DOCTOR OF PHILOSOPHY

Investigations into the epidemiology of ovine psoroptic mange (scab) in Great Britain : with special reference to otoacariasis and the taxonomy of the genus Psoroptes

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Investigations into the Epidemiology of Ovine Psoroptic Mange (Scab) in Great Britain

(with Special Reference to Otoacariasis and the Taxonomy of the Genus *Psoroptes*).

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SUMMARY

The epidemiology of ectoparasitic mites within the genus *Psoroptes* infesting domestic British livestock was investigated. The ear mite *P. cuniculi* was found to be common in sheep, goats, rabbits and probably horses. The body mite *P. ovis* was endemic within the National sheep flock, but was a sporadic, imported problem for cattle, with the last case caused by *P. natalensis* and not *P. ovis*. Equine mange (*P. equi*) has been eradicated from Britain. With the exception of *P. ovis,* mites from all other host species were not infestive to sheep. Comparative measurements of the male L_4 outer opisthosomal setae revealed no statistical differences between ovine and bovine *P. ovis* and rabbit *P. cuniculi,* but *P. cuniculi* from the ears of sheep and goats, *P. equi* and *P. natalensis* were all statistically different from populations of sheep *P. ovis* and rabbit *P. cuniculi*. Setal lengths for goat and sheep ear mites were predominantly below 74.0 μm but mites isolated from the ears of rabbits fell into short ("typical ear canker") and long (generally "extra auricular") setal forms. Some "extra auricular" populations of *P. cuniculi* infesting rabbits were shown to contain sub-populations of *P. cuniculi* (non infestive to sheep) and *P. ovis* (infestive to sheep) and these populations could be selected for by ivermectin. A narrow band of bovine and ovine *P. ovis* divided the two forms of rabbit *P. cuniculi*. Subclinical, rapid growth and decline phases of disease were recorded for *P. ovis* infesting sheep, the duration of these phases varying with the virulence of the infesting population. Highly significant differences were observed between setal lengths for low, medium or high virulence population (73.6 μm, 80.2 and 90.5 μm, respectively). The scab mite *P. ovis* was also recorded in the ear canals of 38.6% of infested sheep, and prevalence was greater the higher the virulence of the infesting population. The majority of infestations were recorded in early rapid growth phase when lesions only covered 11.0 to 43.9% of the body and as far away as the mid back (and as early as 28 days after challenge) and only 22.6 % recorded in the decline phase (lesions 98.9% to 100.0% body cover), when the pinnae themselves could be infested. *P. ovis* and *P. cuniculi* are therefore synoxenous, occuring sympatrically on the same host (sheep) and may be syntopic (sharing the same habitat, ie the ear canal). *P. ovis* and *P. cuniculi* infesting sheep are not reproductively or ecologically isolated but are phenotypic variants of the same species. Thus populations high in *P. cuniculi* act almost as commensals of the ear canal and populations high in *P. ovis* act as highly pathogenic agents of mange. It is also suggested that the type species *Psoroptes communis* should be re-instated, with two variants infesting sheep, *P. communis* var *ovis* and *P. communis* var *cuniculi.*

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DEDICATION

To my Father

Terence David Bates.

1927 to 1984

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Chapter One

Psoroptic Mange: An Overview

1.0. Summary.

Mites within the genus *Psoroptes* are cosmopolitan, obligate ectoparasites, causing a debilitating dermatitis, involving hair or wool loss and a pruritic scab formation. Ovine psoroptic mange (sheep scab) is one of the oldest ectoparasite infestations known and for over 1000 years it has evaded effective control in the British Isles. The Report of the Sheep Scab Working Party (1988) demonstrated that the origins of over 18.5% of scab cases remained obscure. Throughout the history of sheep scab many authors have attributed the failure to eradicate or control the disease to an unknown facet of *Psoroptes* biology (eg. prolonged survivability of the mite off the host, a resting stage in the *Psoroptes* lifecycle (similar to the deuteronymph (hypopus) of free living astigmatid mites) or the presence of a "latent or quiescent phase of infestation." The sheep scab mite has been shown to be only infestive to other sheep within 16 to 17 days off the host and the presence of a resting instar or phase in infestation has been disproved. Attention also turned to the role of other hosts, (cattle, wild rabbits, deer, and domestic and feral goats, horses and ponies) as reservoirs of disease. Obsure outbreaks of sheep scab could also be due to the relative "virulence" of the infesting mite population. In January 1989 a case of psoroptic otacariasis was reported within a flock in the Scottish Borders, with no history of clinical sheep scab and the mites did not initiate clinical sheep scab on transfer to the bodies of sheep. Sheep can be host to two species of *Psoroptes*: *P.ovis* infesting the body and *P.cuniculi* infesting the ear canal. These two species have since been shown not to be reproductively isolated, but probably distinct races of the same mite. Is it possible therefore that *Psoroptes* in the ears of sheep could be reservoirs of clinical sheep scab?

2.0. Introduction.

Astigmatid mites in the family Psoroptidae (Sub-class Acari: Super-order Actinotrichida: Order Astigmata) are non burrowing mammalian ectoparasites. Four genera are currently recognised: *Psoroptes* spp parasitising the ears and bodies of herbivores (Sweatman, 1958a), *Chorioptes* spp parasitising the bodies (and occasionally the ears) of herbivores (Sweatman, 1957), *Otodectes* parasitising the ears (and occasionally the body) of carnivores (Sweatman, 1958b) and *Caparinia*, represented in Britain by *C tripilis* parasitising the European hedgehog (*Erinaceous europaeus*) (Fain and Till, 1987). All species within the genus are characterised by strongly developed legs bearing, in all stages, funnel shaped suckers (pulvilli) on long, three segmented pretarsi (peduncles). The legs of *Chorioptes*, *Otodectes* and *Caparinia* terminate in large bell shaped pulvilli on short pretarsi. Mites within the genus *Psoroptes* are cosmopolitan, obligate ectoparasites, causing a debilitating dermatitis, involving hair or wool loss and a pruritic scab formation. Adult female mites are just visible to the naked eye, approximately 750 μm in length (Figure 1.1). When colonising the ear canals of all hosts and the bodies of sheep these mites are pearly white and globular in appearance. However they may appear black to dark red when colonising the bodies or pinnae of other hosts, due to the ingestion of red blood cells.

3.0. Classification

The first recorded classification of *Psoroptes* was by Megnin (1877), describing the body mites of sheep, horses and cattle and the ear mites of rabbits as variants of the one species, *Psoroptes longirostris* (ie. *P. longirostris* var *ovis*, *P. longirostris* var *equi*, *P. longirostris* var *bovis*, *P. longirostris* var *cuniculi*). Raillet (1893) also regarded all *Psoroptes* mites to be variants of a single re-defined species, *Psoroptes communis* (eg *P. communis* var *ovis*). Neveu-Lemaire (1938) yet again regarded *Psoroptes* as variants of one species, but it was re-defined as *Psoroptes equi* (eg *P. equi* var *ovis*).

In 1922 Hirst (1922) described only two species of *Psoroptes*: *P. natalensis* (1919), first described from specimens found on cattle in Natal Province, South Africa

and *P. communis* (Furstenburg, 1861). Hirst (1922) examined large numbers of *Psoroptes* from various domestic hosts and, although he was the first to use the length of the male L_4 outer opisthsomal setae (L_4OOS) to differentiate species, he found little structural differences between them. He suggested that, with the exception of *P. natalensis* which are morphologically different (the L_3 and L_4 outer opisthsomal setae (L_3OOS and L_4OOS) of the male being distinctly flattened and blade like), the remaining mites in the genus should be regarded merely as races or slight varieties of a single species (*P. communis*). Roberts (1952) stated that the ear mite of horses, *P. hippotis* can be differentiated from *P. equi,* the body mite of horses, by the basal arrangement of the outer opisthosomal setae of the male. In *P. equi* the bases of setae L_2, L_3 and L_4 are contiguous, while in *P. hippotis* only L_2 and L_3 are contiguous. No subsequent author has utilised this method of morphological differentiation.

The extensive morphological studies by Sweatman (1958a) reduced the number of specific names to only five. These were recognised by the comparative length of the male L_4OOS and by host and location of the mite on the host; species were divided into those infesting the body and those infesting the ears of their hosts. The classification devised by Sweatman (1958a) is as follows:-

Body Mites

a) *Psoroptes ovis* (Hering 1838), synonym *P. bovis* Gerlach (L_4OOS = 74.0 to 258.0 μm), is a cosmopolitan body parasite of domestic sheep, bighorn sheep (*Ovis canadensis*), cattle and horses (Sweatman, 1958a). *P. ovis* has also been recorded infesting the bodies of giraffe (Burr, 1984) and the dromedary (Gabaj *et al.,* 1992).

b) *Psoroptes equi* (Hering 1838) Gerlach (L_4OOS = 333.0 μm), is a body mite of horses, mules and donkeys (Sweatman, 1958a).

c) *Psoroptes natalensis* (Hirst 1919) (L_4OOS = 250.0 to 350.0 μm), a body mite of domestic cattle, zebu cattle and horses (Sweatman, 1958a) and Indian water buffalo (*Bubalus bubalus*) (Shastri and Ghafoor, 1974).

Ear Mites

a) *Psoroptes cuniculi* (Delafond 1859) (L_4OOS = 64.0 to 164.0 μm), synonyms *P. caprae* and *P. hippotis* (Sweatman, 1958a). The ear canker mite of rabbits, goats, sheep and horses (Sweatman, 1958a). *P. cuniculi* has also been recorded infesting the ears of bighorn sheep (*Ovis canadensis mexicana, O. canadensis canadensis*) (Lange *et al.,* 1980, Kinzer *et al.,* 1983), blackbuck antelope (*Antelope cervicapra*) (Wright and Glaze, 1988), impala (*Aepyceros melampus*) (Rauchbach cited by Yeruham *et al.,* 1985), mountain goat (*Capra ibex nubiana*) (Yeruham *et al.,*1985), mule deer (*Odocoileus hemionus*) (Roberts *et al.,* (1970), stone sheep (*O. dalli stonei*) (Foreyt, 1997), water buffalo (*B. bubalus*) (Shastri and Ghafoor, 1974), white tailed deer (*Odocoileus virginianus*) (Strickland *et al.,* 1970; Schmitt *et al.,* 1982) and yaez (*Capra ibex nubiana* cross *Capra hircus*) (Yeruham *et al.,* 1978).

b) *Psoroptes cervinus* (Ward 1915) (L_4OOS = 145.0 to 354.0 μm), is an ear mite of bighorn sheep and wapiti (*Cervus elephas canadensis*) (Sweatman, 1958a) and the American elk (*Cervus canadensis* Nelsoni) (Hepworth and Thomas, 1962). *P. cervinus* has not been recorded in Britain.

Undefined Species

A *Psoroptes* mite, tentatively described as *P. equi* var *leporis* was isolated from the European hare (*Lepus capensis* (*L. europaeus*) by Vanek and Novakova (1959). *Psoroptes auchinae* (*P. communis* var *auchinae*) has been identified from the ears of alpaca (*Llama pacos*) in South America (Chavez and Guerrero, 1965; Fowler, 1989) and ears and bodies of llamas (*Llama glama*) (Alverado *et al.*, 1966; Foreyt *et al.,* 1992; Guerrero and La Rosa, 1962). Fain (1970) isolated a *Psoroptes* mite from the head of African Buffalo (*Syncerus caffer*) and named it *P. pienaari*. All three mites have not been subjected to modern re-analysis and their actual status is still unknown (Strong and Halliday, 1993).

The classification of the *Psoroptes* described by Sweatman (1958a) thus suggests that many domestic host species can be infested with more than one species of *Psoroptes*, eg. *P. cuniculi* in the ears (goats, horses rabbits and sheep) and the body mites *P. ovis* (sheep, cattle), *P. equi* (horses) and *P. natalensis* (horses, cattle).

4.0. Ovine Psoroptic Mange (Sheep Scab)

Ovine psoroptic mange or sheep scab (also known by the descriptive names of belt, shab, tag or rubbers) is said to be one of the earliest known ectoparasitic infections. Sheep scab has been known since ancient times. It was known to Moses and has been documented by Cato, Virgil, Pleno and Columnella and is refered to in the Bible (Leveticus XXII, 22). The Arabs were aware of the mite as long ago as 1174, but they did not recognised it as the cause of disease (Stockman, 1912). It was not until the early 19th Century that the mite was positively identified as the causitive agent of scab (Walz, 1809), given the name *Psoroptes ovis* (Hering 1835) and it's life cycle deduced in 1857 (Gerlach, 1857). *P. ovis* is a cosmopolitan obligate ectoparasite occurring, with the exception of Australia and New Zealand, in all the sheep rearing countries of the world. Infestations are characterised by a debilitating dermatitis involving wool loss and pruritis and scab formation and for over a thousand years man has struggled to control the disease (Table 1.1).

The first recorded legislation for the control of sheep scab in Great Britain was by King Hywel Dda in 949 AD, prohibiting the sale of scab affected sheep between the months of November and April and that sheep should not be allowed to graze land previously occupied by infested sheep during the last 7 years. Hywel Dda's edict was unsuccessful and by 1297 the English chronicler Hemingburgh stated that Flanders, dependent on English wool for it's own survival, became almost bankrupt due to the sheep scab epidemic in England. In 1298 another chronicler reported an incident where over 500 sheep from a flock of 645 died of scab (Urquart, 1983). It must be borne in mind though, that these deaths may not have been due to sheep scab, as the mite was not identified as the cause until 1809 and therefore differential diagnosis was not possible.

In 1869 sheep scab was made notifiable in Great Britain and records began to be made of outbreaks and their distribution. By 1890 their was a marked increase in cases, with outbreaks fluctuating between 1207 and 3536 per year (Watson, 1976). This increase was to be expected as the Government and the Sheep Industry were positively looking for the disease. The position in the Great Britain was further complicated by large numbers of infested sheep being imported from Ireland, North America and the Argentine (Page, 1969). In 1895 scab was detected in 370 cargoes totalling 420,000 live sheep arriving from North America and the Argentine. Of these 83,000 (19.8%) were shown to be scab infested (Page, 1969). The Sheep Scab Order 1898 required local authorities to employ veterinary surgeons for expert diagnosis and this led to a (probably coincidental) fall in cases, from 2514 in 1898 to 1379 in 1901 (Page, 1969). Following the need to introduce dipping as a means of eradicating the disease, a scab order was made in 1905 culminating in the Sheep Scab Order of 1907. The Order required the compulsory dipping of all infested sheep, empowered local authorities to require a single, double or triple annual compulsory dippings. The legal immersion time for sheep under the 1907 order was only 30 seconds. In 1914 and again in 1920 double dipping orders resulted in the continued reduction in cases and in 1926 the legal immersion time was increased to one minute.

The Sheep Scab Order of 1928 required the notification of disease, compulsory treatment or destruction of infested animals, isolation and treatment of

other animals in the infested and neighbouring flocks and controls over the movement of animals out of the infested area and MAFF approval of dip formulations. Four active ingredients were approved: tar acid/tar oil dips, arsenic dips, lime-sulphur dips and tobacco dips, all of these required a second dip within 14 days to kill emerging eggs (Spence, 1951). The 1928 Sheep Scab Order was ammended in 1948 to allowed dip formulations containing the organochlorine acaricide γ BHC (γ HCH, lindane) to be officially approved for use as statutory single dipping formulations, but allowed the continued use of the old double dipping formulations (Page, 1969 and Spence, 1951). γ BHC dips, together with rigid official enforcement, good sheep husbandry and restricted animal movement were responsible for the (apparent) total eradication of sheep scab from Britain in 1952. Kirkwood (1985a) postulated that eradication was achieved, not because every sheep had been dipped but because every infested sheep had been dipped. The continued presence of chewing lice (*Bovicola (Damalinia) ovis*) proved this.

Scab re-appeared in Britain in 1973 and during the intervening 20 years a whole generation of farmers and veterinary surgeons had never seen the disease (Loxam, 1974), routine precautions against scab were neglected and familiarity with its signs and symptoms were lost (Anon, 1975). The primary outbreak in 1973 was identified as a dealer handling large numbers of sheep, including several recently imported consignments from the Irish Republic. Initially the condition was diagnosed as mycotic dermatitis (*Dermatophilus congolensis*). Delay in confirming disease was due primarily to inexperience (Loxam, 1974), inefficient dipping at lairage (Watson, 1976) or possibly due to an avirulent mite strain. All documented information suggests that the strain imported from Ireland was extremely low in virulence. The VLA Reference Isolate of *P. ovis* was isolated from these early infestations and may be related to the Abbotstown strain maintained by the Department of Food and Forestry (DAFFs), Veterinary Research Laboratories (VRL), Dublin.

γ BHC based dips continued to be used until 31 December 1984, when they were voluntarily withdrawn from the UK, following pressure from France over possible residues in lamb exported from Britain (Henderson, 1991). Up to the mid 1980s lindane was the major acaricide in the war against sheep scab worldwide, and

continues to be used in many countries (including France!). Eventually scab control changed to the use of organophosphate (OP) based formulations (Page, 1969).

The OP formulations containing diazinon or propetamphos were the next generation of insecticides to appear on the market. Diazinon was approved for sheep scab control in 1981 (Kirkwood and Quick, 1981), although it had been licensed for blowfly and lice control since the early 1970's. Propetamphos was approved for scab, lice and blowfly control in 1982 (Kirkwood and Quick, 1982). OP dip formulations began to be incriminated in post dipping illness in stock owners and contractors (Anon, 1989), consequently safer insecticides were being investigated for their efficacy against scab, lice and blowfly strike.

In 1987 the first non OP dip, containing the synthetic pyrethroid (SP), flumethrin, was licensed for scab and lice control (Kirkwood and Bates, 1987). SPs have the advantage of having excellent selectivity and high toxicity to arthropods and the relative safety to mammals. Unfortunately they are extremely ecotoxic.

Since the re-introduction of scab in 1973 there were a variety of policy measures aimed at eradicating the disease for a second time (Table 1.1). The incidence of the disease continued to increase and between 1984 to 1989 a regime of two supervised compulsory dippings was implemented: one in the summer and one in the Autumn. This concentrated period of bi-annual dipping was hoped to totally eradicate sheep scab from Great Britain. Eradication was not achieved, but the regime succeeded in reducing the incidence of scab to an historic low of just 36 cases in a total of 17 counties. In 1989 a working party was set up by MAFF (including the author) to discuss future policies for scab control. The working party posed some important questions, namely: a) why should scab remain notifiable?; b) It is easily controlled, why do anything?; c) It is not zoonotic; d) The compulsory use of OP based dip formulations may be an unnecessary health risk; e) SP based dip formulations may have a severe ecological impact; f) adequate animal health legislation is in position and g) the effects of the European Union and the Single Market. The EC council directive on animal health conditions governing the Intra - Community trade in ovine and caprine animals made no mention of sheep scab!

During the period of bi-annual dipping only 20% of cases were reported directly by the farmer (the rest identified by Government or private veterinary surgeons). This suggested that flocks were not inspected regularly, or the owners were not aware of the symptoms of scab, or they were unwilling to report disease. The growing antipathy to OP dips may also have been important. During the period of bi-annual dipping MAFF also conducted a "fleece survey" to monitor the efficiency of plunge dipping. Reports for the compulsory dipping periods for 1985 revealed that only three out of four sheep were dipped properly (Anon, 1986). In the summer period 77% of Welsh flocks were dipped satisfactorily, 10% were marginal and 11.5% failed. This improved to 86%, 5% and 9% respectively for the winter dipping. Nationally 74% of sheep were dipped effectively compared to 63% in 1984. Thus 26% to 37% of flocks were inadequately treated.

In 1989 compulsory dipping was reduced to the Autumn only, and between 1990 and 1991 there was one "self certificated" (ie. unsupervised) compulsory dip in the Autumn. Autumn dipping saw outbreaks treble to 31 counties. There were 32 million sheep in Britain within 92,000 flocks in 1983, when scab was at its peak. Statistically this only represented 0.2% of UK flocks and 1:10,000 sheep infested. Without Government control this could have been far worse and could cost the sheep industry £600 x 10^6 over thirty years (Kirkwood, 1986). In 1984, 1987 and 1992 scab was recorded in 0.14%, 0.03% and 0.1% of flocks in Great Britain respectively. MAFF, deciding that total eradication was not tenable (and too expensive, with the estimated MAFF cost of two annual dippings at £12 x 10^6 together with £2.1 x 10^6 for Local Authority enforcement) decided that sheep scab should be deregulated and that dipping should no longer be compulsory with the responsibility for scab resting directly with the farmer himself.

Sheep scab was deregulated in June 1992 (MAFF, 1992), despite protests from the British Veterinary Association (BVA), Sheep Veterinary Society (SVS), the National Office for Animal Health (NOAH) and the National Sheep Association (NSA). The only involvement of MAFF being to continue to prosecute farmers on

animal welfare grounds under the Agriculture (Miscellaneous Provisions) Act 1968 (MAFF 1992, Sargison *et al.,* 1995).

In the early 1990s the compulsory plunge dipping of sheep was open to question. Dipping was time consuming, labour intensive and stressfull to the sheep. OP dip formulations have been incriminated in the post-dipping illness in stock owners and dipping contractors (Anon, 1989a) and the disposal of large volumes of used dipwash, both OP and SP, was a potential hazard to the environment. A study by Stephens *et al.,* (1995) postulated that repeated exposure to OP based pesticides appeared to be associated with subtle changes in the human nervous system and that measures should be taken to reduce exposure to OP as far as possible during agricultural operations. With the deregulation of sheep scab, stockowners were no longer obliged to plunge dip and sought alternative methods of scab control to fit in better with their farming programme (Bates, 1993).

Prior to deregulation in 1992 all dip formulations for the control of sheep scab had to be Ministry Approved: ie. cure existing infestations by killing all (100%) extant *P. ovis* immediately and resolve the lesion within 56 days and persist in 1.0 cm fleece and protect against re-infestation for at least three clear weeks. Deregulation also saw the withdrawal of the appoval protocol: under European Union legislation only 95% to 98% cure was required and protection was no longer required (Anon, 1994). Products were no longer approved, only licenced. In 1992 the systemic endectocide, ivermectin (MSD Agvet, derived from *Streptomyces avermitilis*) was licensed. Double subcutaneous injections are effective in curing sheep scab but have little or no protective capacity (Bates and Groves, 1991; O'Brien *et al.,* 1993; Soll *et al.,* 1992).

In 1992 the sales of OP dip were controlled by the "certificate of competence," which although addressed the problem of human health, also complicated the epidemiology of sheep scab. More farmers were turning to non OP products, either because they were genuine OP suffers or they were unwilling to register for the certification course. The sales of flumethrin and ivermectin increased parallel to their mis-use. The requirement not to notify disease, the responsibility to

treat left with the farmer, the lack of veterinary involvement and the increased incidence of chewing lice (*Bovicola ovis*) resulted in the increased use of unlicenced SP pour-ons. Thus exposing mite populations to sub-lethal concentrations of SP.

In South America the sheep scab mite developed resistance to dips containing γ BHC (lindane) in 1962 (Ault *et al.*, 1962) and to diazinon in 1965/66 (Rosa *et al.*, 1970). In 1994 two isolates of *P. ovis*, from two geographically separated areas of the UK (Somerset and Caithness) were found to be resistant to flumethrin at the recommended use rate of 44 ppm and at the stronger tick rate of 66 ppm (Synge *et al.,* 1995 and Bates, 1998). Following the identification of these two isolates, a further two flumethrin resistant strains of *P.ovis* were identified in 1995, both originating from Cumbria (Bates, 1998). It is interesting to note that after over a year of laboratory culture the Caithness and Somerset strains are still as resistant to flumethrin as they were on isolation. In 1995 and 1996 two isolates resistant to the OP, propetamphos were identified, again from the Caithness area of North Scotland (Clark *et al.,* 1996 and Bates, 1998).

In June 1995 a press release issued by MAFF stated that a State Veterinary Service (SVS) survey in 1994 showed that 177 batches of sheep suspected of scab were presented at market (13 serious welfare cases), decreasing to only 47 batches in 1995 (6 serious welfare cases). Added to these figures in 1994 53 other cases of scab were identified by the SVS together with 254 cases investigated by private veterinary surgeons: giving an estimated total of 484 cases of sheep scab in 1994. In 1995 34 other cases of scab were identified by the SVS together with 595 cases investigated by private veterinary surgeons: giving an estimated total of 676 cases of sheep scab in 1995. Caution must be excercised when interpreting these figures. There was no compulsory identification of the scab mite, consequently a percentage of cases could be attributed to chewing lice (*Bovicola ovis*), increasing in prevelence in Britain post scab deregulation (Bates, 1999).

Also in 1995 another SP dip, high cis Cypermethrin (HCC) was licensed for the British market (O'Brien, 1997). Unfortunately populations of *Psoroptes* resistant to flumethrin already showed side resistance to HCC (Bates, 1998). In September

1997 another endectocide, doramectin, was licenced for scab control and was both curative and protective at a single intramuscular injection (Bates *et al.,* 1995 and McKenzie, 1997). Single or double subcutaneous injections of the macrocyclic lactone, moxidectin (derived from *Streptomyces cyaneogriseus*) have been shown to both cure and protect against sheep scab (O'Brien *et al.,* 1994b, 1996; Williams and Parker, 1996; Parker *et al.,* (1999)).

Since compulsory annual dipping was abandoned in Britain (and the Republic of Ireland) the problem of sheep scab has received much attention (O'Brien, 1996a). The infrastructure involved in these schemes, that accompanied the relevant treatment regime, has mainly disappeared. It is now impossible to quantify the extent of spread. However it is unquestionable that there has been an increase geographically and numerically in outbreaks of the disease (O'Brien, 1996).

The implementation of new legislation (the Sheep Scab Order (1997)) and recent advances in the development of new acaricides and acaricide application techniques, together with the growing concerns in operator safety, the environment, acaricide resistance and animal welfare have shown the need to fully understand the biology of the *Psoroptes* mite infesting sheep. Sheep scab can occur in even the best managed flocks and since deregulation and the removal of compulsory dipping the disease has been reported throughout the country.

5.0. Pathology

It is still not known exactly how *Psoroptes* mites feed. Scanning electron microscope studies of *P. cuniculi* and *P. ovis* have revealed an arrangement of chelicerae, with a pre-oral trough and a pharyngeal lumen similar to solid feeding mites (eg *Acarus siro*), as well as a sponge like "lapping" organ (the pseudorutella) (Rafferty and Gray, 1987). Blake *et al.*, (1978) suggested that the mites cut, tear and abrade the host epidermis with the toothed, chelate, chelicerae, causing the flow of serous exudate. Rafferty and Gray (1987) postulated that the pseudorutella also helps to abrade the skin and channel fluid via the pre-oral trough to the pharynx. Salivary glands and associated ducts have not been observed in a detailed study of the mouthparts of *P. ovis*, salivary secretion are not therefore involved in the formation of the feeding lesion. Rafferty and Gray (1987) concluded that *Psoroptes* spp mouthparts are adapted for both liquid and solid feeding. *Psoroptes* spp are therefore pool feeders (telmophages) feeding on lysed tissue rather than vessel feeders (solenophages) feeding directly on blood vessels or lymphatics.

P.ovis infesting cattle and laboratory rabbits are known to ingest blood serum and erythrocytes (Wright and Deloach, 1980, 1981), but this has not been observed in *P. ovis* infesting sheep. DeLoach and Wright (1981) summarised *Psoroptes* feeding by stating that ingestion of whole blood is not specific to a particular *Psoroptes* spp but may be common to *Psoroptes* when feeding on rabbits. Wright and DeLoach (1981) postulated that the thinness of the skin in the rabbits ear and the larger number of peripheral blood vessels allowed blood to be ingested more readily. Cattle skin is thicker and more difficult to penetrate together with fewer and deeper capillaries reducing the opportunity for the mites to penetrate and ingest erythrocytes. The chelicerae of *P. ovis* measure between 97.0 to 158.0 μm in length but the skin of sheep and cattle is 58 μm and 73 μm thick (Kirkwood 1985a).

