QUANTITATIVE STUDIES OF THEILERIA PARVA
IN THE BOVINE HOST.

By

DAVID EWART RADLEY, B.V.M.& S. (EDIN.),
M.R.C.V.S., D.T.V.M. (EDIN.)

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SUMMARY

A preliminary quantitative study on the population growth rate of Theileria parva in the bovine host failed to confirm the constant growth rate independent of size of infective dose reported by earlier workers. Experiments in methodology were performed therefore to standardize the techniques for obtaining (a) accurate estimates of infection rates from biopsy smears, (b) representative samples from a superficial lymph node source. A further experiment was carried out to establish that such samples were representative of the total parasitic biomass. These techniques were applied in a wider ranging quantitative study using four infective doses at ten fold intervals for infecting animals. The resultant growth rates were again dependent on the size of the infective dose. A definitive experiment, using five infective doses at ten fold intervals confirmed the divergence of growth rates. It was also shown that the severity of the clinical reaction, and the survival time was dependent on the size of the infective dose. The implications of these results in relation to immunization of cattle against T. parva are discussed.

The standardized methods were applied also in a chemoprophylactic study to observe the growth rates of T. parva as affected by different regimens of tetracycline. Other possible applications of these standardized methods are discussed.

CONTENTS

CHAPTER 1.

INTRODUCTION

Theileria parva Theiler, 1904 may be defined as a
protozoal tick-borne parasite of Bovidae, characterized
in the vertebrate host by invasion of cells of the
lymphocytic and erythrocytic series. The syndrome
produced in cattle is called East Coast Fever (E.C.F.).

All Bos taurus, and Bos indicus reared in E.C.F.
free areas, are highly susceptible to infection with
T. parva. In susceptible cattle morbidity rates of
87.6% (Brocklesby, Barnett and Scott, 1961) and 93.2%
(Wilde, Brown, Hulliger, Gall and MacLeod, 1968) are
reported and both groups of workers are in agreement on
the mortality rate of 95.5%. However, in enzootic
areas the indigenous cattle, as calves, may possess an
innate immunity which is genetical and not related to
maternal antibodies. These calves may therefore
survive natural challenge and become immune to the
disease. Calfhood mortality rates from E.C.F. in
these circumstances may vary between 4 and 10% (E.A.V.R.O.,
1956; and Barnett, 1957). If, however, the distribution
of infected ticks is uneven and these calves are not
challenged, they will grow into adults which are as
susceptible as cattle from any other area. Problems
occur particularly when cattle of higher productivity but
with no innate calfhood resistance are imported into
these enzootic areas. There is still no known effective

chemotherapeutic agent which will alleviate the disease
once clinical signs have appeared, no vaccine is
available, and control of E.C.F. has to be effected by
measures aimed at the tick vector.

The East African Livestock Survey (1966) sponsored
by the Food and Agricultural Organisation of the United
Nations noted the limiting effect of E.C.F. on
agricultural development and recommended that research
on the problem should be given first priority. As a
result, a United Nations Special Fund financed the
F.A.O. Tick-borne Disease Project based at the East
African Veterinary Research Organisation, Muguga, Kenya.
The terms of reference for this Project were to
investigate the feasibility of producing a vaccine for
E.C.F. Some of this work in which the author
participated, has been reported (E.A.V.R.O., 1969;
Brown, Malmquist, Cunningham, Radley and Burridge, 1970;
Purnell, Backhurst, Bailey, Boarer, Branagan, Cunningham,
Joyner, Pierce, Radley and Wood, 1970; Purnell, Branagan,
Bailey, Joyner and Radley, 1970; Cunningham, Brown,
Purnell, Radley, Burridge, Musoke and Sempebwa, 1970a).
The majority of the experimentation, however, has yet to
be published.

The author also carried out the experiments to be
presented in this thesis which were designed to augment
the F.A.O. Project research. Part of the thesis has been
published in abstract form (Radley, 1970).

CHAPTER 2.

REVIEW OF LITERATURE

Only the literature pertaining to this thesis will be reviewed but excellent texts of the diseases caused by T. parva and related species are to hand (Henning, 1956; Neitz, 1957 and 1959; Wilde, 1967; Barnett, 1968).

The problems of classification, which arise because of the incomplete knowledge of the life cycle, will be discussed briefly. Insufficient evidence is available for the presence or absence of a sexual stage and sporogony and, because of this, differing opinions exist about the placings of the higher taxa in phylum Protozoa. Levine (1961) suggested that the families Babesidae and Theileridae should be placed as an appendage to Protozoa pending clarification of the life cycle. However the Committee on taxonomy and taxonomic problems of the Society of Protozoologists (Honigberg, Balamuth, Bovee, Corliss, Gojdics, Hall, Kudo, Levine, Loeblich, Weiser and Wenrich, 1964) decided that since there was no evidence for a sexual phase or sporogony the piroplasms were to be placed in the subphylum Sarcomastigophora. This latter classification is possibly too dogmatic because it is based on negative evidence, and the suggestion of Levine to leave the matter in abeyance could be the best solution until more evidence is available.

Controversy also exists about the family Theileridae
at the species level. Until recently the Theileria
species affecting cattle in East Africa besides T. parva
were considered to be T. lawrencei Neitz, 1955 and
T. mutans Theiler, 1906; then Brocklesby (1964) provided
evidence suggesting that T. lawrencei was not a valid
species and should be considered a strain of T. parva.
Matson and Hill (1967), however, maintained that
theileriosis in Rhodesia was caused by T. lawrencei.
Brocklesby (1969) submitted new evidence supporting the
suggestion of Du Toit (1930) that all the species
infecting cattle are strains of T. parva. This simpli-
fication would therefore absorb into the single species
T. mutans and T. lawrencei.

The life cycle of T. parva in cattle may be stated
as follows. The principal invertebrate host and vector
of the parasite is the tick Rhipicephalus appendiculatus
Neumann, 1901. This arthropod acquires infection by
feeding on an animal undergoing an E.C.F. reaction.
After ecdysis the next instar of the tick attaches and
feeds on a bovine host and the theilerial parasite, which
has been dormant in the salivary glands of the tick, is
stimulated to multiply very rapidly by binary fission.
The protozoan then undergoes a process of maturation to
form infective particles (sometimes referred to as
sporozoites or infective units). These infective
particles (IPs) are liberated into the saliva of the tick
by rupture of the acinar cells, and gain entry into the

bovine tissue. The parasite cannot be detected for several days until it next appears in lymphoblasts of the lymph node draining the area of the tick bite. This lymphocytic stage of the parasite is intracytoplasmic and is called the macroschizont. The numbers of infected lymphocytes increase in the local drainage lymph node (L.D.L.N.) and macroschizonts can be detected in other lymph nodes one or two days later. The actual mode of multiplication of these macroschizonts is in question. There are two main schools of thought, and the one generally accepted is that macroschizonts may rupture and split up into individual elements which can either reinvade lymphocytes or remain free in tissue fluids. Free macroschizonts are often seen in smears from lymph node tissue. Reichenow (1941) first put forward the alternative mode of multiplication. He postulated that the macroschizont divided into two parts during cell division, each daughter cell being parasitized. This interpretation of the multiplication was later supported by the in vitro studies of Hulliger, Wilde, Brown and Turner (1964), Hulliger (1965), Zablotskii (1967) and Malmquist and Brown (1970). This school of thought regards the free macroschizonts as having arisen from cell destruction during the process of smearing.

A third but tentative mode of multiplication was put forward by Jarrett and Brocklesby (1966). It was suggested that it may not be uninucleate elements which invade other lymphocytes but that the free macroschizonts

may do so. There is evidence for and against all these hypotheses and it is possible that the parasite does not confine itself to any one of them.

The next stage is the transformation of the macroschizont into the microschizont. The microschizont eventually causes rupture of its' host cell resulting in the liberation of micromerozoites which are destined to enter the erythrocytes. These intraerythrocytic stages or piroplasms as they are often called, are thought to be responsible for the infection of ticks.

Leaving these controversial matters of the protozoan aside, certain aspects of the disease E.C.F. will be reviewed. Although the morbidity rates for susceptible cattle are formidable there have been observations recorded in the literature where the severity of the reaction and the recovery rate were related to the number of infected ticks. Lowe (1933) observed that by reducing the degree of tick infestation in an enzootic area by dipping without hand-dressing, calves became mildly infected with E.C.F., and the mortality rate was reduced from 60 - 70% to 5 - 10%.

This finding is in contradistinction to the conclusion reached by Lewis and Fotheringham (1941), and Lewis (1950) that the reaction of the disease and mortality rate caused by one infected tick are similar to those induced by several infected ticks. Lewis (1950) states that "non-fatal reactions cannot be attributed merely to low numbers of ticks."

Work reported by Wilson (1950) and E.A.V.R.O.(1956)
confirmed the observations of Lowe (1933). Barnett
suggested that the calfhood innate resistance of the
Bos indicus animals used in these experiments enabled
the calves to withstand infection, but the Bos taurus
animals used by Lewis (1950), and Lewis and Fotheringham
(1941), having no innate resistance, easily succumbed to
the disease. On the question of size of infective dose
Barnett (1957) stated "It seems fairly certain that there
will be a direct mathematical relationship between the
size of the infecting dose and the recovery rate," but
went on to say that this finding had no application as
infections with fractions of a tick were impractical. A
few years later Barnett and Brocklesby (1961 and 1966a
found that the mortality rate of Bos taurus could be
related to the number of ticks used to transmit a strain
called T. parva (Icely). By restricting the number of
infected ticks carrying this "mild strain" the mortality
rate was reduced from 60% (30 to 100 ticks) to 0% (3 or
5 ticks). Unfortunately T. parva (Icely) became more
virulent on passage (Brocklesby and Bailey, 1968).

Wilde, Hulliger and Brown (1966) and Wilde (1967)
drew conclusions from these earlier observations and
put forward the hypothesis "that a threshold of infective
material exists, below which the parasite will not
become established, but above which infection will be
induced...... It can be speculated, therefore, that
in the region of the threshold there is a range of

dosage that will evoke a response varying from
nonclinical establishment of the parasite to fatal
attack of the disease.   The threshold can only be
ascertained by titration of free sporozoites in
susceptible cattle and such titration demands as a
prerequisite that a uniform suspension of viable
sporozoites be prepared."   He was led to deduce "that
the quantum of infective material determines whether an
animal becomes infected and dies, becomes infected and
recovers, or remains uninfected."   This hypothesis is
commonly referred to as the "quantum of infection
hypothesis," and was supported by results of experiments
done by Wilde, Brown, Hulliger, Gall and MacLeod (1968)
using very small amounts of salivary gland tissue
derived from infected ticks.   However in attempts to
titrate across this threshold Wilde and his co-workers
found that, although they were able to infect
susceptible cattle with an emulsion of salivary glands
representing, theoretically, 0.001 of a tick, emulsions
of the same glands representing one tick and more failed
to infect.   Their explanation was that they were unable
to produce a uniform suspension of infective particles
and that many would remain in large clumps in the salivary
gland tissue.   The implications of this line of approach
were obvious and if a method of producing a uniform
suspension could be found the quantum of infection
hypothesis could be fully investigated.

However, Jarrett, Crighton and Pirie (1969) using

10, 100 and 1000 ticks to infect <u>Bos taurus</u> cattle
concluded: .... "With the infecting doses used, the
clinical disease was independent of the number of
organisms as the major pathogenic feature, lymphocytol-
ysis, is induced at the same rate in infections of 10,
100 or 1000 ticks because the <u>rate</u> of growth of the
organism is the same.   The time taken to destroy
successively 10^{10}, 10^{11}, and 10^{12} lymphocytes is equal
with all three dose levels but occurs on different days
after infection."

At the beginning of the FAO Project in 1967 it was
decided that a suspension of infective particles of
<u>Theileria parva</u>, which would infect cattle by
inoculation, and which could be preserved viably at low
temperatures could be used:

    a. to produce a reproducible challenge for cattle,

    b. to establish its' infectivity by titration in
       cattle and at the same time studying the severity
       of the disease related to the size of the infective
       dose.

    c. for immunization trials after prior exposure to
       attenuating agents.

    d. for chemoprophylactic studies using
       tetracyclines.

When the author joined the FAO Project in 1968 the
team had developed two techniques which would yield a
suspension of infective particles which would
reproducibly infect experimental cattle.   One method
was by trituration of infected ticks which had been

suitably prepared to allow maturation of the infective
particles in the tick's salivary glands (E.A.V.R.O.
1968), and the other method was the in vitro feeding
technique involving the collection of tick saliva in
capillary tubes (Purnell and Joyner, 1967; Joyner and
Purnell, 1968; Purnell, Branagan and Radley, 1969).

Until the recent work of Jarrett et al. no
quantitative study of T. parva in the bovine host had
been made.   In their experiments, E.C.F. was initiated
in cattle by allowing infected adult R. appendiculatus
to feed naturally on the ears of cattle.   Unfortunately
reproducibility is questionable in any experiment using
ticks since the number of infective particles
inoculated while ticks are feeding will vary with the
 infection rate of the salivary glands, which in turn
depends on the tick batch used and the age of the ticks
after moulting (Barnett, 1957; Purnell and Joyner, 1968).
However, by using a suspension of infective particles to
infect cattle this variability would be reduced since
accurate measurement of the volume of suspension could
be made.   Here then was one area where an advance could
be made.   In order to calculate the daily increase of
parasitized lymphocytes in an animal undergoing an
E.C.F. reaction Jarrett et al. biopsied the prescapular
lymph node and prepared thin films for microscopical
examination.   They selected an area of this film at
random and made a linear count of 400 lymphoid cells and
their associated macroschizonts.   The result was
expressed as a percentage and termed the macroschizont

index (MSI). Arising from their work, several
questions may be asked:

a. Was one linear count sufficient to be
representative of that smear? e.g. would one
linear count in the middle of the film be similar
to a count performed along the edge of the film?

b. Would there be a large error counting only
400 cells and parasites when the MSI equals 1
(frequency of schizonts = 1 in 100 lymphoid
cells)?

c. Was one biopsy sample from the prescapular
lymph node representative of that node? i.e. is
the distribution of parasitized cells even within
the node when several biopsies from that node are
compared?

d. Was the node being sampled representative of
the distribution of parasitized cells throughout
the whole lymphoid organ? Could it be possible
that one part of the lymphoid organ harboured and
encouraged growth of the parasite more than
elsewhere?

Jarrett et al. brought up an important aspect of
the parasite's life cycle in their discussion. They
suggested that the macroschizont has a limited
replication in the bovine host. If the pathogenic
phase of the life cycle, the macroschizont, is
inevitably destined to transform into microschizont
and ultimately the relatively non-pathogenic phase, the
piroplasm, the disease is self-limiting provided the

host can survive the cumulative effects of the
macroschizont stage.

If limited replication does occur we must ask
several questions. Is this factor controlling
replication something intrinsic in the parasite's
genetic make-up?   Is it something controlled by the
host?   Or is it governed by the interaction of the
host and parasite?

There is some evidence against limited replication
in tissue culture of the parasite (Hulliger, Wilde,
Brown and Turner, 1964; Malmquist, Nyindo and Brown,
1970; Malmquist and Brown, 1970).   This evidence shows
that the parasite is capable of prolonged and possibly
unlimited replication in the in vitro bovine system.
However, inoculation of the tissue culture cells into
a bovid did show that some parasites had a limited
replication, since microschizonts and piroplasms were
produced (Brown, Malmquist, Cunningham, Radley and
Burridge, 1970).

Another argument against limited replication
being intrinsic to the parasite itself would be the
serial direct transmissions done by Brown (personal
communication, 1969; and reported by Wilde, 1967).   He
was able to transmit macroschizonts of T. parva from
one animal to another for five passages, and, although
prepatent periods became increasingly shorter using an
inoculum of 10^{10} schizonts at each transmission, there
still remained a time interval from infection of the
original donor to death of the last passage animal of

63 days.   If Brown had varied the number of
macroschizonts it might have been possible to passage
these for an indefinite period.   This would be evidence
that the limitation of replication is not intrinsic to
the parasite.

Hulliger, Brown and Wilde (1966) after in vitro
studies suggested that this transformation may be due in
part to the parasite's response to high temperature.
Multiplication of the host cell was slowed down but at
the same time the parasite's division increased.   It
would appear then, from the above discussion, that
macroschizonts may not have a limited replication in
certain circumstances but during the course of an E.C.F.
reaction transformation from macroschizont to
microschizont will occur and this is probably due to the
host/parasite interaction.

On the basis of the work showing a relationship
between size of infective dose and the clinical response
in the host, the work on the kinetics of replication,
and the techniques developed by the F.A.O. Project team,
the author embarked on quantitative studies as a
contribution to the project as a whole.   If a small
enough number of infective particles could be introduced
in the bovine host, become established, but because of
limited replication, fail to cause a severe or fatal
disease, a method of immunization might have been found.

Throughout the work to be presented, the author
collected, prepared, examined and analysed all the
material and data personally.

CHAPTER 3.
GENERAL MATERIALS AND METHODS

i. Experimental cattle

Bos taurus animals were supplied from farms
situated in E.C.F. free areas of Kenya which applied a
strict regimen of tick control.   These cattle were
presumed to be highly susceptible.   During experiments
these cattle were housed in tick-proof accommodation
(Binns, 1956).

ii. Source of infected ticks

A colony of Rhipicephalus appendiculatus was
maintained in the laboratory as described by Bailey
(1960) and Branagan (1969) and infected adults were
supplied to the author.

iii. Source of T. parva

The strain of T. parva under investigation was
that designated T. parva (Muguga), first isolated from
the field 20 years previously (E.A.V.R.O. 1951).   It
has been maintained in the laboratory by tick passages
through bovine hosts.   This strain has been well
documented by previous workers (Barnett, 1957 and 1960;
Barnett, Brocklesby and Vidler, 1961; Brocklesby, 1962;
Brocklesby, Barnett and Scott, 1961).   The morbidity
and mortality rates of Bos taurus cattle exposed to
T. parva (Muguga) were recorded as 87.6 and 95.5 per
cent respectively (Brocklesby et al., 1961).   There is
no evidence to date indicating any change in character

of this strain.

iv. Preparation of infective inoculum

Infected adult R. appendiculatus (50% male, 50% female) were fed for a minimum of three but never more than four days on rabbits' ears to allow maturation of the infective particles (Martin, Barnett and Vidler, 1964; Purnell and Joyner, 1968). Ticks not attached after two days were removed from the ears and discarded. The partially fed ticks were removed after the fourth day following application, cleaned and triturated in Eagle's minimal essential medium* (MEM) + 3.5% bovine plasma albumin** (BPA) using a pestle, mortar and ground glass (Cunningham et al., 1969, as quoted by Branagan, 1969). The suspensions were allowed to sediment for approximately one hour and the supernate then pipetted off and divided into aliquots according to the experiment. The suspension of infective particles may also be viably preserved in liquid nitrogen at -196°C as a stabilate (as defined by Lumsden and Hardy, 1965). These techniques for preparation of stabilates are reported EAVRO (1969), Cunningham, Brown, Purnell and Branagan (1970 b).

Fresh preparations of suspension were used by the author in all experiments but one (see Chapter 8).

v. Preparation of smears

a. Biopsy material

The technique used is standard practice

* Burroughs Wellcome & Co., London.

** Fraction V, Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex.

for many laboratories.   A small drop of biopsy
material (cellular material suspended in lymph from
a lymph node, or peripheral circulating blood from a
punctured ear vein) was placed at one end of a clean
glass slide.

Figure I

Biopsy sample

B   A
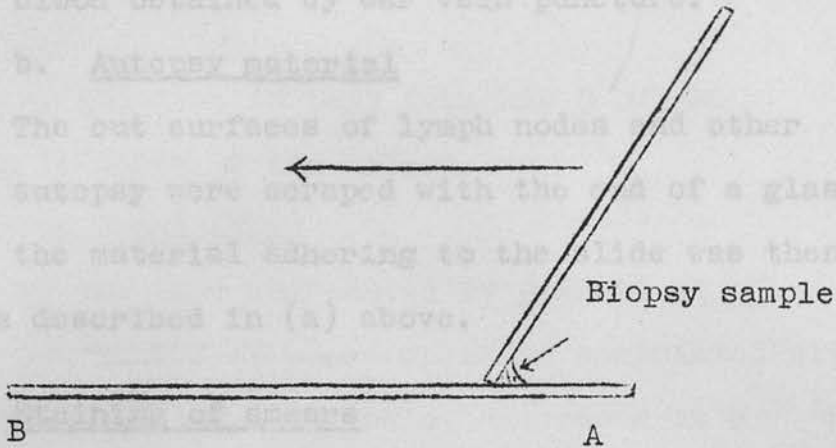
Another glass slide is brought into contact with
the material as shown in Fig. I.   The inclined slide
is pushed along the horizontal slide (in the direction
indicated by the arrow), pulling the biopsy sample
behind it.   The thickness of the smear is dependent on
the angle at which the spreading slide is inclined to
the horizontal one.   With practice smears were made
which were one cell thick but the spacing between cells
varied, i.e. in area A the cells were very close
together, whereas in area B the cells were displayed
singly.

The procedure for lymph node biopsies was as
follows.   The skin overlying the nodes was shaved,
and swabbed with 70% methyl alcohol prior to biopsy.
A 1½" hypodermic needle (the gauge of which is altered
- see later chapters) was inserted at right angles

through the skin and into the lymph node.   On
withdrawal of the needle, cellular material present in
the lumen, was then expressed onto a slide and a smear
made in the above manner.

Smears of peripheral blood were made in the same
way using blood obtained by ear vein puncture.

b.   Autopsy material

The cut surfaces of lymph nodes and other
organs at autopsy were scraped with the end of a glass
slide and the material adhering to the slide was then
smeared as described in (a) above.

vi.   Staining of smears

Smears of biopsy material and scraping samples
were air-dried and fixed in 70% methyl alcohol for five
minutes.   They were then immersed in the vertical
position in Coplin jars containing 5% Giemsa + Azur II
(Shute, 1966) diluted in buffered distilled water
pH7.2 for 45 minutes.   The stain was diluted just
before requirement and used for staining only one set
of slides.   The stained smears were differentiated
individually in tap-water, dried and examined under the
light microscope, total magnification x 1000 (x 10
eye-piece; x 100 objective).

vii.   Terminology

Local drainage lymph node (L.D.L.N.)

This lymph node drained the site of inoculation,
and was the node biopsied for establishment of
prepatent period.

Prepatent period

This is defined as the time taken in days from
inoculation of infective particles on Day 0 of an
experiment to the first detection of macroschizonts in
the L.D.L.N.

Survival time

This is the time in days from Day 0 of the
experiment to death of the animal.

Macroschizont Index (MSI)

This is the term introduced by Jarrett et al.
(1969) as the number of macroschizonts associated with
a given number of lymphoid cells, expressed as a
percentage.   In this text microschizonts were
included in MSI estimations.   The word "associated" is
used because the macro - or microschizont may be
extracellular.

The prescapular lymph node on the opposite side to
the site of inoculation was chosen as the source of
biopsy material for MSI estimation.

viii. Statistical Methods

Estimation of total numbers of macroschizonts

The total number of lymphoid cells during the
hyperplastic stage of E.C.F. was estimated by Jarrett
et al. (1969) to be 3.1×10^{11} per 100 lbs. live body
weight.   Thus the daily total of macroschizonts in an
animal was calculated and plotted by relating the MSI
to the total number of lymphocytes in the body.

Observations on growth curves

Regression lines of growth curves were obtained
using the method of least squares, plotting the
logarithm total parasites (Y) on time in days (X).
The line of best fit for the observations was obtained
using the formula $Y = a + bX$.

In certain instances the MSI estimations showed
daily increases and then levelled off, the level of
this plateau varying with each individual animal.
Death occurred at any time along the growth curve and
sometimes it was several days after the plateau was
reached before the animal succumbed to the disease.
Occasionally, where recovery occurred, the plateau was
of short duration and a decline in macroschizont
numbers was observed. As this study was primarily
aimed at the investigation of the increase in
macroschizont numbers the observations made during
plateau or decline in numbers were recorded but not
included in the statistical analysis.

Growth rate of the parasite (T_{10})

This is expressed as the time taken in days for a
1 $\log_{10}$ unit increase in macroschizont numbers to occur.
For ease of description this parameter was given the
symbol T_{10} by Jarrett et al. i.e. $T_{10} = 3$ means there
will be a ten-fold increase in macroschizont numbers
every three days. The T_{10} value is the reciprocal of
the slope (b) of the line of best fit.

Statistical comparison of growth curves and their means

Analysis of variance was used to determine the

mean slope of the growth curve for a group of animals
and to show the parallelism within the group using the
variance ratio test.

By using the formula below data from different
analyses of variance showed parallelism or non-
parallelism of the mean slopes between groups.

$$t_n = \frac{b_1 - b_2}{\sqrt{\dfrac{6_1^2}{Cxxc_1} + \dfrac{6_2^2}{Cxxc_2}}}$$

where n = Sum of degrees of freedom (Residual)

     b = Slope of mean growth curve

    6^2 = Estimated mean square (E.M.S.)

  Cxxc = Summation of sums of squares of
deviations from the sample mean.

The methods used for linear regression are those
described by Bishop (1966) with one modification.
Bishop states that the degrees of freedom for the
correlation coefficient are (n - 1) where n = number
of observations.   (n - 2) was the formula given
originally (Fisher, 1963) and is generally accepted.
This latter formula was therefore used in this thesis.

