability of an arboreal lifestyle for this route of infection. To survive the seasonal dispersal of infected bats from summer camps, *Toxocara pteropodis* 'hibernates' in the livers of pregnant females.

The need of *T. pteropodis* eggs and nycteribiid fly pupae to develop in the environment means that successful transmission and hence survival of these parasites requires an adequate host population in a suitable habitat. Both organisms disappear from captive bats. They do not occur in *P. tonganus* of the Cook Islands (pers. obs.) which, according to local folklore, were introduced by canoe from Samoa hundreds of years ago. On Niue Island in the Pacific only one mite, *Meristaspis calcarata*, has been found on *P. tonganus* (Wilson and Wodzicki 1977) and *T. pteropodis* has not been found (pers. obs.). For *Toxocara* to survive transoceanic migrations, a large colony of infected bats must move *en masse* to ensure sufficient numbers roost together for worm transmission. On Christmas Island in the Indian Ocean, *P. melanotus* (*natalis*) is free of *T. pteropodis* (C. Tidemann, pers. comm.), suggesting that bats invaded the island gradually, whereas the presence of *T. pteropodis* in Vanuatu (Baylis 1936) indicates a larger-scale initial colonisation by *P. geddiei*.

The pathogenicity of these parasites is unkown. The haemoflagellates are unlikely to cause disease in otherwise healthy bats. Cestodes and filarioid nematodes generally are quite benign. Two captive, suckling juvenile bats died from the erratic migration of adult *T. pteropodis* (pers. obs.) but this worm's effect in wild populations is unknown. In comparison with the depredations of humans upon bats and their environment, the combined effects of all these parasites probably are insignificant. As has been shown for *T. pteropodis* (Prociv *et al.* 1986), the risks of these parasites to human health is also likely to be negligible.

I am deeply indebted to Dr R. Domrow of the Queensland Museum for identifying mites and kindly providing most of the information on these organisms.

REFERENCES

AYALA, S. C., BRADBURY, J. AND BRADBURY, S., 1981. *Hepatocystis* in *Hypsignathus monstrosus* (Pteropinea) in Gaboon. I. *Hepatocystis* malaria in a hammerhead bat population in Gaboon, West Africa. *Ann. de Parasitol.* **56**: 21-22.

BAYLIS, H. A., 1936. A new ascarid from a bat. *Ann. Mag. Nat. Hist., Ser. 10* **17**: 360-365.

BOULARD, Y., LANDAU, I., MILTGEN, F., BACCAM, D. AND HUMPHERY-SMITH, I., 1985. Observations ultrastructurales sur la sporogonie d'*Hepatocystis levinei*, Haemoproteide, parasite de chiroptères australiens chez *Culicoides rubeculosus*. *Protistologica* **21**: 93-105.

CRICK, J., TIGNOR, G. H. AND MORENO K., 1982. A new isolate of Lagos bat virus from the Republic of South Africa. *Trans. R. Soc. Trop. Med. Hyg.* **76**: 211-213.

DOHERTY, R. L., STANDFAST, H. A., DOMROW, R., WETTERS, E. J., WHITEHEAD, R. H. AND CARLEY, J. G., 1971. Studies of the epidemiology of arthropod, borne virus infections at Mitchell River Mission, Cape York Peninsula, North Queensland. IV. Arbovirus infections of mosquitoes and mammals, 1967-1969. *Trans. R. Soc. Trop. Med. Hyg.* **65**: 504-513.

EMANUEL, M., MACKERRAS, I. M. AND SMITH, D. J. W., 1964. The epidemiology of leptospirosis in North Queensland. 1. General survey of animal hosts. *J. Hyg. (Camb.)* **62**: 451-484.

EWERS, W. H., 1973. A host-parasite list of the protozoan and helminth parasites of New Guinea animals. *Int. J. Parasitol.* **3**: 89-110.

GARD, G., MARSHALL, I. D. AND WOODROFE, G. M., 1973. Annually recurrent epidemic polyarthritis and Ross River Virus activity in a coastal area of New South Wales. II. Mosquitoes, viruses and wildlife. *Am. J. Trop. Med. Hyg.* **22**: 551-560.