Histological examination of "snap frozen" scab biopsies suggested that *P. ovis* on sheep feed exclusively on skin lipid (Sinclair and Kirkwood, 1983), but limited enzyme assays of mite extracts showed no significant lipase activity (Bates *unpublished data*). Lipid is therefore probably not a significant part of the mite's diet: it may be ingested but not necessarily digested. It is now thought that the mite grazes

the skin around the moist periphery of the lesion, taking in nutrients with the serous exudate, skin secretions and lipid. Mathieson (1995) confirmed experimentally that *P. ovis* ingests serum components likely to be present in the surface exudate associated with clinical sheep scab. Exact knowledge of how the mite feeds is essential in the development of new control methods. The frequency of feeding is still unknown. Do adult females feed and engorge once, directly after mating/moulting or do they feed continuously or intermittantly? This information is essential in assessing the efficacy of systemic acaricides and the development of immunological methods of control..

It is now generally agreed that the progressive lesion seen in cases of scab is not directly the result of mite feeding but is in fact a form of allergic dermatitis initiated by the mite allergens (most likely the faeces). Antigenic material contained in the faeces cause an inflammatory response in the skin. In this inflamed condition skin breakages could occur, mainly as a result of host scratching but also through small haemorrhages caused by the abrasive action of the mite's mouthparts. These skin breakages result in the leakage of serum, with accompanying scab formation and skin thickening (Rafferty and Gray, 1987). *P. ovis* exploits the allergic reaction: the heat and humidity produced by the inflammation forming the micro-climate needed for mite survival and the leakage of serous exudate forming the basis of the mites nutrition. In short the mite cannot survive without the inflammation.

As in all acarines, the chief nitrogenous catabolite of *P. ovis* is the highly insoluble purine, guanine. Excreta is voided in the form of a distinct, dry, solid faecal pellet, surrounded by a peritrophic membrane (PM). The PM remains intact in air but readily breaks down in water (Evans, 1992). In astigmata the PM is considered to be secreted by cells of the mid gut wall (Mathieson, 1995). The arthropod PM typically consist of a meshwork of chitin containing microfibres embedded in a matrix, the principle constituents of which are proteins, glycoproteins and mucopolysaccharides (Spence, 1991 and Peters, 1992). The entire digestive system of *P. ovis* contains a significant population of luminal bacteria and these can be found in large numbers in the faecal pellets (Mathieson, 1995), breakdown products from these bacteria may also be allergenic.

The clinical symptoms of scab in the early (subclinical) stages include restlessness, rubbing against fence posts etc, soiled and stained areas of wool (particularly on the shoulders), head tossing and deranged or tagged fleece. These could also be the symptoms of other ectoparasite infestations (eg chewing lice *(Bovicola ovis)*, blowfly strike (*Lucilia* spp), fly bites, even scrapie!). Sheep with sub-clinical scab can also look perfectly normal and can easily be introduced to a flock via market purchases. In the later stages of *Psoroptes* infestations, the rubbing and head tossing become more excessive, areas of wool loss appear together with open, bleeding wounds (Figure 1.2). Sheep rapidly lose condition and epileptiform fitting may be evident (Bygrave *et al.,* 1993). Numbers of infested sheep within the flock can vary from one or two in the early days of infestation, to the whole flock as the disease takes hold (depending on their immune status of each individual sheep). Throughout the flock there will be animals with non established lesions (that will eventually die out), young subclinical lesions together with animals with obvious extensive disease. All sheep should be considered to be infested and the whole flock should be treated for scab. One missed sheep could reinfect the whole flock.

6.0. The Effects of Sheep Scab.

The presence of sheep that are not healthy within a flock clearly has welfare implications for those animals and their companions. Sheep infested with sheep scab suffer immensely, so it is rightly an animal welfare issue (Good, 1996). *P.ovis* infestations cause intense irritation and animals can become exhausted and rapidly debilitated from continual scratching, rubbing etc. The entire animal can become covered in scab within a matter of weeks. Fleece loss can occur due to destroyed wool follicles, rubbing off, or lifting away with the rising scab, leaving the animal totally naked. Sheep scab is a visual disease causing great irritation and suffering for infested sheep and should be eradicated on welfare grounds alone. Sheep scab can have profound effects on the health, welfare and economics of infested flocks. In Argentina psoroptic mange is the most damaging ectoparasitic disease affecting domestic livestock. In 1989 the estimated annual losses ranged between $\$100 \times 10^6$ in cattle and $\$150 \times 10^6$ in sheep (Nunez, 1989).

Ram Fertility

Spence (1949) observed live mites on the scrotum of rams. Thickening of the skin can hinder scrotal temperature regulation and in extreme cases, lead to testicular atrophy and reduced fertility (Rhodes, 1976). The latter condition is apparently reversible, as sperm production and fertility are re-established after treatment (Urquhart *et al.,* 1987).

Conception

Tups infested on the ventral surfaces may be unwilling to mount ewes or if they do they remain for only a short period of time. Tups may also transfer mites to the backs of ewes and these in turn may not accept the tup. Conception rates may therefore be low (even though time was adequate to pass on a raddle mark). Ewes may infest tups on the venter. Plunge dipping for the cure or prevention of scab may also have an effect. Any nutritional or mechanical disruption within the first month of gestation will cause unattached ova to float out of the ewe (Dymond, 1984).

Gestation

Infestations during pregnancy could affect the development of the foetus. The ewe may too pre-occupied with rubbing and scratching to ingest or metabolise sufficient nutrient. Nutritional stress in the second month of pregnancy can lead to foetal re-absorption (Dymond, 1984). Lambs may also be born weak or still born. Milk production may also be affected and thus affect early lamb development.

Lamb Growth

If the lamb survives it can be infested from the mother. Lambs below one month tend not to present clinical sheep scab, although mites maybe present on the skin (Bates, *unpublished observations*). Clinical symptoms develop as the lamb grows. Kirkwood (1980) recorded a 30% loss in weight gain in growing lambs. Dipping in a diazinon dip resolved the condition and a weight increase was noted. Sargison *et al.,* (1995) demonstrated that lambs born to infested mothers are 10% lighter at birth. Cole and Guillot (1987) demonstrated that calves severely infested with *P. ovis* had a significantly lower daily gain to feed ratio and energy retention compared to control calves. They found that *P. ovis* increased the maintenance energy

requirements of calves by >50% and for each 10% increase in body surface affected by *P. ovis* the maintenance energy requirement increased by 0.5 Mcal/day.

Death

Death may occur through debility and exhaustion (Downing, 1936), dehydration (a direct result of the feeding of large numbers of mites) (Roberts *et al.,* 1971), secondary bacterial infections (eg pneumonia or septacaemia through self inflincted wounds (Roberts *et al.,* 1971) or hypothermia. Infested sheep are also have lowered resistance to other infections and parasites (eg. parasitic gastroenteritis and *Pasteurella*). Sheep heavily affected by scab are sometimess subject to epileptiform fits (Downing, 1936 and Bygrave *et al.,* 1993) leading to internal haemorrhage.

Effects on Wool and LeatherProduction

Fleece loss can occur due to destroyed wool follicles, rubbing off, or lifting away with rising scab, leaving the animal naked. Spence (1949) observed that the regrowth of wool was unlike normal wool in texture and pigmentation. In Herdwicks the re-growth is grey/brown, in Downland sheep re-growth was sooty brown or black and in Welsh Mountains it was bright orange/tan. Losses of 0.2 kg of fleece per animal have been calculated (Kirkwood, 1980). Olaechea *et al.,* (1997) recorded a deterioration in the tear of the wool and the carding index in infested fleeces, downgrading wool values in Argentina.

Compulsory dipping for the control of sheep scab not only reduced the prevelence of the disease to a low number of cases but it also controlled other sheep ectoparasites that could potentially damage sheep skin (eg. blowfly strike (*Lucilia sericata*), chewing lice (*Bovicola* (*Damalinia*) *ovis*) and pasture ticks (*Ixodes ricinus*)). Since the relaxation in sheep scab policy in 1989 tanners and fellmongers saw an increase in parasite damage and a decline in sheep skin quality. Since the end of compulsory dipping in 1992, the increase in the level caused by ectoparasites has been dramatic. The industry is now reporting that, over the period beginning with the relaxation of compulsory dipping regulations in 1989, the total number of skins damaged by all sheep ectoparasites has increased approximately fivefold. The proportion of skins suffering from the specific type of scarring associated with sheep

scab has increased from 1.2% to 15% of skins (over 25% at the worst time of year) (Pearson, 1996). Lower quality skins can only be used to produce lower grades of leather, commanding reduced prices. There is a potential loss in leather returns of £15 to 20 million or £1.0 per lamb per. Although this money may not be reflected directly in what the farmer receives for his animal, over a period of time the reduced value of the skin has to be passed back up the chain (Pearson, 1996).

7.0. Obscure Outbreaks of Sheep Scab

The Report of the Sheep Scab Working Party (1989) demonstrated that three hundred and nine British flocks were shown to be infested with scab between 1983 and (May) 1988, during the period of two annual compulsory plunge dips. Although the origins of the outbreaks were fully explained in over 73% of cases, the origins of infestation remained obscure in 72/389 (18.5%) of flocks (Table 1.2) and disease recrudesced in 3/389 (0.7%) of flocks. The development of acaricide resistant strains of *P. ovis,* during this period, was not suspected in the recrudescence of disease and the high incidence of outbreaks of obscure origin suggests a method or reservoir of infestation unknown to the Veterinary Field Service. Throughout the history of sheep scab many authors have attributed the failure to eradicate or control the disease to an unknown facet of *Psoroptes* biology.

One such area of investigation has been the survivability of the mite off the host and the potential for re-infestation from the environment. This is very important when considering the efficacy of the new generation of systemic acaricides. It is generally considered that the transmission of sheep scab can be either through direct sheep to sheep contact or indirect, through contact with residual mites in tags of wool or scab attached to brambles, fencing, farm machinery, animal housing etc. An infestation can be initiated by only one egg laying female or hundreds of mites, depending on the mite burdens on other infested sheep or in the environment, together with the relative period of contact. Infestations can spread rapidly through lowland flocks with restricted grazing but may be slower through hill flocks, that are thinly spread over common grazing and infrequently mustered (Spence, 1951).

The Report of the Sheep Scab Working Party (1988) demonstrated that sheep scab outbreaks in Britain originated from lateral spread from contiguous flocks, strays etc (33.9%), movement of sheep via market (22.3%), direct sheep movements (15.9%) and persistent infestations on unenclosed land (1.0%). Although this direct transmission was the predominant method, an element of indirect transmission is present in all outbreaks, ie via mites deposited at marts, in livestock lorries etc.

Although *Psoroptes* spp mites are obligate parasites, they are still capable of surviving off the host for significant periods of time. *P. ovis* has been attributed to ridiculously long survival times off the host. In the USA Salmon and Stiles (1903) reported mites remaining infestive off the sheep for as long as 1 to 2 years and Imes (1916) stipulated that infested pens and buildings should be burnt or at least not re-stocked for a year. Also in the USA, Dill (1920) reported that mites could live several years in protected areas under cool damp conditions, but only 30 to 40 days on the range. Wheeler (1921) observed that three sheep contracted scab from a corral vacated for 5 years and assumed mites could survive off the host for that time. The animals were more than likely carrying sub-clinical infestations on introduction to the corral. It is now certain that the mite can live for over 30 days off the host, given the correct environment, but will only be infestive to sheep for 15 to 16 days (O'Brien *et al.,* 1994). Wilson *et al.,* (1977) stated that mites off the host succumbed to starvation and dessication, with dessication being the more important. In a field situation predation by other arthropods may also be significant.

Associated with the survival of the mite off the host was the unsuccessful search for a *Psoroptes* resting stage (deuteronymph or hypopus). Within the Astigmata it is possible to distinguish a maximum of four developmental stages, namely i) pre-larva, ii) larva, iii) nymph and iv) adult. The pre-larva is a regressive developmental stage, an embryonic cuticle (the deutovial membrane) within the egg shell. The nymphal stage can be protonymph, deuteronymph or tritonymph. The deuteronymphal stage is generally a hypopus or resting stage. Hypopi are frequently found amongst the free living Astigmata but rarely among the parasitic forms, where it is generally suppressed. The life cycle of *P. ovis* therefore consists of the egg, pre-larva (which takes place within the egg), hexapod larva (which are not sexually

dimorphic), octopod male or female protonymphs, male or female tritonymphs and adult males or females. Sweatman (1958) describes the morphological differentiation of each instar. The life cycle may be as short as 10.7 days (Downing, 1936) or as long as 16.0 days (Stockman, 1910).

Attention also turned to the survivability of *Psoroptes* on the host itself. Populations of *P. ovis* were thought to diminish (often to extinction) or actively migrate to the "cryptic or latent sites" (ie. the ears, the infra-orbital fossae (IOF), the inguinal pouches, the crutch, the perineum and the interdigital fossae) at the onset of summer (Roberts *et al.,* 1971; Roberts and Meleney, 1971; Downing, 1936; Shilston, 1915 and Stockman, 1910), remaining quiescent until the onset of autumn/winter. This phenomena of "latent phase" or "suppressed scab" was first described by Downing (1936) and later expanded by Spence (1949) who showed that mites will enter the cryptic sites 45 to 60 days after the onset of the active phase of disease (when extensive encrustation and denudation has rendered the body surface unsuitable). The migration of *P. ovis* to the cryptic sites is not in dispute, but the intentional seasonality of the migration is open to question. Spence himself observed that a small population of an artificial challenge reached the cryptic sites by random dispersal and not by direct migration and that mites can be seen in the latent sites any time of the year. Spence (1949) also observed that populations of mites in the cryptic sites were scarce in the summer but relatively abundant in the winter. Kirkwood (1985b), examining sheep artificially infested with the VLA, Reference Isolate of *P ovis*, found the cryptic sites infested only on sheep having extensive disease, and then more often in the winter than the summer. Roberts *et al.,* (1971) recorded only 7% of sheep examined with detectable infestations in one or more cryptic site during the summer compared to mites oversummering on the broad body surfaces of 32% of sheep examined.

Attention also turned to the role of other hosts, (cattle, wild rabbits, deer, and domestic and feral goats, horses and ponies) as reservoirs of disease.

Obsure or recrudesced outbreaks of sheep scab could also be due to the relative "virulence" of the infesting mite population. Roberts *et al.,* (1971) recorded that infestations could escape detection for over a year. Long periods of latency and a

sudden increase in vigour and pathogenicity of a mite strain could account for unexplained outbreaks of disease (Roberts and Meleney, 1971).

In January 1989 a case of psoroptic otacariasis was reported within a flock in the Scottish Borders, with no history of clinical sheep scab and the mites did not initiate clinical sheep scab on transfer to the bodies of sheep. Wild rabbits were immediately blamed for passing on ear mites to sheep. As mentioned at the beginning of this overview, sheep can be host to two species of *Psoroptes* (Sweatman, 1957a): *P.ovis* infesting the body and *P.cuniculi* infesting the ear canal. These two species have since been shown not to be reproductively isolated, but probably distinct races of the same mite (Wright *et al.,* 1983). Is it possible therefore that *Psoroptes* in the ears of sheep could be reservoirs of clinical sheep scab?

Evidence to support this theory comes from the history of sheep scab in Australia and New Zealand. Sheep scab was first recorded in Australia in 1788 and New Zealand in 1840 and rapidly spread through the colonial flocks (Spence, 1951). Control was originally based upon dipping in lime/sulphur formulations, but this failed. Ultimately scab was eradicated from both countries by shooting all infested animals (Kirkwood, 1986). This being so, all reservoirs of *Psoroptes* in the ear canals would also have been destroyed, an impossible result through a policy of plunge dipping. Australia and New Zealand are still scab free to this day!

8.0. Objectives

This thesis investigated:

1. The prevalence of *Psoroptes* spp. infesting domestic livestock in the UK and their role in the epidemiology of sheep scab (Chapters 2.0 and 4.0).

2. Variations within populations of *Psoroptes ovis* infesting sheep and rabbits, their relative rates of pathogenesis, their comparative capacity to produce otoacariasis and the potential to re-infest previously exposed sheep (Chapters 3.0 and 4.0).

3. Morphological differentiation of species of *Psoroptes* and the frequency that representitives of each morphological type occur in any population of *Psoroptes* (Chapter 4.0).

4. The possability that *P.ovis* and *P.cuniculi* are variants of the same species and that sheep are their own reservoirs of infestation. Ear mites inhabit a part of the sheep where acaricidal dipwash is unlikely to penetrate and thus mites could survive to re-infest the body (Chapter 4.0).

Table 1.1.

The History of Sheep Scab Control in Great Britain

(949 AD to 1997)

Year	Number of Cases	Remarks
949	-	First Sheep scab legislation, Edict of Hywel Dda
1174	-	Arabs aware of the mite.
1297	-	Flanders almost bankrupt due to scab in the UK.
1798	-	Act of Parliament de-stocking contaminated pasture.
1800	-	First account of plunge dipping for scab
1809	-	Mite identified as cause of scab.
1830	-	William IV introduces first scab legislation.
1843	-	William Cooper manufactures the first commercial (arsenic-sulphur) dip formulation.
1857	-	Life cycle deduced.
1869	-	Sheep scab made notifiable in the UK.
1870	2973	First records of outbreaks and distribution. Coal tar, creosote, cryselic acid and rotenone dips become available.
1889	1207	
1895	3536	
1898	-	Local Authorities given power to enforce veterinary inspection.
1905	< 918	Compulsory dipping of infested flocks introduced.
1914	226	Legislation relaxed due to WWI
1918	448	
1926	-	Immersion time increased from 30 to 60 seconds.
1928	744	Compulsory dipping of the National Flock, movement restrictions and dip approval.

Table 1.1 (Continued)

The History of Sheep Scab Control in Great Britain.

(949 AD to 1997)

Year	Number of Cases	Remarks
1935	477	
1940	228	Scab eradicated from lowland counties.
1944	245	
1945	131	
1946	103	
1947	112	
1948	76	Sheep Scab Order (1948) approves γ BHC at a single dip. Scab eradicated from Peak District and Wales.
1949	45	Scab eradicated from Yorkshire and Lancashire.
1950	26	
1951	12	
1952	1	
1953	0	Sheep scab eradicated.
1958	0	First commercial formulation of dieldrin.
1962	0	Scab confirmed at Birkenhead Docks. Quarantined and dipped.
1968	0	Dieldrin withdrawn from market.
1973	43	Sheep scab reintroduced to the UK.
1974	17	Infested area declared around Forest of Bowland.
1975	103	
1976	101	Compulsory national autumn dip
1977	54	Compulsory national autumn dip

Table 1.1 (Continued)

The History of Sheep Scab Control in Great Britain.

(949 AD to 1997)

Year	Number of Cases	Remarks
1978	43	Compulsory national autumn dip
1979	65	Compulsory national autumn dip
1980	33	No national compulsory dipping. Case of psoroptic mange in cattle.
1981	66	Compulsory national autumn dip only. Diazinon approved for scab control.
1982	93	Compulsory national summer dip only. Propetamphos appoved for scab control.
1983	157	Compulsory national summer dip only. Case of psoroptic mange in cattle.
1984	131	Twice annual (summer and winter) dipping begins. Infested areas declared in Cumbria/Co.Durham and the Brecon Beacons. γ BCH voluntarily withdrawn from market.
1985	74	Twice annual dipping year 2
1986	38	Twice annual dipping year 3. No scab cases confirmed in Scotland.
1987	49	Twice annual dipping year 4. Flumethrin approved for scab control.
1988	36	5th and final year of twice annual dipping. Sheep scab policy reviewed
1989	65	Compulsory national summer dip only. First case of psoroptic otoacariasis.

Table 1.1 (Continued)

The History of Sheep Scab Control in Great Britain.

(949 AD to 1997)

Year	Number of Cases	Remarks
1990	103	Compulsory national "Self certified" autumn dip only.
1991	116	Compulsory national "Self certified" autumn dip only.
1992	79 (to June)	Sheep scab deregulated in June. Diagnosis no longer carried out and Scab Approval Protocol abandoned. Ivermectin licenced for scab control.
1994	484	First case of flumethrin resistance reported. Sheep scab returns to Scotland.
1995	676	First case of propetamphos resistance reported HCC licensed for scab control.
1997	?	Sheep Scab Order (1997) introduced. Doramectin licensed for scab control.

Table 1.2

Origins of Sheep Scab Infestations 1983 to 1988[1]

Origin of Infestation	Number of Cases	Percentage
Lateral spread (from contiguous flocks, strays etc).	132.0	33.9
Movement of sheep via market.	87.0	22.3
Obscure.	72.0	18.5
Direct sheep movements.	62.0	15.9
Under investigation (May 1988).	28.0	7.4
Persistent infestations on unenclosed land.	4.0	1.0
Recrudescence	3.0	0.7
Total	309.0	

1. Source: Anon (1989). Report of the Sheep Scab Working Party.

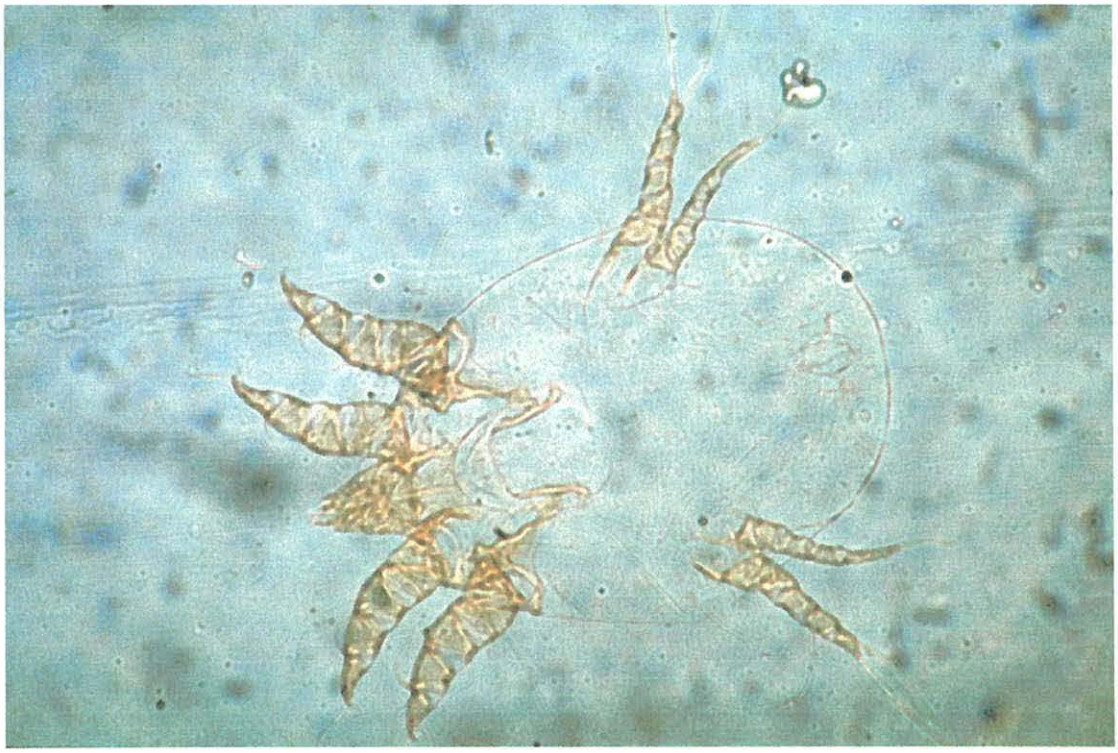

Figure 1.1: Adult female sheep scab mite (*Psoroptes ovis*) (VLA Reference Isolate). Mounted in Berleses Fluid (Gum Chloral). Light microscopy magnification x100.

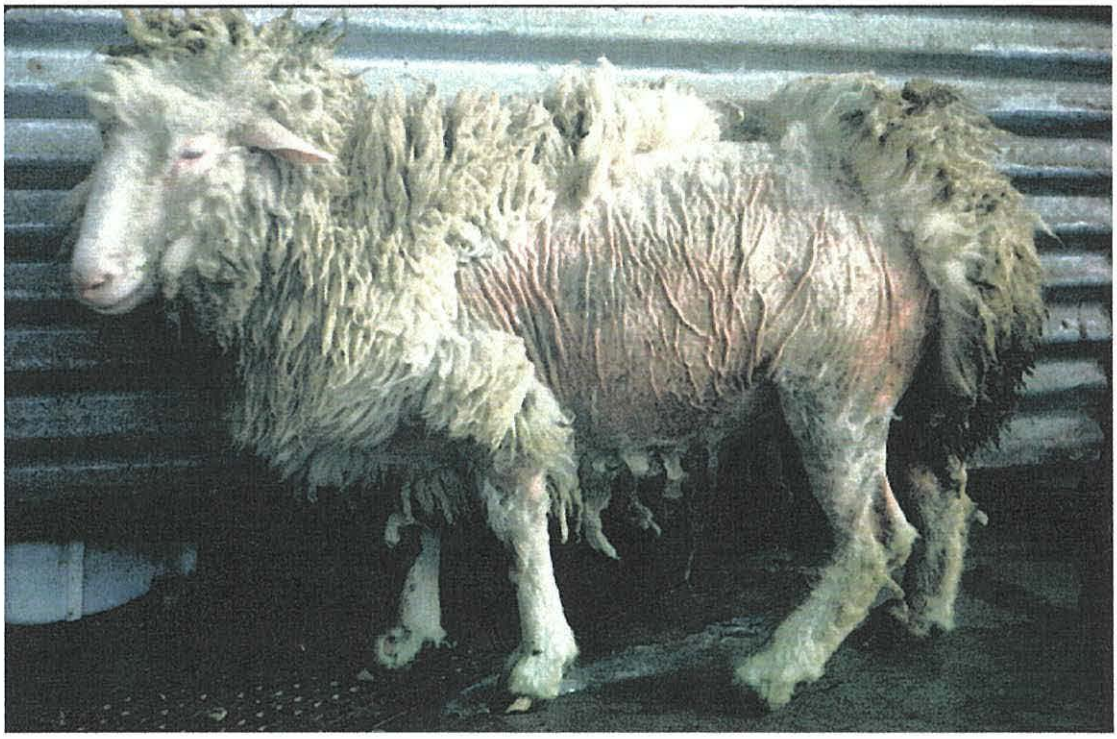

Figure 1.2: Adult ewe with ten to twelve week old infestation of *Psoroptes ovis* (VLA, Reference Isolate) (Sheep Scab).

Chapter 1.9

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Chapter Two

Investigations into the Epidemiology of Natural Reservoirs of *Psoroptes* spp in Britain.

Introduction

Sweatman (1958) divided the genus *Psoroptes* into those that infest the body of the host (ie. producing mange. eg *P. ovis*, *P. equi* and *P. natalensis*) and those that infest the ears of their hosts (ie. producing otoacariasis. eg *P cuniculi* and *P cervinus*). In wild ruminants and solipeds *Psoroptes* infestations are restricted to the ear or ear canal, and are generally well tolerated. They may be regarded as normal relatively benign ear parasites or commensals. In short haired animals (eg. goats and the "hairy" sheep of Africa) body disease is virtually unknown.

This chapter investigates the prevelence and epidemiology of psoroptic mange (other than sheep scab) and otoacariasis (in sheep, goats, horses, cattle, rabbits and camelids) in the United Kingdom. In order to clarify the epidemiology of ear and body *Psoroptes* the nomenclature of Sweatman (1958) was used throughout this Chapter, in that mites isolated from the ears of sheep not presenting body mange were defined as *Psoroptes cuniculi*. A variation occured in Chapter 2.6 where *Psoroptes* mites isolated from the ear canals of sheep with concommitant active body mange (sheep scab) were defined as *P.ovis*, as they were indisputably derived from the body mange populations. The validity of Sweatman's nomenclature will be challenged in Chapter 4.0.

Chapter 2.1

Field Investigations into the Prevalence and Epidemiology of *Psoroptes cuniculi* in Sheep.

1.0. Summary

Seven sheep flocks (2,676 animals) with subclinical psoroptic otoacariasis were investigated. *Psoroptes* mites were isolated from 3.1 % of sheep examined and flock prevalence ranged between 1.3 and 23.9 %, with the highest infestations within pedigree flocks. Infestations (unilateral or bilateral) were found within all age classes. Adult, and shearling rams (21.5 and 14.2 % respectively) were the age/sex classes most affected and lambs 2 and 8 days old, were the youngest to be infested. There was no evidence of vertical transmission. Infestation involved a variety of sheep breeds. No other known host for *Psoroptes* present on the premises were shown to be infested. Non parasitic forage mites were also isolated from the ear canals and may contribute to the overall clinical picture.