On statistical advice received (Freeman, 1969,
personal communication), it was decided that the numbers
of lymphocytes and their associated macroschizonts to be
counted to give a statistically valid estimate of the
MSI was as shown in Table 1.   The numbers of

lymphocytes quoted for each range of infection rates

were the mathematically acceptable minimum.

TABLE 1. (CHAPTER 3)

NUMBERS OF LYMPHOCYTES AND ASSOCIATED MACROSCHIZONTS TO BE
COUNTED IN ESTIMATIONS OF MACROSCHIZONT INDICES FROM LYMPH
NODE BIOPSY SMEARS.

Estimated count of lymphocytes and associated macro-schizonts expressed as a percentage	Recommended minimal number of lymphocytes to be counted
1 - 10	1000
10 - 20	800
20 - 30	600
30 - 40	500
40 - 60	400
60 - 100	200

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CHAPTER 4.

PILOT EXPERIMENT OF QUANTITATIVE STUDIES

Jarrett et al. hypothesise that the growth rate of
the parasite is "an unbroken exponential phase of
multiplication" which "starts immediately on injection."

This experiment was designed

a.   to confirm the conclusions of Jarrett et al.
(1969) that the parasite had a constant growth
rate of $T_{10} = 3$, independent of the infective dose.

b.   to throw some light on the prepatent period
of the disease.   The intention was to reduce the
prepatent period to minimal duration by
inoculating a large number of infective particles.
Observations on the growth curves of the parasite
in the subsequent patent infections might thus be
expected to give a more accurate estimate of the
number of infected particles which became
established.

To facilitate the establishment of large numbers
of infective particles in calves, the aliquots of
suspension were mixed with 10^9 autologous leucocytes
from each calf prior to inoculation (E.A.V.R.O. 1967;
Brown, Cunningham, Joyner, Purnell, Branagan, Corry and
Bailey, 1969).

The major differences in methods used by Jarrett
et al. and the author were:- Jarrett et al. initiated
E.C.F. infections with 1000, 100 and 10 infected ticks

which would inject infective particles over a period of
days reaching a peak in numbers around Days 4 or 5, but
the experiment reported here involved the injection of
infective particles on Day 0 only.   Also in this
experiment autologous leucocytes were used in one group.

    i.   Materials and methods (See Table 2)

    1.   Cattle
           pairs
        Two ~~paris~~ of Friesian calves, six months old
were used, one pair (Group 1) received large numbers of
infective particles, and the second pair (Group 2),
inoculated with a much smaller number, acted as controls
to the infectivity of the suspension.

    2.   Autologous leucocytes

        Venous blood (600 ccs) from each of the calves
numbered 667 and 669 (Group 1) was taken in heparin
(20 units/ccs of blood).   The blood was centrifuged at
2,000 r.p.m. for 15 minutes in a refrigerated centrifuge
(Mistral 6L) operating at $4^{\circ}C$.

        The plasma and platelet layers were discarded
by pipetting, and the buffy coats carefully removed
using a wide bore pipette.   These leucocytes were washed
four times in calcium and magnesium ion free phosphate
buffered saline by alternate washing and centrifugation
at 1,000 r.p.m. for 5 minutes.   After the final wash
the leucocytes were suspended in 10 ccs. Eagles M.E.M. +
B.P.A. 3.5%.

    3.   Ticks

        4,800 infected adult R. appendiculatus were
pooled from the following E.A.V.R.O. batch numbers:

TABLE 2. (CHAPTER 4)

VOLUME AND ROUTE OF ADMINISTRATION OF INFECTIVE SUSPENSIONS OF T.PARVA
(MUGUGA)

GROUP	ANIMAL NUMBER	LIVE WEIGHT IN POUNDS	INOCULUM FOR EACH ANIMAL		
			VOLUME OF INFECTIVE SUPERNATE	NUMBER OF AUTOLOGOUS WBC IN 10ccs.MEM/BPA ADDED TO INFECTIVE SUPERNATE	ROUTE OF INOCULATION
1	667	171	64 ccs.	2.8×10^9	34ccs. intravenous*
	669	166			40ccs. subcutaneous**
2	671	183	0.1 ccs.	none	subcutaneous**
	672	149			

* Injection via jugular vein.
** Injection at base of neck, 2 inches anterior to
point of shoulder.

1145)
1147)   5 months after ecdysis
1149)

1151)
1152)
1153)   4 - 5 months after ecdysis
1156)

        All the ticks selected had dropped as engorged
nymphae from cattle which had at least 5% of their
erythrocytes infected.

        The ticks were applied to 12 rabbits, 100 males and
100 females to each ear.   Approximately 80% attachment
was recorded one day later and 4,300 were attached after
two days.   The remaining 500 ticks were removed and
discarded.   On the fourth day the 4,300 ticks were
manually removed from the rabbits's ears, cleaned and
triturated in 280 ccs. MEM/BPA 3.5%.   The contents of
the mortar were decanted into a measuring cylinder and
allowed to sediment for one hour after which period
132 ccs. of supernate were removed by pipetting.   A
volume of 0.4 ccs. of supernate was taken for Group 2
animals and the remainder divided into two aliquots.
To these latter suspensions were added autologous
leucocytes as shown in Table 1.

        The supernate/leucocyte mixtures were held for ten
minutes at room temperature prior to inoculation to
enable the infective particles to become associated with
the leucocytes (E.A.V.R.O. 1969).   Inoculations were
carried out as shown in Table 2, all subcutaneous ones
being made at the base of the neck, 2 inches anterior to

the point of the right shoulder. The right prescapular
node was the L.D.L.N. and the left prescapular node was
the source of material for MSI estimations. The actual
delivery time of the intravenous material was about
½ minute.

4. Observational procedure

Biopsy smears using 1½" 14 gauge needles were
taken from the right and left prescapular lymph nodes
one hour before and 2 and 4 hours after inoculation
(12 noon, Day 0), then throughout the experiment before
8.30 a.m. daily.

From this material the prepatent period and
M.S.I.s were estimated. Blood smears were examined
for intraerythrocytic piroplasms and rectal temperatures
recorded daily.

ii. Results

The results are summarized in Tables 3 - 6 and
MSI estimations are recorded in Appendix Table 1.

When the intravenous inoculations were carried
out, both calves (667 and 669) developed respiratory
distress and one (667) collapsed for a transient period
of time. The shortest prepatent period was 5 days
(animal 669). The rectal temperature of animal 669
first exceeded 103°F on Day 7 and the other three
animals on Day 10. Piroplasms appeared in the blood
smears from three animals on Day 13 but animal 669 died
overnight between Days 12 and 13 and no piroplasms were
seen in its smears up to and including Day 12.

The mean survival time for Group 1 was 13.5 days, and the fatal case in Group 2 (672) survived 17 days. Calf 671 however recovered, macroschizonts not being detected in biopsy smears after Day 23, and piroplasms after Day 38.

MSI estimations were recorded from Days 8 - 12 for Group 1. In Group 2, calf 671 reached a peak MSI of 29.71 on Day 17 and then macroschizonts decreased in numbers daily thereafter (4% on Day 18, 2.9% Day 19, 7% Day 20). Calf 672 showed a steady increase in MSI from Day 12 to Day 16.

Linear regression of all four sets of data gave very significant correlation coefficients and parallelism of growth curves within groups (Table 5).

Comparison of the mean slopes between groups revealed a significant difference, that is to say the growth curves between Group 1 and Group 2 were not parallel (Table 6). This divergence is shown in Figure 2A & B.

iii. Conclusion

The transient collapse and respiratory distress of Group 1 animals was probably due to shock caused by the rapid injection of this foreign material.

From the analysis of results it would appear that the hypothesis of Jarrett et al. of a constant growth rate (T_{10} = 3) which is independent of infective dose does not hold. In this experiment the individual T_{10}s vary from 2.36 to 5.72. Also, the higher the infective dose the lower the T_{10} and vice versa. A working

hypothesis may be formulated that using a suspension
of infective particles the resultant growth rate is
dependent on the infective dose inoculated.

As the results of this experiment did not confirm
Jarrett's hypothesis, a decision was made to define
more accurately the base-lines for repeated accurate
measurements and then to repeat the experiment.

GROUP	ANIMAL NUMBER	MACROSCHIZONTS IN LOCAL DRAINAGE LYMPH NODE	TEMPERATURE OF 103°F OR OVER	INTRA-ERYTHROCYTIC PIROPLASMS	DEATH
1	667	8	19	13	14
	669	5	7	-	13
2	671	6	10	13	-
	672	7	10	13	17

TABLE 3. (CHAPTER 4)

PREPATENT PERIOD, ONSET OF FEVER AND PARASITAEMIA, AND SURVIVAL
OF TWO GROUPS OF ANIMALS INOCULATED WITH DIFFERENT SIZES OF
INFECTIVE DOSES OF <u>T.PARVA</u> (MUGUGA)

| GROUP | ANIMAL NUMBER | MACROSCHIZONTS IN LOCAL DRAINAGE LYMPH NODE | TIME IN DAYS TO | | |
			TEMPERATURE OF 103°F OR OVER	INTRA-ERYTHROCYTIC PIROPLASMS	DEATH
1	667	6	10	13	14
	669	5	7	–	13
2	671	6	10	13	–
	672	7	10	13	17

TABLE 4. (CHAPTER 4)

DAILY MACROSCHIZONT INDICES DATA TABULATED FOR LINEAR REGRESSION

GROUP	ANIMAL	DAY (X)	MSI	LOG TOTAL MACROSCHIZONTS (Y)
		8	1.20	9.8035
		9	11.30	10.7709
	667	10	27.20	11.1584
		11	47.50	11.4014
		12	76.00	11.6053
1				
		8	1.50	9.8837
		9	12.10	10.7903
	669	10	19.00	10.9863
		11	58.25	11.4728
		12	73.50	11.5740
		12	3.00	10.2330
		13	7.40	10.6253
	671	14	10.63	10.7825
		15	12.00	10.8351
		16	17.17	10.9908
		17	29.71	11.2279
2				
		12	6.70	10.4889
		13	21.60	10.9972
	672	14	43.25	11.2989
		15	54.00	11.3952
		16	76.50	11.5464

TABLE 5. (CHAPTER 4)

RESULTS OF LINEAR REGRESSION AND ANALYSIS OF VARIANCE OF THE DAILY LOGARITHMIC INCREASES OF MACROSCHIZONTS IN TWO GROUPS OF ANIMALS INOCULATED WITH DIFFERENT SIZES OF INFECTIVE DOSES OF $\underline{T.\ PARVA}$ (MUGUGA).

GROUP	ANIMAL	LINE OF BEST FIT $Y = a + bX$	r	P	T_{10} VALUE	PARALLELISM
1	667	6.71 + 0.42X	0.941	<0.020	2.36	F = 0.01 d.f. 1, 6 P > 0.05
	669	6.88 + 0.41X	0.951	<0.020	2.46	
2	671	8.25 + 0.17X	0.968	<0.010	5.72	F = 0.15 d.f. 1, 7 P > 0.05
	672	7.63 + 0.25X	0.950	<0.020	3.98	

TABLE 6. (CHAPTER 4)

STATISTICAL COMPARISON OF MEAN GROWTH CURVES OF MACROSCHIZONTS
OF TWO GROUPS OF ANIMALS INOCULATED WITH DIFFERENT SIZES OF
INFECTIVE DOSES OF T. PARVA (MUGUGA)

GROUP	MEAN SLOPE	MEAN T_{10} VALUE	PARALLELISM BETWEEN GROUPS
1	0.41	2.41	$t = 2.55*$
			d.f. 13
			$P < 0.05$
2	0.20	4.93	

* Significant difference i.e. not parallel.

FIGURE 2 A & B (CHAPTER 4)

A   Mean growth curves of T. parva (Muguga)
     using two different sizes of infective
     doses.

B   Transposition of mean growth curves to
     demonstrate divergence.

A

Group 1

Group 2

10^{12}

10^{11}

10^{10}

MACROSCHIZONTS

8   9   10   11   12   13   14   15   16   17

DAYS   POST-INOCULATION

B

Group 1

Group 2

Y

X
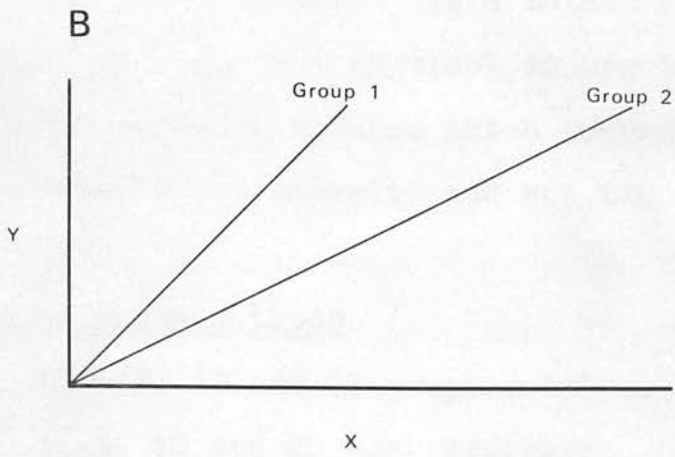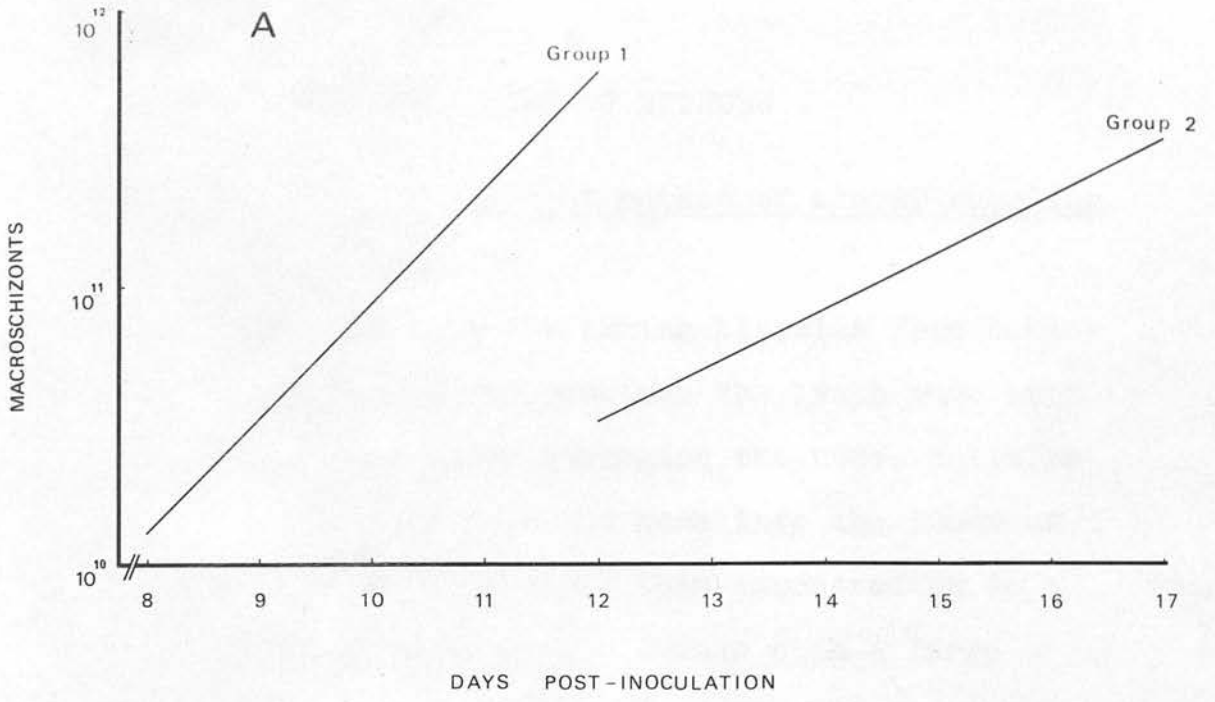
CHAPTER 5

STANDARDIZATION OF METHODS

A) Determination of the best method of biopsy sampling
from a lymph node

The normal practice for taking biopsies from bovine
lymph nodes in vivo is to puncture the lymph node with
a 14 gauge needle and, by massaging the node, cellular
material is expressed from the node into the lumen of
the needle.   This material is then expressed on to a
glass slide and a smear made.   Since such a large
needle produces a fair degree of trauma, it was thought
that this physical insult to the lymph node tissue
would result in large haemorrhagic tracts and a
concomitant cellular response.   Daily samplings with
such a needle would rapidly cause alterations in the
lymph node tissue and conclusions drawn from examination
of these smears could be false.   If a method could be
devised which would keep this physical injury to a
minimum, then any cellular changes which occurred would
most likely be due to the parasite and not the host's
reaction to trauma.

    i.   Materials and Methods

       (1) 17, 18, 19 and 22 gauge needles.

       (2) 1, 5, 10 and 20 ccs. syringes.

       (3) clean dry slides.

       (4) biopsy source: animal reacting to East
       Coast Fever with an enlarged prescapular node.

       Each type of needle was used, with all four

sizes of syringe to make biopsy samples. The
samples were smeared on slides as described in
Chapter 3 and the smears examined
microscopically for cellular consistency.

ii. Results

All the combinations of syringes and needles
proved to be satisfactory for producing samples of
a suitable consistency. Since the 22 gauge needle
produced very little trauma in the lymph node this
was empirically the needle of choice. The ease
with which the different syringes could be handled
became the criterion for choice of a particular size
of syringe. The author found that the 20 ccs.
syringe was a little clumsy when attached to a 22
gauge needle. When the plunger of the 1 cc.
syringe was withdrawn there wasn't a sufficient
vacuum created to withdraw a large enough sample
for smearing.

The 5 or 10 ccs. syringes with 22 gauge
needles were easily manipulated, the needle could
be inserted into the lymph node, with the syringe
attached, causing the animal very little pain.
Withdrawal of the plunger in either case gave a
satisfactory vacuum for removal of cellular
material into the lumen of the needle. By
releasing the plunger gently and withdrawing the
syringe and needle from the lymph node this
material remained in the lumen and could be
expressed on to the slide by depressing the plunger.

iii. Conclusion

Since using a 22 gauge needle attached to a 5 or 10 ccs. syringe would cause relatively little trauma and little or no pain to the animal, and moreover was easily manipulated by the operator, and produced suitable biopsy samples, it was decided that this combination was the one of choice and was used in all subsequent experiments.

B) Determination of a representative count for a smear

It has been noticed in previous work that two separate counts of parasites to a given number of lymphocytes could yield differing results. It was thought that an experiment designed as follows would determine how many counts as set out in Fig. 3 were needed to give a true representation of the infection rates in that smear.

i. Materials and methods

Nine linear counts of lymphocytes and parasites were made on smears as shown in Fig. 3.

Fig. 3.
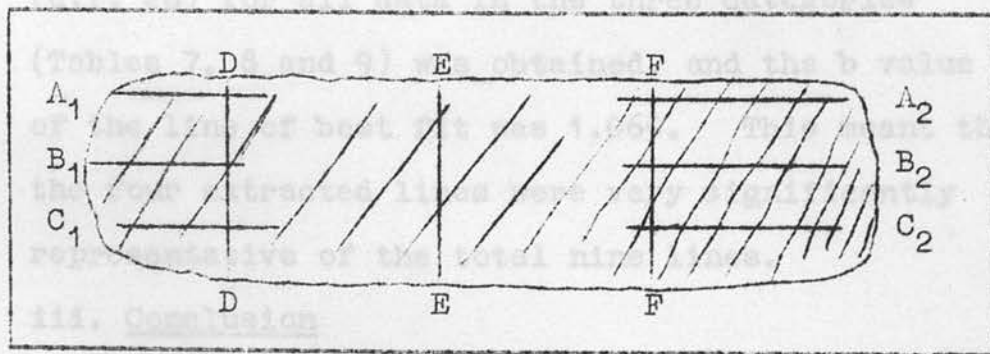
The shaded area is the smear to be examined and the lines are arbitrary lines showing the areas which were counted. Ten such smears from each of the following estimated infection rates:- 1 - 10%, 10 - 50%, and 50 - 100% were examined.

ii. Results

The results are given in Appendix Tables 2 - 4, and summarized in Tables 7 - 9. Difficulty in counting macroschizonts was encountered in some slides in the areas A2, B2, C2, E and F because in this thick area of the smear the cytoplasm of the lymphoblasts was not spread out. For this reason the counts done in areas A1, B1, C1 and D were compared with the total nine lines. It can be seen that any one line was not consistently representative throughout the series for all smears.

The arithmetic mean of the four lines designated A_1, B_1, C_1, and D, however, always fell within 2 x s.d. units of the mean for the total nine lines. Furthermore using linear regression analysis, a correlation coefficient of 0.992** (d.f. 28) for all data in the three categories (Tables 7, 8 and 9) was obtained, and the b value of the line of best fit was 1.069. This meant that the four extracted lines were very significantly representative of the total nine lines.

iii. Conclusion

From the practical point of view MSI estimations carried out at the thin end of a smear were the

** Highly significant

TABLE 7. (CHAPTER 5)

STATISTICAL COMPARISON OF FOUR MSI's WITH TOTAL NINE MSI's OF
TEN SMEARS.

CATEGORY 1 - 10%

SLIDE	NINE LINES		FOUR EXTRACTED LINES	
	Arithmetic mean	Standard deviation	Arithmetic mean	Standard deviation
1	1.82	0.59	1.70	0.69
2	8.04	1.28	8.50	2.09
3	8.08	1.71	8.93	1.74
4	4.26	0.50	4.63	0.52
5	6.90	0.22	6.90	0.17
6	4.18	0.74	4.35	1.03
7	1.71	0.54	2.15	0.11
8	2.30	0.16	2.40	0.49
9	9.29	1.10	8.81	0.97
10	7.39	1.01	6.98	1.18

TABLE 8. (CHAPTER 5)

STATISTICAL COMPARISON OF FOUR MSI's WITH TOTAL NINE MSI's OF
TEN SMEARS.

CATEGORY 10 - 50 %

SLIDE	NINE LINES		FOUR EXTRACTED LINES	
	Arithmetic mean	Standard deviation	Arithmetic mean	Standard deviation
1	36.07	5.07	37.85	6.37
2	22.00	4.34	24.92	4.60
3	44.89	12.07	52.00	10.79
4	20.09	9.70	19.96	3.37
5	48.03	4.73	51.13	5.12
6	48.86	5.04	51.38	4.08
7	27.37	4.18	24.62	4.54
8	42.86	3.50	42.06	1.82
9	24.87	5.32	25.29	4.15
10	25.00	2.66	25.41	3.31

TABLE 9.   (CHAPTER 5)

STATISTICAL COMPARISON OF FOUR MSI's WITH TOTAL NINE MSI's of
TEN SMEARS.

CATEGORY 50 - 100%

SLIDE	NINE LINES		FOUR EXTRACTED LINES	
	Arithmetic mean	Standard deviation	Arithmetic mean	Standard deviation
1	63.22	15.12	52.63	9.78
2	52.72	2.44	51.75	2.30
3	69.06	13.66	78.13	12.77
4	102.94	13.24	109.50	7.80
5	66.39	6.39	69.75	3.47
6	69.17	10.71	73.50	6.06
7	77.06	16.54	91.13	9.18
8	86.39	10.27	91.13	9.84
9	95.39	17.36	104.88	23.73
10	57.67	3.11	51.25	3.65

easiest to perform.   The mean of four such
estimations were shown to represent the total nine
and so this method was used whenever MSI estimations
were calculated.

C) Determination of representative sample from a lymph

node

Since statistically representative counts for a
smear were now possible, it was necessary to find out
if one smear from a lymph node would be representative
of that node.

i.   Materials and methods

Three animals undergoing E.C.F. reactions with
prescapular nodes showing infection rates within the
three categories (1 - 10%, 10 - 50% and 50 - 100%)
were chosen.   The lymph nodes were biopsied as
shown in Fig. 4 and smears made as usual.   The
biopsies from a lymph node were made on one
occasion (the procedure taking about ten minutes).
This ensured that any differences in parasite
numbers would be due to distribution of the
macroschizonts within the lymph node and not due to
an overall increase in numbers with time.

Four linear counts (A1, B1, C1 and D) were
done on each of ten smears from a particular lymph
node and compared.

Fig. 4.

Biopsy sites of a lymph node.
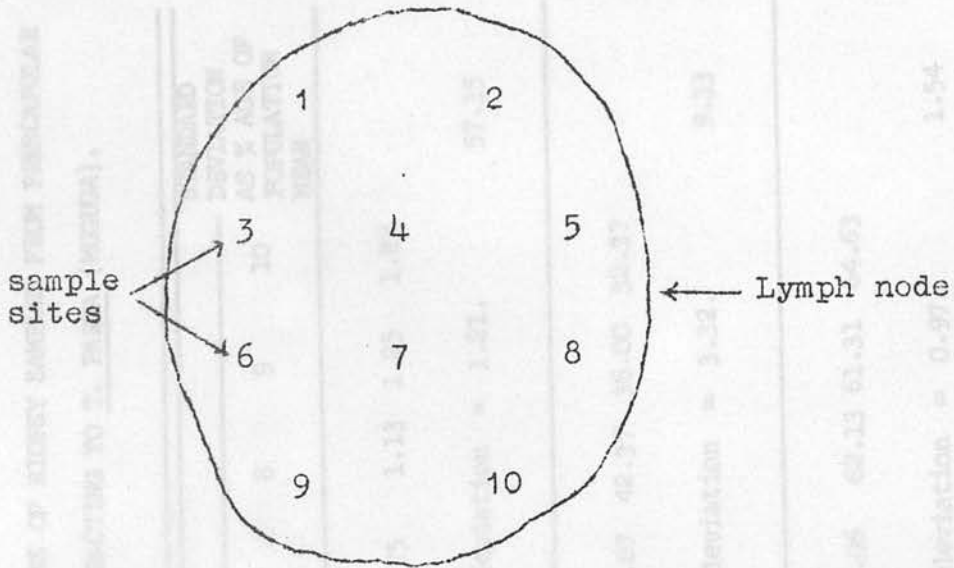
ii. Results (See Table 10, and Appendix Table 5)

The percentage of the standard deviation to the mean varies with the category and for category 1 - 10% the percentage is 57% (see Table 10).   In one case (Sample 3) the sample mean was outside 2 x s.d. of the population mean, whereas the other nine samples were within 1 x s.d. of the population mean.   Sample 3 therefore is not representative of the population mean at the 5% level but if it is combined with Sample 2 and the mean taken this combination mean would fall within the population mean limits.