GARNHAM, P. C. C., 1966. *Hepatocystis* of bats and squirrels. Pp. 873-897 *in* 'Malaria parasites and other haemosporidia'. Blackwell Scientific Publications: Oxford.

JOHNSTON, T. H., 1916. A census of the endoparasites recorded as occurring in Queensland, arranged under their hosts. *Proc. R. Soc. Qld.* **28**: 31-79.

JOHRI, G. N., 1960. Studies on some cestode parasites. V. Two new species of cestodes belonging to the family Hymenolepididae Fuhrmann, 1907. *J. Parasitol.* **46**: 251-255.

JOHRI, L. N., 1934. Report on a collection of cestodes from Lucknow (U.P., India). *Proc. Indian Mus.* **36**(II): 153-177.

KRISHNASAMY, M., SINGH, M. AND IYAMPERUMAL, R., 1981. *Makifilaria inderi* gen. et sp. nov. (Filarioidea: Onchocercidae) from the Island Flying Fox *Pteropus hypomelanus* Temminck in Malaysia. *S.E. Asian J. Trop. Med. Pub. Hlth.* **12**: 185-188.

MACKERRAS, M. J., 1959. The haematozoa of Australian mammals. *Aust. J. Zool.* **7**: 105-135.

MARINKELLE, C. J., 1979. *Trypanosoma (Megatrypanum) megachiropterorum* sp.n. from the flying fox, *Pteropus tonganus* Quoy & Gaimard. *J. Protozool.* **26**: 352-353.

MAYOUX, A., BRYGOO, E. R. AND SIMOND, J. P., 1971. Bilan d'une année d'étude de *Pteropus rufus* a Madagascar son role comme réservoir de virus d'enterobacteries. *Arch. Inst. Pasteur Madagascar* **40**: 29-37.

PAL, S. R., ARORA, B., CHHUTTANI, P. N., BROOR, S., CHOUDHURY, S., JOSHI, R. M. AND RAY, S. D., 1980. Rabies virus infection of a flying-fox bat, *Pteropus poliocephalus* in Chandigarh, Northern India. *Trop. Geogr. Med.* **32**: 265-267.

PARONA, C., 1889. Sopra alcuni elminti di vertebrati birmani raccolti da Leonardo Fea. *Ann. Mus. Civ. Stor. Nat. Genova Ser. 2* **7**: 763-780.

PROCIV, P., 1985. Observations on the prevalence and possible infection source of *Toxocara pteropodis* (Nematoda: Ascaridoidea) in Queensland flying-foxes. *Aust. Mammal.* **8**: 319-323.

PROCIV, P., MOORHOUSE, D. E. AND MAK, J. W., 1986. Toxocariasis — an unlikely cause of Palm Island mystery disease. *Med. J. Aust.* **145**: 14-15.

PRUDHOE, S. AND MANGER, B. R., 1969. A collection of cestodes from Malayan bats. *J. Nat. Hist.* **3**: 131-143.

RAMACHANDRAN, C. P., RAMALINGAM, S. AND POOPALACHELVAM, M., 1966. A filarial parasite from the flying-fox (*Pteropus vampyrus*). *Med. J. Malaya* **20**: 338.

REID, L. A., 1985. Studies on *Hepatocystis pteropi* and *Trypanosoma pteropi*. B. Sc. (Hons) Thesis: University of Queensland, St Lucia.

SCHMIDT, G. D. AND ROBERTS, L. S., 1985. Parasitic Arachnids: Order Acari, the Ticks and Mites. Pp. 715-741 *in* 'Foundations of parasitology' (3rd Ed.), Times Mirror/Mosby: St Louis.

SYMES, C. B. AND MATAIKA, J. U., 1959. Observations of *Microfilaria fijiensis* Yeh, Symes & Mataika, 1958 from fruit bats (*Pteropus hawaiiensis*) in Fiji. *J. Helminthol.* **33**: 223-232.