Symptoms in adult sheep ranged from aural haematomae/fibrosis (cauliflower ears), violent head shaking and ear rubbing, leading to excoriation and wounding of the ear and eye base. Symptoms in lambs included plaques of scab (often bloody) on the external ear cleft, excoriation of the ear base, ear scratching with the hind feet and inflammation of the external aspects of the horizontal canal. In all cases the internal pinnae were clear of typical Psoroptic scabs. *Psoroptes* mites were isolated from 28.6% of damaged ears and also from 7.8% of undamaged ears. There was no evidence of classical sheep scab in any flock.

Post mortem examination revealed a hollow waxy tube containing live mites, approximately 1.0 cm long, at the distal end of the external auditory canal (EAC), close to the tympanic membrane.

2.0. Introduction

It has been known for some time that *Psoroptes* mites exist in the ears of sheep, without the clinical signs of body mange. Psoroptic otoacariasis has been recorded in Brazil (Faccini and Costa, 1992), France (Henry, 1917), Germany (Zurn, 1877), India (Shastri and Deshpond, 1983), Israel (Yeruham *et al*, 1984/85), South Africa (Van der Merwe, 1949), Britain (Bates, 1991a and Morgan, 1991, 1992). Henry (1917) found psoroptic otacariasis in 47.0% of emaciated sheep without clinical sheep scab. Unfortunately he did not state if the mites were isolated from the pinnae or from the external auditory canal. Hirst (1922) described *Psoroptes* spp in the ears of sheep, without occuring on the body.

This chapter investigates the prevelence and epidemiology of subclinical ovine psoroptic otoacariasis within seven British sheep flocks.

3.0. Flock and Case Histories

Case A. Duns, Berwickshire, Southern Scotland, examined January 1989. The Border Leicester flock consisted of in-lamb ewes and rams on the main property for winter housing and shearling rams and ewes, both weaned in August 1988, at separate grazings. Headshaking and aural haematomae/fibrosis had been observed for at least 3 years, particularly on housing for February lambing. There was no history of ear damage or head shaking in any of the younger animals. Shearlings were worm drenched with Oramec (0.08% ivermectin, MSD Agvet) in June, July and August and the ewes and rams August only. The ewes and rams were also treated with cypor pour-on (2.5% cypermethrin, Grampian Pharmaceuticals) the previous June for headlfly control. The last compulsory scab dipping was in October 1988 using Topclip Gold Shield Scab Approved Sheep Dip (60% diazinon, CIBA GEIGY Agrochemicals). The in-lamb ewes were clipped (1.0 to 2.0 cm at investigation) before housing and bedded on straw. Straw and hay bales were also stored within the lambing shed. Shearlings were full fleeced and out to grass. A commercial hill flock of over 2000 Scottish Blackface ewes was also maintained by the flock owner.

Case B: Llanelidan, Clwyd, North Wales, December 1989. A flock of Welsh Mountain/halfbred ewes and Suffolk rams. Three of the 4 rams exhibited severe bilateral aural hematomae and were isolated from the ewe flock and housed in the cattle shed. Three of the rams had been purchased as shearlings and been resident on the premises for at least 4 years. The fourth ram was only purchased (from Scotland) in September 1988. All the rams were running with the ewes from late September and the entire flock was dipped in early October 1989 in Ectomort Sheep Dip (8.0% propetamphos plus 16% phenol, Grampian Pharmaceuticals). Straw and hay bales were stored within the shed along with the rams; as were the cattle but these were not examined.

Case C: Reedham, Norfolk, South East England, June 1990. The flock consisted of 24 adult rams (15 Suffolk, 4 Meatlinc and 1 Texel) housed on the main property and over 2000 commercial half bred ewes, known to have been served by the rams the previous Autumn, grazing local marshland. In June 1990, a group of rams were seen headshaking and rubbing their ears against fence posts, gates etc, and a number were affected with mild to severe unilateral or bilateral aural haematomas/fibrosis. Replacement ewes were purchased each year direct from Scotland and put to the tup in late October, lambing in March. Scab dipping was carried out in early October 1989, using Coopers Powerpack Summer dip (diazinon 45% and chlorfenvinphos 45% Coopers Pitman Moore). All sheep were shorn in the first week in June and no summer dipping was carried out in 1989, vetrazine pour-on (6.0% cyromazine, CIBA GEIGY Agrochemicals) being used for blowfly control. No other insecticides/acaricides were used. Ewes were worm drenched at lambing time using Deosan wormaway (7.5% levamisole hydrochloride, Diversey Limited.)

Case D: Wymondham, Leicestershire, Central England, January 1991. A closed, part pedigree Charolais, part commercial half bred flock. Five ewes exhibited unilateral haemotomae/fibrosis. Lambing was in progress and the majority of animals were housed on straw in loose boxes, barns or within a polythene tunnel. All sheep were treated with 5.0 ml of Coopers P B Dressing (5.0% piperonyl butoxide and 2.0% butyl aminobenzoate) in each ear, prior to investigation. Compulsory scab dipping had been carried out in late October 1990 using Coopers Border Dip (5.6%

propetamphos plus 20% phenol, Coopers Pitman Moore), but the flock was also dipped in early June 1991 in Bayticol (6.0% flumethrin, Bayer UK Ltd). At the time of examination the majority of the flock were in full fleece but some ewes and shearling rams were shorn for later pedigree show entry. Oramec drench was used as an anthelmintic for the first time in the summer of 1990 and no pour-on insecticides were used.

Case E: Taynton, Gloucestershire, South West England, May 1991. A number of ewes within a pedigree Wensleydale flock were observed to have unilateral or bilteral aural haemotomae/fibrosis and the lambs showed scabbing at the base of one or both ears. The holding had just finished lambing and the majority of sheep were now out to grass. At the time of examination the flock was in full fleece and was last dipped the previous winter using Coopers Border Dip. Ewes were drenched with Oramec in December 1990 prior to housing and again in April 1991 with Pancur SC (fenbendazol, Hoechst) and lambs in March. The problem had occurred over the last 2 years. A flock investigation had previously been carried out by the Bristol Veterinary School (Morgan, 1992).

Case F: Kirriemuir, Angus, Scotland, May 1991. A Scottish Blackface/Blackface cross hill flock and Suffolk/Blueface Leicester pedigree breeding flock. Periods of violent scratching of the ear base or flanks, together with head shaking had been occurring for several years before notification. One Suffolk and one Bluefaced Leicester tup, showing excessive symptoms, had been previously treated by the owner prior to investigation using Ivomec (1.0% ivermectin, cattle formulation, MSD, Agvet), administered subcutaneously at a rate of 200 $\mu g\ kg^{-1}$ b/wt. The last scab dip was in September 1990 using Youngs Jason Winter Dip (8.0% propetamphos plus 10.0% phenols, Grampian Pharmaceuticals) and worm drenched throughout 1990/91, using Nilzan gold (levamasol hydrochloride, Coopers Animal Health). A 2.5% cypermethrin pour-on was used routinely in the summer for headfly control. No other insecticides or acaricides were used. The flocks had just finished lambing and all animals were fully fleeced on inspection. Cattle were also kept on the holding and goats had been kept with the sheep in the past.

Case G. Moreton in Marsh, Gloucestersire, South West England, May 1991. Twenty to thirty recently shorn ewes within a Scottish halfbred/greyface flock were observed to have aural haematomae/fibrosis and bouts of headshaking. The problem was worse the year of examination (1991). No dipping history was available but Oramec drench had been routinely used in the past.

4.0 Associated Sheep Scab Cases

There had been no reports of classical sheep scab within any investigated flock or on contiguous premises. Only the Reedham flock had been directly involved in sheep scab tracing. In October 1989, 53 breeding ewes were purchased by a neighbouring farm and classical sheep scab was confirmed six months later (April 1990), directly after lambing.

5.0. Materials and Methods

5.1 Flock Examinations

Wherever possible the whole flock was examined for sheep scab, headshaking, aural lesions and other evidence of infestation. Morgan (1992) scored ear lesions 1 to 5 according to severity. The severity of ear damage in these studies was recorded as, absent (-); mild (+), with slight thickening of the pinna (score 2 in the Morgan scale); moderate (++), with severe thickening or fibrosis of the pinnae, without abscessation (score 3 on the Morgan scale) or severe (+++), ranging from thickened pinnae with abcessation to chronically thickened and deformed cauliflower ears, painful to touch (Scores 4 and 5 on the Morgan scale). In the case of pedigree flocks lambing records were examined for evidence of vertical transmission.

5.2 Ear Sampling

The horizontal aspects of the external auditory canal (EAC) of both ears of all sheep were sampled using 7.5 centimetre long swabs with a 1.0 by 0.5 centimetre cotton head, manufactured for human aural cleansing (Cotton Buds, Boots Ltd, Nottingham, England). Swabs were inserted into the EAC until resistance was met, then gently twisted and removed. Care was taken that the swab entered the EAC and not the blind pouches of the tragus. Young lambs, ranging from 24 hours to 1 to 2

weeks old, were also sampled, although swabs were not forced into the EAC. Swabs were stored in plastic tubes, labelled where possible with the ear tag, tatoo or identification number. In most cases separate tubes were used for each ear but where flocks were large left and right swabs were stored together. Swabs were examined under a dissecting microscope with overhead lighting (x40), for the presence of live or dead mites. Once examined the ear swabs were "digested" in boiling potassium hydroxide, centrifuged and the sediment examined as laid out by MAFF (1986) and the presence of mites recorded. Animals were classed as infested on the isolation of live or dead *Psoroptes* ova, larvae, nymphs, attached couples, adult males, adult females and/or exuvia. The instar identified according to the criteria of Sweatman (1958). All skin scrapings were examined and processed in a similar manner.

5.3 Other *Psoroptes* Hosts

Where possible all other potential *Psoroptes* hosts (rabbits, horses and cattle) present on the premises were examined and ear sampled. Wild rabbits were common on nearly all the farms and 20 dead animals were submitted from the Berwickshire holding for examination. Goats had also been kept at Kirriemuir along with the sheep but were no longer resident at the time of examination.

5.4 Post Mortem Examinations

The heads of eighteen cull or casualty sheep (including four known to be infested) were examined from three infested premises and one head from a lamb from Kirriemuir was examined at the Veterinary Investigation Centre at Pennicuik, Edinburgh. The infra orbital fossae (IOF) and the internal aspects of the pinnae of each head were examined for scab lesions. The pinnae were then removed as close to the skull as possible, exposing the opening to the EAC. The contents of the EAC was then scraped out and examined for live *Psoroptes* mites using a dissecting microscope (American Optical) with overhead lighting (x 40). The EAC contents was then digested in boiling 10% potassium hydroxide for ten minutes and allowed to cool. The digest was then centrifuged at 2000 rpm for ten minutes and examined under a dissecting microscope (American Optical: x 100) with understage lighting. Ears were recorded as infested on demonstration of live or dead *Psoroptes* ova, larvae, nymphs,

adult males, adult females, attached couples or exuvia. Instars were identified according to the criteria of Sweatman (1958).

6. Results

6.1. Flock Examinations

Although data was gathered from 2,676 sheep from seven different infested flocks with similar case histories, differences in husbandry on the individual farms should be considered when comparing or combining data.

Clinical Symptoms

Clinical symptoms ranged from aural haematomae/fibrosis (cauliflower ears), head shaking, ear rubbing, aural plaques, scratching and inflammation of the ear base. Haematomae, fibrosis, cauliflower ears occured in all flocks examined but were only evident in adult ewes (see Table 1) and were first noticed from 1986, 1989 or within the last few years. Mild headshaking occurred to some extent in younger animals but violent manifestations were confined to a small number of adults. Violent incidences were observed over four hour periods, within ewes at Duns, Wymondham, Kirriemuir and Morton in Marsh and in the rams at Llanelidan, Reedham and Kirriemuir. A number of lambs demonstrated headshaking at Wymondham and Kirriemuir but were not as violent as adult animals.

A number of ewes within the Duns flock exhibited unilateral aural haematomae but lesions were not present in the shearlings (Figure 2.9.1). At Llanelidan all three isolated four year old rams manifested severe (+++) bilateral lesions, painful to touch. The Reedham flock contained four rams presenting ear lesions, two with unilateral severe (+++) lesions in the left or right ear (Figure 2.9.2) and the remaining two having bilateral mild (+) lesions in the left ear and severe (+++) lesions in the right of one ram and mild (+) in the left and moderate (++) in the right of the other. Several ewes at Wymondham exhibited mild (+) to severe (+++) unilateral or bilateral lesions. The ages of the affected animals ranged from three years (one ewe), four years (three ewes) and five years (one ewe). At Taynton two ewes demonstrated slight (+) damage and one older ewe severe (+++) unilateral or

bilateral lesions. A further ewe manifested bilateral damage: mild (+) in the right and severe (+++) in the left. Ear damage was only seen in two hill and four Suffolk ewes within the Kirriemuir flock. The severity of damage at at Morton in Marsh was restricted to ten ewes; five with lesions in the left ear, four in the right and one with bilateral lesions (severe (+++) in the left ear and mild (+) in the right).

Attempts to relieve irritation resulted in other forms of traumatic damage. At Duns two ewes scratched and rubbed the ear bases against hurdles and at Kirriemuir a number of ewes scratched their heads along a line drawn from base of the ear to the base of the eye. Abrasions and scabs as a result of this action were evident throughout the flock. A number of lambs at Wymondham, Taynton and Kirriemuir scratched the base of the ear with their hind foot and two adult Suffolk rams mutually rubbed their ear bases against that of the other. Thirty percent of lambs at Tynton manifested 'plaques' of scab on the external ear cleft (Morgan, 1992). This was also seen, often bloody, within a number of lambs at Kirriemuir and a further 30% showed hairless areas at the ear base. In all cases the internal aspects of the pinae were clear of scab. In a large proportion of lambs at Taynton the external aspects of the horizontal canal of the EAC were inflamed and solid to touch. At Kirriemuir and Morton in Marsh the tips and edges of a number of lambs pinnae were covered in thin, grey, waxy scabs and skin scrapings were negative for mites (*Dermatophilus* infection being suspected). At Duns and Morton in Marsh a number of ewes rubbed their flanks against hurdles and fences but without the buccal response typical of classical scab. At Duns some circumscribed areas of wool loss were noticed on the rumps of some ewes and one poor conditioned ewe at Reedham had extensive wool loss and waxy scabbing of the back (possibly severe *Dermatophilus* infection). Skin scrapings were taken and were negative for *Psoroptes* in both cases.

At Kirriemuir and Morton in Marsh the day of examination was hot and sunny, with abundant fly activity around the penned sheep. Head flies (*Hydrotea* spp) were present in large numbers on both holdings (particularly Kirriemuir), and stable flies (*Stomoxys calcitrans*) were present in large numbers at Morton in Marsh. Both species were seen actively worrying the sheep. The nasal bot fly (*Oestrus ovis*) was also reported to be prevalent. Consequently any observations on scratching and/or headshaking observed could be confused with that resulting from fly irritation.

Ear Sampling

A breakdown of the percent prevalence of psoroptic otoacariasis within the investigated flocks as a function of age and sex is shown in Table 2.1.2. Ear swabbing revealed a prevalence of psoroptic ear mites ranging between 1.3 and 23.9 percent, with the highest infestations found in pedigree flocks (Table 2.1.2). Ear infestations either unilateral or bilateral, were found within all age classes of sheep. Overall adult, followed by shearling rams (21.5 and 14.2 percent respectively) were the age/sex classes most affected (Table 2.1.2). The youngest sheep infested were two ewe lambs, tw and eight days old, at Wymondham. The results showed that infestations were not breed specific with Suffolk, Border Leicester, Bluefaced Leicester, Wenslydale, Charollais, Meatlinc, Greyface/Mule, and Charollais or Suffolk cross breeds found to be infested.

A total of 13 sheep (5.7%) of the Duns flock were shown to be infested: three were housed ewes (one four year old and two 2 year old) and ten shearling rams. No shearlings ewe was found to be infested. Stock records showed no evidence of vertical transmission. Both infested rams at Llanelidan were five years old and had been resident on the farm for four years. In both rams a combination of live and dead mites were recovered. *Psoroptes* were not isolated from the remaining ram with severe ear lesions, the ram at grass or from the ewe flock. Live *Psoroptes* were isolated from ten rams at Reedham (nine Suffolk and one Meatlinc), representing 41.6% of the breeding rams. Eight animals had unilateral infestations, five in the right and three in the left ear, and two were bilateral, one with heavy infestations in both ears. *Psoroptes* mites were isolated from the ears of 13 sheep (9.0% of the flock) at Wymondham. Infested animals consisted of two ewe lambs (two and eight days old), four shearling and seven adult ewes (one three year old, four 4 year old, one five year old the other of unknown age). All but one were unilateral infestations. All mites were identified after KOH digestion. Again stock records showed no evidence of vertical transmission. All five ewes with ear damage were infested and a further eight animals with clinically normal ears were also found to be infested. At Taynton 11 sheep were shown to be infested (23.9% of the total flock), consisting of three ewes and eight lambs. None of the four ewes with ear damage were shown to be infested,

all mites were isolated from clinically normal ears. The majority of infestations were unilateral but three sheep (two ewes and one lamb) were infested in both ears. Thirty sheep were found to be infested at Kirriemuir (1.9% of the total flock). Infested animals within the Suffolk breeding flock consisted of one ram, one ewe and four lambs. A Bluefaced Leicester ram was also shown to be infested. Dead mites only were isolated from the Suffolk and Bluefaced Leicester rams treated with ivermectin. Infestation within the hill flock was found in 19 shearling/adult ewes and four lambs. Four sheep were shown to be infested (3.2% of the total examined) at Morton in Marsh; two ewes and two lambs. All infestations were unilateral and mites were only recovered from undamaged ears. One infested ewe was infested in a clinically normal right ear although the uninfested left ear had severe (+++) haematoma/fibrosis. All instars of *Psoroptes* were isolated from all flocks and were globular and pearly white and prone to quick dessication. Ear damage was not indicative of infestation, mites were also isolated from 7.8% of undamaged ears.

6.2. Pathology of Psoroptic Otoacariasis

Case A: A four year old Border Leicester ewe (L39 a known "head shaker") died of unknown causes and the head was submitted for examination. Examination revealed no clinical symptoms of scab and the IOF and the internal and external aspects of the pinnae were free of scab lesions. Only one *Psoroptes* nymph was isolated from the ear from the initial ear swabbing. At post mortem examination a hollow waxy tube, approximately 1.0 cm long was removed from the distal end of the external auditory canal (EAC) of the right ear, close to the tympanic membrane (Figure 2.9.4). Live *Psoroptes* were isolated from within the tube: 28 motile mites of all instars and 19 unhatched eggs, some actually embedded in the wax matrix. The left canal contained a thinner, more scab like tube from the same location as the right ear. This tube contained 29 motile *Psoroptes* of all stages and ten unhatched eggs. A further fifteen heads from cull animals were examined, including two known infested shearling rams. Unfortunately all these animals (except one shearling ram) had their ears removed at the Kelso abattoir. The remaining shearling was killed "on farm" on welfare grounds after injuries received through fighting. Post mortem examination of this ram was negative.

Case C: An infested five year old Suffolk ram from the Reedham flock died from the effects of excessive water intake. The contents of both EACs consisted of waxy tubes containing 50 to 70 *Psoroptes* mites.

Case E: One ewe died of pneumonia. Waxy "tubes" with live *Psoroptes* were removed from each EAC.

Case F: A six week old lamb was submited to the Veterinary Investigation Centre at Pennicuik, Edinburgh. Waxy tubes with live *Psoroptes* mites were isolated from both ears.

6.3. Other *Psoroptes* Hosts

All horses, cattle and rabbits examined were negative for *Psoroptes* in the ear or on the body.

6.4. Other Arthropods Recovered

Active motile non parasitic forage mites (Acaridae) from hay, straw or feed concentrates or their immobile deutonymphs (hypopi) and free living soil or pasture mites were also isolated from the EAC (Table 2.1.3), together with a variety of other small arthropods.

Table 2.1.1: Incidence of aural damage (fibrosis, haematomae, cauliflower ears) within individual flocks as a functrion of age and sex.

	Numbers of affected sheep (percent) for each flock examined								
Farm site/ type of flock	A Pedigree	B Commercial	C Commercial	D Ped/Comm	E Pedigree	F Pedigree	F Commercial	G Commercial	Within Age Group
Lambs	-	-	-	0/80 (0.0)	0/27 (0.0)	0/136 (0.0)	4/761 (0.5)	0/33 (0.0)	0/1037 (0.0)
Shearling Ewes	0/52 (0.0)	-	-	0/13 (0.0)	0/3 (0.0)	2/78* (2.6)	4/527* (0.8)	-	0/68 (0.0)
Adult Ewes	5/110 (4.5)	0/147 (0.0)	0/402 (0.0)	5/55 (9.1)	4/14 (28.6)			10/105 (9.8)	6/605(0.99)* 24/830 (2.9)
Shearling Rams	0/65 (0.0)	-	-	0/5 (0.0)	-	-		-	0/70 (0.0)
Adult rams	0/2 (0.0)	3/4 (75.0)	4/24 (16.7)	0/2 (0.0)	0/2 (0.0)	0/30 (0.0)		-	7/65 (10.8)
Prevalence within flock	5/229 (2.2)	3/151 (2.0)	4/426 (0.9)	5/155 (3.2)	4/46 (8.7)	2/244 (0.84) 6/1533 (0.39)	4/1289 (0.82)	10/135 (7.4)	37/2676 (1.38)

- = No data available (age/sex not present):(x) = percent prevalence: A = Duns; B = Llanelidan; C = Reedham; D = Wymondham; E = Taynton; F = Kirriemuir; G = Morton on Marsh. * = No differentiation made between adult and shearling ewes.

Table 2.1.2: The prevalence (percent) of *Psoroptes* mites recovered from ears within individual flocks as a function of age and sex.

Farm site/ type of flock	A Pedigree	B Commercial	C Commercial	D Ped/Comm	E Pedigree	F Pedigree	F Commercial	G Commercial	Within Age Group
Lambs	-	-	-	2/80 (2.5)	8/27 (29.6)	4/136 (2.9)	4/761 (0.5)	2/33 (6.0)	20/1037 (1.9)
Shearling Ewes	0/52 (0.0)	-	-	4/13 (0.0)	0/3 (0.0)	1/79* (1.3)	19/527* (2/92)	-	10/68 (14.7) 20/606*
Adult Ewes	3/110 (2.7)	0/147 (0.0)	0/502 (0.0)	7/55 (12.7)	3/14 (21.4)			2/92 (2.2)	(3.3) 14/820 (1.8)
Shearling Rams	10/65 (15.3)	-	-	0/5 (0.0)	-	-		-	10/70 (14.2)
Adult rams	0/2 (0.0)	2/4 (50.0)	10/24 (41.6)	0/2 (0.0)	0/2 (0.0)	2/30 (6.7)	0/1 (0.0)	-	14/65 (21.5)
Prevalence within flock	13/229 (5.7)	2/151 (1.3)	10/426 (2.3)	13/155 (9.0)	11/46 (23.9)	7/245 (2.8) 30/1533 (1.9)	23/1288 (1.8)	4/125 (3.2)	83/2676 (3.1)

- = No data available (age/sex not present):(x) = percent prevalence: A = Duns; B = Llanelidan; C = Reedham; D = Wymondham; E = Taynton; F = Kirriemuir; G = Morton on Marsh. *= No differentiation made between adult and shearling ewes.

Table 2.1.3: The incidence of forage mites (Acaridae) in addition to *Psoroptes* mites, within the external auditory canals of sheep in flocks with psoroptic otoacariasis and it's correlation with age/sex.

Number of Infested Sheep (Percent) for Each Flock Examined							
Farm site	A	B	C	D	E	F	G
Lambs	-	-	-	0/80 (0.0)	0/27 (0.0)	4/766 (0.5)	9/33 (27.3)
Shearling ewes	0/52 (0.0)	-	-	0/13 (0.0)	0/3 (0.0)	-	-
Adult ewes	0/110 (0.0)	2/147 (1.4)	12/402 (2.9)	1/55 (1.8)	0/14 (0.0)	7/605 (1.2)	0/92 (0.0)
Shearling rams	10/52 (19.2)	-	-	0/5 (0.0)	-	-	-
Adult rams	0/2 (0.0)	0/4 (0.0)	1/24 (4.2)	0/2 (0.0)	0/2 (0.0)	1/28 (3.6)	-

- = no data available (age/sex not present). A = Duns. B = Llanelidan.
C = Reedham. D = Wymondham. E = Taynton. F = Kirriemuir.
G = Morton on Marsh.

Chapter 2.2

Prevalence of *Psoroptes cuniculi* in Sheep: An Abattoir Survey

1.0. Summary

Over 24 months between 1989 and 1991 2,952 sheep heads from a local abattoir were examined for psoroptic otoacariasis. Forty-two (1.4 %) were found to be infested with psoroptic ear mites. There was no evidence of clinical sheep scab and no sheep presented haematomae or fibrosis of the pinnae nor any other traumatic damage described in cases of psoroptic otoacariasis (Chapter 2.1). The recovery of psoroptic ear mites appeared seasonal, with no mites recorded between May and July. The majority of infested sheep, originated from livestock markets located in areas with long histories of classical scab, but it must be borne in mind that livestock is not automatically sold at the local mart but can be transported considerable distances before sale. The prevelence of psoroptic ear mites appeared to increase each year, with 0.3%, 1.8% and 3.9% of sheep examined infested in 1989, 1990 and 1991 respectively.

2.0. Introduction

Chapter 2.1, demonstrated the prevelence and epidemiology of sub clinical ovine psoroptic otoacariasis (*P. cuniculi*) within individual sheep flocks based upon gross clinical signs. This chapter reports the prevelence of psoroptic otoacariasis within the British national flock following an abattoir survey.

3.0. Materials and Methods

3.1. Source of Material

Sheep heads were collected from an abattoir in Guildford, Surrey, between May 1989 and April 1991 and the external auditory canals (EACs) examined for the presence of *Psoroptes* mites. Heads were examined from sheep gathered from eleven livestock markets, situated in five counties in England (Cumbria, Hereford and Worcester, Oxfordshire, Shropshire and Surrey), three counties in Wales (Dyfed, Gwent and Powys) and one in Scotland (Borders). On occasions consignments of sheep from different markets arrived at the abattoir on the same day; consequently it was impossible to identify the exact source of the animals being examined.

3.2. Post-Mortem Examination

The infra orbital fossae (IOF) and the internal aspects of the pinnae of each head were examined for scab lesions. The pinnae were then removed as close to the skull as possible, exposing the opening to the EAC. The contents of the EAC was then scraped out and examined for live *Psoroptes* mites using a dissecting microscope (American Optical) with overhead lighting (x40). The EAC contents was then digested in boiling 10% potassium hydroxide for ten minutes and allowed to cool. The digest was then centrifuged at 2000 rpm for ten minutes and examined under a dissecting microscope (American Optical) with understage lighting at a magnification of x40. Ears were recorded as infested on demonstration of live or dead *Psoroptes* ova, larvae, nymphs, adult males, adult females, attached couples or exuvia. Instars were identified according to the criteria of Sweatman (1958).

4.0 Results

Over the 24 months of the survey 2952 sheep heads were examined, predominantly lambs and store lambs. Forty-two (1.4 %) of sheep examined were found to be infested with psoroptic ear mites. There was no evidence of clinical sheep scab on the heads and no lesions or mites were seen in or on the pinna or in the IOF. No sheep exhibited haematomae or fibrosis of the pinnae nor any other traumatic damage described in cases of psoroptic otoacariasis (Chapter 2.1). The results of this survey are summarised in Tables 2.2.1 and 2.2.2, and confirm that the recovery of psoroptic ear mites appeared seasonal, with no mites recorded between May and July (Table 2.2.2). The majority of infested sheep, originated from livestock markets located in areas with long histories of classical scab (Table 2.2.1), but it must be borne in mind that livestock is not automatically sold at the local mart but can be transported considerable distances before sale. Psoroptic ear mites may be classical scab mites adapted to an aural environment, a higher prevalence of otoacariasis would therefore be expected in endemic scab areas. Unfortunately the livestock markets used in this study were (unintentionally) biased towards endemic areas, a broader survey is thus required to investigate this further.