However in the other categories the standard deviation as a percentage of the mean was low, and sample means never varied more than 2 x standard deviation of the population mean.

TABLE 10. (CHAPTER 5)

ARITHMETIC MEAN MACROSCHIZONT INDICES AND STANDARD DEVIATIONS OF BIOPSY SAMPLES FROM PRESCAPULAR

LYMPH NODES OF THREE ANIMALS (Categories a, b, c) REACTING TO T. PARVA (MUGUGA).

CATEGORY	SLIDE NUMBERS										STANDARD DEVIATION AS % AGE OF POPULATION MEAN
	1	2	3	4	5	6	7	8	9	10	
a) 1 – 10 %	2.35	3.55	5.10	1.20	1.93	1.60	1.75	1.13	1.25	1.28	
	Population Mean = 2.11.					Standard deviation = 1.21.					57.35
b) 10 – 50%	29.29	39.00	35.55	35.38	35.75	36.54	32.67	42.37	36.00	32.37	
	Population Mean = 35.59.					Standard deviation = 3.32.					9.33
c) 50–100 %	63.00	62.13	62.69	62.25	63.50	63.69	64.06	62.13	61.31	64.63	
	Population Mean = 62.94.					Standard deviation = 0.97.					1.54

iii. Conclusion

These statistical analyses indicated that, for
a 95% chance of being correct, two biopsy smears
should be taken from a lymph node when the MSI was
in the range of 1 - 10, but only one smear was
necessary when the MSI was over 10.

This procedure was adopted in all subsequent
experiments.

D) Determination of representative sample from lymphoid
organ

The stage had now been reached where a representative
sample of a smear(s) which was representative of a
prescapular lymph node could be taken.   In order to
relate the MSI of the prescapular node to the total
number of parasites in the body, it was necessary
to indicate that that MSI was or was not representative
of the distribution of the parasite throughout the
lymphoid organ.

This experiment was designed to investigate the
macroschizont distribution within organs containing
lymphoid cells, obtain the mean MSI for any one organ
and compare that mean MSI between organs.   It was
hoped that one or more of the superficial nodes would
be representative.

On considering the enormity of this problem it was
decided that a complete study to evaluate the
distribution of the parasitic biomass within all
organs and between all organs would be impractical,

and this does not include the study of the dynamics of
the lymphocyte population itself. Therefore, in order
to make this experiment possible in the time which was
available, certain assumptions were necessary.

Assumptions

1. With the exception of the local drainage lymph
node (namely the parotid lymph node - see materials and
methods) and the sequential nodes in the chain (namely
atlantal, anterior, mid- and posterior cervicals), one
can assume the dissemination of the parasite and
parasitized lymphocytes to be via the blood-stream.
If this is correct then one can further assume that
there will be even distribution of the parasite between
anatomically symmetrical lymph nodes e.g. the left and
right precrurals should have a comparable percentage of
infected lymphocytes within them.

2. It will also be assumed that nodes which drain
similar tissues, such as muscle and skin (carcase nodes),
will receive a few infected cells from those tissues and
will have comparable MSIs.

3. It is proposed that a sample or samples taken
from one lymph node of a group draining a similar area
will represent that group e.g. samples from one lymph
node of the mesenteric chain will be representative of
that group.

4. It will be assumed that all lymph nodes, other
than the local drainage chain, will behave similarly to
the prescapular lymph node and that the criteria already

established for the sampling of the latter will apply
to these lymph nodes e.g. two samples will be considered
representative for the 1 - 10% category and only one
sample needed when the MSI exceeds 10.

5. It will also be assumed that samples taken
within the three categories (1 - 10%, 10 - 50%, 50 -
100%) will represent that particular category.

6. Lastly it will be assumed that four counts are
representative for each smear.

i.   Materials and methods

Infected ticks

Fifty infected R. appendiculatus, male and
female, which had been feeding for 4 days attached
to rabbits' ears, were removed from the rabbits and
cleaned manually.   These ticks were then
triturated in 20 ccs. Eagles MEM + 3.5% BPA using a
pestle and mortar and ground glass.   After 10
minutes trituration the suspension was allowed to
sediment in a measuring cylinder for one hour.
The supernate (10.75 ccs.) was pipetted off.

Animals

Five Friesian steers (live weights ranging
from 442 lbs. to 463 lbs.) were each inoculated with
1 cc. of the supernate subcutaneously at the base of
the left ear.   These cattle were numbered as
follows:

C925, C927, C973, D103, D108.

Observational procedure

Daily rectal temperatures and palpation of local drainage lymph nodes were made. The right prescapular lymph node of each animal was biopsied daily after the temperature exceeded 103°C. Smears of the biopsy material were made and the MSI mean of each determined. The animals were killed when the prescapular lymph nodes showed infections ranging from 1.33% to 30.73%. Euthanasia was carried out by intravenous injection of an aqueous solution of chloralhydrate (20% W/V).

Sources sampled

a. Lymph nodes:

(1) At least ten smears were made from each of the following nodes (representing the local drainage chain), left parotid, left atlantal, left posterior cervical.

(2) At least two smears were made from each of the following nodes (representing the nodes infected via the blood), right parotid, right prescapular, internal iliac, mesenteric, mediastinal, hepatic, renal.

b. Organs

At least ten smears were made from each of the following organs (representing the sources of lymphoid cells which are not lymph nodes), thymus, spleen, liver, bone marrow, kidney, lung, Peyer's patches of the small intestine.

c. Blood

Samples of jugular blood (15ccs) from each

animal were collected into universal bottles
containing heparin (20 units/cc) immediately prior
to euthanasia.   This blood was then centrifuged at
2,000 r.p.m. for 15 minutes in a Mistral 6L
refrigerated centrifuge (operating temperature 4°C).
The plasma and platelet layers were discarded, and
the buffy coat layer pipetted off.   After mixing
the buffy coat in a bijou bottle with a Pasteur
pipette, a sample was removed, and smeared.

Smears of autopsy material were made as described in
Chapter 3.

ii.   Results
     The results are summarized in Table 11, and
the detailed data in Appendix Tables 6 - 10.
Animals 103, 108, 925, 927 and 973 were destroyed
on Days 14, 15, 15, 15 and 17 respectively.

iii. Conclusion
     With the exception of the left parotid and
left atlantal nodes from animal 103 (see Table 11
and Appendix Table 6) the MSI results indicate
that the right prescapular nodes reflected the
parasitic distribution throughout the lymphoid
organ in every case.   Since the experiment was
dependent on several necessary assumptions
statistical analysis of the results would be
presumptuous.

     The high MSI of the local drainage nodes in
animal 103 should not affect the total parasitic

numbers since these nodes will only have in the region of 10^{10} lymphocytes constituting a mere 1% of the total lymphocyte population.

It is interesting to note that certain organs such as Peyer's patches and thymus had low MSI estimations. The reasons why lymphocytes contained in these organs were not parasitized have not been elucidated but probably the lymphocytes are not suitable target cells or the organs themselves are not suitable breeding grounds for the parasitized lymphocytes.

The main lymphoid sites may be classed as the general lymph nodes and organs such as the lung, liver and spleen. There was very little variation in MSI between these main sites and the right prescapular node so it may be argued that the MSI of the right prescapular node was representative of the total lymphoid organ within the limits of this experiment.

TABLE 11.   (CHAPTER 5)

MEAN MACROSCHIZONT INDICES OF SAMPLES OF LYMPHOID ORGANS FROM OXEN 103, 108, 925, 927 AND 973 AT AUTOPSY

SAMPLE	ANIMAL				
	103	108	925	927	973
a. Local Drainage Chain					
Left parotid	40.50	1.28	6.41	18.59	7.93
Left atlantal	58.49	1.99	59.21	34.23	·9.09
Left posterior cervical	2.86	1.15	25.09	35.41	12.26
b. General Lymph Nodes					
Right prescapular	3.37	1.33	27.76	30.76	10.39
Right parotid	3.56	0.99	37.88	35.52	12.27
Hepatic	5.96	2.14	35.85	32.75	16.89
Renal	3.92	1.32	36.38	32.64	8.99
Internal iliac	4.32	1.61	35.05	34.57	14.11
Mesenteric	0.49	0.84	24.54	10.62	11.27
Posterior mediastinal	6.64	4.00	17.36	32.46	15.47
c. Organs					
Peyer's patch	0.85	0.28	7.80	2.85	11.80
Lung	2.53	1.64	33.23	27.83	11.08
Kidney	1.48	2.32	7.13	36.00	11.52
Bone marrow	1.99	0.26	8.47	13.23	2.69
Liver	4.83	5.78	31.45	44.18	24.32
Spleen	11.09	3.30	28.12	47.60	18.36
Thymus	0.04	0.23	3.42	1.19	11.16
Buffy coat	1.48	0.44	1.29	8.09	1.08

CHAPTER 6

PILOT EXPERIMENT OF QUANTITATIVE STUDIES USING STANDARDIZED METHODS

This experiment was designed to investigate the working hypothesis formulated in Chapter 4 and using the standardized methods developed in Chapter 5. It was thought possible that wider ranging infective doses could show larger divergences in growth curves.

i. <u>Materials and methods</u> (See Table 12)

<u>Cattle</u>

Eight 6-9 months old Guernsey type calves were used in this experiment.

<u>Ticks</u>

A total of 4,800 infected adult R. appendiculatus was selected from tick batch numbers E.A.V.R.O. 1173 and 1174. These ticks, 4 months old since ecdysis, had engorged as nymphs on infected cattle which had 21 - 52% of their erythrocytes infected. Two hundred adult ticks (100 male and 100 female) were fed on each ear of 12 rabbits for 3 to 4 days. The ticks not attached (850) were removed and discarded after two days. The remaining ticks (3,950) were removed on the fourth day after application to the rabbits' ears.

<u>Preparation of infective inoculum</u>

The partially fed ticks were cleaned and then triturated in 250 ccs. Eagle's MEM/BPA 3.5% for

TABLE 12. (CHAPTER 6)

VOLUMES OF INFECTIVE SUSPENSIONS OF <u>T. PARVA</u> (MUGUGA) RECEIVED
BY 4 GROUPS OF CATTLE.

GROUP	ANIMAL NUMBER	LIVE WEIGHT IN POUNDS	VOLUME OF INFECTIVE SUSPENSION
10^2	675	196	100 ccs.
	762	202	100 ccs.
10^1	674	181	10 ccs.
	718	178	10 ccs.
10^0	664	249	1 cc.
	666	270	1 cc.
10^{-1}	670	167	0.1 ccs.
	761	144	0.1 ccs.

30 minutes. The resultant suspension was
decanted to a 500 ccs. measuring cylinder and
allowed to sediment for one hour. After this
period of time 140 ccs. of supernate were pipetted
off and made up to a total of 230 ccs. inoculum by
the addition of 90 ccs. Eagle's MEM/BPA 3.5%.

The animals were inoculated with differing
volumes of the supernate as shown in Table 12.
Thirty minutes elapsed between inoculation of the
first (10^{-1}) and last (10^2) pairs of animals.

N.B.   1.   No leucocytes were added as in Chapter 4.

2.   All inoculations were made subcutaneously

at the point of the right shoulder.

Observational procedure

Biopsy smears were made daily from the right and
left prescapular nodes of all eight calves. The
right prescapular node was the local drainage lymph
node, and the left prescapular node was the source
of material for the quantitative study. Rectal
temperatures were taken and blood smears made daily
throughout the experiment.

ii.   Results (See Tables 13-17, Figures 5-7, and
Appendix Table 11)

Prepatent period

The shortest prepatent period was 5 days
(Group 10^2 animals). The local drainage lymph
nodes first showed evidence of hyperplastic
activity on Day 3 (calf 762) and Day 4 (calf 675)
when large numbers of lymphoblasts and dividing

TABLE 13. (CHAPTER 6)

PREPATENT PERIOD, ONSET OF FEVER AND PARASITAEMIA, AND SURVIVAL
TIME OF FOUR GROUPS OF ANIMALS INOCULATED WITH DIFFERENT SIZES
OF INFECTIVE DOSES OF T. PARVA (MUGUGA)

GROUP	ANIMAL NUMBER	TIME IN DAYS TO		
		MACROSCHIZONTS IN LOCAL DRAINAGE LYMPH NODE	TEMPERATURE OF 103°F OR OVER	INTRA-ERYTHROCYTIC PIROPLASMS	DEATH
10^2	675	5	6	-	11
	762	5	6(1)	-	11
10^1	674	6	7	12	13
	718	6	7	13	13
10^0	664	6	7	13	15
	666	6	7	13	18
10^{-1}	670	8	7	13	24
	761	7	7	13	19

cells were present. Macroschizonts were not detected in smears of Group 10^2 until Day 5 although very extensive searches (approximately 200 fields of 50 lymphocytes - 10,000 cells) were made. Day 5 smears of the L.D.L.N. from calf 762, however, showed so many macroschizonts (31 per 4000) it necessitated further checks of previous smears. No macroschizonts were detected.

Examination of the L.D.L.Ns. for the other groups first revealed small numbers on the days shown in Table 13. The prepatent periods differed only slightly between groups (5-8 days).

Onset of fever

Animal No. 762 had a rectal temperature of over 103°F on Days 1 and 2. The temperature then fell below 103°F on Days 3, 4 and 5, and rose again on Day 6. The other member of Group 10^2 first showed a rectal temperature of over 103°F on Day 6, whilst in Groups 10^1, 10^0, and 10^{-1} it was Day 7.

Intraerythrocytic piroplasms

Piroplasms were never detected in blood smears from Group 10^2 animals both of which died on Day 11. Piroplasms, however, were found in smears from all the other animals, appearing on Day 12 for animal 674 and Day 13 for the other five animals. The percentage of infected erythrocytes increased with time and animal 670 which was the last to die (Day 24) had a peak

parasitaemia of 35% on the previous day.

Survival Time (See Fig. 5 and Table 13)

The survival time did differ to a marked degree e.g. 11 days (Group 10^2) to a mean of 21.4 (Group 10^{-1}).

Growth curves (See Fig. 6A and B, and Tables 14-17, and Appendix Table 11).

Analysis of variance (Table 15) showed that within Groups the individual growth curves were parallel except in Group 10^0.

Comparison of the slopes of the mean growth curves between Groups, however, revealed a highly significant difference between Groups 10^2 and 10^{-1} (Table 17).

iii. Conclusion

Prepatent period

It would appear from the results that five days is the minimum prepatent period which can be expected. Although a thorough search was made for macroschizonts in the lymphoblasts from L.D.L.N. smears of Group 10^2 animals, taken before Day 5, none were found. However, on Day 5 macroschizonts were easy to find in these animals. These findings are discussed in more detail in Chapter 9.

Intraerythrocytic piroplasms

The intraerythrocytic piroplasms appeared in blood smears invariably in all Groups on Day 13 (one exception - Day 12). This constancy

FIGURE 5   (CHAPTER 6)

Relationship of infective dose of T. parva (Muguga)
and survival time.
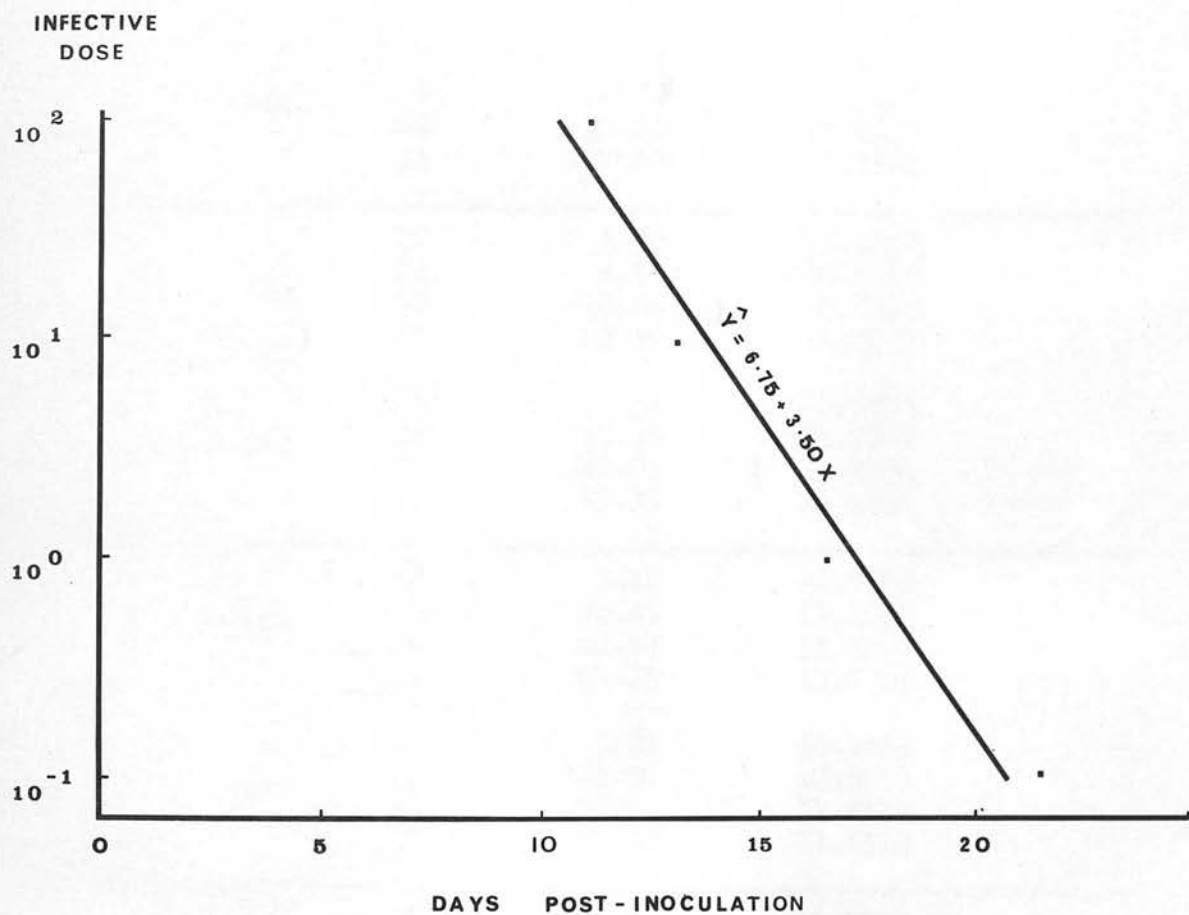
TABLE 14. (CHAPTER 6)

DAILY MACROSCHIZONT INDICES DATA TABULATED FOR LINEAR REGRESSION

GROUP	ANIMAL	DAY (X)	MEAN MSI	LOG TOTAL MACROSCHIZONTS (Y)
10^2	675	8	1.29	9.8943
		9	4.50	10.4378
		10	24.29	11.1703
		11	41.88	11.4065
	762	8	3.57	10.3483
		9	16.47	11.0128
		10	27.29	11.2330
		11	100.63	11.7993
10^1	718	10	1.52	9.9238
		11	4.14	10.3598
		12	12.44	10.8370
		13	23.79	11.1173
	674	10	1.91	10.0294
		11	10.94	10.7882
		12	22.03	11.0934
		13	43.35	11.3856
10^0	666	12	7.12	10.7752
		13	20.41	11.2330
		14	40.54	11.5302
		15	61.25	11.7101
	664	11	3.23	10.3962
		12	11.90	10.9633
		13	37.75	11.4639
		14	84.00	11.8116
10^{-1}	670	12	2.31	10.0792
		13	12.09	10.7966
		14	11.94	10.7910
		15	25.17	11.1139
		16	21.34	11.0453
		17	89.00	11.6637
	761	13	3.39	10.1790
		14	15.47	10.8388
		15	34.38	11.1847
		16	69.38	11.4900

-64-

TABLE 15. (CHAPTER 6)

RESULTS OF LINEAR REGRESSION AND ANALYSIS OF VARIANCE OF THE DAILY LOGARITHMIC INCREASES OF MACROSCHIZONTS IN FOUR GROUPS OF ANIMALS INOCULATED WITH DIFFERENT SIZES OF INFECTIVE DOSES OF $\underline{T. PARVA}$ (MUGUGA)

GROUP	ANIMAL	LINE OF BEST FIT $Y = a + bX$	r	P	T_{10} VALUE	PARALLELISM
10^2	675	5.72 + 0.53X	0.983	< 0.020	1.90	F = 0.59 d.f. 1, 4 P > 0.05
	762	6.75 + 0.46X	0.984	< 0.020	2.19	
10^1	718	5.89 + 0.41X	0.995	< 0.010	2.46	F = 0.11 d.f. 1, 4 P > 0.05
	674	5.79 + 0.44X	0.968	< 0.050	2.29	
10^0	666	7.12 + 0.31X	0.980	< 0.020	3.22	F = 8.61 d.f. 1, 4 P < 0.05
	664	5.23 + 0.47X	0.995	< 0.010	2.11	
10^{-1}	670	7.19 + 0.26X	0.927	< 0.010	3.89	F = 3.01 d.f. 1, 6 P > 0.05
	761	4.72 + 0.43X	0.981	< 0.020	2.34	

TABLE 16.(CHAPTER 6)

MEAN GROWTH RATES OF MACROSCHIZONTS IN FOUR GROUPS OF ANIMALS
INOCULATED WITH DIFFERENT SIZES OF INFECTIVE DOSES OF T. PARVA
(MUGUGA).

GROUP	MEAN SLOPE	MEAN T_{10} VALUE
10^2	0.49	2.03
10^1	0.42	2.37
10^0	0.39	2.55
10^{-1}	0.29	3.39

TABLE 17. (CHAPTER 6)

STATISTICAL COMPARISON OF MEAN GROWTH CURVES OF MACROSCHIZONTS
FROM FOUR GROUPS OF ANIMALS INOCULATED WITH DIFFERENT SIZES OF
INFECTIVE DOSES OF <u>T. PARVA</u> (MUGUGA).