UBELAKER, J. E., 1970. Some observations on ecto- and endoparasites of chiroptera. Pp. 247-261 in 'About bats: A chiropteran biology symposium' ed. by P. H. Slaughter and D. W. Walton. Southern Methodist University Press: Dallas.

WILSON, N. AND WODZICKI, K., 1977. Ectoparasites from fruit bats and rats on Niue Island. *N. Z. J. Zool.* **4**: 383-387.

YEH, L. S., SYMES, C. B. AND MATAIKA, J. U., 1958. On a new filarioid worm *Chiropterifilaria brevicaudata* gen. et sp. nov. from the fruit bat, *Pteropus hawaiiensis* from Fiji. *J. Helminthol.* **32**: 227-232.

Key words: Parasites, flying-foxes, Pteropodidae.

P. Prociv, *Department of Parasitology, University of Queensland, St Lucia, Queensland, Australia 4067.*
Manuscript received 17 November 1986. Aust. Mammal. **10**: 107-110.

APPENDIX IX

FAECAL SYNTHESIS OF VITAMIN B_{12} IN AUSTRALIAN *PTEROPUS* SPECIES (CHIROPTERA: PTEROPODIDAE)

PAUL PROCIV AND RICHARD ANTHONY TRACEY

Prociv, P. and Tracey, R. A., 1987. Faecal synthesis of vitamin B_{12} in Australian *Pteropus* species (Chiroptera: Pteropodidae). *Aust. Mammal.* **10**: 5-9.

In Australian *Pteropus* spp. coprophagy has not been observed but is likely because of its suspected key role in the life cycle of *Toxocara pteropodis*. To determine the potential nutritional benefits of faecal ingestion, vitamin B_{12} was assayed in the faeces of captive *Pteropus* spp. at 0, 10 and 20 days after passage, as well as in samples collected under roost sites and from the recta of animals collected in the field. Faecal specimens kept under warm, humid conditions for 10 days had significant levels of B_{12} but negligible quantities were recovered from similar samples kept under colder, dry conditions. It is concluded that intentional or accidental coprophagy in summer could contribute substantially to the maintenance vitamin B_{12} requirements of Australian *Pteropus* spp.

Key words: *Pteropus*, coprophagy, vitamin B_{12}, nutrition.

P. Prociv, Department of Parasitology, University of Queensland, St. Lucia, Qld, Australia 4067. R. A. Tracey, Division of Haematology, Pathology Department, Royal Brisbane Hospital, Herston, Qld, Australia 4006. *Manuscript received 23 December 1985.*

VITAMIN B_{12}, or cyanocobalamin (hereafter referred to as B_{12}), is essential for the synthesis of nucleic acids and many other metabolic functions. Its ultimate source in nature is the synthetic activity of various microorganisms (Herbert 1965). The intestines of most animals, including humans, harbour bacteria capable of B_{12} synthesis (Mickelsen 1956, Albert *et al.* 1980), and hindgut-fermenting herbivores often satisfy their needs for this vitamin by coprophagy. Fruit passes rapidly through the flying-fox, *Pteropus* spp., intestine, with a transit time as brief as 20 min (Prociv 1983a, Tedman and Hall 1985). Under such conditions, fresh faeces will contain few bacteria, whose multiplication with time may be reflected by an increasing faecal B_{12} content. The Egyptian fruit bat, *Rousettus aegyptiacus*, which is known to feed on fruit, flowers and foliage (Lewis and Harrison 1962, Kulzer 1979) will develop symptomatic B_{12} deficiency in captivity when fed exclusively on cleaned fruit (Green *et al.* 1975). The natural source of B_{12} for *Rousettus* is not known. Australian *Pteropus* spp. also are thought to feed under natural conditions only on fruit and blossom (Nelson 1965) and foliage (Prociv 1983a).