The prevelence of psoroptic ear mites appeared to increase each year, with 0.3%, 1.8% and 3.9% of sheep examined infested in 1989, 1990 and 1991 respectively (Table 2.2.2).

Table 2.2.1: Psoroptic otoacariasis in sheep received by a Surrey Abattoir from eleven livestock markets. Prevalence of infestation per market per year.

Market	County	1989	1990	1991	Total (%)
Welshpool	Powys	-	-	7/100	7/100 (7.0)
Newport or Carmarthen[1]	Gwent Dyfed	-	8/151	-	8/151 (5.3)
St Boswells	Borders	-	4/95	-	4/95 (4.2)
Ross on Wye	Hereford and Worcester	-	0/79	7/100	7/179 (3.9)
Banbury	Oxfordshire	4/148	5/258	-	9/406 (2.2)
Bishops Castle or Welshpool[1]	Shropshire Powys	-	1/48	-	1/48 (2.1)
Welshpool or Guildford[1]	Powys Surrey	-	1/48	-	1/48 (2.1)
Knighton	Powys	-	1/70	-	1/70 (1.4)
Ross on Wye or Guildford[1]	Hereford and Worcester Surrey	1/100	-	-	1/100 (1.0)
Longtown	Cumbria	-	1/202	-	1/202 (0.5)
Guildford	Surrey	0/1069	-	1/100	1/1169 (0.09)
Oswestry	Shropshire	0/100	-	-	0/100 (0.00)
Unknown	?	-	1/100	-	1/100 (0.00)
Total (%)		5/1417 (0.4)	21/1051 (1.9)	15/300 (5.0)	42/2768 (1.5)

1 = Sheep from two livestock markets arriving at abattoir on same day

Table 2.2.2: Monthly and yearly variations in the isolation of psoroptic mites from the external ear canals of sheep heads examined from a Surrey Abattoir.

Sheep with ear mites/Numbers examined (%) per year

Month	1989	1990	1991	Total
January	-	1/70 (1.4)	8/103 (7.8)	9/173 (5.2)
February	-	12/132 (9.1)	0/109 (0.0)	12/241 (5.0)
March	-	2/152 (1.3)	-	2/152 (1.3)
April	-	0/70 (0.0)	8/200 (4.0)	8/270 (3.0)
May	0/554 (0.0)	-	-	0/554 (0.0)
June	0/515 (0.0)		-	0/594 (0.0)
July	-	0/79 (0.0)	-	0/79 (0.0)
August	4/418 (2.7)	0/100 (0.0)	-	4/248 (1.6)
September	0/100 (0.0)	1/100 (1.0)	-	1/200 (0.5)
October	1/100 (1.0)	1/84 (1.2)	-	2/184 (1.1)
November	0/60 (0.0)	4/95 (4.2)	-	4/155 (2.3)
December	-	0/102 (0.0)	-	0/102 (0.0)
Total	5/1477 (0.3)	21/1063 (1.8)	16/412 (3.9)	42/2952 (1.4)

- = No animals examined

Chapter 2.3

Field Investigations into the Prevalence and Epidemiology of *Psoroptes Cuniculi* in Goats:

1.0. Summary

Psoroptic otoacariasis was identified in four out of five (80.0%) of goat herds examined, with 76.0 of a total of 253.0 (30.0%) of goats examined. Prevalence within the four infested herds ranged between 20.0% to 35.0%. No gross clinical symptoms were observed in any herd, other than the occasional individual exhibiting ear scratching with the hind feet. All lesions were confined to the external ear canal. No mites were found in goats under one month old; 12.9% of the infested animals were between one and six months; 64.5% were between 6 and 12 months, 19.4% were 12 to 18 months old and only 3.2% of goats over two years were infested, including one billy goat.

2.0. Introduction

Psoroptes cuniculi (synonym *P. caprae*, Sweatman, 1958) were first reported infesting the ears of goats by Gedoelst in 1909 and Roubaud and Saceghem in 1916 and has since been reported in Australia (Roberts, 1952; Cook, 1981 and Cottew and Yeats, 1982); Bangladesh (Noorudin and Mondal, 1996), Brazil (Bavia *et al,* 1984/85; Faccini *et al,* 1981 and Faccini and Costa, 1992), Canada (Lofstedt *et al.*, 1994), England/Ireland (Littlejohn, 1968 and Bates, 1992), Fiji (Munro and Munro, 1980), India (Shastri and Deshpand, 1983 and Dutta *et al.*, 1996), Israel (Yeruham *et al.,* 1984/85), Italy (Perrucci *et al.*, 1996), New Zealand (Heath *et al.,* 1983, 1989), South Africa (Shilston, 1915), Sudan (Abu-Samra *et al.,* 1981), USA (Wiliams and Williams, 1978 and Friel and Grainer, 1988) and Zimbabwe (Odiawo and Ogaa, 1987).

This chapter sets out to investigate the prevelence and epidemiology of psoroptic otoacariasis (*P. cuniculi*) within the British domestic goat population.

3.0. Materials and Methods

3.1 Case Histories

Case A: In June 1989, Seven out of eight British Saanen yearlings, purchased from the National Institute for the Research into Dairying (NIRD), Shinfield, Berkshire, were found to be infested with *Psoroptes* mites in the external auditory canal. In October 1989 the parent herd was examined for evidence of *Psoroptes* infestation. The herd of 178 dairy goats comprised of 34 kids below one month old; 118 yearlings, 23 goats (12 to 18 months old) and eight goats (6 to 12 years old). The dairy goats had never been treated with any acaricidal or insecticidal compounds or drenched with ivermectin based anthelmintic.

Case B: In January 1991, 17 goats held at Manor Farm, VLA (Weybridge), were examined. The flock consisted of two, nine year old, British Saanen adults (one neutered male and one female) originating from NIRD, Shinfield and one 8 year old neutered male British Saanen, born at Manor Farm, VLA (Weybridge). Two, 2 year old British Toggenburgs (one neutered male and one female), from Gomshall, Surrey. The remaining 12 goats were all yearling female Anglo Nubians, 11 originating from Upton Warren, Bromsgrove and the other from Primrose Hill, Cambridgeshire.

Cases C, D and E: In November 1991, 60 goats at the VLAs Appstree Farm, Ripley, Surrey were examined. The herd comprised of adult (3 to 4 year old) British Saanen females from three different sources: 49 drawn from a bankrupt herd, destined for slaughter, near Chelmsford; Essex (Case C), seven from a commercial herd near Abingdon, Oxfordshire (Case D) and five from a commercial herd in Yorkshire (Case E). The Essex and Oxfordshire goats had been on the VLA farm for only 1 week and had been isolated from each other throughout. The Yorkshire goats arrived later, and again were isolated from both the Essex and Oxfordshire groups. The Essex and Oxfordshire goats were drenched with Oramec (0.08% ivermectin, MSD, Agvet, UK) on the 22nd October. Both groups had moderate infestations of chewing lice (*Bovicola caprae*) and on November 4th were treated with Parasol Pour-on (2.5% cypermethrin, CIBA GEIGY Animal Health, UK), 15 ml per goat in a strip from the base of the ears to the pin bones (tuber coxae), via a standard pour-on gun. The treatment was repeated 10 days later. The goats were ear sampled at the time of both

pour-on treatments. The 7 goats from Yorkshire were not drenched with ivermectin, received only the second pour-on treatment and were only ear sampled once.

3.2 Clinical Examination and Ear Sampling

The external aspects of the pinnae were examined for evidence of infestation and the age and sex of the goat recorded. The external auditory canals (EAC's) were sampled using cotton swabs (Q-Tip Cotton Buds, Boots) as described for sheep in Chapter 2.1. Skin scrapings were also taken from suspect lesions on the broad body surfaces. All swabs and skin scrapings were stored at +4°C for between 2 and 7 days and examined under a dissecting microscope (American Optical, x40) for live or dead mites. The samples were then digested in 10% potassium hydroxide, centrifuged and the sediment examined for dead mites as described in the Manual for Veterinary Laboratory Techniques (MAFF 1986).

Two systems have been developed to grade the severity of psoroptic otoacariasis in goats. Littlejohn (1968) recognised four grades (1+ to 4+) with grades 1+, 2+ and 3+ confined to the external auditory canal (Table 2.3.1). The system of Munro and Munro (1980) was more "user friendly", infestations scored as mild, moderate, severe and extensive, with mild infestations confined to the external auditory canal and divided into three subgrades (Table 2.3.2).

4.0. Results

4.1. Prevalence of Infestation

Psoroptic otacariasis was identified in 4/5 (80.0%) of goat herds examined (Table 2.3.3) with 76.0 of a total of 253.0 (30.0%) of goats examined. Mites were not recovered from Herd B, Manor Farm, VLA (Weybridge). Prevalence within the four infested herds was 35.0% (Herd A, Shinfield), 22.9% Herd C (Essex), 28.5% Herd D (Oxford) and 20.0% Herd E (Yorkshire).

4.2 Breakdown of Infestation With Age

Infestation as a function of age was analysed for Herd A only (Table 2.3.4). No mites were found in goats under one month old; 12.9% of the infested animals were between one and six months; 64.5% were between 6 and 12 months, 19.4% were 12 to 18 months old and only 3.2% of goats over two years were infested, including one billy goat.

4.3. Clinical Signs.

Psoroptes mites recovered from all goat herds ranged from a single unhatched egg identified by the two "bosses" of the prelarval stage, to multi-instar recovery. No gross clinical symptoms were observed in any herd, other than the occasional individual exhibiting ear scratching with the hind feet. All EAC lesions were scored 1+, 2+ or 3+ according to Littlejohn (1968) system or mild (score I to III) according to Munro and Munro (1980). No animal exhibited any lesion in the internal aspects of the pinna or concha and all suspect body lesions were negative for *Psoroptes,* although *Chorioptes* mites were found on several scrapings and ear swabs of Herd A.

Table 2.3.1: The Scoring System of Littlejohn (1968).

Score	Infestation
+	Small single layer of scab lining the large sulcus at the base of the concha.
++	Intermediate
+++	Abundant laminated scab formation occluding meatus.
++++	Infestation consisting of extensive lesions easily visible without close inspection.
+++++	Infestation occluding the entire pinna.

Table 2.3.2: The Scoring System of Munro and Munro (1980).

Score	Infestation
Mild (I)	Soft, whitish/yellow debris, free in the lumen of the auditory canal.
Mild (II)	Firmer accumulations with annular ridged bands lying on the membranes of the auditory canal.
Mild (III)	Dry, moderately adherent scab varying in colour from yellow to brown.
Moderate	Crusty or laminated scabs in the auditory canal with large numbers of grey white mites underneath or between the lamina.
Severe	Complete occlusion of the auditory canal with laminated exudate with the pinnae covered with thin layers of laminated scab, up to 13.0 mm thick. Goats exhibit varying degrees of scaliness around the poll.
Extensive	Severe type of ear lesion with characteristic scab lesions on the neck, back, legs and abdomen.

Table 2.3.3: Prevalence of psoroptic otoacariasis in five UK goat herds

Goat Herd	Breed	No. Goats	No. Infested	% Infested
A. Shinfield	British Saanen	178.0	62.0	35.0
B. Manor Farm	British Saanen Anglo Nubian Toggenburg	15.0	0.0	0.0
C. Ripley/Essex	British Saanen	49.0	11.0	22.4
D. Ripley/Oxon	British Saanen	7.0	2.0	28.5
E. Ripley/Yorks	British Saanen	5.0	1.0	20.0
Total		253.0	76.0	30.0

Table 2.3.4: Breakdown of Infestation with respect to age (Herd A)

Age	Percent Recovery of *Psoroptes cuniculi*							% Infested	
	(n)	Negative	<5 Mites	5-10 Mites	11-20 Mites	21-50 Mites	>50 Mites	In age class	Total
<1 month	34	34 (100)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1-6 months	29	21 (72.4)	8 (27.6)	0.0	0.0	0.0	0.0	27.6 (n=8)	12.9
6-12 months	84	44 (52.4)	26 (31.0)	6 (7.1)	4 (4.7)	2 (2.4)	2 (2.2)	40 (47.6)	64.5
12-18 months	23	11 (47.8)	10 (43.5)	2 (8.7)	0.0	0.0	0.0	12 (52.1)	19.4
6-12 years	8	6 (75.0)	0.0	0.0	2 (25.0)	0.0	0.0	2 (25.0)	3.2

Chapter 2.4

The Prevalence of *Psoroptes cuniculi* in Horses: Field and Abattoir Investigations

1.0. Summary

The ears contents of five horses sharing grazing with sheep flocks affected with psoroptic otoacariasis (Chapter 2.1), 50 heads examined post mortem at a Sussex abattoir and the ear contents of twenty road casualty ponies and horses from the New Forest, Hampshire were examined for evidence of psoroptic otoacariasis. No *Psoroptes* mites, alive or dead were seen in the ear contents of the seventy five horses examined, either after direct microscopical examination or after caustic digestion.

2.0. Introduction.

Equines can be infested with three species of *Psoroptes*: ie. *P. cuniculi* (*P. hippotis*) infesting the ears (otoacariasis) and *P. equi* and *P. natalensis* infesting the body (mange) (Sweatman, 1958).

P. cuniculi (*P. hippotis*) infestations of the ear canal has been demonstrated in Australia (Lucas, 1946; Johnston, 1963; Shaw, 1966; Arundel, 1978 and Pascoe, 1980), Great Britain (Gerring and Thomsett, 1980), France (Henry, 1920) and the USA (Montali, 1976). Henry (1920) recorded mites in the ears of horses, asses, mules in France with up to 70% of horses affected without showing symptoms of body mange. In Australia mites have been isolated from horses in Victoria, New South Wales, Western Australia and Queensland (Arundel, 1978). A survey of horses in Queensland undergoing theatre surgery showed 20% to be infested with psoroptic ear mites (Pascoe, 1981).

Henry (1920) described mites from the ears of horses as a different species (*P. hippotis* (≡ *P. cuniculi*)) from those causing body mange (*P. equi*). Equine psoroptic mange has been recorded in Germany (Dietz and Wiesner, 1984), Libya (Gabaj *et al.*, 1992), South Africa (Zumpt, 1961), Sudan (Abu Samra *et al.*, 1981, 1987) and Great Britain (Kirkwood, 1986). Rainbow (1906) cited the existence of *P. equi* in Australia, but this record is in doubt: symptoms superficially resembling those caused by *P. equi* have been observed, but the mite itself has never been identified in Australia (Seddon, 1952, 1968).

In Great Britain the Parasitic Mange Order (1938) defined parasitic mange as the infestation of the broad body surfaces by *P. equi* (or *Sarcoptes scabiei*) and was made notifiable in 1911, due to it's economic effects on the working horse, especially during wartime. *P. equi* was the commonest source of infestation in horses, with 159 cases reported in the UK between 1938 and 1946. Severe outbreaks of equine psoroptic mange occured in Germany during the Second World War and the disease was still notifiable in both East and West Germany up until 1984 (Dietz and Wiesner, 1984). The last case of equine psoroptic (body) mange in Britain was at Stirchley, near Birmingham in February 1948, although a case of psoroptic otoacariasis (still

defined by the Order as Parasitic Mange) was recorded in July 1980 on a horse at Sandridge, Hertfordshire (Gerring and Thompsett, 1980). The last case of equine sarcoptic mange was recorded on a nine year old bay gelding near Cardiff in October 1977. Since then equine mange has been eradicated and the Parasitic Mange Order revoked in October 1983 (Anon, 1983), primarily due to the decreased agricultural and military importance of the horse and the successful use of γ BHC (lindane/HCH) washes.

This chapter reports a limited investigation into the prevelence of psoroptic otoacariasis (*P. cuniculi*) within a small sample of British domestic and non domestic horse population.

3.0. Materials and Method

3.1. Sources of Material

Material for examination was derived from three sources: 1). horses sharing grazing with sheep flocks diagnosed as being infested with psoroptic otoacariasis (Chapter 2.1), 2.) post mortem examination of horses ears and ear canals at a Sussex abattoir and 3). examination of the contents of the EAC of horses and ponies, all road casualty victims, from the New Forest, Hampshire.

Horses Sharing Grazing with Infested Sheep Flocks.

The ears of five horses present on sheep farms where subclinical ovine psoroptic otoacariasis had been confirmed (Cases A and E, Chapter 2.1) were swabbed, as described in Chapter 2.1.

Abattoir Survey.

The heads of fifty horses were examined *in situ* at a Sussex abattoir, according to the method described in Chapter 2.2.

EAC Contents of New Forest Ponies and Horses.

The EAC contents of 20 horses and ponies, all New Forest road casualty victims (Table 2.4.1) taken according to the method described in Chapter 2.2, were submitted to the VLA (Weybridge) for examination.

3.2. Examination of Ear Swabs and EAC Contents.

Ear swabs and EAC contents were examined microscopically according to the methods described in Chapters 2.1 and 2.2.

4.0 Results

No *Psoroptes* mites, alive or dead were seen in the EAC contents of the 75 horses examined, either after direct microscopical examination or after digestion in boiling 10% potassium hydroxide.

Table 2.4.1: Prevelence of equine psoroptic otoacariasis in Great Britain: EAC contents of New Forest ponies and horses

Date	Age	Sex	Presence of *Psoroptes* spp
26.02.90	Adult	Mare	-
26.02.90	Adult	Mare	-
26.02.90	Adult	Mare	-
26.02.90	Adult	Mare	-
26.02.90	Adult	Mare	-
26.02.90	Adult	Mare	-
26.02.90	Adult	Mare	-
18.06.90	3 years	Stallion	-
18.06.90	10 years	Mare	-
18.06.90	10 years	Mare	-
18.06.90	Adult	Mare	-
18.06.90	7 years	Mare	-
18.06.90	8 years	Mare	-
18.06.90	7 months	Filly	-
18.06.90	7 months	Filly	-
26.07.90	Adult	Mare	-
26.07.90	Adult	Mare	-
26.07.90	Adult	Mare	-
20.11.95	Adult	Mare	-
24.11.95	Adult	Mare	-
28.11.95	Adult Donkey	Female	-

Chapter 2.5

Investigations into the Prevalence and Epidemiology of *Psoroptes cuniculi* in Rabbits

1.0. Summary

Between 1988 and 1992 approximately 4,130 wild rabbits and eighty New Zealand White domestic rabbits were examined for psoroptic otoacariasis. No evidence of *P. cuniculi* infestation was recorded in any wild rabbit examined. Only infested domestic rabbits were received at Weybridge, the majority with lesion scores ranging between 1.0 and 6.0. In animals with mild infestations (score 1.0) mites were observed deep in the external auditory canal, sometimes associated with a waxy (ceruminous) "plug. Five percent of domestic rabbits manifested extra-auricular ("ectopic") disease, presenting extensive psoroptic mange of the entire pinnae (both score 6.0), the base of the ears, the cheeks, dewlap and face. Lesions and mites were also present between the digits of both hind feet.

2.0. Introduction

Ear canker, caused by *P. cuniculi,* is a common disease of domestic rabbits throughout the World. The infestation was first described in Paris in 1858 by Delafond and since then *P. cuniculi* infestations of domestic rabbits have been recorded in Africa (Hirst, 1922), Australia (Seddon, 1952, 1968), Egypt (Ezzat, 1955), France (Delafond, 1858 and Guilhon, 1990), Germany (Von Ribbeck and Ilchmann, 1969), Libya (Gabaj *et al.*, 1992), India (Rai, 1988), United Kingdom (Hirst, 1922), USA (Hirst, 1922) and the Sudan (Abu Samra *et al.*, 1981). In Egypt mange (sarcoptic or psoroptic) in rabbits is considered to be second to coccidiosis in importance, with high losses reported (Ezzat, 1955). In India Rai (1988) reported infestation rates of 15 %, 11 % and 7 %. In the Britain the parasite is extremely common in pet rabbits and commercial rabbit colonies, either for meat of laboratory rabbit production.

Infestations appear confined to domestic rabbits. Surveys of ectoparasites of wild rabbits in Australia have not recorded *P. cuniculi* (Mykytowycz, 1957; Williams, 1972), but it is not clear whether these surveys included an examination of the ears (Strong and Halliday 1993).

There has been only one report of psoroptic otoacariasis occuring in wild rabbits. Guilhon (1990) recorded wild rabbits in France exhibiting mild erythema at the opening of the external auditory canal, with slight crusting, and one or more pairs of psoroptic mites present. He stated that mites were capable of infecting other animals in the burrow and that the equilibrium between parasite and host breaks down under conditions of domestication. Domestic rabbits often escape or are released and join populations of wild rabbits. In suburban Surrey it is not unusual to see escaped black and white domestic (feral) rabbits grazing with wild rabbits (Bates *personal observation*).

This chapter sets out to investigate the prevelence of psoroptic otoacariasis *(P. cuniculi*) within the British domestic and non domestic rabbit population.

3.0. Materials and Methods

3.1. Wild Rabbits.

Survey 1988 to 1992 (ears and scalp)

Between 1988 and 1992 a survey was carried out at the VLA (Weybridge) to investigate the prevelence of *P. cuniculi* in wild rabbits in the South of England.The ears and scalps of over 4000 wild rabbits were submitted by the MAFF, Pest Infestation Laboratory, Worplesdon, Surrey. Ears were excised to reveal the lumenal remains of the EAC and the entire ear examined under a dissecting microscope (American Optical x 40) with overhead lighting for live *Psoroptes* mites and/or the presence of ear canker. Suspicious lesions were removed by skin scraping.

Survey 1990 (entire head)

Between February and April 1991 the entire heads of one hundred and ten rabbits were submitted to Weybridge by Surrey Pest Control Operatives. The internal aspects of the pinnae of each head were examined for scab lesions (ear canker) and the EAC examined according to the method described in Chapter 2.2.

Rabbits Submitted from Farms with Ovine Psoroptic Otoacariasis.

In March 1989 thirty rabbits were shot on a farm in Duns, Berwickshire, Scotland and the whole carcases submitted to Weybridge for examination. The farm was known to have psoroptic otoacariasis within the sheep flock (Case A, Chapter 2.1). The heads were examined and post mortemed according to methods described in Chapter 2.2.

3.2. Domestic Rabbits

Domestic (laboratory) Rabbits 1990.

Between February and May 1990 eighty New Zealand White rabbits were purchased from commercial rabbitries supplying animals for meat or for laboratory use. Infested animals were requested in order to collect *P. cuniculi* for antigen production and purchased via a "dealer," to protect the identity of the producer. Consequently any investigation into the prevelance or epidemiology of the disease in the Britain was impossible. The ears of the rabbits were examined and ear canker

lesion scored according to the method described by Guillot and Wright (1981) (Table 2.5.1).

3.3. Microscopical Examination

The contents of the ear canals and all skin scrapings were examined according to the methods described in Chapters 2.1 and 2.2.

4.0. Results

4.1. Wild Rabbits

No evidence of *P. cuniculi* infestation was recorded in any wild rabbit examined in the two surveys (ears and scalp/entire head) 1988 to 1992 or from animals sharing grazing with sheep flocks known to be affected by psoroptic otoacariasis.

4.2. Domestic Rabbits

Only infested animals were received at Weybridge, the majority with lesion scores ranging between 1.0 and 6.0. In animals with mild infestations (score 1.0) mites were observed deep in the external auditory canal. A waxy (ceruminous) "plug" was sometimes observed in the canal. Microscopic examination of these plugs revealed all instars of *Psoroptes* mites living within the waxy secretion. The mites were observed to actively burrow into the wax. The majority of the observed mites were pearly white in appearance, but occassionaly black to dark red adult female mites were observed. In heavy infestations (scores 3.0 to 6.0) the opening to the canal was completely occluded by purulent material, with no live mites observed, but the pinna itself exhibited large numbers of dark red/black mites.

Of the eighty infested rabbits, four (5.0%) manifested extra-auricular ("ectopic") disease. Two were purchased in February, one in March and one in May 1990, and euthanased directly on arrival at Weybridge. Both animals examined in February presented extensive mange of the entire pinnae (both score 6.0), the base of the ears, the cheeks, dewlap and face (Figure 2.9.5). Scab lesions the inner apects of both pinnae and lesion were laminated and approximately 1.0 cm thick. Large numbers of dark red to black mites were present, both in the ear and under the body

lesions. The animal identified in March was in an extremely poor condition and presented scab lesions covering the face from the base of the ears to the eyes. The eyes themselves were purulent and partially closed. The animal identified in May presented severe mange, involving the inner apects of both pinnae (both score 6.0) and the base of both ears. Lesions and mites were also present between the digits of both hind feet, covering the length of the digits and 1.0 cm up the paw. The animal also suffered from malocclusion of all four incisors.

Table 2.5.1: The Lesion Scoring Method of Guillot and Wright (1981).

Lesion Score	Observation
0.0	No scab or mites
0.5	Irritation in canal, no mites seen
1.0	Small numbers of scabs in ear canal. Mites present.
2.0	Outer ear canal filled with scab. Mites present.
3.0	Scabs in outer ear canal and proximal quarter of pinna.
4.0	Half pinna affected. Mites present.
5.0	Three quarters pinna affected. Mites present.
6.0	Entire pinna affected. Mites present.

Chapter 2.6

Laboratory Investigations into the Prevalence and Epidemiology of the Sheep Scab Mite (*Psoroptes ovis*) in the Ear Canals of Infested Sheep:

1.0. Summary

Two hundred and seventy seven, closewool breed sheep with active artificial infestations of the sheep scab mite (*P. ovis*), were examined for the presence of live mites in the external auditory canal. Live *P. ovis* were observed in the ear canals of 38.6% of infested sheep. Infestation were seasonal, with 64.2% recorded in November and December. Of the six isolates of *P. ovis* examined 90.0% of sheep infested with the Bacup isolate were infested in the EAC, followed by the Porlock (69.4%), Little Melton (50.0%), Caithness (41.0%), St. Brenard (29.1%) and VLA Reference (24.0%) isolates.

Sheep identified with ear mites presented lesion areas ranging between 20.9% to 100.0 % body cover. Two phases of infestation were observed: i) the early stages of active disease, with lesion areas ranging between 11.0% to 43.9% body cover, accounting for 60.4% of ear infestations and ii) the peak of active disease/decline with areas ranging between 98.9% to 100.0% body cover, accounting for 22.6 % of ear infestations. All other lesion areas accounted for 17.9% of infested ears.

Mites did not colonise either the EAC or the pinna in 56.9% of ears examined. The EAC or the pinna were exclusively infested in 29.2% and 6.9% of ears examined, respectively, and 6.9% of ears were infested simultaneously in both the EAC and the pinna. The mean lesion area for sheep exclusively infested in the EAC was approximately 43% body cover. The mean lesion areas for sheep infested exclusively in the pinnae or with simultaneous infestations in both the pinnae and the EAC were approximately 83.8% and 80.6% body cover respectively. Pinneal damage (haematomae/fibrosis) was observed in 2.5% of ears examined but ear damage was not indicative of infestation, with 21.4% of damaged ears neither infested in the EAC or the pinna. Of the remaining damaged ears, 57.1% were infested exclusively in the pinna, with the remaining 21.4% infested exclusively in the EAC.

Results demonstrated that the lower the mite burden, the increased incidence of infestation within the EAC. Burdens of 0.0 to 50.0 mites per lesion accounting for 58.6% of infestations in the EAC. The mean mite burden for sheep uninfested in both the EAC and the pinnae was 77.2 mites per lesion and 72.5 mites per lesion for sheep

exclusively infested in the pinna. The mean mite burden for sheep exclusively infested in the EAC was 109.4 mites lesion^{-1} and 25.0 mites lesion^{-1} for sheep with mites in both the EAC and pinna.

The incidence of otoacariasis increased the nearer the lesion edge approached the ears, with 40.0% of infestations recorded when the leading edge of the lesion had reached the head and/or the base of the ears. Another 40.0% of infestations were recorded when the leading edge had reached between the withers and the base of the neck. Yet 20.0% of sheep were infested in the EAC when the leading edge of the lesion was as far away as the mid back.

2.0. Introduction

Zurn (1877) reported scab mites (*P. ovis*) in the ears of sheep to be a common occurrence. Imes (1916), Miller (1925) and Verney (1926) stated that mites can sometimes be found in the ears and that recrudescence of disease could be due to inadequate dressing of these sites.