GROUPS	10^2	10^1	10^0	10^{-1}
10^2		t = 1.14 d.f. 8 P >0.05	t = 1.88 d.f. 8 P >0.05	t = 3.17** d.f. 10 P = 0.01
10^1	t = 1.14 d.f. 8 P >0.05		t = 0.57 d.f. 8 P >0.05	t = 1.69 d.f. 10 P >0.05
10^0	t = 1.88 d.f. 8 P >0.05	t = 0.57 d.f. 8 P >0.05		t = 1.44 d.f. 10 P >0.05
10^{-1}	t = 3.17** d.f. 10 P = 0.01	t = 1.69 d.f. 10 P >0.05	t = 1.44 d.f. 10 P >0.05	

** Highly significant difference i.e. not parallel

MACROSCHIZONTS

DAYS POST-INOCULATION
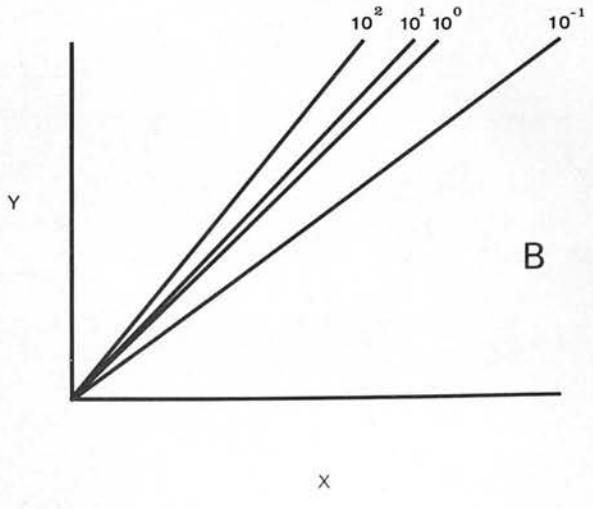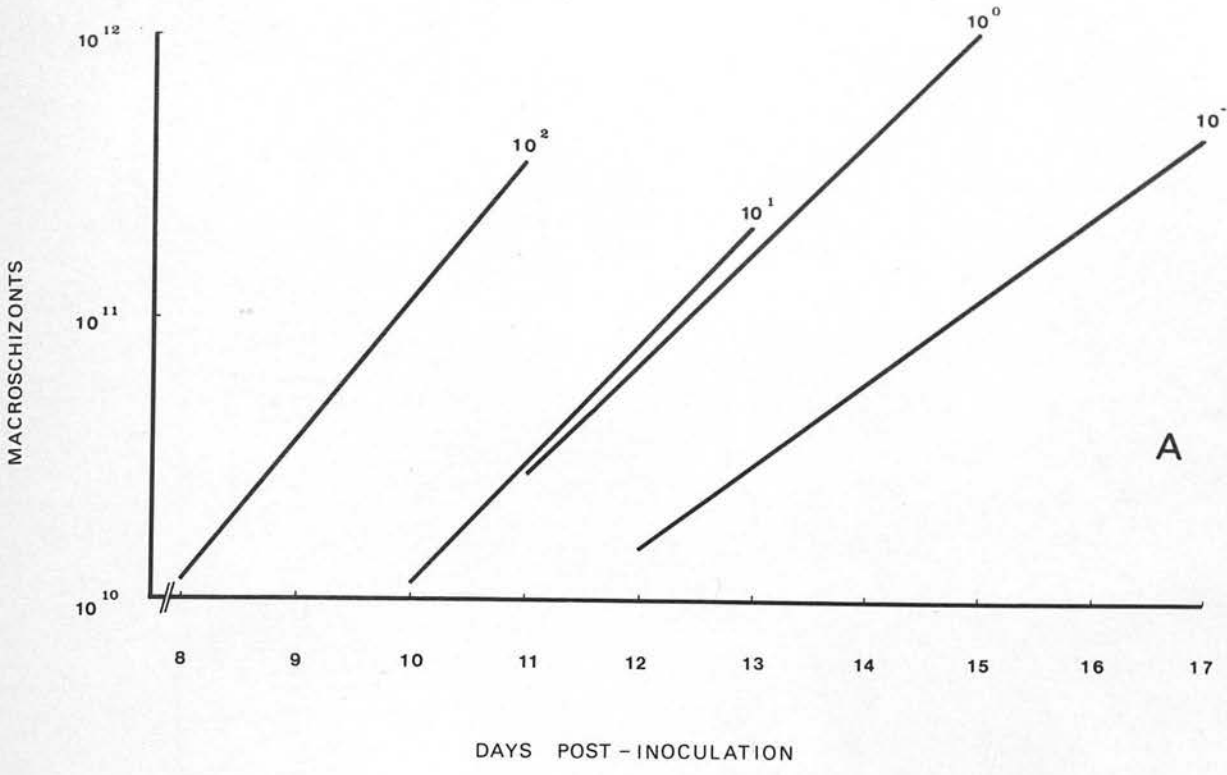
FIGURE 6   (CHAPTER 6)

A.   Mean growth curves of T. parva (Muguga)
      using four infective doses.

B.   Transposition of mean growth curves to
      demonstrate divergence.

FIGURE 7   (CHAPTER 6)

Relationship of infective dose and growth rate
(T_{10}) of _T. parva_ (Muguga)

INFECTIVE DOSE (T_{10} VALUES)

$\hat{Y} = 1.52 + 0.43 X$
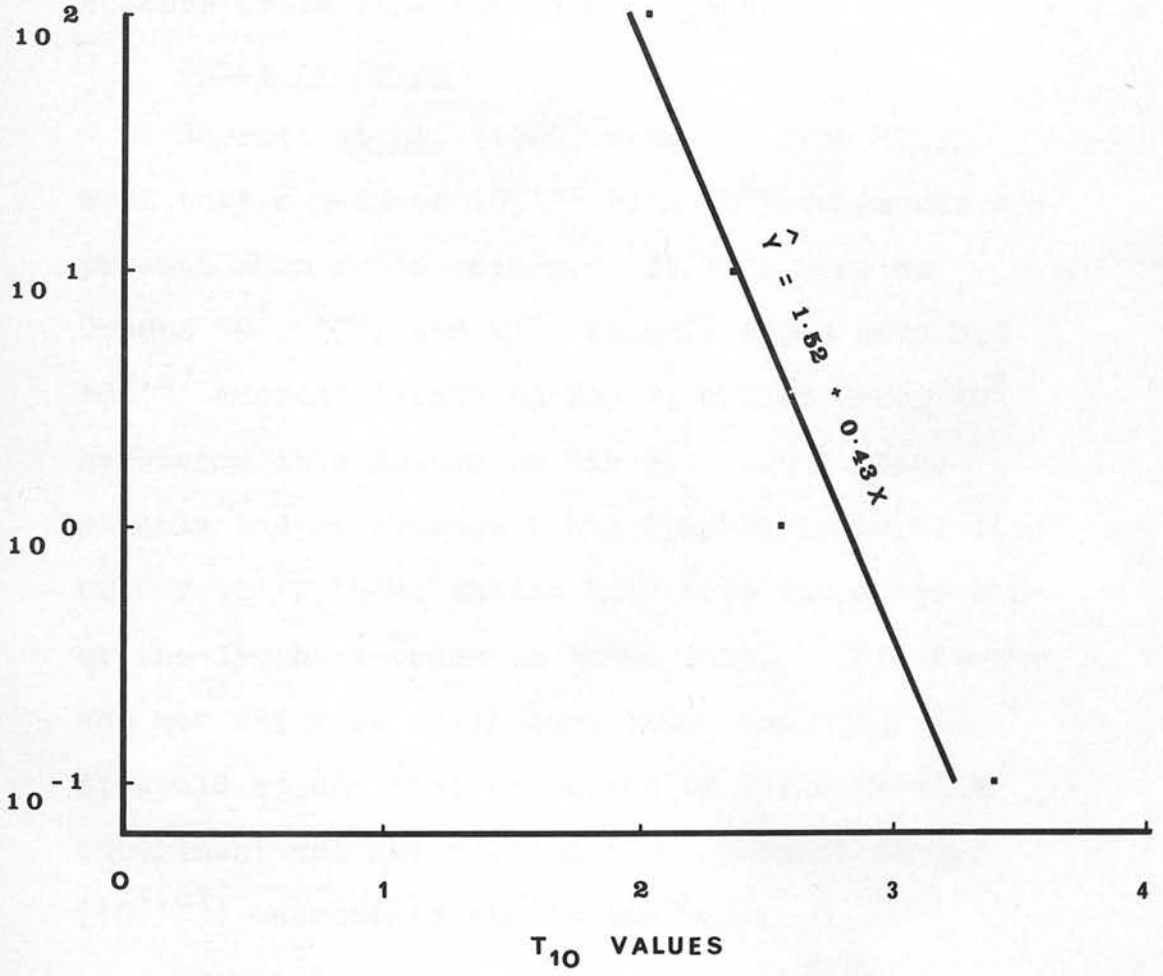
suggests (as was concluded by Jarrett et al.) that
the commencement of piroplasm production is time-
dependent and not dose-dependent.

This fixed period has also been observed by
the author when animals were inoculated with tissue
culture cells infected with T. parva.

Onset of Fever

Jarrett et al. (1969) conclude from their
work that a mean of $10^{9.87}$ (7×10^9) schizonts are
present when fever occurs.   If this were so
Groups 10^1, 10^0, and 10^{-1} animals would have had
$10^{9.87}$ macroschizonts on Day 7, whilst Group 10^2
harboured this number on Day 6.   Since these
animals had an average total lymphocyte population
of 6×10^{11}, there should have been 1% parasitosis
of the lymphoid organ on these days.   This figure
was not attained until much later (see Fig. 6).
It would appear that the onset of fever in this
experiment was not related to a constant number
($10^{9.87}$) macroschizonts in the body.

Survival time

As shown in Figure 5 there is an inverse
relationship between the volume of inoculum and
the survival time.   This finding adds weight to
the hypotheses of Barnett (1957) and Wilde (1967).

Growth curves

As yet there is no means of counting the
infective particles in a suspension and further it
is impossible to know the numbers which become

established in the bovine host.   In an attempt to
calculate the numbers of infective particles
introduced by ticks, Jarrett et al. extrapolated
their growth curves into the occult period and
found that the number of parasites in the body on
Day 4 (day on which 50% of infective particles are
mature) agreed closely with the theoretical number
of infective particles calculated from work done
by Martin et al. (1964) and Purnell and Joyner
(1968).   This calculation, however, assumes 100%
establishment of infective particles.

Assuming their argument to be correct, in the
experiment presented here extrapolation would give
the largest input of infective particles for the
group receiving the smallest inocula.   Conversely
the group receiving the largest inocula would have
received the smallest number of infective particles.

It is obvious then that extrapolation of
regression lines into the unobserved period is
quite erroneous and therefore useless as a method
for counting the number of infective particles in
a given suspension.

It is thought by the author that perhaps the
parasite is undergoing a lag or resting phase
during the prepatent period where no significant
change in numbers is occurring.   This would be a
reasonable assumption since the parasite has just
been introduced to a completely new physiological
medium and a period of adaptation will be

necessary. Once adjusted to its new environs the
parasite will be able to multiply at the rate
observed.

The evidence which is more factual is the
growth curves calculated in the patent period. A
thousand-fold difference in volume of inocula gave
very different multiplication rates of the parasite.
This divergence supports the working hypothesis
given in Chapter 4 and contradicts the findings of
Jarrett et al. (1969). As stated before the
methods used by the author to infect cattle do
differ from those of Jarrett et al.

The application of ticks will result in a
continuous ingress of infective particles reaching
a peak on Day 4 and the resultant cumulative growth
curve from each daily input of infective particles
may be akin to "compound interest". The
indications are that inoculation of infective
particles results in the establishment of the
parasite very quickly (E.A.V.R.O. 1969), and any
not fortunate enough to find a suitable host cell
within a very short time, probably a few hours,
will die. This means that, in the experiments
recorded in this thesis, the growth curves are
observations derived from infective particles
establishing themselves within a few hours of
inoculation and not several days.

An explanation of the divergence of growth

curves may be made as follows:- when a large number
of infective particles becomes established in the
bovine host, the host reaction against the
parasite will have minimal effect on the increase
in macroschizont numbers.   In other words the
host reaction is masked by the surfeit of parasites
and the multiplication rate nears its absolute
potential.   However, with decreasing numbers of
infective particles inoculated the host can assert
itself over the parasite killing relatively more
parasites than with the larger inocula.   This
inhibition of the parasite's increase in numbers
will manifest itself as a slowing down of the
multiplication rate.

  These conclusions at this stage were
regarded as tentative, since only pairs of animals
were studied per group.   If however, the
indications were correct one could hope that
animals given even smaller numbers of infective
particles should be able to overcome the
infection completely.

CHAPTER 7

DEFINITIVE EXPERIMENT OF QUANTITATIVE
STUDIES USING STANDARDIZED METHODS

This experiment was designed to confirm the results

of Chapters 4 and 6.

In this wider ranging experiment the numbers of

cattle in each group were doubled and an additional

group of smaller infective dose incorporated. However

it was felt that inoculations of 0.01 cc. would be

difficult and inaccurate, and dilutions of infective

supernate would be better.

i.   Materials and methods (See Table 18)

Ticks

A total of 9,500 infected adult (male and

female) ticks were pooled from tick batch numbers

E.A.V.R.O. 1344, 1346, 1347 and 1349.   These ticks

were 4 months old since ecdysis and had dropped

engorged as nymphae from cattle which had 9 - 45%

of their erythrocytes infected.

These ticks were fed on 12 rabbits, 400 ticks

per ear, for 3 to 4 days.   The unattached ticks

(2,150) were discarded on the second day, and

7,350 were removed on the fourth day after

application.

Cattle

Twenty yearlings (15 males, 5 females),

arranged in 5 groups of 4 as shown in Table 18.

TABLE 18. (CHAPTER 7)

VOLUME AND ADMINISTRATION OF INFECTIVE SUSPENSIONS
OF T. PARVA (MUGUGA).

GROUP	ANIMAL NUMBER	LIVE WEIGHT IN POUNDS	VOLUME OF INFECTIVE SUSPENSION RECEIVED BY EACH ANIMAL	ORDER OF INOCULATION
10^2	348	407		11
	360	435	100 ccs.	20
	365	400		12
	368	505		15
10^1	349	457		9
	351	430	10 ccs.	2
	352	400		6
	376	477		1
10^0	347	476		8
	366	411	1 cc.	7
	377	448		14
	379	446		16
10^{-1}	354	409		10
	358	445	0.1 cc. [+]	4
	372	470		3
	374	410		19
10^{-2}	346	459		5
	361	371	0.01 cc. [++]	13
	364	445		18
	370	463		17

+ 1 cc. of 1/10 dilution of infective supernate.
++ 1 cc. of 1/100 dilution of infective supernate.

Preparation of infective inoculum

The ticks were cleaned, and then triturated in 450 ccs. Eagle's MEM/BPA 3.5% for 50 minutes. The mixture was allowed to sediment for 45 minutes and 340 ccs. of supernate removed by pipetting. This volume was made up to 460 ccs. by the addition of 120 ccs. Eagle's MEM/BPA 3.5%.

After thorough mixing 1 cc. of supernate was pipetted into 9 ccs. Eagle's MEM/BPA 3.5%, and 1 cc. of this dilution pipetted after mixing into 9 ccs. Eagle's MEM/BPA. 1 cc. of these two dilutions, 1/10 and 1/100 respectively, was the inoculum used for Groups 10^{-1} and 10^{-2}. The remainder of the undiluted supernate was inoculated into the experimental cattle as shown in Table 18.

All injections were made subcutaneously at the point of the left shoulder, the procedure taking forty minutes.

Observational procedure

Prepatent period

Twice daily examination (before 8.30 a.m. and 4 p.m.) of the L.D.L.Ns. (left prescapular nodes) were made until macroschizonts were detected in the biopsy smears, then once daily samplings thereafter.

MSI estimations

Biopsies were taken daily from the right prescapular lymph nodes commencing the day after macroschizonts were detected in the L.D.L.N., and

MSIs estimated.

Blood smears

These were taken daily from Day 10 onwards.

Temperature

Daily rectal temperatures were recorded.

ii. Results (See Tables 19-27, Appendix Tables 12-16, and Figures 8-12)

Prepatent period (See Table 19)

The prepatent periods varied for individual animals ranging from 5 days to 13 days. Macroschizonts were very easily detected (often 2 or 3 per field) in animal 368, Group 10^2. The arithmetic means for each group varied from 5 days (Group 10^2) to 9.75 days (Group 10^{-2}). The relationship of the length of prepatent period and infective dose is shown in Figure 8.

Onset of fever (See Table 19)

The first day that rectal temperatures were elevated over $103^{\circ}F$ varied from Day 6 (348 Group 10^2) to Day 16 (346 Group 10^{-2}). The mean number of days to temperature varied from 6.75 to 12.50 days post-inoculation. The relationship between time to first elevation of temperature and infecting dose is seen in Fig. 2.

Intraerythrocytic piroplasms (See Table 19)

On examination of the Day 10 and 11 blood smears a few large piroplasms were seen in two animals (352 and 377). On the next day (Day 12) very small comma-shaped piroplasms and large

piroplasms were seen in these two animals and a
third animal (376) showed only the former type.
Every other animal revealed small piroplasms on
Day 13.

Survival time (See Table 19)

The time in days post-inoculation to death
varied from 13 (animals 360 and 365 in Group 10^2)
to 24 (animal 364 in Group 10^{-2}).   Two calves in
Group 10^{-2} (346 and 370) recovered from E.C.F. the
last macroschizonts being seen on Day 20 in 346 and
Day 21 in 370.

The arithmetic mean of survival times varied
from 14.25 days (Group 10^2) to 21.50 days (two
fatal cases in Group 10^{-2}).   The relationship
between survival time and infecting dose is seen
in Figure 10.

Growth curves

Analysis of linear regression (Tables 20-25)
showed significant correlation coefficients for 19
out of 20 individual growth curves.   The one
exception (animal 358) had only three significant
MSI estimations and although the correlation
coefficient was not significant at p 0.05 it was
significant at p 0.10.

The variance ratio tests showed that the
individual growth curves were parallel within all
groups (Table 25).

The individual T_{10} values varied from 2.04
(animal 365 Group 10^2) to 4.04 (animal 346 Group 10^{-2}).

TABLE 19. (CHAPTER 7)

PREPATENT PERIOD, ONSET OF FEVER AND PARASITAEMIA, AND SURVIVAL
TIME OF FIVE GROUPS OF ANIMALS INOCULATED WITH DIFFERENT SIZES
OF INFECTIVE DOSES OF T. PARVA (MUGUGA).

GROUP	ANIMAL NUMBER	TIME IN DAYS TO			
		MACROSCHIZONTS IN LOCAL DRAINAGE LYMPH NODE	TEMPERATURE OVER 103°F	INTRA-ERYTHROCYTIC PIROPLASMS	DEATH
10^2	348	5	6	13	15
	360	5	7	13	13
	365	5	7	13	13
	368	5	7	13	16
10^1	349	6	8	13	14
	351	6	7	13	18
	352	6	9	12^+	14
	376	5	10	12	13
10^0	347	6	11	13	14
	366	6	9	13	15
	377	6	7	12^+	18
	379	7	9	13	16
10^{-1}	354	7	9	13	20
	358	8	13	13	17
	372	7	9	13	22
	374	7	9	13	15
10^{-2}	346	13	16	13	-
	361	9	13	14	19
	364	8	11	14	24
	370	9	10	13	-

+ Large piroplasms (T.mutans type?) seen on two previous days
but day 12 was first recording of T.parva type piroplasms.

FIGURE 8   (CHAPTER 7)

Relationship of infective dose and prepatent period

of T. parva (Muguga)

INFECTIVE DOSE

$Y = 3.50 + 1.10X$

DAYS   POST - INOCULATION
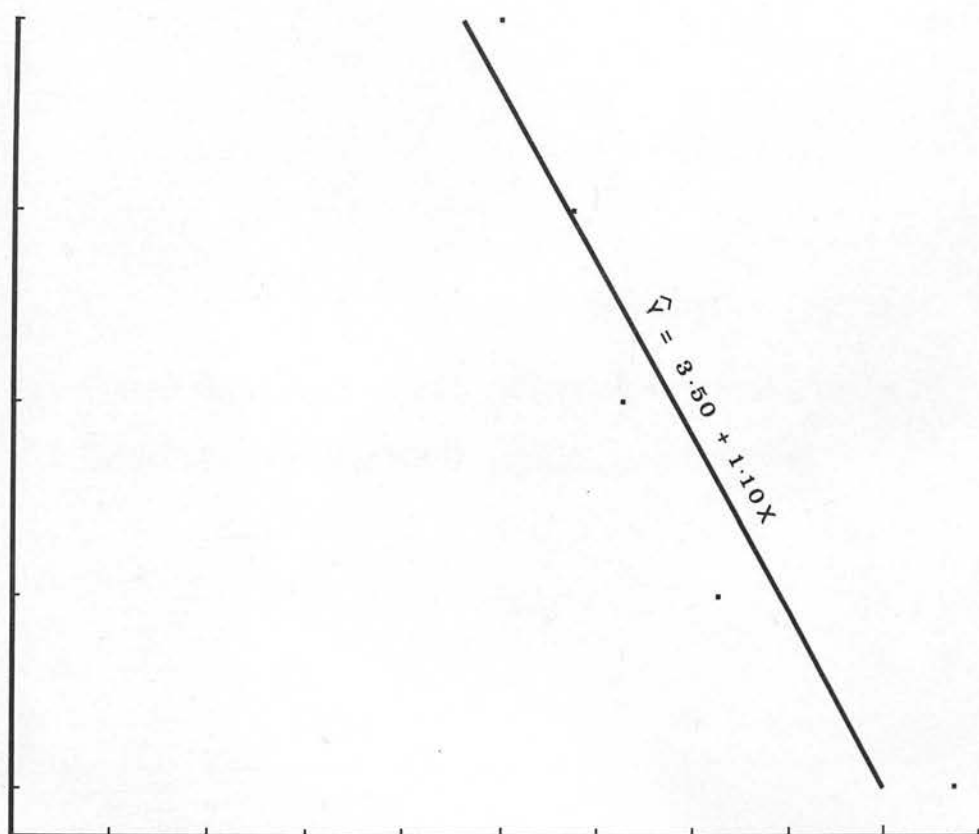
FIGURE 9 (CHAPTER 7)

Relationship of infective dose and onset of fever in T. parva (Muguga) infections.

$Y = 5.45 + 1.30 X$

DAYS POST -INOCULATION
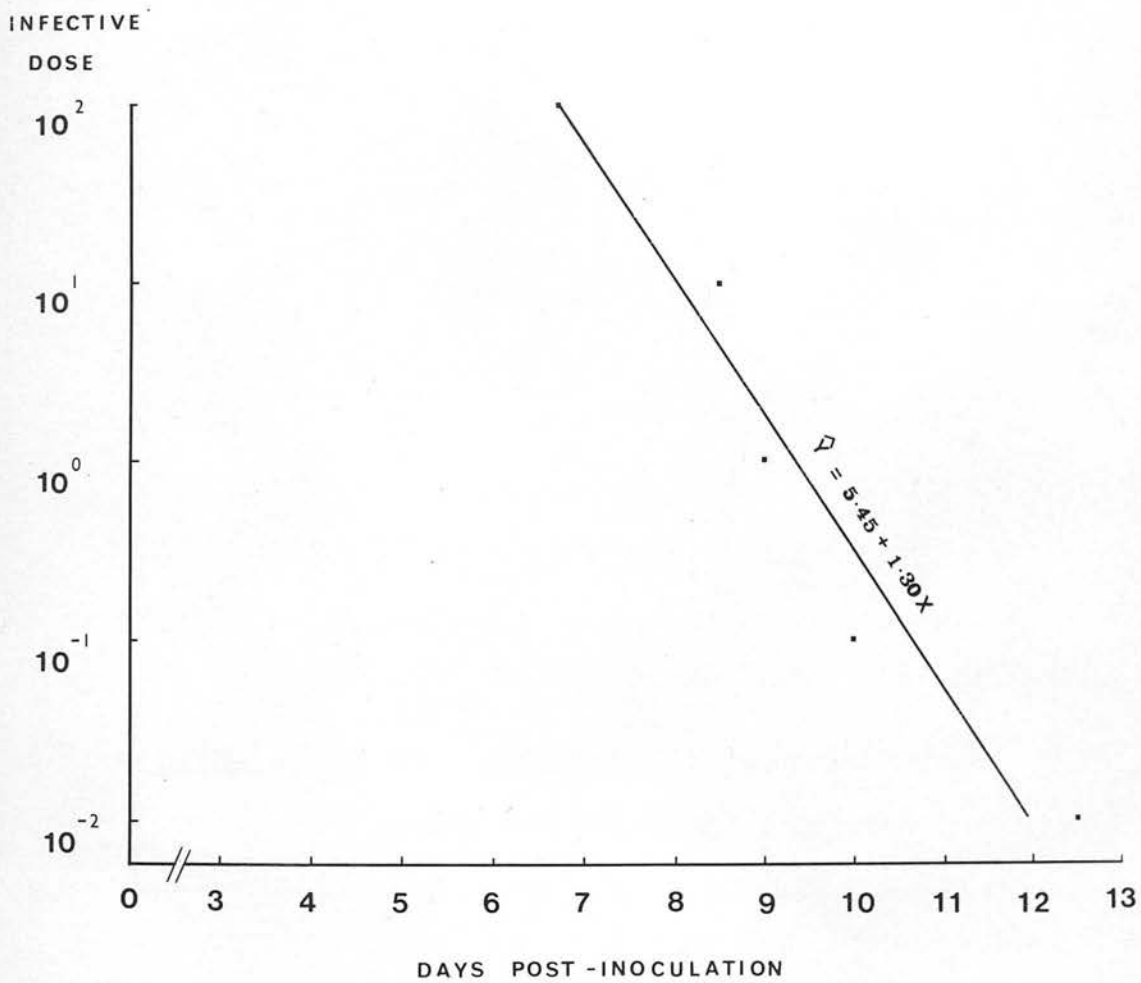
FIGURE 10   (CHAPTER 7)

Relationship of infective dose of T. parva
(Muguga) and survival time.