The life cycle of *Toxocara pteropodis*, an ascaridoid nematode found in the genus *Pteropus*, probably depends on faecal ingestion by adult flying-foxes (Prociv 1983a). The eggs of this nematode are passed in the faeces of suckling juvenile *Pteropus poliocephalus* and become infectious after at least 10 days of embryonation on leaves. Captive bats were observed to avoid fruit freshly contaminated with their own faeces but were less averse to food contaminated with stale faeces.

Folivory also has been observed in related genera, such as *Eidolon helvum* (Funmilayo 1979) and *Rousettus aegyptiacus* (Kulzer 1979). It is possible that these species either accidentally or intentionally ingest their own faeces while eating or chewing leaves. To determine if coprophagy could be a source of the vitamin to flying-foxes, faeces of these animals were assayed for B_{12} content.

MATERIALS AND METHODS

CAPTIVE ANIMALS

A mixed colony of the Grey-headed Flying-fox, *Pteropus poliocephalus*, Black Flying-fox, *P. alecto*, and Spectacled Flying-fox, *P. conspi-*

cillatus, had been kept in a flight cage for 2 years and maintained on a diet of fruit supplemented with sugar, vitamins and reconstituted skimmed cows' milk. In December 1983 (summer conditions) four male *P. poliocephalus* (group I) and four male *P. alecto* (group II) were placed in two separate, small cages. After 2 days of an exclusively fruit diet, fresh faeces were collected from each cage into two sterile beakers. Each sample was thoroughly mixed, then 5 g portions were placed into each of 30 labelled, pre-weighed, sterile, clear 60 ml plastic containers. Five samples from each of these two groups were processed within 3 h of collection for B_{12} assay (see below). The remaining 20 open containers were placed on a tray, sealed inside a large clear plastic bag containing an open jar of water to maintain high humidity and left on a bench at room temperature (average daily range 24-30°C). Ten days later, five samples from each group of stored faeces were removed, weighed and B_{12} extracted; the remaining 10 were weighed and tested on the 20th day.

In June 1984 (winter conditions), four female *P. scapulatus* (group III), which had been captured 3 months previously, were placed with their 2 month-old offspring in a small cage and their faeces collected as above. The faecal containers were covered with gauze and left at room temperature (average daily range 12-18°C) on the same bench as above. At the same time, pooled faeces were collected from another cage holding two *P. alecto* males and two *P. poliocephalus* males (group IV) and processed as described above.

WILD ANIMALS

Semi-liquid contents were gently scraped from the recta of five *P. poliocephalus* adults which had been shot in their roost 4 h previously. Vitamin B_{12} was extracted from a 5 g pooled sample.

In February 1985 faecal specimens were collected from mangrove leaves beneath a roost of *P. poliocephalus* at Indooroopilly Island on the Brisbane River. These samples were placed into four sterile plastic containers according to their estimated age, *i.e.* very fresh (soft with moist surface, probably less than 24 h old), several days old (soft, moist interior with dry surface), slightly older faeces (moist inside but crusted exterior) and stale (dry and hard throughout). These samples were weighed and B_{12} extracted within 6 h of collection.

EXTRACTION AND ASSAY OF VITAMIN B_{12}

Each sample was soaked in 50 ml distilled water for 3 h, comminuted with a sterile spatula then boiled for 10 min. After boiling, the volume was topped up to 50 ml with distilled water, and then the specimen was filtered through sterile filter paper in a sterile glass funnel. Ten millilitres of each filtrate was stored in a dark sterile glass container at 5°C, and B_{12} levels were assayed within 48 h.

Each filtrate was subjected to competitive radioassay using a ^{57}Co Vitamin B_{12} Assay Kit with purified B_{12} binder (Diagnostic Corp., Los Angeles, California, U.S.A.) according to the instructions supplied with the kit, these being a modification of the method of Liu and Sullivan (1972). B_{12} concentrations were determined by counting in an Auto Gamma Scintillation Spectrometer Model 5260 (Packard Instrument Co. Inc., Illinois, U.S.A.), linked to a CDC 1700 computer (Control Data Corporation, St Paul, Minnesota, U.S.A.). It was assumed that B_{12} was sufficiently water-soluble and heat-resistant for the purposes of this experiment (see Herbert 1965) so that the B_{12} content of each faecal sample was readily calculated from its concentration in the filtrate. Filtered, boiled, distilled water and known B_{12} standard solutions were used as controls.