Sheep scab is a winter disease, with the majority of cases occuring between September and April, although a significant number of cases do occur in the summer months (Bates, 1991b). Populations of *P ovis* were thought to diminish (often to extinction) during the summer or actively migrate to the "cryptic or latent sites" (ie. the ears, the infra-orbital fossae (IOF), the inguinal pouches, the crutch, the perineum and the interdigital fossae) at the onset of summer, remaining quiescent until the onset of autumn/winter (Roberts *et al.,* 1971; Roberts and Meleney, 1971; Downing, 1936; Shilston, 1915 and Stockman, 1910). This phenomena of "latent phase" or "suppressed scab" was first described by Downing (1936) and later expanded by Spence (1949). The work of Spence (1949) showed that mites will enter the cryptic sites 45 to 60 days after the onset of the active phase of disease (when extensive encrustation and denudation has rendered the body surface unsuitable). Early disease in the cryptic sites is characterised by little inflammation and encrustation and without the characteristic touch (hypersensitivity) response (THR) and scratching associated with classical sheep scab. The general progression of disease is similar to but slower than body disease, with crusts slowly accumulating and eventually terminating infestations, by driving mites out to perish. The reduced pathological reaction could be attributed to the greater tolerance of skin or to the production of wax reducing mite numbers by mechanical entanglement. The cryptic phase may be asymptomatic, but mites can be found by careful examination. In the USA Roberts *et al.,* (1971) recorded that infestations could escape detection for over a year and that these long periods of latency and a sudden increase in vigour and pathogenicity of a mite strain could account for unexplained outbreaks of disease (Roberts and Meleney, 1971). Summer decline in *P. ovis* populations has been attributed to a) climate (extreme cold, as an environmental stressor, directly altering host resistance) (Kelley 1980); b) host nutrition (improvements in body condition and the oily state of the skin and fleece, rendering the environment unsuitable for mite survival (Downing, 1936 and Shilston

1915); c) shearing (stopping the progress of disease, either temporarily or permenently, by removing the micro-climate) or d) the strain of *P ovis* (highly pathogenic strains withstanding summer population reduction more than avirulent populations) (Roberts and Meleney, 1971).

Spence (1949) found the ears and the infra orbital fossae (IOF) the most frequented cryptic site in the summer (82.5% and 81.3% respectively), followed by the inguinal pouches (56.9%), vulval folds (36.9%), interdigital fossae (29.0%), perineum (17.4%) and the general body (13.9%). Unfortunately he did not differentiate between the pinnae or the EAC. Spence also demonstrated that despite encrustation infestation in the ear often escapes detection and can only be demonstrated by post mortem examination, with large numbers of mites found in the EAC.

This chapter investigates the prevalence of *P. ovis* in the external auditory canals of sheep presenting moderate to severe artificial infestations of psoroptic body mange (sheep scab), relative to the presence or absence of aural damage (haematomae/fibrosis), lesion area, mite burden, distance of the leading lesion edge from the ears, season and mite strain.

3.0. Materials and Methods

3.1 Source of Infestation

Two hundred and seventy seven, closewool breed sheep artificially infested with *P. ovis*, were examined for the presence of live mites in the external auditory canal. The sheep were a mixture of heavily infested cull sheep or untreated control sheep from acaricide efficacy studies with lesion areas ranging between 0.0 and 100% body cover. The sheep were infested on the withers by either tying-in infested scab and wool or by placing 25 adult female *P. ovis* directly onto the skin, within a 1.0 cm^2 area of plucked of wool. In each case the area of challenge was marked using a stock marker ring (Agrimark, Pfizer). Six isolates derived from natural outbreaks in Caithness (isolated in 1994), St. Brenard (isolated in 1989), Bacup (isolated in 1991), Little Melton (isolated in 1990) and Porlock (isolated in 1994), and since maintained

in vivo at the VLA, Weybridge (Appendix One), were compared to the VLA Reference Isolate (originally isolated prior to the eradication of scab from Great Britain and augmented with mites from the first foci of infestation in Lancashire on re-introduction of the disease in 1973). The Caithness and Porlock isolates were the original flumethrin (synthetic pyrethroid, (SP) resistant isolates described by Synge *et al.*, (1995).

3.2. Parasitological Assessments

Mite Counts

Mite counts were were carried out before slaughter. The wool at the periphery of the lesion was parted at 5.0 cm intervals, starting at the mid-neck and proceeding along the right hand side of the lesion, continuing until the initial parting was reached. At each count, the total numbers of adult female *P. ovis* were counted individually up to 10 and estimated in units of 5 thereafter. Numbers of partings thus varied in relation to the lesion area.

Assessment of the Lesion Area.

The length of the lesion was measured along the backbone and the width measured from the point nearest to the ventral surfaces on both flanks. Measurements were taken using a washable plastic tailors tape measure and the result expressed as length x width, giving an estimation of lesion area in cm^2.

Examination of the Ears and Infra-Orbital Fossae (IOF).

The infra orbital fossae (IOF) and the internal aspects of the pinnae of each animal were examined for scab lesions and for ear damage (haematomae/fibrosis). The severity of ear damage was recorded as, absent (-); mild (+), with slight thickening of the pinna; moderate (++), with severe thickening or fibrosis of the pinnae, without abscessation or severe (+++), ranging from thickened pinnae with abcessation to chronically thickened and deformed cauliflower ears.

All sheep were ear swabbed, according to the method described in Chapter 2.1, prior to infestation. The external auditory canals of the cull sheep infested with the St. Brenard, Bacup, Little Melton and VLA Reference Isolate were examined at post mortem according to the method described in Chapter 2.2. The Caithness and Porlock isolates were ear swabbed prior to plunge dipping, approximately 42 days post infestation.

4.0. Results

No sheep was shown to have pre-existing *Psoroptes* infestations of the pinnae or the EAC. Live *P. ovis* were isolated from the EAC of 107 of the 277 (38.6%) of infested sheep examined (Table 2.6.1). Of these 38/107 (35.5%) were unilateral infestions in the left ear, 19/107 (17.8%) were unilateral infestations in the right ear and 50/107 (46.7%) were bilteral infestions in both the left and right ears. 170/277 (61.4%) were negative for psoroptic otacariasis in both ears. Infestation of the EAC followed a seasonal pattern (Figure 2.6.3), with a peak of infestation (64.2%) in November and December. The incidence of otoacariasis was not affected by the sex of the host, with 29.7% of infested sheep comprising of entire or neutered males and 33.9% females. Of the six field isolates of *P. ovis* examined 9/10 (90.0%) of sheep infested with the Bacup isolate were infested in the EAC, followed by the Porlock, Little Melton, Caithness, St. Brenard and VLA, Reference isolates respectively (25/36 (69.4%), 6/12 (50.0%), 17/41 (41.0%), 30/103 (29.1%) and 22/90 (24%) respectively (Table 2.6.1).

Sheep identified with ear mites presented lesion areas ranging between 20.9% to 100.0 % and although sheep could be infested with lesions anywhere within this range, two "peaks" of prevalence were observed (Table 2.6.2 and Figure 2.6.2): a) the early stages of active disease, with lesion areas between 11.0% to 43.9% body cover, accounting for 64/106 (60.4%) of ear infestations and b) the peak of active disease/decline with areas ranging between 98.9% to 100.0% body cover, accounting for 24/106 (22.6 %) of ear infestations. All other lesion areas accounted for 18/106 (17.9%) of infested ears.

Mites had not colonised either the EAC or the pinna in 189/332 (56.9%) of ears examined. The EAC or the pinna were exclusively infested in 97/332 (29.2%) and 23/332 (6.9%) of ears examined, respectively. Similarly, 23/332 (6.9%) of ears examined were infested simultaneously in both the EAC and the pinna. The mean lesion area for sheep exclusively infested in the EAC was 3885.0 cm^2 (± 3795.9), approximately 43% body cover. The mean lesion area for sheep infested exclusively in the pinnae was 10,995.0 cm^2 (± 3186.7) and 10,580.2 cm^2 (± 3542.4) for sheep infested in both the EAC and pinna, approximately 83.8% and 80.6% body cover respectively. Pinneal damage (haematomae/fibrosis) was observed in 14/554 (2.5%) of ears examined. Ear damage was not indicative of infestation, with 3/14 (21.4%) of damaged ears neither infested in the EAC or the pinna. Of the remaining damaged ears, 8/14 (57.1%) were infested exclusively in the pinna, with the remaining three (21.4%), infested exclusively in the EAC.

Results show that the lower the mite burden, the increased incidence of infestation within the EAC (Table 2.6.3 and Figure 2.6.2). Burdens of 0.0 to 50.0 mites per lesion accounting for 58.6% of infestations in the EAC. The mean mite burden for sheep uninfested in both the EAC and the pinnae was 77.2 (± 123.5) mites per lesion and 72.5 (± 72.5) mites per lesion for sheep exclusively infested in the pinna. The mean mite burden for sheep exclusively infested in the EAC was 109.4 (± 114.6) mites $lesion^{-1}$ and 25.0 (± 66.1) mites $lesion^{-1}$ for sheep with mites in both the EAC and pinna.

The incidence of otoacariasis increased the nearer the lesion edge was to the ears, with 22/55 (40.0%) of infestations recorded when the leading edge of the lesion had reached the head and/or the base of the ears. Another 22/55 (40.0%) of infestations were recorded when the leading edge had reached between the withers and the base of the neck. Yet 11/55 (20.0%) of sheep were infested in the EAC when the leading edge of the lesion was as far away as the mid back (20.0 to 55.0 cm from the ears).

Isolate	No. Sheep Examined	Lesion % body cover			Otoacariasis	
		Mean	Min	Max	(n)	%
St. Brenard (1989)	94	38.9	7.9	100.0	31.0	32.9
VLA Ref. (1973)	84	48.3	0.0	98.9	19.0	22.6
Caithness (1994)	41	83.2	41.5	80.3	17.0	41.5
Porlock (1994)	36	55.5	0.0	97.1	25.0	69.4
Ltl.Melton. (1990)	12	82.2	39.2	91.3	6.0	50.0
Bacup (1991)	10	94.1	86.7	91.3	9.0	90.0
Total	**277.0**	**67.3**	**0.0**	**100.0**	**107.0**	**38.6**

Table 2.6.1: Prevalence of the sheep scab mite (*P. ovis*) in the external auditory canal (EAC) of heavily infested cull sheep, challenged with six isolates of *P.ovis*.

Isolate	Infestation of the EAC or pinna/Mean lesion area (cm^2) ±sd.			
	EAC-/Pinna -	**EAC-/Pinna +**	**EAC+/Pinna-**	**EAC+/Pinna+**
VLA Ref.	5149.0 ±4340.4	None recorded	6027.2±4551.2	7276.6±4005.8
St.Brenard	4336.9±4225.4	9388.5±3160.9	4499.9±4138.1	12,379.3±4607.1
Porlock	4078.5±2371.0	None recorded	2202.3±672.8	None recorded
Little Melton	6153.0±2403.0	12,057.0**	12,057.0*	10,580.5±1590.4
Bacup	12,057.0*	None recorded	12,057.0*	12,190.6±612.9
Caithness I	4408.6±2371.4	None recorded	2994.6±364.7	None recorded
(n)	75	7	66	19
Mean (lesion area)	4970.5±4050.0	10,995.0±3186.7	3885.0±3795.9	10,580.2±3542.4
Mean (% body cover)	4970.5±4050.0	10,995.0±3186.7	3885.0±3795.9	10,580.2±3542.4

Table 2.6.2: Prevelance of six isolates of the sheep scab mite (*P. ovis*) in the external auditory canals (EAC) of cull sheep, correlation with lesion area.* = Single sheep. ** = Two sheep with same lesion area.

Isolate	**Infestation of the EAC or pinna**/(Mean mite burden ±sd (range)			
	EAC-/Pinna -	**EAC-/Pinna +**	**EAC+/Pinna-**	**EAC+/Pinna+**
VLA Ref.	47.9 ± 102.5 (0.0 to 455.0)	Not Recorded	30.1± 41.3 (0.0 to 122.0)	Not Recorded
St.Brenard	88.7 ± 80.0 (0.0 to 280.0)	72.5 ± 72.5** (0.0 to 145.0)	117.4 ± 128.4 (0.0 to 540.0)	100.0 ± 100.0 (0.0 to 200.0)
Porlock	96.8 ± 148.0 (0.0 to 490.0)	Not Recorded	119.0 ± 94.9 (0.0 to 399.0)	Not Recorded
Little Melton	Not Recorded	Not Recorded	Not Recorded	Not Recorded
Bacup	0.0*	Not Recorded	0.0	0.0
Caithness I	130.5±177.8 (0.0 to 486.0)	Not Recorded	198.8 ± 127.2 (0.0 to 431.0)	Not Recorded
(n)	50	2	65	2
Mean	77.2 ± 123.5 (0.0 to 490.0)	72.5 ± 72.5 (0.0 to 145.0)	109.4 ± 114.6 (0.0 to 540.0)	25.0 ± 66.1 (0.0 to 200)

Table 2.6.3: Prevelance of six isolates of the sheep scab mite (*P. ovis*) in the external auditory canals (EAC) of cull sheep, correlation with mite burden. * = Single sheep. ** = Two sheep with same lesion area.

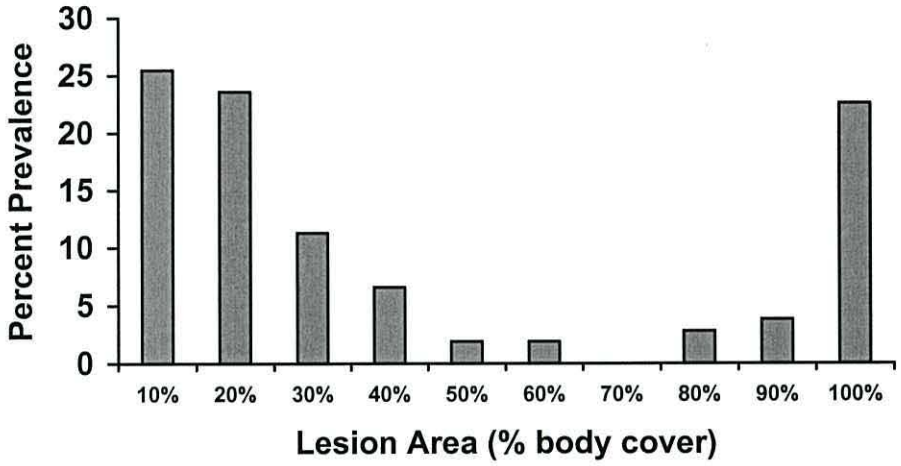


Figure 2.6.1: The prevalence of all isolates of the sheep scab mite (*P ovis*) recorded in the external auditory canals of cull infested sheep, with respect to lesion area (percent body cover). Data presented as a percent of the total infested in the EAC (n = 106).

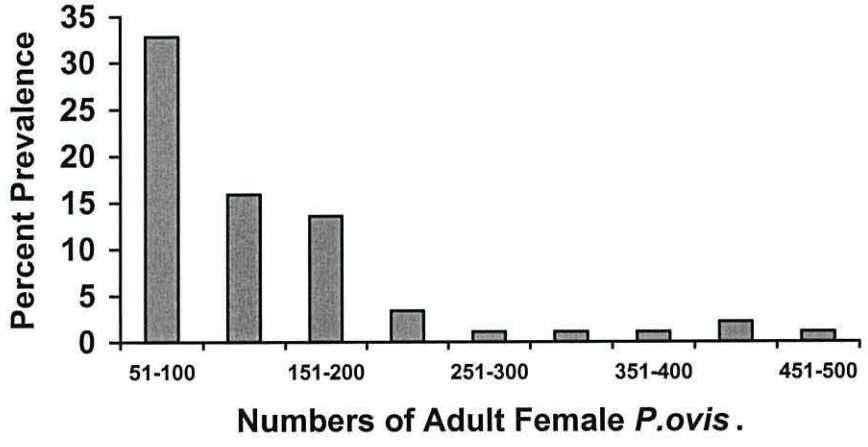
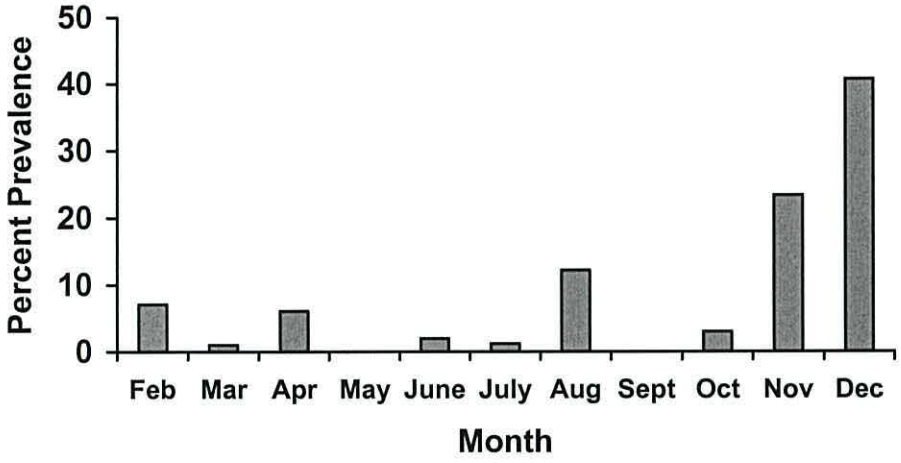


Figure 2.6.2: The prevalence of all isolates of the sheep scab mite (*P ovis*) recorded in the external auditory canals of cull infested sheep with respect to mite burdens. Data presented as a percent of the total infested in the EAC (n = 88).



Figue 2.6.3: The prevalence of all isolates of the sheep scab mite (*P ovis*) recorded in the external auditory canals of cull infested sheep with respect to season. Results presented as percent of the total infested in the EAC (n = 98) between January and December 1989 to 1995.

Chapter 2.7

Investigations into the Prevalence of Bovine Psoroptic Mange in Great Britain

1.0. Summary

The prevelence of bovine psoroptic mange in Great Britain was investigated by examining i) the day books and diagnostic records archived at the VLA, Weybridge between 1962 and 1997, ii) the Veterinary Investigation Data Analysis (VIDA) returns from seventeen English VLA Regional (formerly Veterinary Investigation) Centres, between 1986 and 1990, iii) a literature search to identify published reports of field cases of bovine psoroptic mange in Great Britain and iv) a survey sponsored by Pfizer Animal Health 1996 to 1997. The results demonstrated that between 1962 and 1997, 61.4% of bovine mange in Great Britain was caused by *Chorioptes bovis* and 30.0% caused by *Sarcoptes scabiei* var *bovis*. The remaining 8.6% of cases were due to isolated outbreaks of *Psoroptes* spp imported from mainland Europe. Bovine psoroptic mange is not therefore endemic to Great Britain.

2.0. Introduction

Hirst (1922) described two species of *Psoroptes* infesting the bodies of cattle: *P. communis* var *bovis* (Furstenberg, 1861) and *P. natalensis* (Hirst, 1919). Sweatman (1958) defined *P. ovis* (Hering) as a body mite of domestic sheep, bighorn sheep, cattle, horses and possibly the donkey and mule and considered the body mite of sheep (*P. ovis*) to be synonymous with the body mite of cattle, previously known as *P. bovis* Gerlach. *P. ovis* infestations of cattle have been recorded in Argentina (Nuñez, 1989), Czechoslovakia (Sevcikova *et al.,* 1987), Belgium (Losson, 1996), India (Gill *et al.,* 1989), Italy (Genchi *et al.,* 1995), Libya (Gabaj *et al.,* 1992) and USA (Hourrigan, 1979).

P. natalensis was first described from specimens found on cattle at Richmond, Natal Province, South Africa (Hirst, 1922). Sweatman (1958) defined *P. natalensis* as a body mite of domestic cattle, zebu cattle, Indian water buffalo and horses. *P. natalensis* has since been recorded infesting cattle in Brazil (Sweatman, 1958), France (Sweatman, 1958), India (Shastri and Ghafoor, 1974), New Zealand (Sweatman, 1958), South Africa (Hirst, 1922 and Sweatman, 1958), South America (Rocha *et al.*, 1952), and Uruguay (Sweatman, 1958). Sweatman (1958) also recorded *P. natalensis* infesting horses at Onderstepoort, South Africa. *P. ovis* can be differentiated morphologically from *P. natalensis* by the comparative morphology and lengths of the adult male L_4 outer opisthosomal setae (L_4 OOS).

Early psoroptic mange begins as moist plaques of hair over the withers, followed by intense pruritus with active rubbing against fixed equipment, leading to loss of hair, serum exudation, ulceration, bleeding and eventually thickened, scabby lesions, oozing blood and serum, progressing over the withers and tailhead, before extending along the back and down the flanks and legs (Linklater and Gillespie, 1984). Pyodermatitis is common due to secondary bacterial infections. Psoroptic mange of cattle can be life threatening to calves under one year old but deaths rarely occur in older animals, although Losson (1996) stated that infested cattle are predisposed to pulmonary infections and may die. Cattle mange can spread rapidly within confined situations of a feedlot but transmission at pasture is slower, especially in the summer when there is no close body contact and mites are in the ("alleged")

quiescent phase (Meleney and Christy, 1978). Like sheep scab, bovine psoroptic mange is considered a winter disease, but clinical outbreaks are sometimes observed in July - August (Hirst, 1922 and Losson, 1996). Also in comparison to the prevalence of sheep scab, heavily infested cattle are readily detected, but lightly infested cattle are difficult to detect, especially during the early stages of disease (or summer latency), when lesions are very small (Fisher *et al.,* 1986, Bates, 1997). Mixed infestations with *Chorioptes bovis* or *Sarcoptes scabiei* var *bovis* are common, complicating control measures (Losson, 1996).

Bovine psoroptic mange is present on mainland Europe and in Belgium an estimated 400,000 cattle are treated each year (Pouplard *et al.,* 1990). Belgian White and Blue cattle (B.W.B) represent around 50% of the Belgian national herd and are highly susceptible to *Psoroptes,* with infestations being generalised and chronic (Losson, 1996). In general beef breeds are more susceptible, dairy breeds (eg. Holstein) are more resistant: experimental infestations are difficult to perform and self cure is the rule (Losson, 1996). Bovine psoroptic mange was once notifiable in the USA and is still considered to be a major parasite of cattle.

At the beginning of the First World War all but a small percentage of bovine mange in Britain was due to *Psoroptes* sp. Psoroptic mange then became rarer, with sarcoptic mange becoming more prevelent (Hirst, 1922), only to become widespread once again in the 1940s (Anon, 1949). The disease then again decreased in prevelence and since 1975 has only been reported six times: 1975 (MAFF Quarantine Station, Dundee), 1976 (Cumbria), 1978 (Selkirkshire), 1980 (Sussex) all possibly connected via animal movements (Taylor, 1978) and the last cases occured in the Scottish Borders in 1980 and 1983 (Linklater and Gillespie, 1984). Evidence suggests that on all occasions the disease was imported from mainland Europe and although difficult to eradicate did not pass to other animals. The single European market and the relaxation of EU import regulations in 1993 may have resulted in an increase in the incidence of psoroptic mange in cattle. Warble fly (*Hypoderma* spp) was eradicated from the UK for several years before it's (temporary) re-introduction into the national herd in 1993 (Tarry and Sinclair, 1995).

This chapter reports investigation into the prevelence of bovine psoroptic mange (*P. ovis* or *P natalensis*) within the national herd in relation to other forms of bovine mange (eg. chorioptic or sarcoptic).

3.0. Materials and Methods

The prevelence of bovine psoroptic mange in Great Britain was investigated by the following methods.

3.1. Diagnosis reports filed at the VLA, (Weybridge) (1962 to 1998).

Day books and diagnostic records between 1962 (the oldest material available) to 1997, archived at the VLA, Weybridge were examined for entries concerning the differential diagnosis of bovine mange (chorioptic, psoroptic or sarcoptic) in Great Britain.

3.2. VIDA Returns (1986 to 1990).

Veterinary Investigation Data Analysis (VIDA) returns from seventeen English VLA Regional (formerly Veterinary Investigation) Centres, between 1986 (the oldest material available) and 1990 (the most up to date version available) were also examined for records concerning the differential diagnosis of bovine mange (chorioptic, psoroptic or sarcoptic) in Great Britain.

3.3. Case investigations of bovine psoroptic mange in Great Britain.

A literature search was conducted to identify published reports of field cases of bovine psoroptic mange in Great Britain.

3.4. A survey sponsored by Pfizer Animal Health 1996 to 1997.

Selected veterinary practices, distributed throughout Great Britain, were requested to take skin scrapings from cattle presenting skin lesions. Skin scrapings were taken, according to the method described by MAFF (1986), and submitted together with the scalpel blade and a submission form, to the VLA (Weybridge) for examination. Scrapings were examined under a dissecting microscope with overhead lighting (American Optical, magnification x40) for the presence of live mites. If live mites were not observed the scrapings were digested in boiling 10% potassium hydroxide, and examined for the presence of *Chorioptes* sp, *Psoroptes* sp or *Sarcoptes* sp mites according to the methods described in Chapter 2.1.

4.0 Results

The results of these investigations, shown in Table 2.7.1, demonstrate that between 1962 and 1997, 61.4% of bovine mange in Great Britain was caused by *Chorioptes bovis* and 30.0% caused by *Sarcoptes scabiei* var *bovis*. The remaining 8.6% of cases were due to isolated outbreaks of *Psoroptes* spp imported from mainland Europe (Taylor, 1978 and Linklater and Gillespie 1984).

4.1. VLA (Weybridge) Diagnosis Reports 1962 to 1997.

The results for the examination of VLA (Weybridge) Diagnosis Reports are shown in Table 2.7.1. Only 13 cases of bovine mange were recorded at the VLA (Weybridge) between 1962 to 1997, 10/13 (76.9%) of these cases were caused by *Chorioptes bovis* and the remaining 3/13 (23.0%) of cases caused by *Sarcoptes scabiei* var *bovis*. Psoroptic mange was not recorded.

4.2. VIDA Returns 1986 to 1990.

The results for the examination of VIDA returns are also shown in Table 2.7.1. There were 39 records of cattle mange between 1986 and 1990 with 23/39 (59%) of the cases identified as *C bovis* and 16/39 (41%) identified as *S scabiei* var *bovis*. Once again there were no records of psoroptic mange in English cattle during this period.

4.3. Case investigations of bovine psoroptic mange in Great Britain.

The literature search revealed only six outbreaks of bovine psoroptic mange in Great Britain, documented by Taylor (1978) and Linklater and Gillespie (1984). Taylor (1978) recorded a case in Sussex in 1980, originating from a herd in Selkirkshire in the Borders of Scotland in 1978. This outbreak itself originating from a Charolais bull originating from a herd in Cumbria in 1976. Mange was thought to be introduced to the Cumbrian herd through a Charolais heifer imported from France, via the MAFF quarantine Station, Dundee in February 1975. The 1983 case recorded by Linklater and Gillespie (1984) was located in the St Boswells area of the Borders of Scotland, also originating from cattle imported from France (Figure 2.9.6).

4.4. Pfizer Survey 1996 to 1997.

The results of the Pfizer Survey are also presented in Table 2.7.1. Thirty six skin scrapings were submitted to the VLA, Weybridge from 28 different cattle herds. Herds originated from Oxfordshire (6), Dorset (5), Gloucestershire (2), Lanarkshire (4), Somerset (4), Norfolk (2), Wiltshire (2), Cornwall (1), Warwickshire (1) and West Yorkshire (1). Cattle mange was identified in twelve herds (42.8%) with *C bovis* identified in 10/12 (83%) of affected herds and *S scabiei* var *bovis* indentified in the remaining 2/12 (17%) of affected herds.

Source	*Chorioptes* spp	*Psoroptes* spp	*Sarcoptes* spp
a. VLA Records (1962 - 1974)	10/13 (76.9%)	0/13	3/13 (23.0%)
b. Investigated Cases (1979 - 1980)	0/6	6/6 (100.0%)	0/6
c. VIDA Returns (1986 - 1990)	23/39 (59.0%)	0/39	16/39 (41.0%)
d. Pfizer Survey (1996 - 1997)	10/12 (83.0%)	0/12	2/12 (17.0%)
Total (1962 - 1997)	43/70 (61.4%)	6/70 (8.6%)	21/70 (30.0%)

Table 2.7.1. Prevelence of chorioptic, psoroptic and sarcoptic bovine mange in Great Britain (1962 to 1997). Sources: a) Examination of diagnosis reports filed at the VLA from 1962 to 1974; b) Documented case investigations; c) Examination of the Veterinary Investigation Data Analysis (VIDA) returns from seventeen English V I Centres, between 1986 and 1990 and d) a veterinary practioner survey sponsored by Pfizer Animal Health 1996 to 1997.