INFECTIVE DOSE

$\hat{Y} = 11.48 + 1.83 X$
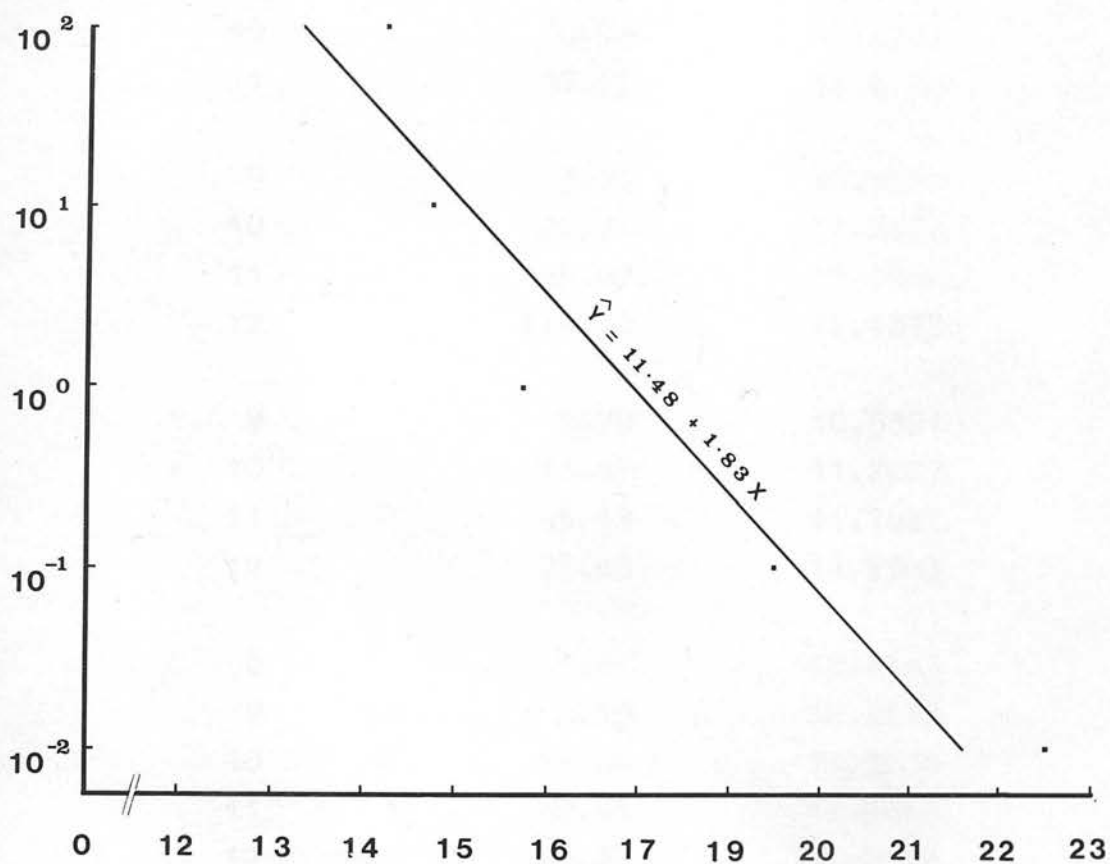
DAYS POST INOCULATION

TABLE 20. (CHAPTER 7)

DAILY MACROSCHIZONT INDICES DATA TABULATED FOR LINEAR REGRESSION

GROUP 10^2

ANIMAL	DAY (X)	MEAN MSI	LOG TOTAL MACROSCHIZONTS (Y)
	9	4.29	10.7332
348	10	14.25	11.2553
	11	37.75	11.6776
	9	3.98	10.7300
	10	20.71	11.4472
360	11	45.47	11.7882
	12	114.00	12.1875
	9	2.79	10.5391
	10	13.47	11.2227
365	11	45.13	11.7482
	12	79.63	11.9943
	8	1.67	10.4183
	9	4.19	10.8182
368	10	15.44	11.3838
	11	40.94	11.8082
	12	82.88	12.1139

TABLE 21. (CHAPTER 7)

DAILY MACROSCHIZONT INDICES DATA TABULATED FOR LINEAR REGRESSION GROUP 10[1]

ANIMAL	DAY (X)	MEAN MSI	LOG TOTAL MACROSCHIZONTS (Y)
	9	1.37	10.2900
	10	8.05	11.0569
349	11	20.13	11.4564
	12	38.60	11.7388
	13	59.50	11.9269
	14	104.00	12.1703
	9	1.48	10.2945
	10	7.08	10.9741
351	11	21.56	11.4579
	12	34.90	11.6665
	13	51.94	11.8395
	9	1.09	10.1303
	10	6.56	10.9101
352	11	20.03	11.3945
	12	43.38	11.7324
	13	73.63	11.9605
	10	2.54	10.5752
	11	11.23	11.2201
376	12	30.50	11.6542
	13	48.69	11.8579

TABLE 22. (CHAPTER 7)

DAILY MACROSCHIZONT INDICES DATA TABULATED FOR LINEAR REGRESSION

GROUP 10°

ANIMAL	DAY (X)	MEAN MSI	LOG TOTAL MACROSCHIZONTS (Y)
	11	4.24	10.7980
347	12	19.42	11.4579
	13	35.20	11.7168
	14	58.13	11.9345
	11	3.08	10.5922
	12	10.02	11.1038
366	13	30.00	11.5809
	14	48.69	11.7910
	15	69.00	11.9425
	10	1.32	10.2625
	11	6.98	10.9868
377	12	19.47	11.4330
	13	36.75	11.7084
	14	60.20	11.9227
	12	2.77	10.5821
	13	11.48	11.1987
379	14	31.50	11.6385
	15	59.00	11.9106

TABLE **23.** (CHAPTER 7)

DAILY MACROSCHIZONT INDICES DATA TABULATED FOR LINEAR REGRESSION
GROUP 10^{-1}

ANIMAL	DAY (X)	MEAN MSI	LOG TOTAL MACROSCHIZONTS (Y)
	11	2.52	10.5051
	12	7.82	10.9969
354	13	14.19	11.2553
	14	35.25	11.6513
	15	64.88	11.9159
	16	101.00	12.1072
	15	1.04	10.1584
358	16	1.90	10.4182
	17	4.67	10.8089
	11	1.03	10.1761
	12	7.03	11.0128
	13	14.94	11.3385
372	14	31.70	11.6656
	15	68.50	12.0000
	16	106.50	12.1903
	11	1.76	10.3502
374	12	9.66	11.0899
	13	20.56	11.4166
	14	30.50	11.5877

TABLE 24 (CHAPTER 7)

DAILY MACROSCHIZONT INDICES DATA TABULATED FOR LINEAR REGRESSION GROUP 10^{-2}

ANIMAL	DAY (X)	MEAN MSI	LOG TOTAL MACROSCHIZONTS (Y)
346	18	1.22	10.2380
	19	2.24	10.5024
	20	3.81	10.7332
361	14	1.39	10.2041
	15	3.16	10.5599
	16	3.82	10.6425
	17	7.24	10.9206
	18	35.10	11.6064
364	13	1.73	10.3784
	14	3.39	10.6702
	15	17.75	11.3892
	16	43.50	11.7782
	17	68.13	11.9731

TABLE 25. (CHAPTER 7)

RESULTS OF LINEAR REGRESSION AND ANALYSIS OF VARIANCE OF THE DAILY LOGARITHMIC INCREASES OF
MACROSCHIZONTS IN FIVE GROUPS OF ANIMALS INOCULATED WITH DIFFERENT SIZES OF INFECTIVE DOSES
OF T. PARVA (MUGUGA)

GROUP	ANIMAL	LINE OF BEST FIT $Y = a + b X$	r		P	T_{10} VALUE	PARALLELISM
10^2	348	$6.50 + 0.47X$	0.998	<	0.050	2.12	
	360	$6.59 + 0.47X$	0.985	<	0.020	2.12	$F = 0.25$
	365	$6.24 + 0.49X$	0.980	<	0.020	2.04	d.f. 3,8 $P > 0.05$
	368	$6.93 + 0.44X$	0.995	<	0.001	2.28	
10^1	349	$7.40 + 0.35X$	0.963	<	0.010	2.85	
	351	$7.09 + 0.38X$	0.960	<	0.010	2.64	$F = 0.61$
	352	$6.29 + 0.45X$	0.973	<	0.010	2.23	d.f. 3,12 $P > 0.05$
	376	$6.40 + 0.43X$	0.974	<	0.050	2.34	
10^0	347	$6.89 + 0.37X$	0.961	<	0.050	2.73	
	366	$7.00 + 0.34X$	0.970	<	0.010	2.95	$F = 0.54$
	377	$6.41 + 0.40X$	0.969	<	0.010	2.47	d.f. 3,10 $P > 0.05$
	379	$5.36 + 0.44X$	0.985	<	0.020	2.26	
10^{-1}	354	$7.10 + 0.32X$	0.991	<	0.001	3.14	
	358	$5.26 + 0.33X$	0.993	>	0.050	3.08	$F = 0.47$
	372	$6.24 + 0.38X$	0.971	<	0.010	2.62	d.f. 3,10 $P > 0.05$
	374	$6.06 + 0.40X$	0.952	<	0.050	2.48	
10^{-2}	346	$5.79 + 0.25X$	0.999	<	0.050	4.04	$F = 1.90$
	361	$5.72 + 0.32X$	0.954	<	0.020	3.16	d.f. 2, 7 $P > 0.05$
	364	$4.79 + 0.43X$	0.982	<	0.010	2.33	

Unfortunately the growth curve for animal 370 in
Group 10^{-2} could not be plotted since measurement
of the MSI was only significant on one day.

Results of tests comparing the mean slope of
the growth curves are seen in Table 26 showing
significant divergence between Groups 10^2 and 10^{-1},
10^2 and 10^{-2}. Figures 10A and B demonstrate this
non-parallelism. There is also non-parallelism
of 10^2 and 10^1, 10^2 and 10^0 at $p < 0.10$ level of
significance.

The arithmetic means of the growth curves for
these groups is at variance with the statistical
means (see Table 27). The relationship between
size of infective dose and growth rate (T_{10}) is
shown in Figure 12.

iii. Conclusion
    Prepatent period

As in Chapters 4 and 6 the shortest prepatent
period was five days. As can be seen in Figure 8
there was a linear relationship between the size of
the infective dose and the prepatent period; the
larger the infective dose the shorter was the
prepatent period. This statistical relationship
was not significant in Chapters 4 and 6 probably
because fewer animals and fewer Groups were used
thus limiting the number of observations (and
therefore the degrees of freedom for the linear
regression).

TABLE 26. (CHAPTER 7)

STATISTICAL COMPARISON OF MEAN GROWTH CURVES OF MACROSCHIZONTS
FROM FIVE GROUPS OF ANIMALS INOCULATED WITH DIFFERENT SIZES OF
INFECTIVE DOSES OF <u>T. PARVA</u> (MUGUGA).

GROUPS	10^2	10^1	10^0	10^{-1}	10^{-2}
10^2		t = 1.83 d.f. 20 P > 0.05	t = 2.01 d.f. 18 P > 0.05	t = 2.80* d.f. 16 P < 0.02	t = 2.55* d.f. 15 P < 0.05
10^1	t = 1.83 d.f. 20 P > 0.05		t = 0.16 d.f. 22 P > 0.05	t = 0.82 d.f. 20 P > 0.05	t = 0.62 d.f. 19 P > 0.05
10^0	t = 2.01 d.f. 18 P > 0.05	t = 0.16 d.f. 22 P > 0.05		t = 0.66 d.f. 18 P > 0.05	t = 0.62 d.f. 17 P > 0.05
10^{-1}	t = 2.80* d.f. 16 P < 0.020	t = 0.82 d.f. 20 P > 0.05	t = 0.66 d.f. 18 P > 0.05		t = 0.14 d.f. 15 P > 0.05
10^{-2}	t = 2.55* d.f. 15 P < 0.05	t = 0.62 d.f. 19 P > 0.05	t = 0.62 d.f. 17 P > 0.05	t = 0.14 d.f. 15 P > 0.05	

* Significant difference i.e. not parallel.

FIGURE 11   (CHAPTER 7)

A.   Mean growth curves of T. parva (Muguga)
     using five infective doses.

B.   Transposition of mean growth curves to
     demonstrate divergence.

MACROSCHIZONTS

DAYS   POST - INOCULATION

$a = 10^{-2}$ $b = 10^{1}$ $c = 10^{0}$ $d = 10^{-2}$ $e = 10^{-1}$
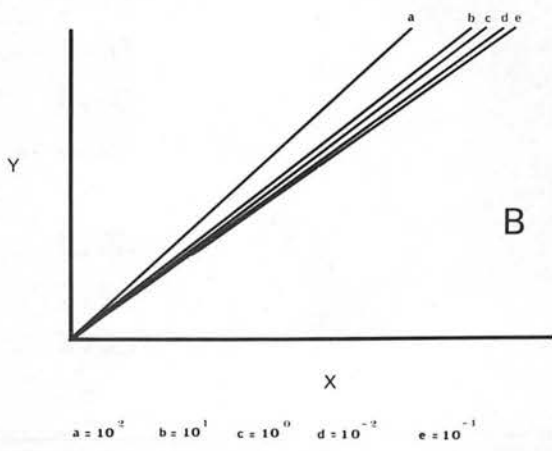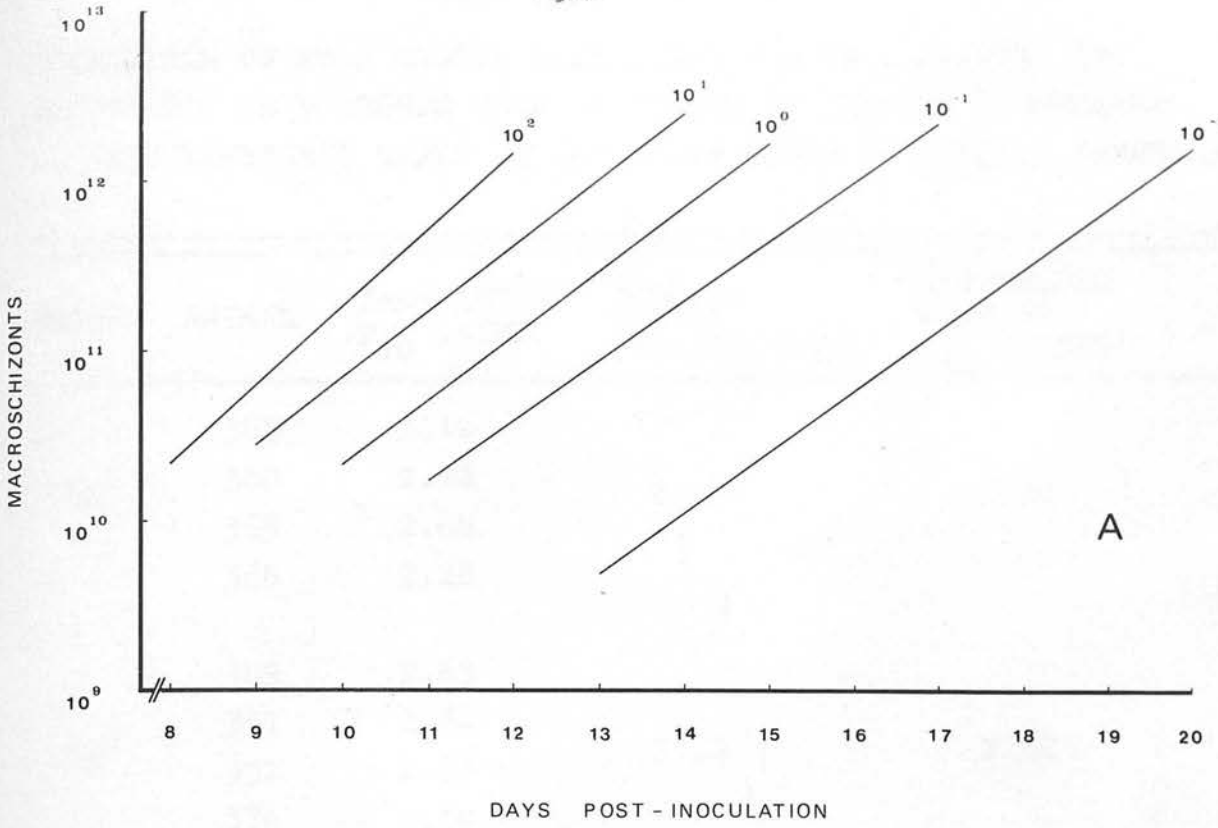
TABLE 27. (CHAPTER 7)

COMPARISON OF MEAN GROWTH RATE (ANALYSIS OF VARIANCE) AND
ARITHMETIC MEAN GROWTH RATE OF GROUPS OF ANIMALS INOCULATED
WITH DIFFERENT SIZES OF INFECTIVE DOSES OF T.PARVA (MUGUGA).

GROUP	ANIMAL	INDIVIDUAL T_{10} VALUE	MEAN T_{10} VALUES (STATISTICAL)	ARITHMETIC MEAN OF T_{10} VALUES
10^2	348	2.12		
	360	2.12	2.17	2.14
	365	2.04		
	368	2.28		
10^1	349	2.85		
	351	2.64	2.57	2.52
	352	2.23		
	376	2.34		
10^0	347	2.73		
	366	2.95	2.61	2.60
	377	2.47		
	379	2.30		
10^{-1}	354	3.13		
	358	3.07	2.81	2.83
	372	2.62		
	374	2.48		
10^{-2}	346	4.04		
	361	3.16	2.76	3.18
	364	2.33		
	370	-		

FIGURE 12   (CHAPTER 7)

Relationship of infective dose and growth rate

(T_{10}) of <u>T. parva</u> (Muguga).

INFECTIVE
DOSE

$\hat{Y} = 1.94 + 0.24X$

T_{10} VALUES
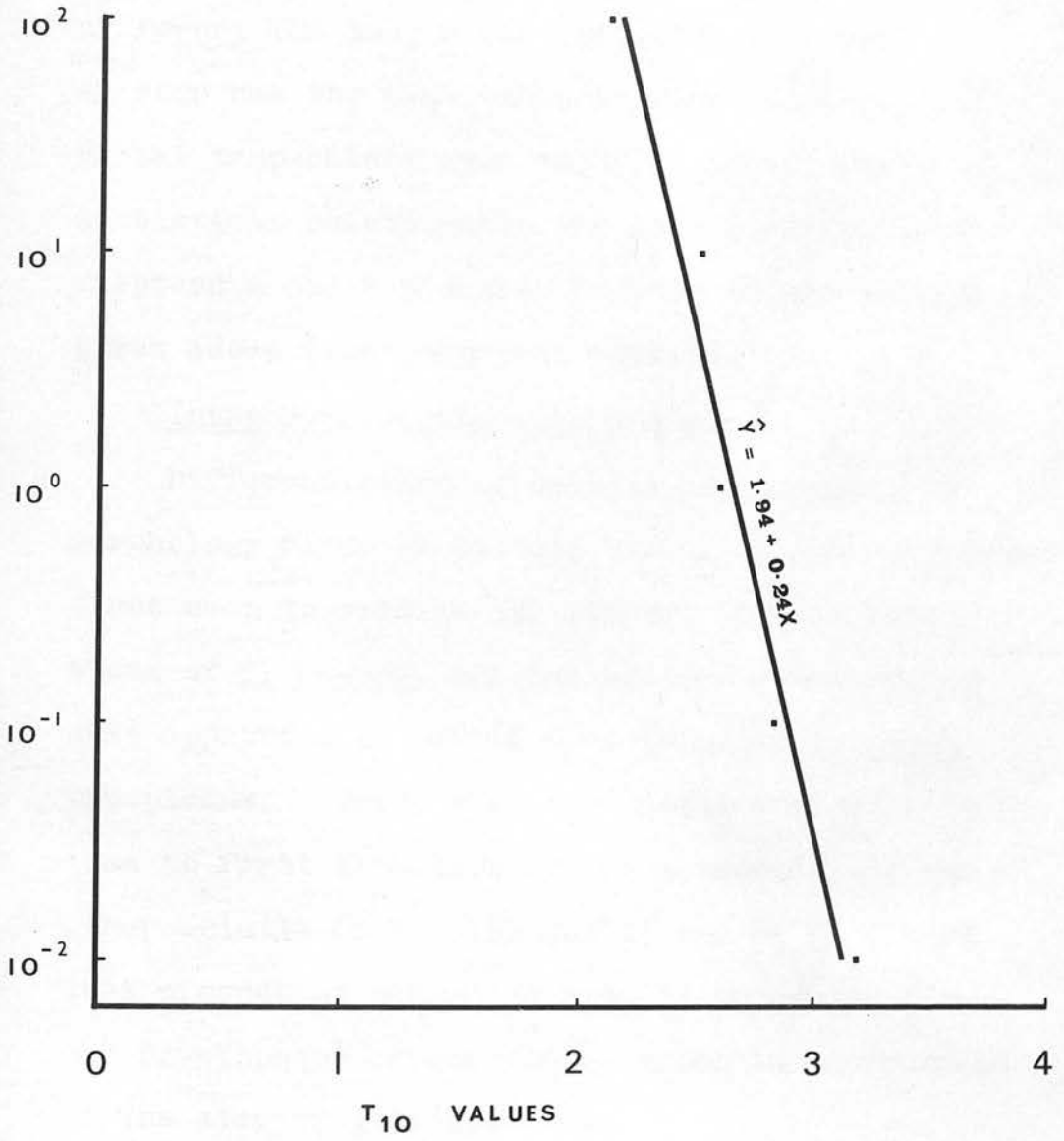
Onset of fever

The time taken for onset of fever varied in this experiment more than it did in Chapters 4 and 6. Figure 9 demonstrates the linear correlation between the size of infective dose and the onset of fever; the larger the infective dose the shorter was the time taken to first elevation of rectal temperature over 103°F. Again, the statistical relationship was not significant in Chapters 4 and 6 probably because of the reasons given above (see prepatent period).

Intraerythrocytic piroplasms

Differentiation of species of Theileria on morphology alone is unsound but the large piroplasms first seen in animals 352 and 377 were probably those of T. mutans, and the smaller comma-shaped ones appearing on Day 12 were probably T. parva piroplasms. There was very little variation in time to first detection of piroplasms in all the other animals (Table 19) and it may be concluded that a constant period of time is necessary for the development of piroplasms which is independent of the size of infective dose.

Survival time

The survival time increased with decreasing size of infective dose in this experiment, confirming the observations made in Chapters 4 and 6. It would appear that Group 10^{-2} animals were given an

infective dose which was small enough for some
animals (2/4) to overcome the parasite but the
other animals succumbed fatally.

Growth curves

The divergence of the mean slopes of the
growth curves depending on the size of infective
dose was demonstrated again, thus confirming
earlier experimentation in this thesis.   The
mean growth curve for Group 10^{-2}, however, was
not truly representational since one individual
growth curve (animal 370) could not be evaluated.
If the MSI had been over 1 for one more day in this
animal, the T_{10} would have been large thus increasing
the divergence more than is demonstrated in Figure
11.   Furthermore the statistical calculation of
the mean slope for Group 10^{-2} places more weight on
the slopes for animals 364 and 361 than it does for
animal 346 because in the latter calf only three
observations were possible.   The calculation for
the arithmetic mean, however, gives equal weight to
each animal's growth curve and this probably reflects
the situation better.   The linear correlation
between size of infective dose and the arithmetic
mean growth rate (T_{10}) as shown in Figure 12
validates the working hypothesis put forward in
Chapter 4.

CHAPTER 8

EXPERIMENT TO SHOW ONE APPLICATION OF
QUANTITATIVE STUDIES

This experiment was one of a series of chemoprophylactic experiments carried out by the F.A.O. Project and the series, not yet complete, will be the subject of a future publication. The author shared the work-load equally with his colleagues, and in addition personally collected and examined material for the quantitative studies.

The materials and methods of the experiment will be given and a summary of the results. However, only the macroschizont indices and the resultant growth curves will be given in detail. The discussion will not impinge on the chemoprophylactic series but will deal with the application and limitations of the techniques developed in the thesis.

The experiment was designed to consolidate the results obtained earlier in the chemoprophylactic series which indicated that cattle could be immunized by infecting with a limited dose of stabilate material followed by a short regimen of tetracycline therapy early in the prepatent period. The treatment of patent infections was also to be tried.

    i.   <u>Materials and methods</u> (See Table 28).

        <u>Cattle</u>

        Twenty-five Ayrshire steers, 318 - 424 lbs. (144 - 193 kg) live body weight, were divided into five groups of five animals each. Four groups

TABLE 28. (CHAPTER 8)

TETRACYCLINE REGIMENS USED FOR GROUPS OF ANIMALS INOCULATED WITH
EQUAL VOLUMES OF A SUSPENSION OF T. PARVA (MUGUGA).

GROUP	ANIMAL NUMBER	LIVE BODY WEIGHT IN LBS. (Kg.)	DOSE TETRACYCLINE IN CCS.	ORDER OF INOCULATION WITH STABILATE
1 (Day 0) group	465	346 (157)	15.8	10
	481	369 (168)	16.8	6
	484	407 (185)	18.6	8
	490	402 (183)	18.4	7
	493	420 (191)	19.2	9
2 (Days 0,1) group	462	335 (152)	15.2	12
	472	343 (156)	15.6	15
	475	367 (167)	16.8	11
	483	369 (168)	16.8	13
	486	361 (164)	16.4	14
3 (Days 0, 1,2) group	471	356 (162)	16.2	18
	474	376 (171)	17.2	20
	480	410 (186)	18.6	16
	491	397 (180)	18.0	17
	492	417 (189)	19.0	19
4 (Treated 2x daily in patent period)	467	324 (147)	14.8	24
	473	318 (144)	14.4	1
	476	388 (176)	17.6	21
	485	388 (176)	17.6	23
	494	338 (154)	15.4	3
5 (Untreated controls)	466	322 (146)	14.6	22
	468	417 (189)	19.0	25
	469	348 (158)	15.8	4
	477	424 (193)	19.4	5
	482	336 (153)	15.4	2

were to be treated, and the fifth group acted as
untreated controls.

Drug regimen

Five mg/Kg body weight of pyrrolidinomethyl
tetracycline* was administered intramuscularly.

a. __Prophylactic__ Single daily doses,
commencing approximately 20 minutes after
the infective inoculum was administered.

Group 1 received one dose only - Day 0;

Group 2 received two doses - Day 0, Day 1

Group 3 received three doses - Day 0, Day 1,
Day 2.

b. __Therapeutic__ Group 4 - five cattle were
treated twice daily (2 x 5 mg/Kg) for five
days starting from the day when macroschizonts
were detectable and there was a concomitant
elevation of rectal temperature over 103°F.
This group of cattle was selected randomly as
clinical disease developed from the ten
animals not treated prophylactically. The
control group, Group 5, comprised the
remainder.

Preparation of inoculum

The contents of two tubes of stabilate 10
(5.6 cc. frozen infective supernate) were rapidly
thawed, pooled, and diluted to 1/10 by the
addition of 50.4 ccs. of foetal calf serum
containing 7.5% glycerol (Cunningham et al. 1970b).
After allowing twenty minutes to elapse for

* Reverin, Hoechst (5 mg/cc).

equilibration of the glycerol, the inoculations were carried out in the following manner. Cattle of Groups 1, 2, 3, 4 and 5 were infected by inoculation of 1 cc. of 1/10 dilution of stabilate. All inoculations were made subcutaneously in front of the right ear (the right parotid being the local drainage lymph node).

Observational procedure

Daily biopsy smears were made and examined from the right parotid lymph nodes (L.D.L.N.s) starting on Day 1 to establish the prepatent periods and from the left prescapular lymph nodes for MSI estimations starting the day after macroschizonts were detected in the L.D.L.N.

Daily rectal temperatures were recorded.

ii. Results

The results are shown in Tables 29-36, Appendix Tables 17-20 and Figure 13.