RESULTS

The findings from the four groups (I-IV) of captive bats are shown in Table 1.

In group I there was significant accumulation of B_{12} by day 10 (t-test, $P<0.005$). The further rise in concentration by day 20 resulted from desiccation of the samples as there was no increase in total B_{12} content. The rise in group II by day 10 was also significant ($P<0.02$). There was no significant difference between Groups I and II on the tenth day.

Group No.	Day 0			Day 10			Day 20		
	Sample wt (g)	Total B_{12} (ng)	B_{12} conc. (ng g^{-1})	Sample wt (g)	Total B_{12} (ng)	B_{12} conc. (ng g^{-1})	Sample wt (g)	Total B_{12} (ng)	B_{12} conc. (ng g^{-1})
I	5.00	2.12 ± 0.21 1.45 - 2.55	0.42 ± 0.04 0.29 - 0.51	3.18 ± 0.18 2.50 - 3.60	36.2 ± 6.0 15.0 - 48.2	11.13 ± 1.56 6.00 - 15.1	1.38 ± 0.23 0.85 - 2.10	31.7 ± 14.8 9.0 - 90.0	20.5 ± 6.17 7.54 - 42.9
II	5.00	1.94 ± 0.50 0 - 2.55	0.38 ± 0.10 0 - 0.54	2.94 ± 0.17 2.40 - 3.45	40.6 ± 10.3 12.3 - 73.5	13.6 ± 3.46 9.35 - 25.3	1.01 ± 0.13 0.60 - 1.35	20.4 ± 5.76 7.10 - 35.7	19.9 ± 4.54 8.81 - 24.2
III	5.00	0	0	0.85 ± 0.02 0.80 - 0.91	0	0	0.77 ± 0.03 0.69 - 0.88	0.47 ± 0.47 0 - 2.35	0.68 ± 0.68 0 - 3.4
IV	5.00	0	0	0.62 ± 0.02 0.57 - 0.65	0	0	0.53 ± 0.01 0.50 - 0.56	2.37 ± 0.69 0 - 3.75	4.50 ± 1.32 0 - 6.80

Table 1. Weights, total B_{12} contents and B_{12} concentrations (mean ± SEM; range) of faecal samples at 0, 10 and 20 days after collection from four groups of captive *Pteropus* species. Groups I (*P. poliocephalus*) and II (*P. alecto*) were collected in summer; III (*P. scapulatus*) and IV (mixed *P. poliocephalus* and *P. alecto*) in winter. Lowest limit of accuracy of assay techniques was 30 ng $B_{12}l^{-1}$ of filtrate, which corresponded to a total sample B_{12} content of 1.5 ng. All samples below this level were recorded as 0 and included in calculations as such.

In the dry winter samples, insufficient quantities of B_{12} to measure were present at day 10 and only small amounts were present at day 20. The apparently higher B_{12} content in the faeces of the mixed bats (group IV) than in those of the *P. scapulatus* group (III) was not significant ($P<0.2$).

The summer samples kept at high humidity (groups I and II) had not dried completely by day 10 ($P<0.001$), whereas the winter samples (III and IV) effectively were completely desiccated by day 10.

The B_{12} concentration in the pooled rectal contents from bats collected in the field was 3.4 ng g^{-1}. Levels in faecal specimens collected from leaves in the summer camp were as follows: fresh, <0.3 ng g^{-1}; estimated 2-3 days old, 1.1 ng g^{-1}; estimated 5-10 days old, 8.7 ng g^{-1}; desiccated, <0.3 ng g^{-1}.

DISCUSSION

These results indicate that B_{12} is synthesised by bacteria in fruit bat faeces. In a warm, humid atmosphere, this occurs within the first 10 days, whereas under cooler, dry conditions, generation of B_{12} is either negligible or excessively delayed.