Chapter 2.8

Investigations into the Prevalence of *Psoroptes sp* Infesting Wild and Captive Herbivores in Britain

1.0. Summary

The prevalence of psoroptic otoacariasis or mange on captive or wild non-domesticated hervivores in Great Britain was investigated by examining i) day books and diagnostic records, archived at the VLA, Weybridge between 1962 and 1997 and ii) a literature search identifying published reports of field cases of psoroptic mange or otoacariasis of captive or wild, non-domesticated hervivores in Great Britain. Only three "non native" mammalian hosts for *Psoroptes* spp. were recorded in the day books and diagnostic records: i) Barbary sheep (*Ammotragus lervia*) presenting clinical mange at Flamingo Park Zoo, Manchester in 1974 and ii) alpacas (*Lama pacos*) in Cumbria 1999 and llamas (*Lama glama*) in Sussex, 1998.

2.0. Introduction

The ear mite *P cuniculi* has been recorded infesting the ears of a number of wild herbivores (mainly in the USA), including: blackbuck antelope (*Antelope cervicapra*) in the USA (Wright *et al.,* 1988), impala (*Aepyceros melampus*) at the Municipal Zoo in Tel Aviv, Israel (Rauchbach cited by Yeruham *et al.,* 1985), mountain goat (*Capra ibex nubiana*), Isreal (Yeruham *et al.*, 1978), mule deer (*Odocoileus hemionus*) USA (Roberts *et al.,* 1970), stone sheep (*Ovis dalli stonei*) Canada (Foreyt, 1997), water buffalo (*Bubalus bubalus*) India (Shastri and Ghafoor (1974), white tailed deer (*Odocoileus virginianus*) USA (Strickland *et al.,* 1970 and Schmitt *et al.,* 1982) and yaez (*Capra ibex nubiana* cross *Capra hircus*) Israel (Yeruham *et al.,* 1978). Undefined species of *Psoroptes* (presumably *P cuniculi*) have been recorded infesting the ears of antelope and gazelle (exact species or country of origin not specified) (Caparini, 1887 and Canestrani, 1894. Cited by Sweatman, 1958) and wild goats (*Capra hircus*) in the Pyrenees (Pezas, cited by Ward 1915). The ear mite *P cervinus* appears confined to North America where it has been recorded infesting the ears of American elk (*Cervus canadensis* Nelsoni), wapiti (*Cervus elephas canadensis*) and Bighorn sheep (*Ovis canadensis mexicana, O. canadensis canadensis*) (Hepworth and Thomas, 1962;Sweatman, 1958 and Ward, 1915).

Bighorn sheep in the USA have also been reported to be infested in the ears with *P. cuniculi* (Lange *et al.,* 1980, Kinzer *et al.,* 1983). Meleney *et al.,* (1980) described *Psoroptes* spp infesting bighorn sheep (*O. canadensis mexicana*) as an ear mite which sometimes invades the surrounding body areas with devastating (sometimes fatal) results and that the mites have many characteristics of *P. ovis, P. cuniculi* and *P. cervinus*. *Psoroptes* spp have been recovered from Desert bighorn (*O. canadensis mexicana*) and Rocky Mountain bighorn (*O. canadensis canadensis*) in Arizona, California, Idaho, New Mexico, Nevada, Oregon, Washington and Wyoming (Boyce, 1990a; Foreyt *et al.,* 1985; Lange *et al.,* 1980 and Muschenheim *et al,* 1990). Lesions were most prominent on the ears, face and neck, but extensive lesions did occur in severe infestations often resulting in mortality (Lange *et al.,* 1980; Welsh and Bunch 1983 and Foreyt *et al.,* 1985). Photographs by Lange *et al.,* (1980) of infested bighorn ears show symptomology similar to *P. cuniculi* infestations of rabbits (with typical dry, laminated aural scab). This form of ear mange is rarely as extensive in

domestic sheep in Britain. Lange *et al.,* (1980) also recorded the mites to be "deep brown" in colour suggesting the mite has access to blood. Mites in the ears of sheep in the UK are always "pearly white" suggesting the *P. ovis* in the concha of the pinna does not have access to or feed on red blood cells (Bates, *unpublished observations*) *Psoroptes* spp isolated from Bighorn sheep do not cause disease in sheep or cattle but can infest rabbits restricted from grooming (Wright *et al.,* 1981).

A further three *Psoroptes* sp found in un-domesticated herbivores have yet to be subjected to modern re-analysis and their actual status is still, at present, unknown (Strong and Halliday, 1992). A *Psoroptes* mite, tentatively described as *P equi* var *leporis* was isolated from the European hare (*Lepus capensis* (*L europaeus*)) by Valek and Novakova (1959) and Fain (1970) isolated a *Psoroptes* mite from the head of African Buffalo (*Syncerus caffer*) and named it *P pienaari.*

Ear mites of New World camelids *P auchinae* (*P communis* var *auchinae*) are well documented and have been recorded from the ears of alpacas (*Lama pacos*) and the bodies and ears of llamas (*L. glama*) (Alverado *et al.*, 1966; Chavez and Guerrero, 1965; Guerrero and La Rosa, 1962; Foreyt *et al.,* 1992 and Fowler, 1989). Alverado *et al.*, (1966) examined skin scrapings from sites other than the ears of llamas and found mites present in the perineum, nose, axillae, groin, neck and legs. Common lesions consisted of "big dry flakes" in the ears, occasionaly secondary infestions could fill the ears with purulent discharge responsible for headshaking and incoordination.

Representitives of all four species of New World camelid can now be found in Britain, either farmed, in zoos and circuses or as companion animals. These are represented by the llama (*L. glama*), the guanaco (*L. guanacoe*), the alpaca (*L. pacos*) and the vicuna (*Vicugna vicugna*). In 1990 there were approximately 100 New World camelid breeders in the UK, owning a total of 700 llamas, 100 alpacas and 300 guanacos (Hook, 1990). The camelid population of the UK has now increased and is currently estimated to be 3000 llamas, 1000 alpacas, 400 - 500 guanacos, 20 vicuna and approximately 100 camels (dromedaries and bacterians), mostly located in the southern half of the country (British Camelids Ltd Owners and Breeders Association, *pers comm*). Nearly all the Old World Camelids (the two humped bactrian camel

(*Camelus bactrianus*) and the one humped dromedary or the Arabian camel (*C dromedarius*)) are located in zoological gardens or wildlife parks, but the majority of New World camelids are kept privately for fibre production with alpacas and gunacos producing top quality fibre or as companion animals.

P. ovis infestations (based upon L_4 Outer Opisthosomal Setal Lengths) have been recorded on a giraffe (*Giraffe camelopardis*) in the Masai Mara Game Reserve in Kenya (Burr, 1984) but mites were not isolated from the external ear. *Psoroptes* mites (species not designated) have also been isolated from clinical mange in Libyan camels (*Camelus dromaderius*) (Gabaj *et al.*, 1992).

3.0. Materials and Methods

The prevalence of psoroptic otoacariasis or mange on captive or wild non-domesticated hervivores in Great Britain was investigated by the following methods.

3.1. Diagnosis reports filed at the VLA, (Weybridge) (1962 to 1998).

Day books and diagnostic records between 1962 (the oldest material available) to 1997, archived at the VLA, Weybridge were examined for entries concerning the diagnosis of psoroptic mange of captive or wild, non-domesticated hervivores in Great Britain.

3.2. Case investigations of psoroptic mange in Great Britain.

A literature search was conducted to identify published reports of field cases of psoroptic mange or otoacariasis of captive or wild, non-domesticated hervivores in Great Britain.

4.0 Results

The literature search failed to identify any cases of psoroptic mange or otoacariasis of captive or wild, non-domesticated hervivores were recorded in Great Britain. Only three "non native" mammalian hosts for *Psoroptes* spp. were recorded in the day books and diagnostic records archived at the VLA, Weybridge between 1962 and 1997.

1. Barbary sheep (*Ammotragus lervia*) presenting clinical mange at Flamingo Park Zoo, Manchester in 1974.

2. Alpacas (*L. pacos*) and Llamas (*L. glama*). Two cases of psoroptic otoacariasis were recorded, in llamas in Sussex and alpacas in Cumbria. These were the first recorded cases of psoroptic infestations in South American camelids in Great Britain.

Chapter 2.9

Investigations into the Epidemiology of Natural Reservoirs of *Psoroptes* spp in Britain.

Discussion

This chapter documented the first ever investigations into the status of the genus *Psoroptes* infesting mammalian hosts in Great Britain. The ear mite, *P. cuniculi*, in the form of subclinical psoroptic otoacariasis, is prevalent in domestic sheep, goats, domestic rabbits (not wild rabbits) and possibly the horse. Clinical and extensive aural and body (extra auricular) infestations of *P. cuniculi* were observed in domestic rabbits and occasionally goats.

In order to clarify the epidemiology of ear and body *Psoroptes* the nomenclature of Sweatman (1958) was used throughout this Chapter. A variation occured in Chapter 2.6 where *Psoroptes* mites isolated from the ear canals of sheep with concommitant active body mange (sheep scab) were defined as *P.ovis*, as they were indisputably derived from the body mange populations. The validity of Sweatman's nomenclature will be challenged in Chapter 4.0.

The body mite, *P ovis*, is widespread in domestic sheep in the form of otoacariasis and body mange (sheep scab), but is no longer endemic within the UK national cattle herd, but isolated infestations have been imported-in from mainland Europe. Equine psoroptic mange (*P. equi*) was last recorded in the UK in 1948 and is now presumed to be eradicated. Psoroptic otoacariasis (*P auchinae* ≡ *P. cuniculi* ?) and mange (*P auchinae* ≡ *P. ovis* ?) were also recorded in imported South American camelids (alpacas (*L. pacos*) and llamas (*L. glama*)) (Windsor *et al.*, 1999) and may become commonplace with the increasing number of domestic camelids in the UK. Captive barbary sheep were the only other recorded hosts for *Psoroptes* in the UK.

Ovine subclinical psoroptic otoacariasis (*P. cuniculi*) was a common parasite within the British national flock, with 1.4% of lambs shown to be infested (Chapter 2.2). Critical investigations of seven field cases (Chapter 2.1) revealed flock prevalence ranging between 1.3 and 23.9 %, with the highest infestations found in pedigree flocks. Commercial ewe flocks, with the exception of flocks where commercial and pedigree ewes were run as joint enterprises, appeared relatively uninfested. Rams appeared the most affected and in commercial flocks were often the only animals infested, therefore playing an important role in the epidemiology of otoacariasis. At the time of these studies, infested commercial breeding rams would

have been kept isolated from the ewe flock for most of the year, disseminating mites within the resident rams. Ewes and rams came together for tupping either directly before, during or directly after the autumn compulsory scab dip (in force at the time), thus blocking the migration of mites to the ears of the ewes. Evidence from Chapter 2.2 suggested that the prevalence of ovine otoacariasis was increasing, with 0.3%, 1.8% and 3.9% of sheep examined infested in 1989, 1990 and 1991 respectively. Results of a telephone survey carried out by Morgan (1992) revealed 28% of sheep owners reporting ear lesions possibly attributed to psoroptic otacariasis. With the deregulation of scab in 1992 and the withdrawal of compulsory dipping, ovine psoroptic otoacariasis could now be even more extensive within both pedigree and commercial flocks. Only the Reedham flock (Case C) had been directly involved in sheep scab tracing with breeding ewes purchased by a neighbouring farm and clinical sheep scab confirmed six months later.

Chapter 2.3 indicated that 80.0% of English goat herds could be affected by psoroptic (*P.cuniculi*) otoacariasis. This is considerably higher than the 22.0% of Australian domestic goat herds recorded by Cook (1981). The recorded prevalence of caprine psoroptic otoacariasis within herds ranged between 21% (Cook, 1981) to 87% (Williams and Williams, 1978). In these studies 30.0% of goats examined were affected, with a prevalence within herds ranging between 20.0% to 35.0%. Caprine psoroptic otoacariasis is thus a very common but often overlooked disease of the goat. No gross clinical symptoms were observed in any infested herd, other than the occasional individual exhibiting ear scratching with the hind feet, supporting the observations in the USA by Schillhorn van Veen and Williams (1980) and in New Zealand by Heath *et al.,* (1983). Similar observation were made in horses by Pascoe (1980), where infested horses were not noted headshakers or "touchy about the poll." These horses could be overlooked as carriers and ear mites may be more common than would be indicated.

In general infestations of the external ear canal by *P. cuniculi* presented few clinical signs in goats and horses, but were responsible for significant abnormal behaviour and self trauma in sheep and lambs and extensive aural mange in rabbits. Yet in Brazil, Faccini and Costa (1992) recorded that the number of mites collected

and mean numbers of mites per host were all considerably higher in goats (2,390 to 15,224 mites collected) than in sheep (674 to 5729 mites collected). Goats therefore appear more tolerant to infestation than sheep, despite larger parasite burdens..

Extreme clinical signs of psoroptic otoacariasis in goats have been recorded by other authors, including ear twitching (Cook, 1981), head shaking (Lofstedt *et al.*, 1994). In Zimbabwe Odiawo and Ogaa (1987) also recorded partial deafness, head tilting, recumbancy, ear scratching and head shaking, walking in circles, occasional "epileptiform fitting" and the ears could be thickened with their edges rolled up with thick keratinized painful areas together with dried bloody exudate. Clinical signs of equine psoroptic otoacariasis may be restricted to ear discharge, but can also include ears held at right angles or giving a lop appearance (Shaw, 1966 and Montali, 1976). Ear drooping is usually associated with severe rubbing of the affected ear or ears. Other common symptoms in horses include scratching ears with the hind feet (Shaw, 1966), rubbing the ear base on stalls etc (Shaw, 1966; Montali, 1976 and Lucas, 1946), head shaking (Shaw, 1966; Montali, 1976; Lucas, 1946 and Gerring and Thomsett, 1980), touchiness of poll (Lucas, 1946).

Clinical symptoms of psoroptic otoacariasis recorded in sheep (Chapter 2.1), ranged from aural haematomae/fibrosis (cauliflower ears), violent head shaking and ear rubbing, leading to excoriation and wounding of the ear and eye base in adult ewes and rams. Symptoms in lambs included plaques of scab (often bloody) on the external ear cleft, excoriation of the ear base, ear scratching with the hind feet and inflammation of the external aspects of the horizontal canal. In all cases the internal pinnae were clear of typical psoroptic scabs. Clinical symptoms of psoroptic otoacariasis could be attributed to self inflicted trauma in relieving irritation, to local immunological reactions to mite antigens (to both *P. cuniculi* or forage mites) or associated with other factors, such as ear tagging. Severe haematomae were painful to touch and a source of considerable discomfort to affected sheep. Ear damage itself was not indicative of infestation: *P. cuniculi* were isolated from 28.6% of damaged ears but also from 7.8% of undamaged ears.

No typical sheep scab lesions were observed in the infra orbital fossae and the internal and external aspects of the pinnae of sheep presenting subclinical psoroptic otoacariasis. Post mortem examination of the ear canals revealed a hollow waxy tube, approximately 1.0 cm long situated at the distal end of the external auditory canal (EAC), close to the tympanic membrane and containing between 28 and 70 live *P. cuniculi* (all instars). Some mites were actually embedded in the wax matrix., together with large numbers of unhatched eggs. Although no post mortem examinations of goats were carried out in these studies, similar lesions have been recorded in the caprine auditory canal, where the auditory canal of heavily infested goats can be plugged with thick, brown, laminated scab, sometimes completely occluding the canal. Damage to the tympanic membrane has never been recorded (Odiawo and Ogaa 1987, Williams and Williams, 1978). Heath (1983) noted the occlusion of the auditory canal in 21% of goats examined. Cottew and Yeats (1983) also recorded that mites congregate at the base of the pinna and as well as the tympanic membrane. Like ovine and caprine psoroptic otoacariasis the equine ear canal can also be plugged with soft ceruminous material containing many mites (Gerring and Thomsett 1980, Montali 1976 and Shaw 1966). Lucas (1946) found numerous mites in the "cavum conchae" and the lumen of the ear canal, which was also plugged with ceruminous material. Johnson (1963) observed a thick malodourous discharge (similar to *Otodectes cynotis* infestations of dogs) and the ears showed a marked hypersensitivity.

Secondary infections have been recorded with respect to *P. cuniculi* infestations of domestic rabbits, including otitis media and otitis interna, torticollis, ulcerous meningocephalitis accompanied by abcessation of the medulla oblongata region and interference with the central nervous system (Von Ribbeck and Ilchmann 1969). *O. cynotis* can occasionally rupture the tympanic membrane and initiate otitis media/interna, particularly in cats (Shell, 1988) and Megnin (1890 cited by Ribbeck and Ilchmann 1969) described changes in the tympanum of infested rabbits attributed to *P. cuniculi*. Duckett (1916, cited by Ribbeck and Ilchmann, 1969), actually saw *P. cuniculi* in the immediate vicinity of the brain of an infested rabbit.

The total length of an ovine (Border Leicester) EAC is 5.0 cm long and 0.6 cm in diameter, with 2.0 cm within the skull. The entire life cycle of the mite is carried

out within this narrow environment. Some form of population control must be in place to prevent over population, and forcing the mites out of the EAC.

In certain circumstances *P. cuniculi* can leave the EAC and infest the body. Zurn (1875) and Henry (1928) described cases of extra auricular psoroptic (body) lesions in rabbits, dispelling the theory that the mite exclusively inhabited the rabbit ear canal. Chapter 2.5 demonstrated that psoroptic ear canker was prevalent within British commercial rabbitries, with the majority of lesions ranging between scores 1.0 and 6.0 (Guillot and Wright, 1981). Extra-auricular lesions were demonstrated in 5.0% of infested rabbits with lesions extending to the base of the ears, the cheeks, dewlap, face and between the digits of both hind feet.

Caprine infestations have also been recorded to involve the entire pinna, or spread to infest the body, presenting lesions, severe pruritus and hair loss at the poll, neck, lips, muzzle, ears, withers, back, abdomen, pasterns and interdigital spaces (Littlejohn, 1968; Munro and Munro, 1980; Abu-Samra *et al.,* 1981 and Lofstedt *et al.*, 1994).

Guilhon (1990) postulated that the development of extra auricular lesions was closely connected with the permanent "conflict" between the mite and the host, in which direct or indirect human intervention plays an important part. Pyrexia may impair local defence mechanisms in the ear, upsetting the microclimate or intercurrent infections may impair cell immunity. Stress, nutritional deficiencies or poor housing can lead to the generalisation of disease (Von Ribbeck and Illchmann, 1969). Body infestations are more frequently seen in old or debilitated animals, younger goats showing milder symptoms (Heath, 1983 and Munro and Munro, 1980). *P. cuniculi* is not considered to be a serious threat to goats, but in Angora goats they can inflict serious damage to the skin and hair and can be considered a threat to the Angora fibre industry worldwide (Graham and Hourigan, 1977).

The spread of psoroptic ear mites may be direct, through mutual grooming, playing, head butting, head shaking or close contact during sleeping or at feeding or drinking. Mites could therefore enter flocks via contact with infested sheep at market

as well as through the purchase of infested stock. Mites can also be transmitted indirectly, through contaminated fomites (eg. equine mites can be spread indirectly via infested harness such as bridles, head collars and grooming equipment).

Disease prevalence appears to be a function of age. In sheep (Chapter 2.1) adult rams were the most affected (21.5%) and in commercial flocks were often the only animals infested, therefore playing an important role in the epidemiology of otoacariasis. The next age/sex class affected were shearling rams (14.2 %). The youngest sheep infested were two ewe lambs, two and eight days old. Transmission of *P. cuniculi* can occur horizontally, soon after birth. Williams and Williams (1978) and Heath (1979) found mites present in 5, 10 to 21, 28.0, 35.0 and 42.0 day old goats. In Chapter 2.3 analysis of *P cuniculi* infestation as a function of goat age revealed the highest infestation (64.5%) in animals between 6 and 12 months, followed by animals between 12 to 18 months old (19.4%), animals between one and six months (12.9%) and over two years old (3.2%). In contrast to Williams and Williams (1988) and Heath (1989) no mites were found in goats under one month old. Friel and Grainer (1988) observed that goats under one year old had a higher mite prevelence, but gross lesions were noted in only three animals. In horses Shaw (1966) observed that infestations were more common in yearlings than in adult horses and postulated that mares were non-suffering carriers.

Parallels to psoroptic otoacariasis can be drawn from *Otodectes cynotis*, the ear mite of carnivores. In cats *O. cynotis* is aquired early in life, with a limited number of mites; later a hostile aural environment develops, preventing colonisation; only animals with defective immune systems or physiology will perpetuate disease (Weisbroth *et al.,* 1974). It has been observed in dogs that the level of pruritus can vary between asymptomatic and extreme discomfort (Thoday, 1980), with numbers of mites unrelated to the severity of the lesion, as hypersensitivity responses are suspected to play a significant role (Scott, 1980). *P. cuniculi* infestations in sheep, goats and horses probably follow a similar pattern. Yearling sheep, goats and horses are continually challenged by mites from a minority of heavily infested carriers; some yearlings successfully prevent colonisation, others having transient infestations and yet others allowing for complete colonisation and becoming carriers themselves. As

forage mites were also isolated from a number of sheep, symptoms may not be exclusive to *Psoroptes* infestation.

Ovine psoroptic otoacariasis is seasonal, with no mites recorded between late May and July, supporting the observations of Heath *et al.,* (1989), who noted a winter peak in New Zealand feral goats. Ovine psoroptic mange (sheep scab) is also a winter disease, with the majority of cases occuring between September and April, although a significant number of cases do occur in the summer months (Bates, 1991b). Populations of *P. ovis* were thought to diminish (often to extinction) or actively migrate to the "cryptic or latent sites" (including the EAC) at the onset of summer, remaining quiescent until the onset of autumn/winter. This phenomena of "latent phase" or "suppressed scab" was first described by Downing (1936) and Spence (1949), who showed that mites will enter the ears and the other cryptic sites 45 to 60 days after the onset of the active phase of disease (when extensive encrustation and denudation has rendered the body surface unsuitable).

The migration of *P. ovis* to the ears is not in dispute, but the intentional seasonality of the migration is open to question. Kirkwood (1985), examining sheep artificially infested with the VLA Reference strain of *P ovis*, found the cryptic sites infested only on sheep having extensive disease, and then more often in the winter than the summer.

The occurrence of the sheep scab mite (*P ovis*) in the ears of sheep is well reported (Zurn, (1877); Imes, (1916); Miller, (1925) and Verney, (1926). Spence (1949) found the ears the most frequented cryptic site in the summer (82.5%) and that ear infestations often escaped detection and can only be demonstrated by post mortem examination, with large numbers of mites found in the EAC. Sweatman (1958) postulated that mites found in the ears can sometimes be found on the bodies of the host, but no evidence that body mites could be found in the ears of the host.

In Chapter 2.6 live *P. ovis* were isolated from the EACs of 38.6% of infested sheep examined and like *P. cuniculi* were located in tubular lesions located in the last centimeter of the EAC. Pinneal damage (haematomae/fibrosis) was observed in 2.5% of ears examined, and like *P. cuniculi* otacariasis, was not indicative of infestation.

Also in comparison to *P. cuniculi* infestations of sheep and goats *P. ovis* in the ear canals of sheep followed a seasonal pattern, with a peak of infestation (64.2%) in the winter (November to December). This is comparable to the natural prevalence of sheep scab and does not indicate a latent phase of disease. Of the six field isolates of *P. ovis* examined: 90.0% of sheep infested with the low virulence Bacup isolate were infested in the EAC, followed by the highly virulent Porlock, Little Melton and Caithness isolates and then the medium virulent St. Brenard and VLA Reference isolates respectively. The high prevalence of the Bacup isolate in the ear canal is probably due to the examination of a single, small group of infested sheep. This being so, infestation of the EAC may be a factor of the relative virulence of the infesting mite population: the more virulent the isolate (and associated high mite numbers) the higher the prevalence of otoacriasis.

The majority of aural *P.ovis* infestations occured in the early stages of active disease, when the body lesion was relatively small (11.0% to 44% body cover), but as the lesion progressed to the decline phase (98.9% to 100.0% body cover) the pinna itself becomes infested, and simultaneous infestations of the canal and pinna occured. Mites can therefore infest the pinnae once the leading edge of the lesion approaches the head, but mites can migrate to the EAC when the leading edge is a considerable distance away (eg. the base of the neck, withers or as far away as the mid back). This evidence supports the hypotheses that there are two distinct sub-populations of mites and that ear mites (defined as *P cuniculi*) may originate from scab mites (defined as *P ovis*), now adapted to an aural environment (and possibly kept there through the constraint of compulsory plunge dipping).

The majority of lambs with *P.cuniculi* otoacariasis identified in the abattoir survey (Chapter 2.2), originated from livestock markets located in areas with long histories of endemic scab, but it must be borne in mind that livestock is not automatically sold at the local mart, but can be transported considerable distances before sale. If psoroptic ear mites are classical scab mites (*P. ovis*) adapted to an aural environment, a higher prevalence of otoacariasis would therefore be expected in endemic scab areas. Unfortunately the livestock markets used in this study were

(unintentionally) biased towards endemic areas, a broader survey is thus required to investigate this further.

Until *Psoroptes* taxonomy is resolved, mites causing subclinical ovine psoroptic otoacariasis should be regarded as possible reservoirs of sheep scab. The survey of sheep scab outbreaks in Britain between 1984 and 1988 (as described in Chapter 1.0) demonstrated that the origins of 18.5% cases were 'obscure' (Anon, 1989). Could these have been infested by *Psoroptes* ear mites colonising the body?

There have been difficulties in differentiating body mites *P. ovis* from the ear mite *P. cuniculi.* Strong and Halliday (1993) stipulated that for identification critical examination of the mites themselves is essential, and not simply based on presumed host and site specificity. In these studies all instars of *Psoroptes* were isolated from the ears of sheep and goats, suggesting that the entire life cycle is carried out within the EAC. In other cases infestations were identified by the presence of only a few instars, and sometimes only a single non sexually dimorphic larva or nymph or an adult male. Thus infestations may not be patent, but the potential for infestation was recorded. It is also not certain that the mites identified would survive to form permanent colonies.

Bovine psoroptic mange (*P. ovis* or *P. natalensis*) is not endemic to Great Britain (Chapter 2.7). Between 1962 and 1997 64.1% of bovine mange was chorioptic; 31.3% sarcoptic and only 4.6% psoroptic mange. The latter were represented by isolated outbreaks imported from mainland Europe and although difficult to eradicate did not pass to other animals. The single European market and the relaxation of EU import regulations in 1993 may result in a subsequent increase in prevelence as was seen for *Hypoderma* spp. (Warble fly) infestations of cattle (Tarry and Sinclair, 1995). The current low prevelence of bovine mange in Great Britain may be associated with treatment for other ectoparasites, eg. warble fly, initially using systemic organophosphates and latterly ivermectin based formulations. In addition the current increase in the use of endectocides (doramectin, ivermectin, moxidectin etc), either as anthelmintics or ectoparasiticides, may also have contributed to the present epidemiology of the disease.

Two species of native deer (red deer (*Cervus elephas*) and the roe deer (*Capreolus capreolus*)) and 4 species of introduced deer (Chinese muntjac (*Muntiacus reevsi*), Chinese Water Deer (*Hydropotes inermis*), Fallow deer (*Dama dama*) and Sika deer (*Cervus nippon*)) are present in Britain (Lawrence and Brown, 1979). Axis (Chetal) deer (*Axis axis*) and White Tailed Deer (*Odocoileus virginianus*) established temporarily from escaped stock in 1940's to 1950's (Lawrence and Brown, 1979). White tailed deer are known reservoirs of *P. cuniculi* (Strickland *et al.,* 1970 and Schmitt *et al.,* 1982). Although *Psoroptes* mites (*P. cervinus* Ward 1915*)* have been isolated from American elk or wapiti (*Cervus canadensis* Nelsoni, *Cervus elephas canadensis*) (Hepworth and Thomas, 1962 and Sweatman 1958) and although wapiti (*Cervus elephas canadensis*), are genetic variants of British red deer (*Cervus elephas*) (Sweatman, 1958), there is no published information on the prevelence of *Psoroptes* spp in British deer. In Ireland Sleeman (1983) examined red, fallow and sika deer and red/sika hybrids for ectoparasites, but did not examine the ears. Deer ectoparasite surveys in England have been carried out by Jackson (1975), examining fallow deer in the New Forest, Hampshire and McDiarmid (1975) examining wild deer throughout the United Kingdom. Neither author specifically examined the ears for mite infestations.