Group 2 animals don't feature in the analysis tables since the MSI estimations never reached significant levels. This also applies to animals 484 (Group 1), and 471, 474, 491, 492 (Group 3). Another animal (469 Group 5) was excluded from linear regression since the peak mean MSI of 2.57 occurred only one day after the first significant MSI estimation.

Animal 477 showed a correlation coefficient 0.936 (P > 0.050) was therefore excluded from the analysis of variance for Group 5.

TABLE 29. (CHAPTER 8)

PREPATENT PERIOD, ONSET OF FEVER, AND TIME TO DEATH/RECOVERY OF ANIMALS INFECTED WITH <u>T. PARVA</u> (MUGUGA) UNDER DIFFERENT REGIMENS OF TETRACYCLINE.

GROUP	ANIMAL NUMBER	MACROSCHIZONTS IN LOCAL DRAINAGE LYMPH NODE	TIME IN DAYS TO		
			TEMPERATURE OVER $103°F$	DEATH	RECOVERY
1 (Day 0) group	465	8	13	25	-
	481	11	14	22	-
	484	8	13	-	19
	490	11	14	19	-
	493	9	11	21	-
2 (Days 0,1) group	462	11	15	-	18
	472	11	13	-	17
	475	12	12	-	19
	483	13	-	-	17
	486	12	-	-	17
3 (Days 0, 1,2 group)	471	11	-	-	14
	474	11	14	-	17
	480	11	16	27	-
	491	12	13	-	16
	492	13	-	-	15
4 (Treated 2x daily in patent period	467	9	11	-	20
	473	8	12	24	-
	476	9	11	18	-
	485	8	12	21	-
	494	9	13	15	-
5 (Untreated controls)	466	9	13	17	-
	468	10	12	20	-
	469	9	14	-	25
	477	10	12	-	26
	482	8	11	21	-

TABLE 30. (CHAPTER 8)

DAILY MACROSCHIZONT INDICES DATA TABULATED FOR LINEAR REGRESSION

GROUP 1

ANIMAL	DAY (X)	MEAN MSI	LOG TOTAL MACROSCHIZONTS (Y)
	13	1.22	10.1173
	14	4.07	10.6385
465	15	8.82	10.9750
	16	15.82	11.2279
	17	21.54	11.3617
	12	2.03	10.3636
	13	5.83	10.8228
	14	10.84	11.0934
481	15	27.13	11.4900
	16	44.13	11.7016
	17	79.50	11.9571
	14	1.99	10.3962
	15	10.24	11.1072
490	16	23.75	11.4728
	17	47.56	11.7745
	18	87.88	12.0414
	12	2.61	10.5302
	13	6.79	10.9460
	14	13.34	11.2380
493	15	26.46	11.5366
	16	40.50	11.7218
	17	56.50	11.8663
	18	88.13	12.0607

TABLE 31. (CHAPTER 8)

DAILY MACROSCHIZONT INDICES DATA TABULATED FOR LINEAR REGRESSION
GROUP 3

ANIMAL	DAY (X)	MEAN MSI	LOG TOTAL MACROSCHIZONTS (Y)
	16	5.98	10.8802
	17	10.38	11.1206
	18	22.08	11.4472
	19	36.20	11.6628
480	20	41.31	11.7202
	21	47.88	11.7839
	22	63.50	11.9063
	23	82.88	12.0212
	24	101.38	12.1106

TABLE 32. (CHAPTER 8)

DAILY MACROSCHIZONT INDICES DATA TABULATED FOR LINEAR REGRESSION
GROUP 4

ANIMAL	DAY (X)	MEAN MSI	LOG TOTAL MACROSCHIZONTS (Y)
	13	3.29	10.5105
	14	17.29	11.2304
473	15	17.92	11.2480
	16	62.88	11.7924
	17	91.63	11.9557
	12	1.86	10.3483
	13	10.75	11.1106
476	14	41.75	11.6998
	15	61.00	11.8645
	12	1.42	10.2304
	13	6.62	10.8998
485	14	15.03	11.2553
	15	33.76	11.6075
	12	1.01	10.0253
494	13	3.01	10.4997
	14	10.45	11.0414

TABLE 33. (CHAPTER 8)

DAILY MACROSCHIZONT INDICES DATA TABULATED FOR LINEAR REGRESSION
GROUP 5

ANIMAL	DAY (X)	MEAN MSI	LOG TOTAL MACROSCHIZONTS (Y)
466	13	1.13	10.0531
	14	3.48	10.5403
	15	10.88	11.0374
	16	24.50	11.3892
468	14	3.56	10.6618
	15	20.59	11.4249
	16	44.06	11.7543
	17	97.75	12.1004
477	15	2.93	10.5843
	16	5.14	10.8280
	17	5.47	10.8555
	18	16.83	11.3424
482	13	1.12	10.0645
	14	4.42	10.6628
	15	9.63	11.0000
	16	15.58	11.2095

TABLE 34. (CHAPTER 8)

RESULTS OF LINEAR REGRESSION AND ANALYSIS OF VARIANCE OF THE DAILY LOGARITHMIC INCREASE OF MACROSCHIZONTS OF GROUPS OF ANIMALS INFECTED WITH $\underline{T.PARVA}$ (MUGUGA) UNDER DIFFERENT REGIMENS OF TETRACYCLINE

GROUP	ANIMAL	LINE OF BEST FIT Y = a + bX	r	P	T_{10} VALUE	PARALLELISM
1	465	6.25 + 0.31X	0.973	< 0.010	3.25	
	481	6.68 + 0.31X	0.993	< 0.001	3.18	F = 4.18 d.f. 3,15 P<0.05
	490	5.03 + 0.40X	0.977	< 0.010	2.53	
	493	7.71 + 0.25X	0.984	< 0.001	4.05	
3	480	8.74 + 0.14X	0.963	< 0.001	6.93	
4	473	6.17 + 0.35X	0.960	< 0.010	2.90	
	476	4.32 + 0.51X	0.967	< 0.050	1.95	F = 1.43 d.f. 3, 8 P>0.05
	485	4.94 + 0.45X	0.985	< 0.020	2.23	
	494	3.92 + 0.51X	0.999	< 0.050	1.97	
5	466	4.22 + 0.45X	0.997	< 0.010	2.22	
	468	4.29 + 0.46X	0.976	< 0.050	2.15	* F = 2.27 d.f. 3, 4 P>0.05
	477	7.10 + 0.23X	0.936	> 0.050	4.35	
	482	5.26 + 0.38X	0.974	< 0.050	2.65	

* ANALYSIS OF VARIANCE OF GROUP 5 EXCLUDING 477.

Tests for parallelism of individual growth curves within Groups revealed non-parallelism in Group 1 at $P < 0.05$ but were parallel $P > 0.01$. The other two groups (Groups 4 and 5) showed no significant variance i.e. individual curves were parallel (Table 34).

The mean T_{10} values ranged from 2.33 (Group 5) to 7.14 (Group 3).

Comparison of the mean slopes of group growth curves is shown in Tables 35 and 36, Groups 4 and 5 have parallel mean slopes but all other comparisons show a highly significant difference (non-parallelism). This divergence of growth curves is demonstrated in Figures 13A and B.

iii. Conclusion

Since 2 of 5 animals recovered in the control group it would appear that a very low infective dose had been administered. The comparison of the growth curves between Group 4 and Group 5 showed parallelism which suggests that the chemotherapeutic effect of pyrrolidinomethyl tetracycline, given at this dosage, had no measurable effect on the growth rate of the parasite when the disease had reached the clinical stage.

Comparison of other Groups with Group 5 showed marked non-parallelism. This means that the growth rate of the parasite had been significantly depressed by the drug regimen used (in fact depressed so much in Group 2 that the MSI estimations

TABLE 35. (CHAPTER 8)

COMPARISON OF MEAN SLOPES AND GROWTH RATES OF MACROSCHIZONTS IN
ANIMALS INFECTED WITH T. PARVA (MUGUGA) UNDER DIFFERENT REGIMENS
REGIMENS OF TETRACYCLINE.

GROUP	MEAN SLOPE OF REGRESSION LINES	MEAN T_{10} VALUE
1 (Day 0)	0.30	3.33
3 (Days 0,1,2)	0.14	7.14
4 (Treated 2x daily in prepatent period)	0.42	2.38
5 (controls)	0.43	2.33

TABLE 36.   (CHAPTER 8)

STATISTICAL COMPARISON OF MEAN GROWTH CURVES OF MACROSCHIZONTS
FROM ANIMALS INFECTED WITH T. PARVA (MUGUGA) UNDER DIFFERENT
REGIMENS OF TETRACYCLINE.

GROUPS	1	3	4	5
1		t = 7.63** d.f. 23 P < 0.001	t = 3.23** d.f. 23 P < 0.01	t = 7.73** d.f. 19 P < 0.001
3	t = 7.63** d.f. 23 P < 0.001		t = 7.17** d.f. 15 P < 0.001	t = 16.55** d.f. 11 P = < 0.001
4	t = 3.23** d.f. 23 P < 0.01	t = 7.17** d.f. 15 P < 0.001		t = 0.24 d.f. 12 P > 0.05
5	t = 7.73** d.f. 19 P < 0.001	t = 16.55** d.f. 11 P = < 0.001	t = 0.24 d.f. 12 P > 0.05	

** Highly significant difference, i.e. not parallel.

FIGURE 13   (CHAPTER 8)

A.   Mean growth curves of T. parva (Muguga)
      under different regimens of tetracycline.

B.   Transposition of mean growth curves to
      demonstrate divergence.
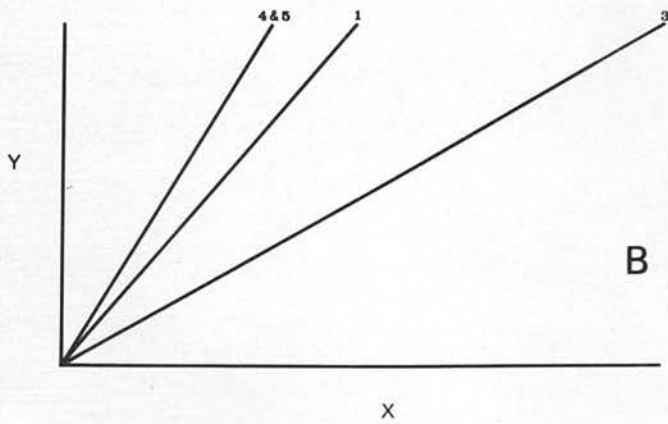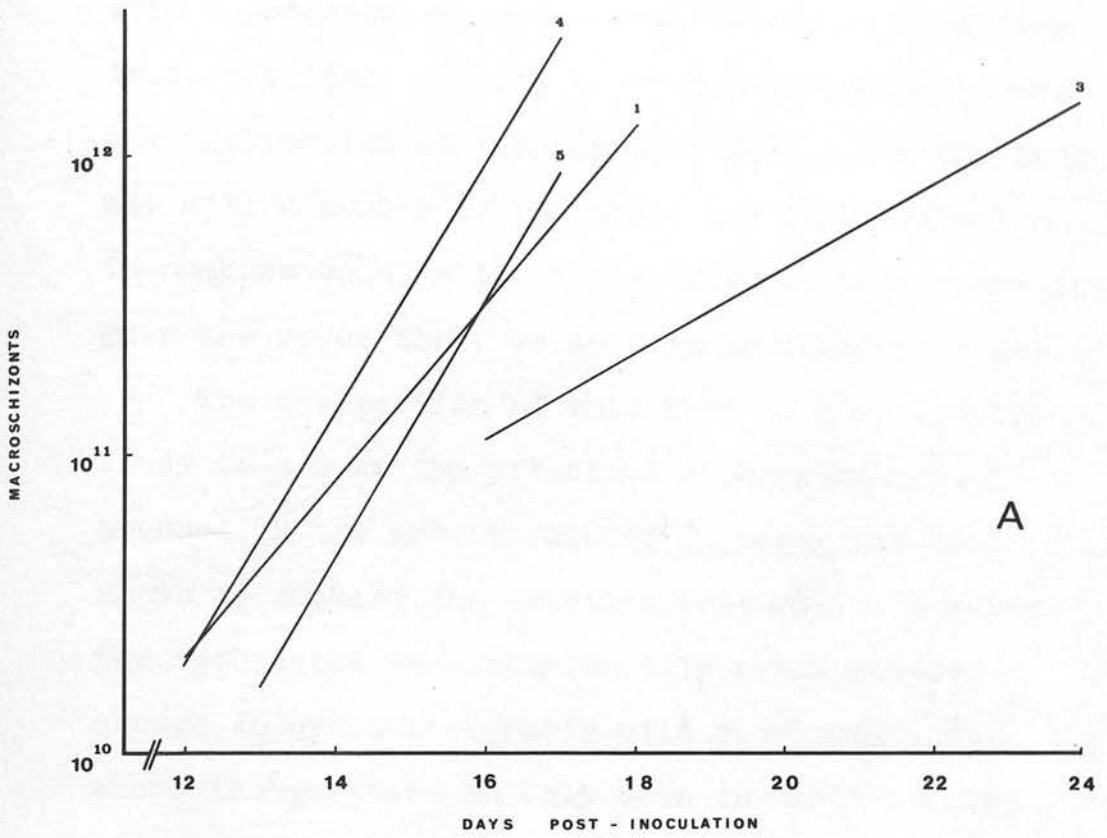
never reached significant levels). The reason for
this depression of growth rate is not evident from
these results. It may be that the drug depresses
the replication of the parasite per se, or the drug
may kill a number of parasites and this reduction
in numbers enables the host's defence mechanisms to
gain the upper hand, as seen in previous chapters.

The application of this kind of quantitative
study to assess the effect of a pharmacological
product on the growth rate of T. parva has been
shown to augment the existing criteria. However
the techniques are unfortunately not sensitive
enough to evaluate certain mild reactions i.e.
where the parasite is only seen in small numbers
or even not at all.

CHAPTER 9

DISCUSSION

At the outset of this research, the objectives
were to supplement the existing knowledge of the
behaviour of T. parva in the bovine host, and,
furthermore, to augment the F.A.O. Project in its
endeavours to find a feasible method of immunization
against E.C.F., by using quantitative studies. The
extent to which these objectives were achieved will be
discussed in this chapter.

Growth curves

In the first experiment, using the technique of
inoculating suspensions of infective particles to
initiate E.C.F. reactions, the author was unable to
confirm the constant growth rate of the parasite as
claimed by Jarrett et al. (1969). The growth curves
of the different groups were widely divergent and it
was decided, therefore, that the methods used should
be standardized before the experiment was repeated to
investigate that variance.

Consequently, a method of biopsy sampling of a
superficial lymph node, using a small bore needle, was
developed in order to reduce physical injury to a
minimum, and by critical examination of such biopsy
smears, it was shown possible to obtain a mean
macroschizont index which would be representative of
the smear. Using these baselines, it was concluded in
a further experiment that two such biopsy smears when

the infection rate was between 1 and 10%, but one smear
when over 10%, were necessary to give a statistically
representative mean macroschizont index for the lymph
nodes examined.   The experiment comparing macroschizont
indices within and between lymphoid sources indicated
that counts done on samples from a superficial node such
as the prescapular would reflect the total numbers of
macroschizonts present in the body at the time of
sampling.   Since certain assumptions were necessary in
the latter experiment, statistical analysis of the
observations was not possible.   However there were
very strong indications that such samples would be
representative of the total situation.   The overall
indications were that by daily sampling of a peripheral
lymph node not involved in the local drainage chain, it
was possible to monitor the increase in the number of
macroschizonts and infer that this represented the
total parasitic biomass.

These standardized methods were used in the
experiment designed to validate previous results.
Again, statistical comparison of growth curves showed a
significant divergence between groups, the rate of
increase in parasite numbers being dependent on the
size of infective dose administered.

Conclusive evidence for this statement was shown in
the definitive experiment using infective doses which
varied ten thousand fold.   In the animals given very
large numbers of infective particles, the growth rate

of the parasite was in the region of a ten-fold
increase every two days. With decreasing numbers of
infective particles administered, however, the time
interval taken for a ten-fold increase to occur
lengthened, until the situation was reached eventually
where the parasite numbers never attained countable
proportions. This evidence suggests to the author
that the degree to which the growth curves are
depressed in an animal is not only a reflection of the
number of parasites introduced but also a measure of
the ability of the animal's defence mechanisms to
combat the insurgent parasite.

The reasons why there are individual variations of
growth rates within a group of animals given the same
infective dose are speculative. In any biological
system there is inevitably individual variation to
susceptibility to disease. Part explanation of this
variation would be the differing capabilities of the
defence mechanisms of individual animals to cope with
the parasite. However, coupled with this fact will be
the degree of efficiency attained in producing a
uniform suspension of infective particles. The method
used for trituration of infected ticks requires
refinement and it is thought that in these experiments,
particularly in the lower infective dose range, coarse
clumps of infective particles may or may not have been
present.

The F.A.O. team considered that the technique of

harvesting infective particles directly from the tick
saliva would give a more uniform suspension of IPs
which could be accurately titrated.   An experiment
was therefore designed to evaluate the feasibility of
immunizing cattle against T. parva using this method
(Cunningham, Brown, Purnell, Radley, Burridge, Musoke,
Sempebwa, 1970a).   The author took an active part in
this experiment but since it was a team effort it has
not been included in the body of this text.

The main conclusions drawn from this experiment
which are relevant to this thesis were "infections of
predictable severity can be produced in groups of
cattle by varying the volume of preserved suspension
inoculated.   We therefore consider that we are in a
position to investigate the possibility of immunizing
cattle against T. parva infection using 3 different
approaches:

(i)   Inoculation of a small number of preserved
infective particles, sufficient to produce a mild
reaction and subsequent immunity to challenge with
infected ticks.

(ii) Inoculation of infective particles attenuated
by exposure to gamma rays.

(iii) Inoculation of preserved infective material
followed by chemotherapy."

The applications of the quantitative studies
described in this thesis are several.   Since most of
the future experimentation using infective particle

suspensions is reliant on stabilate material, initial
titration and the subsequent study of growth curves
will characterize that particular stabilate.
Characterization of stabilate material is considered
to be an essential prerequisite to chemoprophylactic
studies, immunization trials, experimentation on breed
susceptibility and further pathogenesis studies.   This
kind of application has its limitations because of the
low numbers of parasites present in mild reactions.
However since the individual variations appear to be
greater when small infective doses are used difficulty
in interpretation of results using any criteria will
be encountered.   It is therefore recommended that
infective doses giving a uniform growth rate within a
group be used as standard for much of the future work,
then any slight deviation from this can be detected
easily with the growth curve study.

Prepatent period

Since E.C.F. infections may vary from acute to
inapparent the prepatent period may vary from a
minimum of 5 days to a figure which cannot be estimated
by available methods of detection.   The 5 day minimum
has been seen in three of the experiments recorded here
and speculation about the life cycle of the parasite
during this stage remains open.   Several explanations
are possible.   The parasite may be in an unrecognizable
form during the prepatent period but assumes normal
morphology on Day 5 e.g. the parasite may not be
stained by Giemsa before Day 5, or it may be associated

with and camouflaged by, the host cell nucleus before
Day 5 but enters the cytoplasm on that day.   On the
other hand, the parasite may be present throughout the
prepatent period in its recognizable form in a small
focus and sampling techniques are not thorough enough
for its detection.   Possibly the parasite has a
developmental phase in a type of cell or tissue other
than the local drainage lymph node, but first appears
in that node due to prior conditionihg (it was the
first lymphoid site to be exposed to the infective
particles).

Another explanation may be that the parasite numbers
are not increasing rapidly, if at all, during the
prepatent period since the parasite will be adapting
itself to new environs.

As was shown in the definitive experiment there is
a mathematical relationship between the size of infective
dose and the length of the prepatent period.   This is
a linear correlation where the prepatent period lengthens
with decreasing size of infective dose.   The linear
correlation was not significant in the two pilot
experiments and therefore the use of the prepatent
period as a criterion for size of infective dose is best
reserved for experiments when many observations are
available.

Onset of fever

The linear correlation of the onset of fever and
the size of infective dose was only significant in the
definitive experiment, and so it may be a useful

criterion for assessing the size of the infective dose
only in experiments involving large numbers of cattle.

Intraerythrocytic piroplasms

The results of experiments presented in this thesis
confirm the conclusions of Jarrett et al. (1969) that
"the switch to piroplasm production appears to be time-
dependent and not dose-dependent." This means that
irrespective of the number of infective particles which
become established in the bovine host there is a
constant period necessary before piroplasms are
produced, that the parasite has a limiting replication
based on time, but once this production of the
relatively nonpathogenic stage begins it will act as a
safety valve. This fact coupled with the host's
ability to curtail the increase in macroschizont
numbers probably accounts for the recoveries from E.C.F.
when small infective doses are used.

Survival time

In the two experiments using the standardized
methods an inverse linear relationship between the size
of infective dose and the survival time was demonstrated.
In the preliminary and in the definitive experiments it
was observed that inoculation of a very small
infective dose could result in death or recovery of the
animals. This observation suggested that even smaller
infective doses could result in higher recovery rates
which was later confirmed (Cunningham et al., 1970a).

To sum up the situation so far, it can be said that
decreasing size of infective dose indirectly depresses

the growth rate of the parasite, which results in a lengthening of the prepatent period, a delay in onset of fever, and a prolonging of survival time.

Since it is possible to produce the complete gamut of acute to inapparent reactions by varying the size of infective dose, it may be postulated that strains of T. parva with different virulence are simply due to varying numbers of infective particles within the tick populations. This implies that there is only one strain of T. parva.

Jarrett et al. (1969) hypothesized that there could be different strains of T. parva which would have different growth rates, also within a strain there would be clones of parasites having a scatter of growth rates about the mean. This infers that piroplasms from slow replicating parasites would cause mild infections on transmission. However, the evidence of Barnett and Brocklesby (1966b) was to the contrary. Ticks infected by feeding on cattle recovered months after infection with E.C.F. caused the typical fatal syndrome, implying that the parasite was still capable of rapid replication. In spite of this observation, the inadvertent selection of faster growing clones on tick passage was put forward by Brocklesby (1969) as being the explanation for various transformations of Theileria spp. recorded. In view of the work presented in this thesis, the author would like to suggest that another explanation is now possible: if a particular strain is of mild virulence it is likely

that the ticks transmitting this "strain" harbour few
infective particles and on passage in the laboratory,
where optimal conditions for transmission are aimed for,
the infection rate in the tick salivary glands will
probably increase.   Concomitant with this increase in
infectivity of the ticks on passage will be an increase
in growth rate of the parasite in the bovine host, and
therefore it is assumed that the strain has transformed
and become more virulent.   The tendency of most
recorded transformations have been from mild to severe
and it is therefore suggested that this may be due to
the attainment of increased infection rates of the
ticks.

In conclusion, this thesis, and the work of the
F.A.O. project, support earlier observations of Lowe
(1933), Wilson (1950), Barnett (1957), Barnett and
Brocklesby (1966a), and in particular the "quantum of
infection" hypothesis first put forward by Wilde (1967).
The research has supplemented the existing knowledge of
the behaviour of T. parva in the bovine host, has
augmented the research of the F.A.O. team, and it is
hoped, provided a useful investigational tool for future
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    Use of tissue culture in the study of <u>Theileria</u>
    <u>annulata</u>.

    Veterinariya, Moskva. <u>9</u>, 66 - 69.

APPENDIX

APPENDIX TABLE 1.   (CHAPTER 4)

MACROSCHIZONT INDICES OF DAILY LYMPH NODE BIOPSY SMEARS TAKEN FROM TWO GROUPS OF ANIMALS'

INOCULATED WITH DIFFERENT SIZES OF INFECTIVE DOSES OF T. PARVA (MUGUGA).

GROUP	ANIMAL	DAYS POST - INOCULATION									
		8	9	10	11	12	13	14	15	16	17
1	667	1.20	11.30	27.20	47.50	76.00					
1	669	1.50	12.10	19.00	58.25	73.50					
2	671					3.00	7.40	10.63	12.00	17.17	29.71
2	672					6.70	21.60	43.25	54.00	76.50	

APPENDIX TABLE 2.   (CHAPTER 5)

NINE MACROSCHIZONT INDICES OF BIOPSY SMEARS FROM TEN PRESCAPULAR LYMPH NODES

CATEGORY 1 - 10%

LINE	1	2	3	4	5	6	7	8*	9	10
A_1	1.90	7.00	10.00	5.50	6.70	3.20	2.00	2.40	8.13	5.00
A_2	2.30	8.40	7.70	3.90	7.40	4.00	1.10	2.10	7.88	8.50
B_1	2.80	12.00	8.70	4.30	7.80	3.90	2.30	3.20	10.00	7.60
B_2	1.90	6.10	6.00	3.70	6.10	4.60	1.00	2.60	9.88	6.80
C_1	1.00	6.90	6.20	4.20	6.70	4.30	2.10	2.00	9.50	7.20
C_2	1.20	8.70	5.80	4.40	5.80	3.60	2.30	1.50	10.00	7.30
D	1.10	8.10	10.80	4.50	6.40	6.00	2.20	2.00	7.62	8.10
E	1.80	8.60	8.20	3.80	7.40	4.00	1.00	2.00	11.13	8.50
F	2.40	6.60	9.30	4.00	7.80	4.00	1.40	2.90	9.50	7.50

SLIDE NUMBER

Each macroschizont index is the linear count of 1000 (except where indicated) lymphocytes and associated macroschizonts expressed as a percentage.

* 800 lymphocytes + associated macroschizonts.