Green *et al.* (1975) prevented signs of deficiency in *Rousettus aegyptiacus* by the intramuscular injection of 200 ng of B_{12} once weekly. The average body mass of adult *R. aegyptiacus* is 126 ± S.E. 3 g (Noll 1979), compared with 750 ± 12 g for adult male *P. poliocephalus* (Prociv, unpublished). That daily B_{12} maintenance is not linearly related to body weight is indicated by human requirements of 1000 ng day^{-1} (Herbert 1965). Nevertheless, assuming such a direct relationship, *P. poliocephalus* would require about six times that of *Rousettus i.e.* 160 ng day^{-1}, which would be recoverable from 8 g of a 20-day-old summer faecal sample (groups I and II, Table 1). Samples from leaves contained less B_{12} (8.7 ng g^{-1}) so larger quantities would need to be ingested to satisfy maintenance requirements. Failure to detect B_{12} synthesis in *P. scapulatus* faeces kept under cool and dry conditions similar to those of their winter nursery camps indicates that coprophagy in these circumstances would provide little B_{12}.

As milk contains measurable quantities of B_{12} (Herbert 1965) absence of the vitamin from the faeces of nursing *P. scapulatus* indicates efficient intestinal absorption by the young, or by the mothers which ingested the faeces of their offspring while grooming (pers. obs.).

Maintenance of the *T. pteropodis* life cycle requires adult bats to ingest faeces older than 10 days (Prociv, 1983a). In *P. poliocephalus, P. alecto* and *P. conspicillatus*, the young are born in spring and infection of adult bats with the nematode occurs from mid to late summer in humid coastal areas (Prociv 1985), when coprophagy could provide a substantial B_{12} intake as well as ensuring continuation of the *Toxocara* life cycle. In *P. scapulatus*, the young are born in autumn and reared in camps in cold, dry, inland regions (Prociv 1983b). This species does not appear to be a natural maintaining host of *T. pteropodis* (Prociv 1985), although adult *P. scapulatus* mix with the three other species in summer camps and may then be exposed to hepatic infection with third-stage larvae of *T. pteropodis* (Prociv 1985).

The higher concentrations of B_{12} in bat rectal contents than in the fresh experimental samples requires explanation. Although *Pteropus* spp. have rapid intestinal transit during feeding, the gut contains a residuum from the end of one meal to the beginning of the next (pers. obs.). This would allow sufficient bacterial proliferation to produce detectable B_{12} levels within rectal contents. Furthermore, the material scraped from the dead wild bats may have been contaminated with gut secretions and mucosal cells which would contribute to the B_{12} level.

It is unlikely that photodegradation would account for the lower levels in faeces collected in the field because firstly, the bats' faeces are opaque and secondly, the degradation products are B_{12} congeners which are reactivated by the cyanide buffer used in the assay procedure (Herbert 1965). As B_{12} is water soluble (Herbert 1965) and the period before the samples were collected was one of frequent rainfall, some of the vitamin content may have been leached from the faeces on leaves.

Although not observed with *Pteropus* spp., accidental or intentional faecal ingestion could satisfy the B_{12} requirements of these animals in summer and liver stores could maintain needs over the rest of the year. Furthermore, this conclusion would be in keeping with findings on the life cycle and host distribution of *T. pteropodis* in Australia.

REFERENCES

ALBERT, M. J., MATHAN, V. I. AND BAKER, S. J., 1980. Vitamin B_{12} synthesis by human small intestinal bacteria. *Nature* (Lond.) **283**: 781-782.

FUNMILAYO, O., 1979. Ecology of the straw-coloured fruit-bat in Nigeria. *Rev. Zool. Afr.* **93**: 589-600.

GREEN, R., VAN TONDER, S. V., OETTLE, G. J., COLE, G. AND METZ, J., 1975. Neurological changes in fruit-bats deficient in Vitamin B_{12}. *Nature* (Lond.) **254**: 148-150.