Representitives of all four species of New World camelid can now be found in the Britain, either farmed, in zoos and circuses or as companion animals and there numbers are increasing. Psoroptic otoacariasis (*P. auchinae*?) of south American camelids has only recently been identified in Great Britain (Windsor *et al.*, 1999). Clinical mange has been recorded on llamas in Peru (Alverado *et al.*,1966) but has not been recorded in Great Britain, with *Chorioptes* spp. often mis identified in clinical cases (Bates, *unpublished observations*). Gelded llamas are also gaining popularity in the UK as "guard llamas" for sheep flocks, protecting the flock from predators (Safran, 1997). This use will bring llamas in direct contact with sheep and the classification of *P auchinae* must be fully investigated. Psoroptic otoacariasis or mange has not been recorded on Old World camelids in Great Britain.

Plunge dipping in diazinon, propetamphos or flumethrin wash appeared to be ineffective against ovine psoroptic otoacariasis. Scab regulations required the

immersion of the head at least once, but this rarely happened in practice. Even if the head was immersed correctly, trapped air within the ear may prevent adequate penetration of dipwash within the ear canal. Studies at the VLA, Weybridge dipping sheep in wash containing a blue dye, demonstrated incomplete penetration of the EAC after head immersion (Bates, *unpublished data*). Mites could therefore survive dipping and exposure to sublethal concentrations of acaricide may select for acaricide resistance within the ear mite population. Cypermethrin pour-ons rely on the active ingredient binding to intercellular lipid within the stratum corneum (Jenkinson *et al.*, 1986), moving with the flow of lipid/sweat away from the point of application. It is unlikely therefore that active ingredient will translocate to the head and penetrate the ears (Bates, 1993a). Psoroptic ear mites can be effectively controlled by the systemic endectocides : Kinzer *et al.*, (1983); Odiawo and Ogaa (1987); Lofstedt *et al.*, (1994); Wilkins *et al.*, (1980) and Wright and Riner, (1985)).

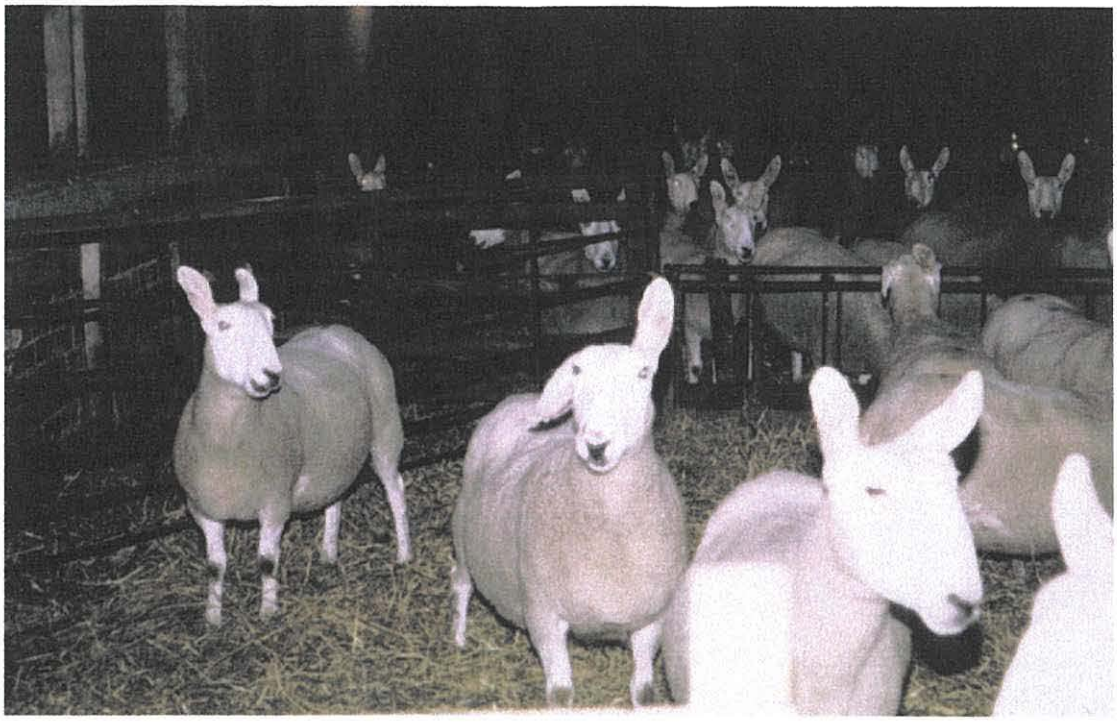


Figure 2.9.1: Natural outbreak of psoroptic otoacariasis in sheep (Chapter 2.1). Border Leicester ewes in Flock A (Duns, Berwickshire, Scotland) demonstrating haematomae associated with natural sub-clinical psoroptic otocacariasis.

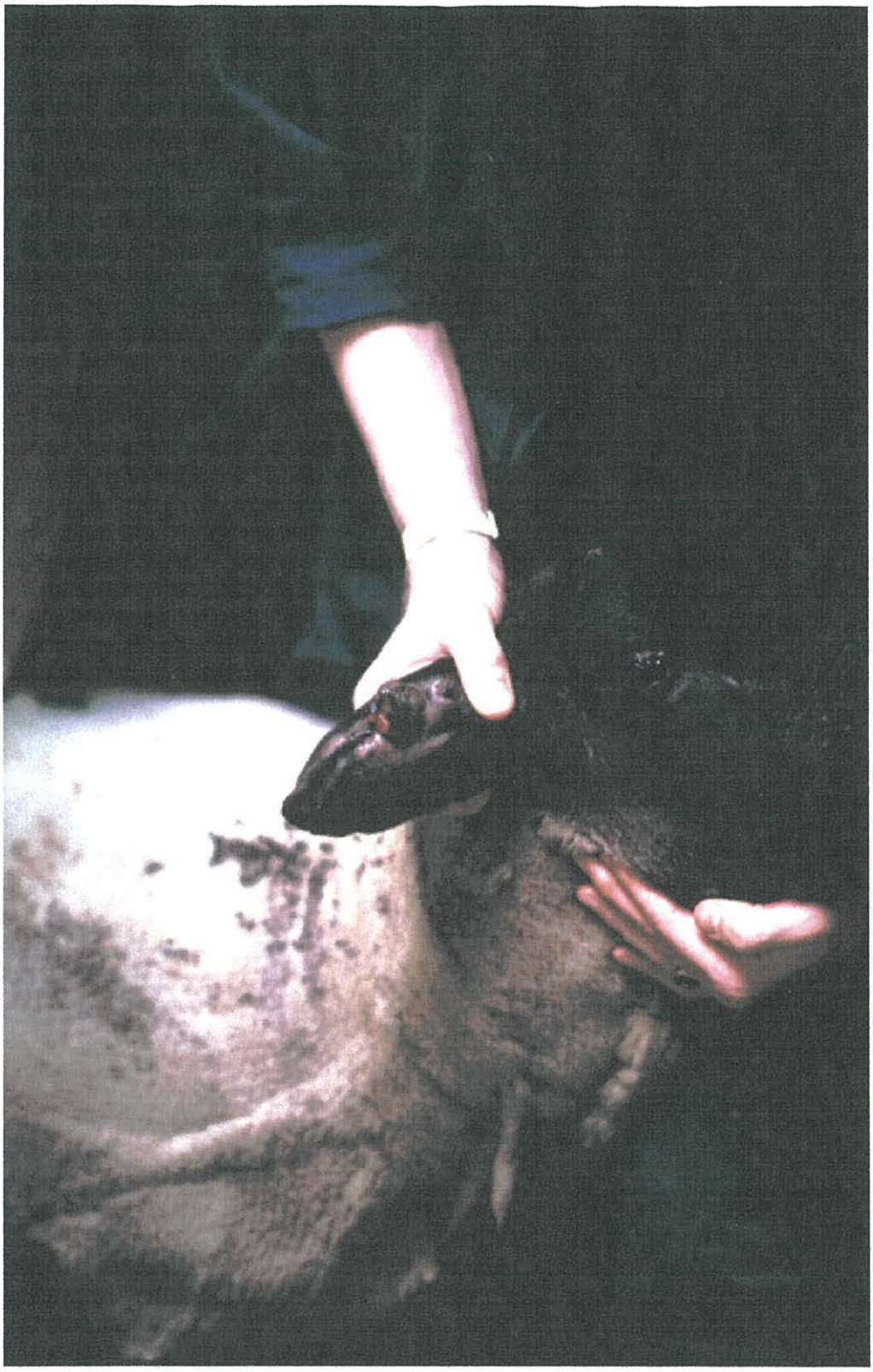


Figure 2.9.2: Natural outbreak of psoroptic otoacariasis in sheep (Chapter 2.1). Left pinna of a Suffolk ram (Flock C, Reedham, Norfolk, England) demonstrating score +++ haematoma, with exudation and bleeding, associated with natural sub-clinical psoroptic otoacariasis.

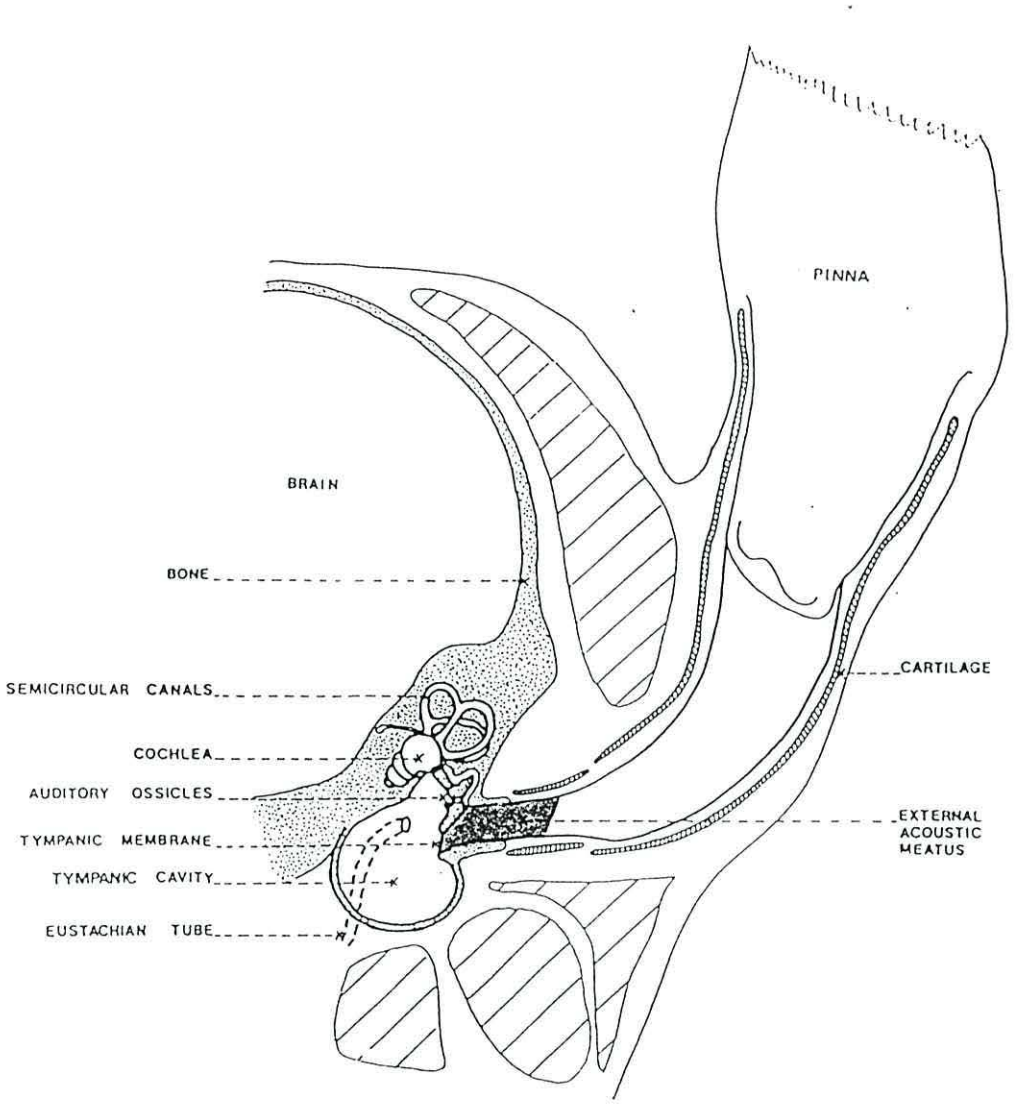


Fig. 15.1. The normal ear.

Figure 2.9.3: Diagramatic cross section of mammalian ear. Area highlighted indicates position of the typical aural lesion lesion ("plug") associated with natural sub-clinical psoroptic otacariasis of sheep (Diagram from Doxel (1988)).

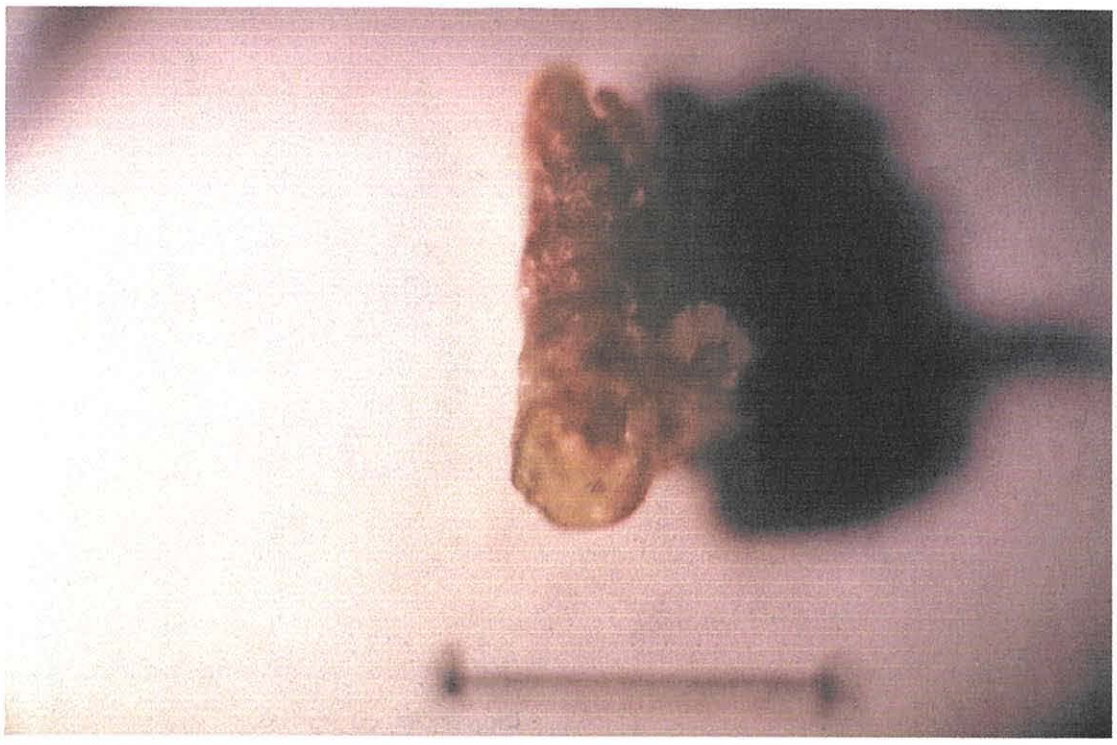


Figure 2.9.3: Natural psoroptic otoacariasis in sheep. Typical aural lesion ("plug"), found in the external auditory canal (EAC) of a ewe (L.39), identified at Duns, Berwickshire, Scotland (Case A, Chapter 2.1). The lesion was located close to the tympanic membrane. Live *Psoroptes* mites were identified within the lumen of the lesion. Scale bar = 1cm.

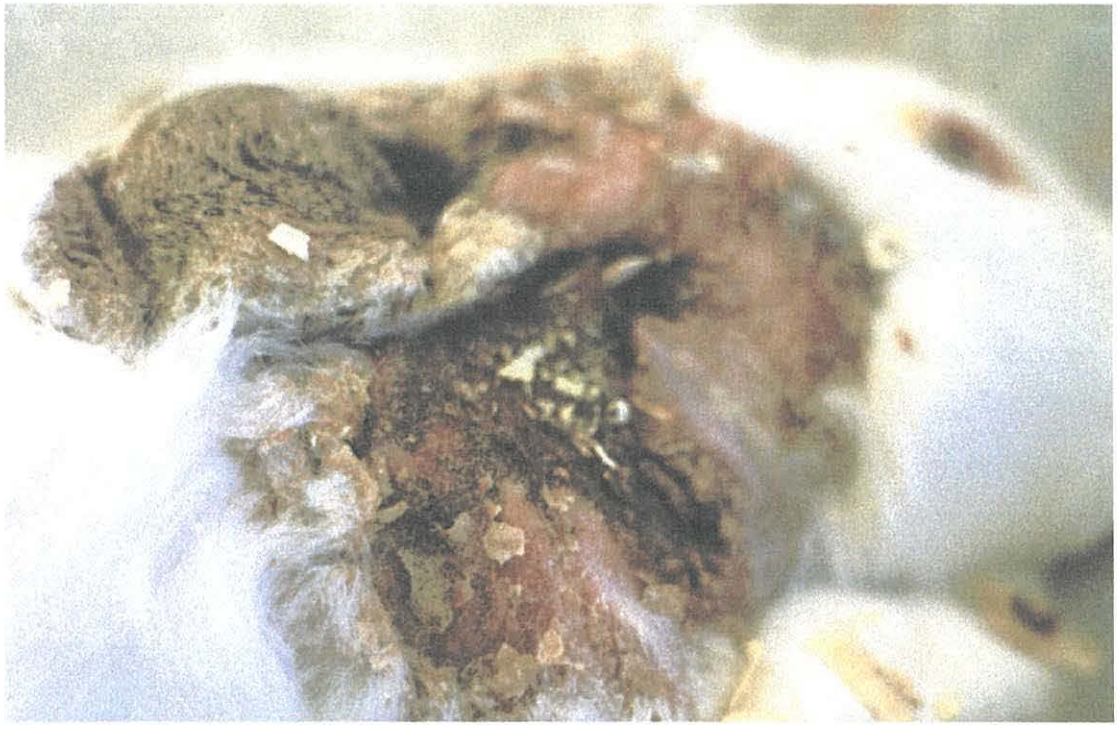


Figure 2.9.5: Natural psoroptic ear canker in a domestic rabbit. Extra auricular ("ectopic") infestation of a New Zealand White doe, natural infestation from a commercial rabbitry (Chapter 2.5). Note brown *Psoroptes* mites on ectopic lesion.

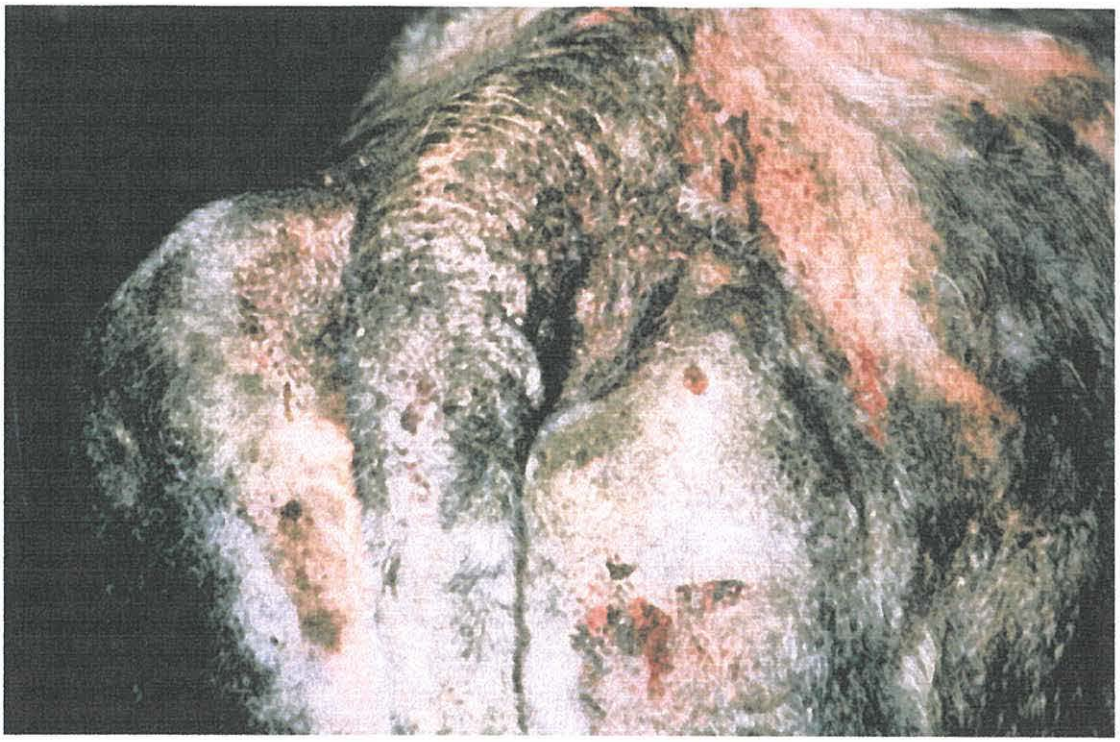


Figure 2.9.6: Bovine psoroptic mange (*Psoroptes natalensis*). Natural infestation from the Borders of Scotland, 1983 (Chapter 2.7).

Chapter 2.10

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Chapter 3.0

Investigations into Population Variation Within Geographical Isolates of the Sheep Scab Mite (*Psoroptes ovis*

3.0. Introduction

Chapter 2.0 investigated the prevalence of *Psoroptes* species infesting domestic and non-domesticated livestock in Great Britain. This chapter critically assesses the clinical aspects of psoroptic mange in sheep (sheep scab) and investigates variations within populations of the causitive agent, *P. ovis* and it's potential to re-infest sheep previously exposed to sheep scab.

Chapter 3.1

Investigations into the Temporal Progression of Ovine Psoroptic Mange (Sheep Scab) on Artificially Infested Sheep

1.0. Summary

Groups of sheep were artificially challenged with either the VLA Reference isolate or a field isolate from St.Brenard in Cornwall. Three phases of infestation were identified. eg the "subclinical", "rapid growth" and "decline" phases. The subclinical phase (ie. characterised by lesion area below 5.0% body cover), lasted 14 to 28 days post challenge (PC) for the St.Brenard isolate and 21 to 28 days PC for the VLA Reference isolate. The subclinical phase also varied with the individual sheep, up to 63 days PC for some sheep challenged with the VLA Reference isolate and 98 days for some sheep challenged with St.Brenard isolate. In contrast to the VLA Reference isolate (where mites failed to establish on a number of sheep), clinical scab developed on all sheep challenged with the St.Brenard isolate.

After the subclinical phase lesion areas and mite populations rapidly increased with time, "the rapid growth phase." Lesions produced by the St.Brenard isolate grew at a greater rate than those produced by the VLA Reference isolate. The mean percent body cover for the VLA Reference Isolate did not achieve 100% on any sheep challenged over the 63 days of the study. The St.Brenard isolate, however, achieved 100% body cover on some sheep 28 days PC and on all sheep by 63 days PC. Overall the mean percent body cover of the lesion produced by the St.Brenard isolate was in the magnitude of 30.5 % after 42 days PC and 100.0 % after 63 days PC, compared to only 7.6% and 60.9 % for the VLA, Reference Isolate over the same periods. Differences in the mean lesion area with time for sheep challenged with the VLA Reference isolate were not significant ($P=0.083$). In contrast the variations in lesion area with time between study groups challenged with the St.Brenard isolate were highly significant ($P<0.001$).

Mite burdens for both isolates varied considerably between individual sheep as well as between the two isolates. In comparison to lesion area, the differences in mite burden with time within groups of sheep infested with the VLA Reference isolate were not significant ($P=0.200$) but differences in the mite burdens with time between groups of sheep infested with the St.Brenard isolate were highly significant ($P=0.003$).

Subclinical lesions presented low mite burdens (ie. mite numbers per sheep below the 25 adult females of the original challenge) for 14 to 35 days in sheep infested with the VLA Reference isolate. From then on mite numbers gradually increased, although climactic populations were not achieved during the time period of the individual studies. A long subclinical phase of 21 to 35 days was observed in two studies and a shorter subclinical phase of 14 to 21 days was observed in the remaining three studies. The duration of the subclinical phase also varied with individual sheep, ranging between 35 to 49 days. An overall subclinical phase of 21 to 28 days was observed in sheep challenged with the St.Brenard isolate. In contrast to the VLA isolate mean and maximum mite burdens climaxed (with exceptionally high numbers compared to the VLA Reference isolate) 49 to 63 days PC, then declined rapidly, often to extinction.

There was a strong positive correlation between lesion area and mite burden for the VLA Reference isolate ($r = 0.7783$), but no correlation was observed between lesion area and mite burden for the St.Brenard isolate ($r = 0.3628$).

The mean touch (hypersensitivity) response (THR) score for sheep challenged with the St.Brenard isolate peaked between 14 and 28 days PC, earlier than than observed for the VLA Reference isolate (35 days). A number of sheep in all groups presented epileptiform seizures (often fatal), between 28 and 120 days with corresponding lesion areas of 1.2% to 58.3%.

Live *P. ovis* were isolated from 25.9% of the EACs of sheep challenged with the VLA Reference isolate, as early as 28 days PC, with the leading lesion edge 28.0 cm from the ears. In contrast, live *P. ovis* were isolated from the EACs of 53.2% of sheep challenged with the St.Brenard isolate, as early as 35 days PC, with the leading lesion edge 17.0 cm from the ears. No sheep challenged with the VLA Reference isolate recorded mites present in any cryptic site at any time throughout the studies. Mites were, however, recorded in the cryptic sites of sheep infested with the St.Brenard isolate.

2.0. Introduction

Early qualitative observations at the VLA, Weybridge on sheep heavily infested with an isolate of *P. ovis* originating from Powys, mid-Wales (Penderyn isolate, Appendix One) demonstrated no obvious behavioural or clinical signs of active scab, despite a lesion covering of 80% of the body, extending over the back, flanks, sides and belly. These observations contradicted the normal pattern of disease observed for the VLA Reference isolate of *P. ovis* and suggested the possibility that populations of *P. ovis* existed in the UK, varying in the pathogenicity.

Roberts and Meleney (1971) demonstrated that variations existed within strains of *P. ovis* in the USA, with "aggressive" strains characterised by an increased pathogenicity and thus more rapid lesion growth. To investigate the presence of similar aggressive and non-aggressive populations of *P. ovis* in Great Britain, a model comparing the relative rates of lesion growth and mite population increase (temporal progression of disease), needs to developed. Knowledge of the progression of the lesion on individual sheep is also important regarding the epidemiology of scab within flocks and the dissemination of disease within common grazings. Defining and quantifying the causes of variation within the natural progression of scab is essential in order to age infestations in cases of animal welfare litigation under the Sheep Scab Order 1997, and to assess the relative efficacies of acaricides, acaricide delivery systems and potential vaccines against the disease.

Early investigators into the pathology of sheep scab (Stockman, 1910; Shilston, 1915; Downing, 1936 and Spence, 1949) recorded only qualitative observations on the progress of disease. The studies presented in this chapter attempted to investigate the temporal progression of sheep scab by recording the quantitative changes in mite population, lesion area, touch hypersensitivity, infestation of the "cryptic" ("latent") sites and the development of psoroptic otoacariasis with time, following artificial challenges of two different geographical isolates of *P. ovis*. In order to achieve these objectives it was necessary to develop a standardised *in vivo* model, accounting for a number of host factors (eg. the age, sex and breed, housing and nutrition of the host, together with the month of infestation), that may affect the progress of disease.