APPENDIX TABLE 3. (CHAPTER 5)

NINE MACROSCHIZONT INDICES OF BIOPSY SMEARS FROM TEN PRESCAPULAR LYMPH NODES

CATEGORY 10 - 50%

LINE		SLIDE NUMBER									
		1*	2	3+	4	5+	6+	7	8+	9	10
A_1		47.80	18.00	69.00	19.83	58.25	50.75	18.66	43.75	25.83	22.66
A_2		30.80	17.00	36.50	21.50	44.50	56.25	27.35	44.75	28.50	24.33
B_1		39.00	29.00	52.00	24.50	52.75	45.25	25.16	44.00	31.17	28.00
B_2		36.80	20.00	49.50	23.50	46.25	43.25	30.33	39.50	22.50	24.66
C	C_1	32.00	23.50	47.50	20.50	44.25	53.00	23.33	40.50	19.50	29.33
	C_2	35.20	21.00	34.00	16.66	41.50	43.50	30.16	43.00	15.00	28.00
D		32.60	29.16	39.50	15.00	49.25	56.50	31.33	40.00	24.67	21.66
E		31.60	17.50	25.00	16.85	48.00	46.25	32.66	39.25	31.67	24.33
F		38.80	22.83	51.00	22.50	47.50	45.00	27.33	51.00	25.00	22.00

Each macroschizont index is the linear count of 600 (except where indicated) lymphocytes and associated macroschizonts expressed as a percentage.

* 500 lymphocytes + associated macroschizonts.
+ 400 " " "

APPENDIX TABLE 4.   (CHAPTER 5)

NINE MACROSCHIZONT INDICES OF BIOPSY SMEARS FROM TEN PRESCAPULAR LYMPH NODES

CATEGORY 50 - 100%

LINE	SLIDE NUMBER									
	1+	2+	3	4	5	6	7	8	9	10
A_1	68.50	55.00	77.50	105.00	66.50	66.00	76.00	95.00	84.00	52.50
A_2	58.50	51.50	62.00	95.00	61.00	83.00	84.00	92.00	88.50	56.00
B_1	41.75	52.00	84.50	117.50	72.50	70.00	92.00	88.50	133.00	61.50
B_2	75.75	52.50	55.50	81.00	68.00	65.50	65.50	79.50	67.50	56.50
C_1	50.00	51.50	92.50	99.00	65.00	76.00	96.50	77.00	100.50	60.00
C_2	70.00	50.50	53.50	86.00	55.00	61.50	54.00	76.00	105.00	57.00
D	50.25	48.50	58.00	116.50	75.00	82.00	100.00	104.00	102.00	55.00
E	93.75	58.00	58.50	105.50	60.50	46.00	52.50	71.00	82.00	57.50
F	60.50	55.00	79.50	121.00	74.00	72.50	73.00	94.00	96.00	63.00

Each macroschizont index is the linear count of 200 (except where indicated) lymphocytes and associated macroschizonts expressed as a percentage.

+ 400 lymphocytes + associated macroschizonts.

APPENDIX TABLE 5. (CHAPTER 5)

MACROSCHIZONT INDICES OF BIOPSY SAMPLES TAKEN FROM THE RIGHT PRESCAPULAR LYMPH NODES OF THREE
ANIMALS (CATEGORIES a, b, c) REACTING TO T. PARVA (MUGUGA).

LINE	SLIDE NUMBER									
	1	2	3	4	5	6	7	8	9	10
a) Category 1 - 10%										
A	2.00	3.20	4.30	1.00	1.20	1.60	1.80	1.20	1.10	1.60
B	2.80	3.00	4.80	1.10	2.80	1.20	1.70	1.00	1.10	1.40
C	1.70	3.90	5.50	1.50	2.50	2.00	1.60	1.00	1.70	1.10
D	2.90	4.10	5.80	1.20	1.20	1.60	1.90	1.30	1.10	1.00

Each macroschizont index is the linear count of 1000 lymphocytes and associated
macroschizonts expressed as a percentage.

LINE	SLIDE NUMBER									
b) Category 10 - 50%										
A	30.17	41.40	34.40	27.50	32.00	34.33	28.33	36.50	27.33	30.33
B	29.33	36.20	37.80	39.83	43.17	41.83	39.83	57.33	28.00	36.33
C	29.67	43.00	31.00	37.17	30.50	32.50	32.50	31.33	26.84	31.83
D	28.00	35.40	39.00	37.00	37.33	37.50	30.00	44.33	61.83	31.00

Each macroschizont index is the linear count of 600 lymphocytes and associated
macroschizonts expressed as a percentage.

LINE	SLIDE NUMBER									
c) Category 50 - 100%										
A	58.25	54.75	55.00	63.00	61.00	68.50	55.75	67.00	62.75	64.25
B	64.00	65.00	66.75	58.00	67.25	63.75	63.50	53.75	71.25	63.00
C	66.00	63.50	67.25	68.75	64.50	65.00	67.50	59.50	57.00	65.50
D	63.75	65.25	61.75	59.25	61.25	57.50	69.50	68.25	54.25	65.75

Each macroschizont index is the linear count of 400 lymphocytes and associated
macroschizonts expressed as a percentage.

APPENDIX TABLE 6. (CHAPTER 5)

MACROSCHIZONT INDICES*OF SAMPLES OF THE LYMPHOID ORGAN FROM OX 103 AT AUTOPSY

a. LOCAL DRAINAGE CHAIN

SAMPLE	SLIDE NUMBER									
	1	2	3	4	5	6	7	8	9	10
Left Parotid (1)	37.00	55.00	53.25	42.80	26.80	36.20	39.00	41.40	56.00	25.40
	41.40	53.75	46.00	33.00	26.00	32.00	41.60	48.00	51.40	47.20
	46.40	50.00	53.50	14.80	16.20	40.60	43.60	43.60	29.60	57.20
	37.20	42.50	51.00	24.00	18.00	44.20	42.20	52.40	39.40	40.20
Left Atlantal (2)	62.50	62.50	52.50	71.00	39.00	53.00	48.50	52.50	18.00	53.50
	91.00	85.50	76.00	75.50	50.00	46.50	64.00	44.50	18.50	65.00
	65.50	72.00	51.50	85.00	56.00	70.00	69.50	45.50	19.50	53.50
	68.00	63.00	61.00	75.00	68.50	80.00	66.50	42.00	21.00	77.00
Left posterior cervical	2.30	4.60	2.60	2.80	4.90	2.00	1.70	1.30	2.70	2.10
	3.80	3.40	2.20	3.00	3.30	4.10	1.30	3.00	2.20	4.40
	2.90	2.70	2.40	2.10	2.50	2.00	2.20	3.30	3.20	3.40
	2.10	2.60	3.80	2.50	2.80	2.80	3.00	2.90	3.40	4.00

b. GENERAL LYMPH NODES

SAMPLE	SLIDE NUMBER	
	1	2
Right prescapular	4.60	2.50
	3.10	4.30
	2.30	3.40
	3.90	2.80
Right parotid	2.60	3.40
	3.00	3.20
	2.00	3.50
	5.70	5.00
Hepatic	7.00	3.60
	6.90	6.20
	5.30	6.20
	5.70	6.70
Renal	4.60	4.10
	2.50	4.90
	3.70	3.90
	4.40	3.30
Internal iliac	3.30	6.20
	4.50	4.00
	3.80	3.90
	4.00	4.80
Mesenteric	0.20	0.40
	0.60	0.90
	0.10	1.00
	0.30	0.40
Posterior mediastinal	6.10	8.70
	6.70	12.90
	4.60	3.60
	5.00	5.50

c. ORGANS

SAMPLE	SLIDE NUMBER	
	1	2
Peyer's patch	0.10	1.20
	0.00	1.60
	0.10	2.00
	0.00	1.80
Lung	2.20	2.40
	2.10	1.20
	5.20	1.60
	3.50	2.00
Kidney	1.30	0.80
	1.80	1.50
	1.60	1.20
	1.60	2.00
Bone marrow	2.80	1.40
	1.60	1.40
	2.10	2.30
	1.90	2.40
Liver	4.80	6.10
	6.20	4.00
	2.90	5.60
	4.20	4.80
Spleen	11.30	9.20
	12.30	14.20
	9.90	12.40
	11.10	8.30
Thymus	0.00	0.10
	0.00	0.00
	0.00	0.00
	0.10	0.10
Buffy coat	1.50	1.90
	1.60	1.40
	1.80	1.10
	1.20	1.30

* Each macroschizont index is a linear count of 1000 lymphocytes (except where indicated) and the associated macroschizonts.

(1) 500 lymphocytes + macroschizonts.
(2) 200 lymphocytes + macroschizonts.

APPENDIX TABLE 7. (CHAPTER 5)

MACROSCHIZONT INDICES[*] OF SAMPLES OF THE LYMPHOID ORGAN FROM OX 108 AT AUTOPSY

a. LOCAL DRAINAGE CHAIN

SAMPLE	SLIDE NUMBER									
	1	2	3	4	5	6	7	8	9	10
Left parotid	0.30	0.50	1.30	1.80	1.20	2.90	1.30	1.30	1.20	1.20
	0.20	0.70	0.80	0.80	2.10	1.40	1.80	1.50	1.10	2.10
	0.70	1.10	1.80	0.60	1.00	2.30	1.60	1.20	1.10	1.90
	0.60	0.90	0.70	0.90	0.90	1.40	1.10	1.60	1.00	3.10
Left atlantal	2.80	1.90	0.60	1.40	2.20	0.50	1.70	2.90	2.20	0.30
	2.50	1.30	2.20	3.50	1.60	4.00	3.70	2.60	0.70	1.80
	2.00	0.80	5.00	1.70	1.10	1.80	2.60	0.80	1.20	0.40
	1.70	1.80	3.30	3.90	0.90	2.70	3.30	1.00	1.40	1.70
Left posterior cervical	0.60	1.20	1.60	0.50	1.30	1.30	0.80	0.90	1.00	0.70
	0.80	1.70	2.80	0.80	1.20	1.10	1.30	0.90	2.30	1.50
	0.80	0.80	0.80	1.60	0.90	0.80	0.80	1.40	1.00	1.10
	1.00	1.10	0.80	1.20	1.30	1.30	1.20	1.60	0.70	1.20

b. GENERAL LYMPH NODES

SAMPLE	SLIDE NUMBER	
	1	2
Right prescapular	1.50	1.10
	1.70	1.00
	0.50	1.20
	2.30	1.30
Right parotid	0.50	1.30
	0.80	0.70
	1.30	1.30
	0.60	1.40
Hepatic	2.10	2.00
	1.70	2.50
	2.40	2.10
	1.50	2.80
Renal	1.50	1.30
	1.00	1.40
	0.80	2.20
	0.90	1.40
Internal iliac	1.10	0.90
	1.70	1.90
	2.30	2.10
	1.40	1.40
Mesenteric	0.70	0.90
	0.70	1.20
	0.70	1.10
	0.50	0.90
Posterior mediastinal	3.00	2.80
	5.70	3.00
	7.00	3.00
	5.50	2.00

c. ORGANS

SAMPLE	SLIDE NUMBER	
	1	2
Peyer's patches	0.30	0.30
	0.40	0.10
	0.50	0.40
	0.10	0.10
Lung	0.80	0.80
	2.80	1.00
	2.40	1.60
	2.20	1.50
Kidney	2.60	3.20
	1.80	1.50
	2.40	2.30
	2.10	2.60
Bone marrow	0.30	0.10
	0.20	0.40
	0.50	0.20
	0.10	0.20
Liver	6.10	7.50
	4.20	8.20
	3.60	6.80
	5.70	4.20
Spleen	2.50	1.70
	2.50	6.40
	4.00	3.30
	3.20	2.80
Thymus	0.00	0.20
	0.00	0.70
	0.00	0.30
	0.10	0.50
Buffy coat	0.60	0.30
	0.10	0.20
	0.70	0.60
	0.50	0.50

* Each macroschizont index is the linear count of 1000 lymphocytes and associated macroschizonts expressed as a percentage.

APPENDIX TABLE 8. (CHAPTER 5)

MACROSCHIZONT INDICES[*] OF SAMPLES OF THE LYMPHOID ORGAN FROM OX 925 AT AUTOPSY

a. LOCAL DRAINAGE CHAIN :

SAMPLE	SLIDE NUMBER									
	1	2	3	4	5	6	7	8	9	10
Left parotid	3.10	3.00	1.50	4.10	3.50	11.40	14.40	14.20	5.00	3.80
	4.40	3.40	2.40	3.90	1.90	16.70	10.50	14.40	4.60	4.50
	3.20	3.10	4.40	3.50	3.00	5.90	14.20	20.10	3.50	3.50
	1.80	2.90	3.50	3.80	3.60	8.30	10.00	15.60	7.80	3.70
Left atlantal (3)	52.40	47.00	40.75	50.25	79.75	57.50	76.00	48.00	73.50	70.75
	59.80	50.25	47.50	57.25	76.50	63.50	59.25	60.00	67.00	61.75
	57.60	59.25	58.75	56.50	45.75	61.25	69.25	61.25	52.50	49.50
	64.60	57.75	54.50	50.00	47.75	62.50	75.25	61.25	62.25	62.00
Left posterior cervical (1)	15.70	25.33	38.00	34.67	23.50	24.17	25.33	26.00	37.17	13.50
	22.00	24.33	15.00	21.50	22.50	22.83	23.83	26.67	25.83	25.50
	17.50	21.33	29.00	14.83	31.50	20.33	27.17	24.67	33.33	20.50
	16.00	28.67	41.00	25.17	28.67	26.67	23.00	23.67	35.67	21.67

b. GENERAL LYMPH NODES

SAMPLE	SLIDE NUMBER	
	1	2
Right prescapular (1)	22.71	26.67
	24.50	20.50
	35.50	26.17
	36.67	29.33
Right parotid (2)	56.00	27.20
	47.60	38.00
	42.40	27.00
	23.40	41.40
Hepatic (2)	38.00	30.40
	39.20	28.80
	50.80	25.60
	43.00	31.00
Renal (1)	34.67	38.00
	40.17	36.50
	31.50	37.83
	36.33	36.00
Internal iliac (1)	31.17	26.00
	54.50	28.67
	23.83	31.50
	44.17	40.50
Mesenteric (1)	19.50	20.33
	19.67	24.33
	34.67	20.50
	35.33	22.00
Posterior mediastinal	15.90	16.40
	16.30	21.30
	16.40	17.80
	14.70	20.00

c. ORGANS

SAMPLE	SLIDE NUMBER	
	1	2
Peyer's patch	8.50	6.80
	7.60	6.80
	9.30	9.20
	6.40	7.80
Lung (2)	36.40	32.60
	32.80	32.00
	32.40	35.20
	31.60	32.80
Kidney	5.20	6.00
	11.00	9.30
	7.40	4.50
	5.80	7.80
Bone marrow	10.80	3.50
	8.80	5.20
	11.90	9.30
	9.50	8.70
Liver (2)	33.00	32.00
	32.40	33.40
	31.80	32.20
	28.40	28.40
Spleen (2)	30.60	20.00
	28.00	33.83
	22.80	36.83
	19.20	33.67
Thymus	3.40	2.60
	4.70	2.90
	3.50	2.40
	4.20	3.60
Buffy coat	1.60	1.60
	1.20	1.00
	1.40	1.40
	1.00	1.10

* Each macroschizont index is the linear count of 1000 lymphocytes (except where indicated) and associated macroschizonts expressed as a percentage.

(1) 600 lymphocytes and macroschizonts.
(2) 500 lymphocytes and macroschizonts.
(3) 400 lymphocytes and macroschizonts.

APPENDIX TABLE 9. (CHAPTER 5)

MACROSCHIZONT INDICES* OF SAMPLES OF THE LYMPHOID ORGANS FROM OX 927 AT AUTOPSY

a.   LOCAL DRAINAGE CHAIN

SAMPLE	SLIDE NUMBER									
	1	2	3	4	5	6	7	8	9	10
Left parotid (1)	11.70	13.33	8.50	17.70	23.20	17.30	10.40	30.00	45.00	20.70
	14.80	20.50	15.90	16.00	14.30	18.30	16.80	23.30	20.40	17.70
	14.80	17.60	19.00	11.60	9.10	15.40	18.90	21.10	27.00	18.10
	16.30	24.80	17.50	19.90	15.90	17.90	15.00	25.00	23.33	19.30
Left atlantal	35.33	25.17	34.33	22.83	33.17	31.00	26.67	31.17	25.33	41.50
	36.83	28.00	35.00	26.50	29.17	31.67	39.17	28.17	41.50	59.67
	28.00	31.50	37.17	24.67	18.67	26.67	36.00	27.67	34.83	33.33
	46.00	36.67	39.67	37.50	34.00	28.17	39.17	32.17	58.67	56.50
Left posterior cervical	28.17	52.00	56.33	27.33	30.50	26.67	30.00	35.00	39.50	35.00
	28.33	28.83	55.17	30.00	30.67	36.33	38.67	44.33	27.00	17.17
	42.17	34.33	32.00	18.67	35.83	33.00	37.83	30.00	22.83	51.00
	42.00	35.83	41.17	28.83	33.67	39.67	46.67	41.00	34.00	38.83

b.   GENERAL LYMPH NODES

SAMPLE	SLIDE NUMBER	
	1	2
Right prescapular	38.67	24.67
	23.00	30.67
	30.00	24.33
	33.83	40.83
Right parotid	44.80	25.67
	41.80	28.00
	34.00	39.67
	37.20	33.00
Hepatic	37.80	40.00
	27.80	29.50
	30.20	36.67
	30.00	30.00
Renal	35.20	28.83
	39.60	31.67
	29.40	27.00
	36.40	33.00
Internal iliac	32.20	24.83
	37.00	26.33
	34.20	27.17
	39.80	55.00
Mesenteric (2)	14.38	9.63
	10.38	7.25
	9.38	11.38
	10.38	12.13
Posterior mediastinal	36.50	27.33
	28.33	32.33
	28.83	37.67
	35.33	33.33

c.   ORGANS

SAMPLE*	SLIDE NUMBER	
	1	2
Peyer's patch (1)	0.80	6.30
	1.20	2.80
	3.80	2.30
	2.60	3.00
Lung	25.83	30.50
	23.16	29.33
	27.17	31.67
	26.17	28.83
Kidney (3)	31.60	41.60
	35.80	36.60
	32.60	42.20
	28.40	39.20
Bone marrow (1)	12.70	15.40
	12.10	13.20
	13.00	16.90
	10.50	12.00
Liver (3)	39.00	48.80
	44.60	44.00
	42.20	46.20
	38.20	50.40
Spleen (4)	45.50	52.50
	48.25	56.00
	33.50	45.00
	51.75	48.25
Thymus (1)	0.90	1.10
	1.60	1.10
	1.20	1.60
	0.70	1.30
Buffy coat (1)	8.40	9.80
	5.70	10.20
	7.50	7.60
	7.60	7.90

* Each macroschizont index is the linear count of 600 lymphocytes (except where indicated) and associated macroschizonts expressed as a percentage.

(1)   1000 lymphocytes + associated macroschizonts.
(2)   800 lymphocytes + associated macroschizonts.
(3)   500 lymphocytes + associated macroschizonts.
(4)   400 lymphocytes + associated macroschizonts.

APPENDIX TABLE 10.   (CHAPTER 5)

MACROSCHIZONT INDICES* OF SAMPLES OF THE LYMPHOID ORGAN FROM OX 973 AT AUTOPSY

a.   LOCAL DRAINAGE CHAIN

SAMPLE	SLIDE NUMBER									
	1	2	3	4	5	6	7	8	9	10
Left parotid	7.50	5.00	9.70	15.30	10.40	11.00	6.20	7.50	8.20	10.50
	5.20	9.10	6.30	14.60	6.80	8.00	7.40	4.90	5.60	4.50
	4.30	3.60	5.30	8.80	3.40	8.50	10.80	12.90	6.10	7.00
	9.20	9.90	5.40	7.10	5.80	9.10	12.00	7.80	8.00	8.50
Left atlantal	7.70	10.90	9.00	15.60	6.60	9.90	13.60	9.20	11.70	4.00
	10.80	6.00	6.50	17.40	5.90	14.50	17.70	6.60	13.00	10.40
	5.70	6.00	8.50	10.00	4.20	9.10	8.00	4.50	7.50	5.10
	12.40	9.40	7.00	12.10	7.00	10.50	9.50	6.50	8.00	5.50
Left posterior cervical	13.50	14.80	10.80	14.40	9.60	17.30	12.30	9.80	11.30	16.20
	13.00	11.50	12.50	14.60	17.60	9.60	9.50	12.30	16.10	13.50
	10.50	10.00	9.80	11.00	11.70	12.30	10.10	8.70	6.00	9.50
	14.10	9.00	9.00	15.70	14.80	16.00	12.20	14.00	8.80	16.80

b.   GENERAL LYMPH NODES

SAMPLE	SLIDE NUMBER	
	1	2
Right prescapular	10.90	11.10
	9.30	10.20
	9.60	8.50
	11.80	11.70
Right parotid	10.40	10.30
	16.40	17.30
	8.20	12.40
	10.80	12.30
Hepatic	17.80	16.30
	14.60	16.00
	14.60	21.10
	15.50	19.20
Renal	13.00	7.80
	7.80	7.40
	6.60	9.00
	11.70	8.60
Internal iliac	13.40	18.40
	6.80	17.50
	7.30	15.00
	14.00	20.40
Mesenteric	11.20	12.10
	11.30	13.00
	10.10	9.60
	11.20	11.60
Posterior mediastinal	14.40	23.00
	14.10	19.10
	13.24	13.10
	15.30	11.60

* Each macroschizont index is the linear count of 1,000 lymphocytes and associated macroschizonts expressed as a percentage.

c.   ORGANS

SAMPLE	SLIDE NUMBER	
	1	2
Peyer's patch	12.40	13.50
	14.40	12.30
	7.40	9.20
	10.80	14.40
Lung	8.70	16.10
	10.60	10.50
	15.80	9.10
	9.20	8.60
Kidney	11.80	15.50
	11.80	12.30
	13.70	9.20
	9.30	10.50
Bone marrow	1.10	3.60
	1.30	5.20
	2.10	2.10
	2.50	3.60
Liver	23.00	29.60
	23.70	23.60
	22.00	25.60
	26.00	21.00
Spleen	18.10	21.33
	13.00	21.00
	16.60	22.67
	15.50	18.67
Thymus	11.80	7.33
	10.00	11.00
	11.10	13.90
	11.50	13.10
Buffy coat	1.10	1.60
	0.80	1.20
	0.40	1.80
	0.70	1.00

APPENDIX TABLE 11. (CHAPTER 6)
MACROSCHIZONT INDICES OF DAILY LYMPH NODE BIOPSY SMEARS TAKEN FROM ANIMALS
INOCULATED WITH DIFFERENT SIZES OF INFECTIVE DOSES OF T.PARVA (MUGUGA)

GROUP	ANIMAL NUMBER	SLIDE NUMBER	8	9	10	11	12	13	14	15	16	17	18	19
10^2	675	1	1.20	7.30	20.67	46.00								
			1.60	5.00	27.17	40.75								
			1.10	2.80	17.83	36.00								
			1.60	4.90	31.50	44.75								
		2	1.00	3.20										
			1.10	3.60										
			1.70	3.20										
			1.00	2.80										
	762	1	4.60	14.25	25.17	126.50								
			5.30	21.25	39.00	73.50								
			3.80	14.25	18.67	113.50								
			3.00	16.13	26.33	89.00								
		2	3.40											
			3.60											
			2.50											
			2.30											
10^1	674	1			2.10	10.13	18.00	31.80						
					2.60	12.88	20.25	72.60						
					1.50	10.88	28.63	33.00						
					1.90	9.88	21.25	36.00						
		2			1.70									
					2.40									
					1.70									
					1.30									
	718	1			1.60	4.50	14.88	27.33						
					1.20	5.00	14.13	27.67						
					1.90	3.80	10.75	18.50						
					2.20	5.00	10.00	21.67						
		2			1.20	4.10								
					1.00	3.00								
					1.00	4.70								
					2.00	3.00								
10^0	664	1					2.90	7.00	41.50	91.00	63.50			
							4.40	9.80	36.50	103.00	78.00			
							3.80	13.50	36.00	61.50	60.00			
							4.70	17.30	37.00	80.50	121.50			
		2					1.70							
							3.40							
							2.50							
							2.30							
	666	1					4.50	13.25	35.33	64.50	61.00	56.00		
							9.00	21.13	39.17	45.00	75.50	73.00		
							7.20	25.13	41.83	67.50	56.00	70.50		
							8.80	22.13	45.83	68.00	75.00	65.00		
		2					5.70							
							7.50							
							7.00							
							7.20							
10^{-1}	670	1					2.30	14.25	11.38	30.83	16.88	73.00	70.50	67.50
							2.00	10.88	12.63	25.50	22.50	100.00	94.50	102.50
							1.90	11.38	12.25	23.50	23.00	84.50	122.00	70.00
							2.10	11.88	11.50	20.83	23.00	98.50	112.50	105.00
		2					2.20							
							1.50							
							4.30							
							2.10							
	761	1					3.20	14.75	30.00	69.50	71.50	85.00		
							3.70	21.00	25.33	83.00	78.00	100.00		
							2.70	12.50	40.00	47.00	57.50	66.00		
							3.20	13.63	42.17	78.00	77.50	82.50		
		2					2.70							
							3.70							
							4.20							
							3.70							

APPENDIX TABLE 12. (CHAPTER 7)