HERBERT, V., 1965. Drugs effective in megaloblastic anemias: Vitamin B_{12} and folic acid. Pp. 1410-1442 *in* 'The Pharmacological Basis of Therapeutics'. Ed by L. S. Goodman and A. Gilman, 3rd Ed. Macmillan Publishing Co.: New York.

KULZER, E., 1979. Physiological ecology and geographical range in the fruit-eating cave bat genus *Rousettus* Gray 1821 — a review. *Bonn. Zool. Beitr.* **30**: 234-275.

LEWIS, R. E. AND HARRISON, D. L., 1962. Notes on the bats from the Republic of Lebanon. *Proc. Zool. Soc. Lond.* **138**: 473-486.

LIU, Y. K. AND SULLIVAN, L. W., 1972. An improved radio-isotope dilution assay for serum Vitamin B_{12} using haemoglobin-coated charcoal. *Blood* **39**: 426-432.

MICKELSEN, O., 1956. Intestinal synthesis of vitamins in the nonruminant. *Vitams Horm.* **14**: 1-95.

NELSON, J. E., 1965. Movements of Australian flying foxes (Pteropodidae: Megachiroptera) *Aust. J. Zool.* **13**: 53-73.

NOLL, U. G., 1979. Postnatal growth and development of thermogenesis in *Rousettus aegyptiacus. Comp. Biochem. Physiol.* **63A**: 89-93.

PROCIV, P., 1983a. Observations on the transmission and development of *Toxocara pteropodis* (Ascaridoidea: Nematoda) in the Australian grey-headed flying-fox, *Pteropus poliocephalus* (Pteropodidae: Megachiroptera) *Z. Parasitenkd* **69**: 773-781.

PROCIV, P., 1983b. Seasonal behaviour of *Pteropus scapulatus* (Chiroptera: Pteropodidae). *Aust. Mammal.* **6**: 45-46.

PROCIV, P., 1985. Observations on the prevalence and possible infection source of *Toxocara pteropodis* (Nematoda: Ascaridoidea) in Queensland flying-foxes. *Aust. Mammal.* **8**: 319-323.

TEDMAN, R. A. AND HALL, L. S., 1985. The morphology of the gastrointestinal tract and food transit time in the fruit bats *Pteropus alecto* and *P. poliocephalus* (Megachiroptera). *Aust. J. Zool.* **33**: 625-640.

334

copper in the glomerular filtrate, necrosis of the proximal tubules, and copper deposited in both proximal and distal tubular epithelial cells.

The intravenous administration to sheep of 60 mg of copper, as a 2.5% copper acetate solution, caused acute diarrhea, vascular congestion, respiratory distress, hemoconcentration, and cardiovascular collapse, culminating in death within six hours.[18] Autopsy demonstrated hepatic and renal congestion and intense inflammation of the upper gastrointestional epithelium.

CHRONIC COPPER POISONING

Human cases have rarely been reported. One 15-month-old infant developed diarrhea, neurological disturbance, and hepatomegaly following prolonged ingestion of water from copper pipes,[19] and a 14-month-old boy, perhaps with Wilson's disease, died of liver failure under similar circumstances.[20]

However, manifestations in animals are well known from the considerable number of studies which have been published. Daily intraperitoneal injections of copper chloride to rats, which survived for two to five months, caused no illness until the five days preceding death, when the animals developed anorexia, diarrhea, lethargy, weight loss, and abdominal distension.[21] Notable autopsy findings included patchy hepatic necrosis, inflammation, regeneration, and periportal fibrosis with copper deposition in lobular peripheries and, in the kidneys, proximal tubular necrosis with copper deposition in tubular cells. In another long-term study, rats were given 0.2% copper acetate in their drinking water for 320 days without ill effects.[22] At autopsy, copper was demonstrated in jejunal mucosal and hepatic parenchymal cells.