MACROSCHIZONT INDICES OF DAILY LYMPH NODE BIOPSY SMEARS TAKEN FROM ANIMALS

INOCULATED WITH DIFFERENT SIZES OF INFECTIVE DOSES OF <u>T.PARVA</u> (MUGUGA)

GROUP 10^2

ANIMAL	SLIDE	DAYS POST − INOCULATION						
		8	9	10	11	12	13	14
348	1	0.80	4.90	16.37	40.50	28.00	41.75	29.25
		1.10	3.60	15.38	26.25	39.40	53.75	32.50
		1.00	4.80	10.00	48.00	26.40	44.25	37.50
		0.90	4.80	15.25	41.25	50.20	49.00	35.00
	2	0.60	4.00					
		0.80	3.40					
		0.70	3.90					
		0.90	4.90					
360	1	0.80	5.70	20.67	40.40	118.50		
		1.20	4.20	20.33	41.00	119.00		
		1.00	5.30	21.00	58.00	100.50		
		1.40	5.10	20.83	45.00	118.00		
	2	0.60	2.40					
		0.60	3.50					
		0.20	1.50					
		0.70	4.10					
365	1	0.90	4.10	12.60	46.00	67.50		
		0.70	4.30	14.75	40.25	57.00		
		1.10	3.00	13.00	55.00	91.00		
		1.20	5.50	13.75	39.25	103.00		
	2	0.50	1.00					
		0.40	1.70					
		0.20	1.50					
		0.40	1.20					
368	1	2.00	3.30	14.00	43.00	70.00		
		1.90	3.80	18.00	29.50	94.50		
		2.00	3.90	14.13	40.50	77.50		
		3.20	6.00	15.63	50.75	89.50		
	2	1.10	3.60					
		0.80	3.20					
		1.30	3.90					
		1.00	5.80					

APPENDIX TABLE 13. (CHAPTER 7)

MACROSCHIZONT INDICES OF DAILY LYMPH NODE BIOPSY SMEARS TAKEN FROM ANIMALS

INOCULATED WITH DIFFERENT SIZES OF INFECTIVE DOSES OF T.PARVA (MUGUGA)

GROUP 10^1

ANIMAL	SLIDE	DAYS POST − INOCULATION					
		9	10	11	12	13	14
	1	1.00	5.30	23.67	34.00	67.00	112.50
		1.40	9.40	17.00	41.20	73.50	95.00
		2.20	6.50	16.17	40.40	52.00	101.00
		2.40	9.20	25.33	38.80	45.50	102.50
349							
	2	1.40	5.90				
		0.60	9.80				
		1.30	8.60				
		0.60	9.70				
	1	1.70	7.20	19.50	31.20	45.25	65.50
		1.60	7.40	22.25	35.20	53.00	55.50
		1.70	5.70	22.25	37.20	57.50	61.00
		2.10	8.50	22.25	36.00	52.00	58.00
351							
	2	1.40	6.10				
		1.00	7.30				
		1.10	7.50				
		1.20	6.90				
	1	1.20	6.70	16.75	43.00	60.50	
		0.60	6.40	21.63	44.50	74.00	
		1.70	5.90	19.88	41.50	70.00	
		0.80	9.30	21.88	44.50	90.00	
352							
	2	1.30	5.20				
		1.10	8.00				
		1.40	4.20				
		0.60	6.70				
	1	0.40	1.90	10.50	30.60	50.25	
		0.30	2.40	12.80	23.80	47.00	
		0.20	1.80	12.60	31.60	49.00	
		0.30	3.80	9.00	36.00	48.50	
376							
	2	0.60	2.10				
		0.10	2.60				
		0.20	2.70				
		0.50	3.00				

APPENDIX TABLE 14. (CHAPTER 7)

MACROSCHIZONT INDICES OF DAILY LYMPH NODE BIOPSY SMEARS TAKEN FROM ANIMALS

INOCULATED WITH DIFFERENT SIZES OF INFECTIVE DOSES OF T.PARVA (MUGUGA)

GROUP 10^0

ANIMAL	SLIDE	DAYS POST - INOCULATION					
		10	11	12	13	14	15
	1	1.20	3.80	25.00	35.60	48.50	
		1.00	2.30	17.17	35.40	57.00	
		0.90	3.30	15.00	33.00	61.50	
		0.90	4.80	20.50	36.80	57.50	
347							
	2	0.60	4.20				
		0.90	7.00				
		0.30	3.40				
		0.70	5.10				
	1	0.50	3.10	9.75	29.67	51.50	55.00
		0.90	3.00	10.25	29.17	38.75	64.00
		1.00	2.10	8.38	29.67	46.50	78.50
		0.50	3.20	8.25	31.50	58.00	78.50
366							
	2	0.70	3.00	9.25			
		0.20	4.80	9.50			
		1.00	2.70	13.50			
		0.50	2.70	11.25			
	1	1.50	7.70	19.00	34.60	45.50	52.00
		0.90	6.80	18.00	40.20	80.50	38.50
		1.60	9.40	21.88	38.60	57.50	57.00
		1.70	5.60	19.00	33.60	72.50	66.00
377							
	2	1.80	7.10				
		1.30	7.80				
		0.80	5.60				
		0.90	5.80				
	1		0.40	4.70	9.50	32.00	70.50
			1.00	4.10	14.80	24.60	57.50
			1.00	2.00	11.50	38.40	51.00
			1.70	3.40	10.10	31.00	57.00
379							
	2		0.50	1.90			
			0.20	2.10			
			0.30	1.50			
			0.40	2.40			

APPENDIX TABLE 15. (CHAPTER 7)

MACROSCHIZONT INDICES OF DAILY LYMPH NODE BIOPSY SMEARS TAKEN FROM ANIMALS
INOCULATED WITH DIFFERENT SIZES OF INFECTIVE DOSES OF T.PARVA (MUGUGA)

GROUP 10^{-1}

ANIMAL	SLIDE	DAYS POST - INOCULATION						
		11	12	13	14	15	16	17
	1	3.20	5.10	17.13	41.50	66.00	127.00	
		2.30	5.20	16.00	29.75	65.50	97.50	
		1.90	3.60	14.63	31.50	53.50	82.50	
		3.00	5.00	9.00	38.25	74.50	97.00	
354								
	2	2.20	11.50					
		2.90	11.40					
		1.80	10.90					
		2.80	9.80					
	1				1.30	1.30	2.80	7.80
					0.30	1.20	1.60	3.80
					0.50	1.70	0.70	5.70
					1.00	1.00	2.30	3.10
358								
	2				0.20	0.90	1.90	4.00
					0.40	0.80	2.50	4.70
					0.30	0.80	1.80	3.40
					0.60	0.60	1.60	4.80
	1	1.20	5.60	15.00	34.60	80.00	120.00	
		1.20	5.20	11.00	29.60	56.00	95.00	
		1.80	6.90	19.38	30.40	77.50	106.00	
		1.50	8.50	14.38	32.20	60.50	105.00	
372								
	2	0.30	6.60					
		1.10	7.10					
		0.40	7.90					
		0.70	8.40					
	1	2.50	6.90	16.75	30.20			
		2.10	8.20	25.13	30.80			
		1.70	8.40	22.25	29.40			
		1.60	8.00	18.13	31.60			
374								
	2	1.90	13.00					
		1.60	12.30					
		1.40	8.90					
		1.20	11.50					

APPENDIX TABLE 16. (CHAPTER 7)

MACROSCHIZONT INDICES OF DAILY LYMPH NODE BIOPSY SMEARS TAKEN FROM ANIMALS

INOCULATED WITH DIFFERENT SIZES OF INFECTIVE DOSES OF T.PARVA (MUGUGA)

GROUP 10^{-2}

ANIMAL	SLIDE	DAYS POST − INOCULATION							
		13	14	15	16	17	18	19	20
	1					0.90	1.00	2.30	3.20
						0.00	0.70	2.40	4.60
						0.20	0.10	2.40	4.20
						0.40	0.40	2.10	3.50
346									
	2					0.60	2.00	1.80	4.80
						0.10	2.20	2.50	3.70
						0.10	1.50	2.20	3.20
						0.20	1.80	2.20	3.20
	1	0.50	1.40	2.90	2.90	8.10	37.80		
		0.40	1.80	5.40	3.00	6.20	21.60		
		0.10	1.30	1.80	2.00	4.80	42.00		
		0.40	1.50	2.20	3 10	6.30	39.00		
361									
	2	0.80	1.10	6.50	5.30	10.20			
		0.50	1.40	2.10	3.00	7.30			
		0.40	1.40	1.80	6.40	5.60			
		0.60	1.20	2.50	4.80	9.40			
	1	2.20	1.70	19.00	45.25	70.50	56.00	25.50	
		1.80	1.90	22.38	39.00	72.50	60.00	48.50	
		1.50	2.40	15.63	49.00	61.00	54.50	57.50	
		1.60	2.20	14.00	40.75	68.50	66.50	51.50	
364									
	2	1.20	4.30						
		2.30	3.60						
		1.70	5.80						
		1.50	4.80						
	1	0.00	0.80	0.20	0.10				
		0.10	1.70	0.10	0.00				
		0.00	1.00	0.00	0.10				
		0.00	0.80	0.00	0.00				
370									
	2	0.20	0.90	0.30	0.00				
		0.00	1.30	0.00	0.00				
		0.10	1.30	0.00	0.00				
		0.00	1.70	0.00	0.00				

APPENDIX TABLE 17.   (CHAPTER 8)

MACROSCHIZONT INDICES OF DAILY LYMPH NODE BIOPSY SMEARS OF ANIMALS INFECTED WITH
T.PARVA (MUGUGA) TREATED DAY O WITH TETRACYCLINE

GROUP 1

ANIMAL NUMBER	SLIDE	DAYS POST - INOCULATION						
		12	13	14	15	16	17	18
	1		1.20	4.40	8.70	16.33	21.50	
			1.50	2.80	7.30	19.67	17.17	
			1.30	3.90	6.80	12.17	25.50	
			1.60	3.70	7.10	15.17	22.00	
465								
	2		1.00	4.80	10.10			
			0.90	4.30	11.50			
			1.20	4.10	9.50			
			1.00	4.50	9.50			
	1	1.80	6.70	12.13	24.67	48.50	74.00	66.50
		2.10	5.50	11.50	27.00	44.75	98.00	73.50
		2.20	5.30	9.25	27.67	40.50	74.00	70.50
		2.00	6.30	10.50	25.63	42.75	72.00	88.00
481								
	2	1.50	4.80					
		1.50	6.50					
		2.50	6.10					
		2.60	5.40					
	1			1.80	7.70	24.67	37.75	84.50
				2.00	11.00	22.83	46.75	70.00
				2.20	10.25	23.50	50.75	99.00
				1.90	12.63	24.00	55.00	98.00
490								
	2			2.60				
				2.10				
				1.30				
				2.00				
	1	1.50	5.30	16.88	22.83	43.25	69.50	81.00
		1.50	5.70	11.63	28.67	34.75	46.00	125.50
		1.70	7.30	12.88	27.67	42.75	56.00	66.00
		1.80	5.10	12.00	26.67	41.25	54.50	90.00
493								
	2	3.20	6.30					
		2.80	8.40					
		4.40	8.30					
		3.90	7.90					

N.B.   ANIMAL NO. 484 HAD NO SIGNIFICANT MSI THROUGHOUT IT'S REACTION.

APPENDIX TABLE 18. (CHAPTER 8)

MACROSCHIZONT INDICES OF DAILY LYMPH NODE BIOPSY SMEARS OF ANIMALS INFECTED WITH T.PARVA (MUGUGA) TREATED DAYS 0, 1 AND 2.

GROUP 3

ANIMAL	SLIDE	DAYS POST - INOCULATION									
		15	16	17	18	19	20	21	22	23	24
	1	0.30	6.20	9.00	23.50	36.00	41.00	46.00	73.00	77.50	112.00
		0.30	6.40	11.13	21.33	33.40	37.25	46.00	54.50	84.50	100.50
		0.20	6.90	11.38	21.83	38.80	45.25	51.75	60.50	86.50	97.00
		0.40	6.60	10.00	21.67	36.60	41.75	47.75	66.00	83.00	96.00
480	2	0.30	5.20								
		0.50	4.70								
		0.30	5.80								
		0.20	6.00								

N.B. ONLY ANIMAL 480 HAD SIGNIFICANT MSI LEVELS IN THIS GROUP.

APPENDIX TABLE 19. (CHAPTER 8)

MACROSCHIZONT INDICES OF DAILY LYMPH NODE BIOPSY SMEARS OF ANIMALS INFECTED WITH
T. PARVA (MUGUGA) TREATED TWICE DAILY IN PATENT PERIOD

GROUP 4

ANIMAL NUMBER	SLIDE	DAYS POST − INOCULATION					
		12	13	14	15	16	17
	1			0.00	2.60	1.40	
				0.10	2.50	1.70	
				0.20	2.10	1.30	
				0.00	2.70	1.20	
467							
	2			0.30	2.40	0.50	
				0.00	2.40	1.10	
				0.00	1.90	1.20	
				0.00	2.80	0.70	
	1		2.40	17.30	20.00	57.50	99.00
			2.30	16.38	19.67	68.50	78.00
			1.70	16.25	16.00	60.00	79.50
			2.60	19.25	16.00	65.50	110.00
473							
	2		4.10				
			4.50				
			3.30				
			5.40				
	1	1.50	11.50	46.50	59.00	31.00	42.00
		1.60	10.50	41.50	62.00	60.00	68.50
		1.30	9.75	35.75	61.00	48.50	61.50
		1.50	10.88	44.25	62.00	59.00	59.00
476							
	2	2.70					
		3.20					
		1.50					
		1.50					
	1	1.40	7.50	15.25	24.17	23.80	26.60
		1.80	6.20	14.38	41.40	24.20	23.00
		1.60	6.90	15.63	37.40	26.60	25.00
		1.60	6.70	14.88	36.00	25.40	22.00
485							
	2	1.90	6.30				
		0.80	5.80				
		1.10	7.10				
		1.10	6.40				
	1	0.30	3.10	9.20			
		1.20	3.90	11.50			
		1.40	2.20	11.00			
		1.00	2.90	10.10			
494							
	2	1.00	3.00				
		1.10	2.50				
		1.00	3.30				
		1.00	3.10				

APPENDIX TABLE 20. (CHAPTER 8)

MACROSCHIZONT INDICES OF DAILY LYMPH NODE BIOPSY SMEARS OF ANIMALS INFECTED WITH
T. PARVA (MUGUGA) UNTREATED CONTROLS.

GROUP 5

ANIMAL	SLIDE	DAYS POST - INOCULATION								
		13	14	15	16	17	18	19	20	21
466	1	1.50	3.50	10.50	23.00					
		0.90	2.90	8.38	24.50					
		2.20	3.30	13.25	25.50					
		0.90	3.50	11.38	24.65					
	2	0.90	4.70							
		0.50	3.40							
		1.60	3.40							
		0.50	3.10							
468	1		3.40	21.10	51.00	87.00				
			4.70	20.24	40.25	97.50				
			3.60	19.75	43.00	104.00				
			2.20	21.13	42.00	97.50				
	2		2.60							
			3.60							
			4.50							
			3.80							
469	1		0.90	2.50	1.70	0.40				
			1.60	2.10	1.50	0.20				
			0.90	2.00	2.30	0.60				
			1.80	2.30	1.90	0.10				
	2		1.40	3.00	1.00	0.30				
			2.30	2.90	1.90	0.40				
			1.30	2.40	1.70	0.50				
			1.50	3.30	2.00	0.20				
477	1			1.90	2.40	5.00	21.17	16.50	2.10	8.40
				1.70	5.40	6.30	13.50	16.83	1.50	7.30
				1.40	6.20	6.50	15.83	17.50	2.20	6.90
				1.00	4.40	5.10	16.83	15.33	1.90	8.20
	2			4.80	5.50	4.80			1.70	5.50
				4.60	5.20	5.50			1.80	5.70
				3.80	6.30	5.40			1.70	4.80
				4.20	5.70	5.10			2.20	5.30
482	1	1.10	3.20	6.50	17.20	13.00	3.80	6.50	17.30	20.67
		1.00	4.10	7.60	14.00	14.50	9.60	9.30	19.80	19.50
		1.10	5.10	6.50	17.40	14.30	3.70	7.60	16.90	21.83
		1.10	5.20	10.40	13.25	13.90	6.80	8.20	18.40	20.17
	2	1.30	4.30	10.00			6.50	10.40		
		0.90	5.10	12.30			7.30	9.20		
		1.20	3.80	10.10			3.80	7.30		
		1.20	4.50	13.60			8.40	9.90		

REPRINTS

Immunization against East Coast Fever. Inoculation of cattle with Theileria parva schizonts grown in cell culture.

C. G. D. BROWN, W. A. MALMQUIST*, M. P. CUNNINGHAM, D. E. RADLEY, M. J. BURRIDGE, F.A.O. Tick-borne Diseases Project, E.A.V.R.O., Muguga, P.O. Kabete, Kenya.

A method for the *in vitro* cultivation of schizonts of *Theileria parva* in transformed lymphoblasts has recently been described (Malmquist, Nyindo & Brown, 1970). Certain novel and interesting features have been observed when these tissue culture schizonts and their host lymphoid cells are introduced to cattle. The results obtained have indicated that such material might have potential as a means of immunizing cattle against East Coast fever in a manner similar to the *T. annulata* tissue culture vaccine of Pipano & Tsur (1965) against the Israeli theileriosis.

Using the first of three tissue culture lines of the laboratory strain of *T. parva* isolated at Muguga, a number of cattle have been inoculated. The following summarises the results obtained:

(i) Cattle may be infected by the suboutaneous or intravenous inoculation of 10^7 or more tissue culture lymphoblasts containing macroschizonts.

(ii) The infection which develops in cattle following injection of viable tissue culture material is not confined to the macroschizont stage of the parasite as in the case of *T. annulata*. Once established in the cells of the recipient animal the parasite completes its bovine life cycle, with macroschizonts, microschizonts, micromerozoites, and intra-erythrocytic forms observable. The piroplasms have been proven infective for *Rhipicephalus appendiculaius* nymphs, the resultant adult ticks inducing a classical East Coast fever reaction when fed on cattle.

(iii) Inoculations of 10^9 infected cells have produced fatal theileriosis in 2 cattle. The post mortem picture in both these animals was typical of the response to the strain of *T. parva* used at Muguga.

(iv) The majority of cattle, which received inoculations of 10^5 to 10^9 tissue culture lymphoblasts containing macroschizonts, have undergone mild or inapparent reactions followed by increased *T. parva* antibody titres. Such cattle have been shown to be resistant to challenge with the laboratory strain of *T. parva* derived from infective *R. appendiculatus*.

(v) It appears that immunogenesis is dependent on the establishment of the parasite in the host cells and its subsequent multiplication there in. Both infectivity and immunogenicity of the tissue culture material for cattle declined with increasing maintenance and/or passage *in vitro*.

Immunisation against East Coast Fever, the relationship between infective dose and the severity of the disease in cattle.

M. P. CUNNINGHAM, C. G. D. BROWN, R. E. PURNELL, D. E. RADLEY, M. J. BURRIDGE, A. J. MUSOKE and C. SEMPEBWA, F.A.O. Tick-borne Diseases Project, E.A.V.R.O., Muguga, P.O. Kabete, Kenya.

A stabilate of infective particles of *Theileria parva*, obtained from infected ticks using an *in vitro* feeding technique (Purnell & Joyner, 1967), was preserved in liquid nitrogen. One ml. volumes of 3 dilutions of this stabilate (1:50, 1:150 and 1:450) were inoculated into 3 groups of 10 cattle. With increasing dilution of stabilate there was a slight decrease in the morbidity rate in the 3 groups (10, 9 and 8) but a marked difference in the mortality rate (7, 4 and 1). All cattle which reacted and recovered following inoculation with the stabilate material resisted a subsequent challenge.

These results support the prediction of Barnett (1957) and Wilde (1967), that the severity of the East Coast fever reaction in cattle is directly preportional to the number of parasites inoculated by infected ticks.

It has now been demonstrated that:

(a)   suspensions of infective particles of *T. parva* can be harvested from ticks and will regularly infect cattle by inoculation,

(b)   these suspensions can be preserved at low temperatures,

(c)   the infectivity of the preserved suspensions can be established by titration,

(d)   infections of predictable severity can be produced in groups of cattle by varying the volume of preserved suspension inoculated.

We therefore consider that we are in a position to investigate the possibility of immunising cattle against *T. parva* infection using 3 different approaches:

(i)   Inoculation of a small number of preserved infective particles, sufficient to produce a mild reaction and subsequent immunity to challenge with infected ticks.

(ii)   Inoculation of infective particles attenuated by exposure to gamma rays.

(iii)   Inoculation of preserved infective material followed by chemotherapy.

velopment of the parasite in the engorged nymph, have been disputed in many respects by all subsequent workers on *T. parva* and allied parasites. Similarly, Reichenow's (1940) interpretations of the development of the parasite in the salivary glands of the tick are disputed by Martin, Barnett and Vidler (1964). The techniques used by the latter authors and their quantitative observations have stimulated much of our work (Purnell and Joyner, 1968).

Our current experimental programme is divided into three distinct parts. Firstly, investigations are in progress on the relationship between the level of parasitaemia in the *T. parva* infected animals upon which immature ticks have engorged and the infection rates observed in the salivary glands of the ticks in their subsequent developmental stage. Concomitant with this work, techniques have been developed for artificial feeding of nymphal ticks on fresh and preserved blood.

Secondly, observations are being made on the relationship between tick-feeding and the development of parasites in their salivary glands. Three distinct stages of the parasite have been observed, including an intermediate cytomere-like stage where budding-off of infective particles from parasitic masses occurs. The application of different staining techniques to the parasites in the salivary glands has clearly indicated their composition. We hope shortly to be in a position to apply some of the elegant techniques of ultra-thin sectioning used by Friedhoff and Weber (1969) on *Babesia* spp., and this should undoubtedly clarify this aspect of the life cycle.

Thirdly, investigations are in progress to discover the most suitable techniques for collecting, identifying and quantitating the infective particles emitted by the ticks. We are able to collect infective particles both in feeding substrates (Purnell and Joyner, 1967) and in tick saliva (Purnell, Branagan and Radley, 1969), and are currently using a variety of methods to identify and quantitate them.

Theileria parva: Quantitation of the parasite in the tick *Rhipicephalus appendiculatus*.

R. E. PURNELL, D. E. BACKHURST, K. P. BAILEY, C. D. H. BOARER, D. BRANAGAN, M. P. CUNNINGHAM, L. P. JOYNER, M. A. PEIRCE, D. E. RADLEY, and B. WOOD, East African Veterinary Research Organization, Muguga, Kabete, Kenya.

Observations on the life cycles of parasites may be made for two reasons, either to further elucidate the processes involved in replication of the parasite so that its taxonomic position may be clarified, or to quantitate a particular part of the life cycle so that the results obtained can be used directly in other experiments. Whilst the former approach is clearly more balanced and therefore more satisfactory and, futhermore, will naturally lead up to the latter, we have been obliged to concentrate on quantitation of the parasite. This is because our results are needed for immediate application to the work of the UNDP/SF Tick-borne Diseases Project, whose objective is the production of a vaccine against East Coast fever of cattle caused by *Theileria parva* infection.

Three previous groups of workers have made observations on the life cycle of *Theileria parva* in its tick host, but there are considerable gaps in our knowledge of the development of the parasite. The original description of this part of the life cycle was by Cowdry and Ham (1932) and their observations, particularly on the de-

lation into the bovid requires a certain length of time before it can be detected in the L.D.L.N. There could be several explanations for this, the most likely being either (a) the parasite is changing its environs from an arthropod's cell to a mammalian cell and a resting phase for adjustment is required or (b) the parasite is present in the L.D.L.N. in a small focus and escapes detection when samples and smears are made.

From the survival times and T_{10}s recorded it is seen that there is a marked difference in these values dependent on the inoculum and number of infective particles received. That the T_{10} value is variable is contradictory to the findings of Jarrett et al. (1969) where an application of 1,000, 100, and 10 ticks in three different experiments, they observed a constant multiplication rate of the parasite, $T_{10} = 3$. The author considers that the results imply that if a large number of infective particles become established in the host, the host reaction against the parasite has minimal effect on the parasite's subsequent increase in numbers. However, with decreasing numbers of infective particles inoculated, the host is able to exert an effect on the parasite which manifests itself as a slowing down of the parasites' replication rate (T_{10} would increase). It may be argued that giving even smaller numbers of infective particles would have resulted in recovery of the animals.

Examinations of the blood smears revealed piroplasms in eight animals on day 13 of the infections, in one animal on day 12, and no piroplasms were detected in the blood of the three animals which died on or before day 13. This quite constant prepatent period to appearance of piroplasms agrees with the conclusions of Jarrett et al. (1969) that the switch to piroplasm production is time-dependent and not dose-dependent.

The results of these experiments are not conclusive since only pairs of animals were used in each group and further investigation is necessary using larger numbers of animals. Also inoculations of smaller numbers of infective particles will be undertaken.

In conclusion, this technique provides a method of measuring the host reaction in controlled circumstances. It will be useful in immunisation trials, chemotherapeutic studies, and other investigations.

Studies on the growth rate of *Theileria parva* in the bovine host.

D. E. RADLEY, F.A.O. Tick-borne Diseases Project, East African Veterinary Research Organization, Muguga, Kenya.

The prepatent periods to appearance of schizonts in the L.D.L.N., were fairly constant both between and within experiments. Also time in days to a rise in body temperature of 103 F or over was fairly constant within experiments.

It would appear then that the parasite on inocu-