Pigs fed 1 g/day of copper as various salts remained well for up to six weeks, when they developed anorexia, jaundice, fever, lethargy, and melena followed by death within two or three days.[23] Laboratory data indicated a rapid hemolysis before death. Autopsy findings included hepatic and renal congestion, pulmonary edema, gastric ulceration, and small intestinal hyperemia and bleeding. Histologically, copper granules were demonstrated in the livers and epithelial cells of renal proximal tubules.

Chronic copper poisoning has been a recognised veterinary problem in sheep,[24] which consequently have been the subject of most experimental work in this area. Tissue copper levels can build up for weeks or months without illness, to be followed by an acute 'hemolytic crisis' manifesting as anorexia, anemia, jaundice, hemoglobinuria, and death.[25] Copper sulphate (1 g/day) given in food caused death in two sheep after three and five weeks.[24] Both were well, with normal blood copper levels, until the terminal hemolytic crisis, when blood copper levels rose rapidly. Hemoconcentration seemed to occur for up to 10 days before the crisis, which was also preceded by a rapid rise in blood methemoglobin content.[26] At the time of the crisis, blood glutathione levels were low, transaminases high, and copper and urea grossly elevated, although serum K^+ and Na^+ were little changed. In sheep which survived, blood copper levels rapidly returned to normal within three days, indicating that the crisis may have resulted from a rapid release of copper from hepatic stores once a threshold level had been reached.[18,26]

Clearly, no particular animal species at present can be considered a reliable predictive model for human copper toxicity. Different species vary markedly in their tolerance of copper loading, perhaps reflecting hepatic metallothionein concentrations.[27] However, hemolytic crises have occurred in humans following excessive absorption of copper.[14-16]

DISCUSSION

It is not possible so long after the event to determine the exact cause of the Palm Island epidemic. However, the absence of convincing reports of chronic algal toxicity in humans or other animals must throw doubt upon the natural occurrence of such an entity. The algal bloom was present in the dam for two months before the outbreak,[1] yet the first case did not develop until five days after its treatment with copper sulphate and acute cases continued to present for another three weeks, *i.e.* 26 days after the water treatment. By that stage, presumably, most of the earlier cases had completely recovered. It is difficult to accept that some individuals were developing acute symptoms while others were already recovering, yet presumably continuing to be exposed to the same toxin. On the other hand, most, if not all, of the epidemiological and clinical features of the outbreak can be explained on the basis of rapidly developing 'chronic' copper poisoning, which in effect is an 'acute' event resulting from the chronic hepatic accumulation of copper over variable periods.[23]

A series of hypothetical scenarios are presented which could account for a high level of copper in the reticulated water and which would be consistent with the epidemiological features of the outbreak.

1. The dam water level was low, so that the actual volume may have been considerably overestimated in the calculation of a copper sulphate dose.

20. Walker-Smith J, Blomfield J. Wilson's disease or chronic copper poisoning? Arch Dis Child 1973; 48; 476-9.
21. Wolff SM. Copper deposition in the rat. AMA Arch Pathol 1960; 69: 217-23.
22. Williams AO. Histochemical localization of copper in the gastrointestinal tract of the rat. J Clin Pathol 1965; 18: 800-2.
23. Allen MM, Harding JDT. Experimental copper poisoning in pigs. Vet Rec 1962; 74: 173-9.
24. Barden PJ, Robertson A. Experimental copper poisoning in sheep. Vet Rec 1962; 74: 252-6.
25. Todd JR, Thompson RH. Methaemoglobin in chronic copper poisoning of sheep. Nature 1961; 191: 89-90.
26. Todd JR, Thompson RH. Studies on chronic copper poisoning: 11. Biochemical studies on the blood of sheep during the haemolytic crisis. Br Vet J 1963; 119: 161-73.
27. Harris ED. Copper in human and animal health. In: Rose J, ed. Trace elements in health: a review of current issues. London: Butterworths, 1983; 44-73.
28. Marsh FP. Toxic nephropathies. In: Weatherall JD, Ledingham JGG, Warrell DA, eds. The Oxford textbook of medicine. Oxford: Oxford University Press, 1983; 18: 100-6.

D LIBRARIES
LIBRARY