Host Sex, Age and Breed

O'Brien (1992) observed no differences in the susceptability of sheep scab due to the sex of the host. Downing (1936) stated that there were variations in scab susceptability with age and preliminary studies at Weybridge have shown that yearlings may manifest a more severe form of scab compared to adult ewes (Bates, *unpublished data*). The breed of the host can also have a profound affect on scab establishment and progress: lowland breeds, with a high density of wool follicles per cm^2 are extremely efficient in holding in the ideal microclimate. Hill breeds, on the other hand, with a low density of wool follicles per cm^2, have a characteristic "open" fleece, inefficient in forming the disease microclimate. Similar observations were made by Downing (1936). Thus the mites take longer to establish under these adverse conditions. Fourie *et al.,* (1997) demonstrated that the progress of sheep scab lesions was almost five times greater in the South African Merino, compared to the Dorper breed. Suggesting that Dorper sheep play an important role in the spread of sheep scab. This is comparable to Britain, where open fleeced breeds graze hill and marginal common grazing areas that have long been foci of infestation.

Host Nutrition

Nunez (1989) demonstrated experimentally that nutritional stress could play an important role in the epidemiology of sheep scab. Local physiological changes in the skin or behavioural changes of the host as a result of its nutritional status or nutritional value (particularly the concentration of vitimins A and B) can affect ecto-parasite numbers. (Harmon *et al.,* (1963); Jurin and Tannock, (1972); Nelson *et al.*, (1977) and Nutting and Rauch, (1961)). Host nutrition may also help in explaining the seasonality of scab by taking into account the availability of good quality grazing.

Seasonality

Sheep scab is primarily a winter disease, but a significant number of cases do occur during the summer months (Bates, 1991b). The phenomena of "summer latency", where mites were presumed to migrate to the latent sites on the onset of summer, has been discussed in detail in Chapter 2.0. This "voluntary" migration has been attributed to a decreased oviposition rate in the summer and difficulties in lesion establishment (Spence, 1949). As of yet these theories are unsubstantiated, but sheep housed for long periods, creating a very dry fleece, fail to accept a challenge of *P. ovis* (Bates, *unpublished observation*). Shearing infested sheep gives marked amelioration of symptoms, but the decline in field cases is already well in operation before shearing begins (Stockman, 1910, Hutyra *et al.*, 1949). Mites will also take longer to establish under the adverse conditions presented by freshly shorn sheep. In contrast to the information presented above, strains of the sheep scab mite (*P. ovis*) have routinely been passaged at Weybridge, between full fleeced sheep throughout the year, with no differences in pathology observed in the summer months (Kirkwood, 1985).

Predisposing Ectoparasite Infestations

Observations at Weybridge have recorded that sheep with pre-disposing infestation of chewing lice (*Bovicola ovis*) will not accept challenges with sheep scab mites, whereas sheep with active sheep scab can be colonised by lice following natural exposure (Bates, 1999). The exact nature of this inter-species exclusion is unknown, but the skin changes initiated by lice feeding/excretion may render it

unfavourable for mite colonisation. Lice, on the other hand, may actively feed on the scab lesion!

Consequently all the *in vivo* studies undertaken in this chapter were carried out on untreated, full fleeced, scab naive, un-housed, yearling sheep, of mixed sex and without comcomitant chewing louse infestations. The origins of the sheep were also known and the studies weighted accordingly. Sheep were introduced from outdoor grazing to a roofed, biosecure building and the sheep fed a standard daily ration of concentrated dried grass pellets. All investigations were carried out within the accepted "sheep scab season".

3.0 Materials and Methods

3.1. Sources of Infestation

In the ten year period between 1986 and 1996 clinical data was collected from twelve separate laboratory studies comparing the St.Brenard field isolate of *P. ovis* with the VLA Reference isolate. The St.Brenard population was isolated in August 1989 from diagnostic material submitted to the VLA, Weybridge from an infested flock at St. Brenard, Cornwall and since maintained at the VLA, Weybridge, by *in vivo* passage on sheep (Appendix One). The original VLA Reference population was isolated from a field case in Great Britain prior to the eradication of sheep scab in 1953 and augmented with mites derived from the first foci of infestation in Lancashire (Worsley and Dunsop Bridge) on the re-introduction of scab in 1973 (Appendix One). At the time of these studies the VLA Reference isolate was relatively well adapted to artificial culture, being passaged for 4,820 to 8,372 days following "augmentation" in 1973. In contrast the St. Brenard field isolate was only cultured *in vivo* for 238 to 2,434 days during the period of these studies.

Sheep were challenged artificially with *P. ovis* either specifically to investigate the potential to compare infestations (Studies 41/1, May 1989 (VLA Reference isolate only), 41/2, March 1990 (both isolates), 41/3, May 1992 (both isolates), 41/4, February 1995 (VLA Reference isolate only) and 41/6, March 1996 (both isolates)). Data was also obtained from untreated control groups (UTC groups: St Brenard

isolate only) used in commercial acaricide efficacy studies: UTC/1a (August 1992), UTC/1b (July 1992), UTC/2 (February 1993), UTC/3 (October 1993), UTC/4 (January 1994) and UTC/5 (June 1995).

3.2. Method of Infestation

Adult female *P. ovis* were collected from the lesion periphery of donor sheep infested with either the VLA Reference or St.Brenard isolates, using a mounted needle and transferred (30mm x 8 mm) glass Durham tubes. Twenty five adult female mites were removed from the tube using a mounted needle and placed directly onto the skin within a 1.0 cm^2 area of plucked of wool, on the withers of each of sheep. The area of challenge was marked using a stock marker ring (Agrimark, Pfizer). In all studies groups of between five and fifteen sheep were challenged in this manner.

3.3. Animals

Scab naive, full fleeced Dorset or Suffolk cross, yearling sheep (ewes or male castrates) were used in all studies. The sheep had never been exposed to the acaricides/insecticides diazinon, propetamphos, flumethrin or high-cis cypermethrin, by plunge or shower dipping; cypermethrin, high cis cypermethrin or deltamethrin by backline treatment or subcutaneous or intramuscular injection or oral drenching by the systemic endectocides ivermectin, doramectin or moxidectin or the anthelmintic closantel. The animals may have previously received Vetrazine (6.0% cyromazine, Novartis Animal Health) as a pour-on for the prevention of blowfly strike (*Lucilia sericata*) and wormed, when necessary, with levamisol or benzamidazole based anthelmintics. Cyromazine, levamisol and benzamidazole have been demonstrated to have no effect on *P. ovis* infestations (Bates, *unpublished data*). All sheep were certified "healthy" prior to infestation and were shown not to have any concomitant chewing louse (*Bovicola* (*Damalinia*) *ovis*) infestations.

During the course of the trial the groups were housed within a covered yard and maintained on expanded metal flooring. Study groups were isolated in pens constructed of solid metal hurdles and breeze block walls, to prevent cross infestation. Sheep were fed a diet of unmedicated dried grass pellets (Torberry Feeds, Torberry

Farm, Hurst, Petersfield, Hampshire, GU31 5RG), approximately one kilogram per sheep per day. Fresh mains water was available *ad lib* via floor troughs.

3.4. Parasitological Assessments

Assessment of Lesion Area

The length of the lesion was measured (using a washable plastic tailors tape measure) along the backbone and the width measured from the point nearest to the ventral surfaces on both flanks. Lesions were measured at intervals between days +7 and +100 post challenge (PC) and the area calculated by multiplying the measured length by the measured width and the result expressed in cm^2.

In order to estimate the percent body cover for each scab lesion the mean surface area for the "trunk" of an average shearling sheep was calculated from nine typical shearlings, directly after shearing. Body length was measured from the base of the neck to the tuba coxae (pin bones) and the girth was measured around the rib cage. The surface area was calculated by multiplying body length by the girth. The mean surface area for the nine shearlings measured was 4778.0 cm^2 (range 4240.0 cm^2 to 5185.0 cm^2).

Mite Counts

Numbers of *P. ovis* were counted by parting the wool at 5.0 cm intervals around the periphery of the scab lesion, starting at the mid-neck and proceeding along the right hand side of the lesion, continuing until the initial parting was reached. At each count, the total number of adult, female *P. ovis* were counted individually up to 10 and estimated in units of 5 thereafter. Numbers of partings thus varied in relation to the lesion area. Mite counts were carried out at intervals between days +7 and +100 PC.

Examination of the Cryptic ("Latent") Sites

The cryptic ("latent") sites described by Spence (1949), the infra-orbital fossae (IOF), pinnae, inguinal pouches, perineum and crutch were examined for the presence of live mites and associated lesions prior to challenge (day 0) and at intervals between days +7 and +100 PC (Study 41) or pre-challenge and at post mortem on termination of the study (all UTC studies). Cryptic site infestations were scored as either present (+) or absent (-). In Study 41 the horizontal aspect of the external auditory canal (EAC) of both ears were swabbed at the time of each lesion assessment according to the method described in Chapter 2.1. In all UTC studies the EAC was examined at post mortem (as described in Chapters 2.2). The severity of ear damage in these studies was recorded as, absent (-); mild (+), with slight thickening of the pinna; moderate (++), with severe thickening or fibrosis of the pinnae, without abscessation or severe (+++), ranging from thickened pinnae with abcessation to chronically thickened and deformed cauliflower ears, painful to touch.

Touch Hypersensitivity Response

Sheep have been shown to show their "gratification", when the affected parts are handled, by turning its head around and smacking its lips in a characteristic manner (Downing 1936). Stockman (1910) witnessed two experimental cases of sheep "after a few minutes thrown into "orgasm", falling to the ground and losing consciousness". Bygrave *et al.*, (1993) further described this behaviour as "epileptiform seizures". This has been erroneously defined as the hypersensitivity reaction. In these studies the reaction is referred to as the Touch (Hypersensitivity) Response (THR) in order to save confusion with hypersensitivity of an immunological nature.

The THR was initiated by massaging the main scab lesion with the fingers for ten seconds. The resultant involuntary response was scored as no response (-), mild (+) with definite, but slight attempts to chew or bite the manipulated lesion (with no direct mouth contact with the body or the lesion itself); moderate (++) as for (+) but with definite mouth contact with the body/lesion and involvement of at least one limb in a scratching response, symptoms subsiding on removal of the stimulus; severe (+++) as for (++) but the response very intense, symptoms continuing on removal of

the stimulus with the sheep remaining standing at all times. An extreme (++++) score was recorded when the sheep presented a short or prolonged "epileptiform seizure". The THR was recorded at intervals between days +7 and +100 PC for Study 41 but was not recorded in the UTC studies.

3.5. Acaricidal Treatment

Sheep in Study 41 were treated from day +40 PC onwards with either IVOMEC® for Sheep (1.0% ivermectin, MSD Agvet, two sub-cutaneous injections at a dose rate of 200 μg per kg sheep body weight, 7 days apart) or plunge dipped in an organophosphate based dipwash (Flyte 1250, 40% propetamphos. Robert Youngs) made up to the maintenance level (125 ppm). Sheep in Commercial (UTC) Studies were treated with a licensed acaricide on termination of the efficacy study.

4. Results

4.1. Analysis of Results

Analysis of variance (ANOVAR) was carried out to compare the study means for the lesion areas and the mite counts for each isolate. Because of the considerable variation in the standard deviations analyses were carried out on the logarithm to the base 10 of the lesion areas and to the square roots of the mite counts.

Lesion areas and numbers of adult female *P. ovis* with time post challenge (PC) for each study were tabulated (lesion areas Tables 3.1.1, 3.1.2 and mite burdens Tables 3.1.3, 3.1.4) and the arithmetic means, standard deviations and the ranges for each study group calculated. The arithmetic means for lesion areas with time for all studies were also represented graphically.

Figure 3.1.1. Comparing the mean lesion areas (% body cover) with time for the VLA Reference isolate and the St.Brenard field isolate.

Figure 3.1.2. Comparing the mean numbers of adult female *P.ovis* with time for the VLA Reference isolate and the St.Brenard field isolate.

Figure 3.1.3. Presenting the mean, minimum and maximum lesion areas (% body cover) with time for the VLA Reference isolate of *P.ovis*.

Figure 3.1.4. Presenting the mean, minimum and maximum lesion areas (% body cover) with time for the St.Brenard field isolate of *P.ovis*.

Figure 3.1.5. Presenting the mean, minimum and maximum numbers of adult female *P.ovis* with time for the VLA Reference isolate of *P.ovis*.

Figure 3.1.6. Presenting the mean, minimum and maximum numbers of adult female *P.ovis* with time for the St.Brenard field isolate of *P.ovis*.

The ratio of *P.ovis* to lesion area was also calculated. Lesion areas were ranked in ascending area into data sets of 0-50 cm^2, 51-100 cm^2, 101-200 cm^2 and then at intervals of 200 cm^2 to a final area of 5000 cm^2 and the corresponding arithmetic mean mite burden calculated and the maximum and minimum burdens recorded for each data set. The relationship between mite burdens to lesion area with time for each isolate is represented graphically, together with the minimum and maximum ranges of mite to lesion ratio (Figures Five and Six). The slope of the curve for each isolate was calculated using a programmable calculator (Texas Instruments Ti-60), together with minimum and maximum ranges of mite to lesion ratio.

4.2. Parasitological Assessments.

Lesion Area (cm^2) With Time.

The general appearance and sequalae of infestation was common to all sheep challenged with both the VLA Reference isolate or the St. Brenard isolate, only the temporal progression and severity of disease varied between sheep and the individual isolates. Within a day of challenge a small raised area developed at the site of challenge, surrounded by a greenish zone of inflamed skin and surmounted by a feeding mite or mites. Later this raised papule was replaced by a small crusty lesion. Scab mites began to aggregate at the periphery of the lesion, which began to spread outwards as the mite population increased. On the margins of the extending lesion was a narrow zone of inflamed, moist skin (often pale green in appearance) where the mites aggregated. This inflammed area surrounded an area where the lesion was composed of soft, moist crusts, becoming paler, harder and dryer towards the central challenge site. Lesions tended to spread downwards towards the axilla before spreading along the back towards the head or tail. Eventually the lesion growth slowed down or stopped completely and the mite population declined rapidly as the encrustation covered the whole body. The general appearance of the lesion also changed, the active moist edge becoming indistinct, dry and scaly.

A number of sheep infested with the VLA Reference isolate failed to develop lesions. The minimum value in all studies involving this isolate was therefore zero The predicted lesion size at a given time could therefore be anywhere within the area bounded solely by the maximum value in Figure 3.1.3. A subclinical phase (lesion area <250 cm^2, $\approx$ 5.0% body cover), lasting 21 to 28 days, was observed in all studies, although a small number of sheep presented subclinical lesions (lesions areas 1.0 to 16.0 cm^2, 0.02% to 0.3% body cover) throughout the 63 days of the studies. After the subclinical phase lesion areas steadily increased with time, with a marked increase 56 days PC. There was little variation in lesion progression with time between the study groups infested with the VLA Reference isolate. Differences in mean lesion areas with time were not significant at the 5.0 % level (P=0.083).

A similar subclinical phase lasting 14 to 28 days PC was observed in the mean values for sheep infested with the St.Brenard isolate. The marked increase in lesion growth 56 days PC, seen in sheep infested with the VLA Reference isolate was also observed in infestations of the St.Brenard isolate. In contrast to the VLA Reference isolate, clinical scab developed on all sheep challenged with the St.Brenard isolate, with minimum values also showing the subclinical and rapid growth phases. Also in contrast to the VLA Reference isolate lesion areas with time varied between study groups, with differences highly significant at the 5.0 % level ($P<0.001$). In two studies (UTC/1/a and UTC/4) the lesions grew steadily over the 42 and 63 days of examination, not varying greatly between sheep initially, but a number of final lesion areas were twice that of lesions on other sheep in the group. In another study (UTC/2) lesion areas remained relatively small ($\approx$ 5.1 % body cover) over the 98 days of assessment.

Overall lesions produced by the St.Brenard isolate grew at a greater rate than those produced by the VLA Reference isolate. The mean percent body cover for the VLA Reference Isolate did not achieve 100% on any sheep challenged over the 63 days of the study. On day 63 the mean percent body cover was 64.0% (min. 55.4% and max. 84.72%). The St.Brenard isolate on the other hand achieved 100% body cover on some sheep 28 days PC and on all sheep in the study by 63 days PC. Overall the mean percent body cover of the lesion produced by the St.Brenard isolate was in the magnitude of 30.5 % after 42 days PC and 100.0 % after 63 days PC, compared to only 7.6% and 60.9 % for the VLA, Reference Isolate over the same periods.

Two sheep infested with the St.Brenard isolate presented "flaking" lesions at the base of the neck (300 cm^2) and above the left fore leg (864.0 cm^2) respectively, 56 days PC. The remaining three sheep in the group all manifested areas of wool loss ranging between 150.0 cm^2 to 361.0 cm^2.

Numbers of Adult Female P. ovis with time.

Live *P. ovis* were located around the periphery of the expanding lesions (the "lesion edge"), either as a single adult female mite or in feeding aggregations of 5.0 to over 100.0 mites of all instars. At peak mite burdens the majority of *P. ovis* could be

found on the edge of the lesion heading towards the axilla. Mite burdens for both the VLA Reference isolate and St.Brenard isolates varied considerably between individual sheep as well as between the two isolates.

In comparison to lesion area, the differences in mite burden with time within groups of sheep infested with the VLA Reference isolate were not significant at the 5% level (P=0.200) but differences in the mite burdens with time between groups of sheep infested with the St.Brenard isolate were highly significant (P=0.003). Variation was rarely consistent with time, ie. sheep showing the highest burden on day 28 may demonstrate the lowest burden 7 days later.

Overall subclinical lesions presented low mite burdens (with mite numbers per sheep remaining below the 25 adult females of the original challenge) for 14 to 35 days in sheep infested with the VLA Reference isolate. From then on mite numbers gradually increased, although climax populations were not achieved during the time spans of the individual studies. A long subclinical phase of 21 to 35 days was observed in two studies (41/1 and 41/2) and a shorter subclinical phase of 14 to 21 days was observed in the remaining three studies (41/3, 41/4 and 41/5). The duration of the subclinical phase also varied with individual sheep. No mites were observed on one animal in Study 41/1 until 49 days PC and one sheep in each of studies 41/2 and 41/4 remained in the subclinical numbers throughout the 35 and 56 days of the study, respectively.

An overall subclinical phase of 21 to 28 days was observed in sheep infested with the St.Brenard isolate. Arithmetic mean and maximum mite burdens climaxed 56 days PC, mites then declined rapidly in number. In study 41/2 three animals were treated with a licensed product (to assess efficacy) 42 days PC, the remaining 2 animals continued to be assessed to day 70 PC. All sheep in the group maintained relatively high mite burdens, increasing with time. On the day of treatment mite burdens within the group ranged between 106.0 and 5180.0 mites per sheep. Mite burdens peaked on the untreated sheep 49 and 63 days PC (5180.0 and 740.0 mites respectively). Burdens then decreased to 521.0 mites (day 63 PC) and 25.0 mites (day 70 PC) on one sheep and 5.0 mites on day 70 PC on the other.

Three sheep in Study 41/3 were treated with a licensed product (to assess efficacy) 42 days PC, again two animals continued to be assessed to day 56 PC. In comparison to Study 41/2 all sheep maintained relatively high mite numbers, increasing with time. On the day of treatment, mite burdens ranged between 101.0 and 400.0 mites per sheep. Mite burdens peaked on the untreated sheep 49 and 56 days PC, with 836.0 and 206.0 mites recovered respectively. All sheep in Study 41/5 were treated with a licensed product 42 days PC. Of the ten sheep infested in the study eight (80%) manifested increasing mite burdens on the day of treatment. One sheep maintained increasing high mite numbers throughout the study, peaking at 410.0 mites on day 42 PC. On the other hand another sheep maintained relatively low mite burdens (never >9 mites) and mites were self eradicated prior to treatment on day 42 PC.

In Studies UTC/1/a and UTC/1/b mite burdens were not constant and, with a few exceptions, animals vied for the position of highest or lowest mite burdens at each weekly assessment. Burdens continued to increase on all sheep in Study UTC/1/a throughout the study, but had began to decrease to extinction on 5/6 (83.3%) of sheep in UTC/1/b. This extinction occured on one sheep 42 days PC, on another 56 days PC and on three others 63 days PC. One animal maintained a low burden throughout the 63 days of the study, with 6.0 adult females observed at the time of treatment.

Mite burdens had also decreased to extinction on 3/5 (60.0%) of sheep in Study UTC/2 by 98 days PC. This decrease in mite numbers was not related to initial high burdens. The remaining two sheep in the study group still maintained relatively high mite burdens on day 98 PC (26.0 and 38.0 mites respectively). In Study UTC/3 mite burdens continued to increase in 5/13 (38.5%) of sheep over the 63 days of the study. The highest mite burdens were observed during these investigations, peak burdens ranging between 234.0 to 5540.0 mites per sheep. In the same study mite burdens decreased in 8/13 (61.5%) of sheep to 15.0 to 70.0 mites per sheep over the 63 days of the study, although mite numbers were still relatively high compared to other studies. Two sheep developed relatively high mite burdens with 540.0 and 430.0 mites per sheep respectively 35 days PC, increasing to 5540.0 and 990.0 mites per

sheep on day 56 PC. In Efficacy Study UTC/4 mite burdens peaked between 28 to 56 days PC, and then decreased to apparent extinction on all sheep by day 70 PC. In this study all sheep developed relatively high mite burdens, with the highest mite burden (155.0 and 274.0 mites per sheep, 35 and 63 days PC respectively), but numbers rapidly dropped to extinction, 7 days later.

A similar rapid drop in mite numbers was observed in another sheep, with 72.0 mites on day 63 PC decreasing rapidly to zero on day 70 PC. All other sheep showed a gradual decrease in mite numbers. In Efficacy Study UTC/5 mite burdens peaked between 28 and 56 days PC, eventually decreasing on all animals in the study. All sheep maintained relatively high mite burdens (range 42.0 to 102.0) throughout the 91 days of the study, with burdens decreasing to extinction on two sheep 91 days PC. One sheep maintaining relatively low burdens, only beginning to increase 91 days PC.

Ratio of Mites to Lesion Area.

The numbers of mites per cm^2 of lesion was initially high with the number of adult female mites relatively large for the small initial lesion. As the lesion progressed mites were only found around the lesion periphery. Consequently mite numbers appeared to decrease as the lesion grew. Comparing the range of lesion areas presented for each isolate with the corresponding mean numbers of adult female *P ovis* demonstrated a strong positive correlation between lesion area and mite burden for the VLA Reference isolate ($r = 0.7783$. $y = 24.8838$, $x = 0.0285$), but no correlation was observed between lesion area and mite burden for the St.Brenard isolate($r = 0.3628$, $x = 622.686$, $y = 0.3628$).

Touch Hypersensitivity

Overall the mean touch (hypersensitivity) response (THR) for all studies comparing the VLA Reference isolate peaked 35 days PC (mean 6.1% body cover), but gradually subsided there after. The THR with time varied with individual sheep. Some sheep could remain unreactive until 35 or 42 days PC, yet other sheep could develop high THR scores as early as day 7 PC (body cover ranging between 0.03 and 0.09 %) and some maintaining these high scores for over 42 days. Three sheep manifested epileptiform seizures (THR score ++++) after 28, 42 or 120 days with

corresponding lesion body cover ranging between 1.2 and 58.3%. One sheep died as a result of an epileptiform seizure, maintaining a high THR score throughout the study.

The mean THR score for sheep infested with the St.Brenard isolate peaked between 14 and 28 days PC, earlier than than observed for the VLA Reference isolate. Two sheep presented epileptiform seizures (one fatal), both after 56 days PC. The fatal seizure occured when the lesion covered 56.9 % of the body cover.

Infestation of the External Auditory Canal

Live *P. ovis* were isolated from the external auditory canals (EACs) of seven sheep challenged with the VLA Reference isolate (25.9% of the total examined). Otoacariasis was recorded in one animal 28 days PC, with the leading lesion edge 28.0 cm from the ears. In the remaining sheep, two recorded otoacariasis 35 days PC (with leading lesion edge 33.0 cm from the ears) and four sheep recorded otoacariasis 42 days PC. Haematomae/fibrosis was not observed in any sheep challenged with the VLA Reference isolate of *P. ovis*.

Live *P. ovis* were isolated from the external auditory canals of twenty five sheep challenged with the St.Brenard isolate (53.2% of the total examined). Otoacariasis was recorded in one animal 35 days PC, with the leading lesion edge 17.0 cm from the ears. Twenty two sheep infested in the EAC presented no associated lesions in the internal aspects of the pinnae. However two of these sheep also presented mild (1+) aural haematomae. The remaining three sheep infestated in the EAC also presented corresponding pinneal lesions. One sheep challenged with the St.Brenard isolate presented pinneal lesions with mites but was uninfested in the EAC.

Infestation of the Remaining Cryptic Sites

The cryptic ("latent") sites were not examined in Study 41/1. No animal challenged with either the VLA Reference isolate or the St.Brenard isolate was infested in any cryptic site prior to challenge.

No sheep infested with the VLA Reference isolate recorded mites present in any cryptic site (pinnae, IOF, inguinal pouches or crutch) at any time throughout the studies. Mites were however, recorded in the cryptic sites of sheep infested with the St.Brenard isolate. Mites were recorded in the internal aspects of the pinnae of eight sheep (17.0% of total examined). Two animals presenting infested pinnae also presented mild (1+) aural haematomae, one sheep presenting bilateral pinneal infestations with corresponding bilateral haematomae.

Twelve sheep infested with the St.Brenard isolate (25.5% of total examined) recorded infestations in the IOF, first observed 56 days after challenge. Twelve sheep (25.5% of total examined) also recorded mites present in the inguinal pouches, again first observed 56 days after challenge. Six sheep (12.8% of the total) presented lesions and mites in the crutch.

	7	14	21	28	35	42	49	56	63
Number of Sheep	24.0	27.0	14.0	24.0	12.0	17.0	22.0	6.0	4.0
Mean % body cover	0.07	0.55	1.60	7.59	6.09	26.31	30.50	21.81	64.00
Minimum % Body cover	0.0	0.0	0.02	0.33	0.02	0.0	0.17	0.0	55.46
Maximum % body cover	0.42	2.50	3.80	17.16	25.12	64.27	52.49	61.15	84.72

Table 3.1.1: Lesion (percent body cover) days after challenge. VLA Reference isolate. Arithmetic mean data for all studies.

	7	14	21	28	35	42	49	56	63
Number of Sheep	15	14	29	44	48	39	25	24	2
Mean % body cover	0.09	3.88	22.44	40.58	81.35	81.36	95.9	100.0	100.0
Minimum % Body cover	0.02	0.04	2.51	2.26	8.28	11.72	21.09	40.6	100.0
Maximum % body cover	0.33	4.60	26.16	100.0	100.0	100.0	100.0	100.0	100.0

Table 3.1.2: Lesion (percent body cover) days after challenge. St.Brenard isolate. Arithmetic mean data for all studies.

	7	14	21	28	35	42	49	56	63	70
Number of Sheep (n)	29	29.0	14.0	24	10	19.0	6	7	6	1
Mean number of P.ovis	4.1	5.0	17.1	45.8	41.5	77.3	45.3	109.8	105.0	155.0
Standard Deviation (sd)	4.8	4.9	14.5	29.4	35.9	49.5	24.9	147.9	132.7	-
Minimum number of P.ovis	0.0	0.0	0.0	0.0	0.0	0.0	5.0	0.0	0.0	-
Maximum number of P.ovis	19.0	22.0	37.0	106.0	100.0	185.0	86.0	455.0	391.0	-

Table 3.1.3. Numbers of adult female *Psoroptes ovis* with time (days) after challenge. VLA Reference isolate. Arithmetic mean data for all studies.

	7	14	21	28	35	42	49	56	63	70
Number of Sheep (n)	15	24	14	30	45	47	42	26	23	7
Mean number of *P.ovis*	5.9	14.8	37.5	63.8	140.2	224.9	304.8	526.3	99.5	4.2
Standard Deviation (sd)	3.5	9.5	24.2	51.1	116.1	302.5	787.5	1302.3	177.0	8.6
Minimum number of *P.ovis*	0.0	0.0	11.0	3.0	21.0	0.0	0.0	0.0	0.0	0.0
Maximum number of *P.ovis*	13.0	46.0	92.0	170.0	540.0	1775.0	5180.0	5540.0	740.0	25.0

Table 3.1.4. Numbers of adult female *Psoroptes ovis* with time (days) after challenge. St.Brenard isolate. Arithmetic mean data for all studies.

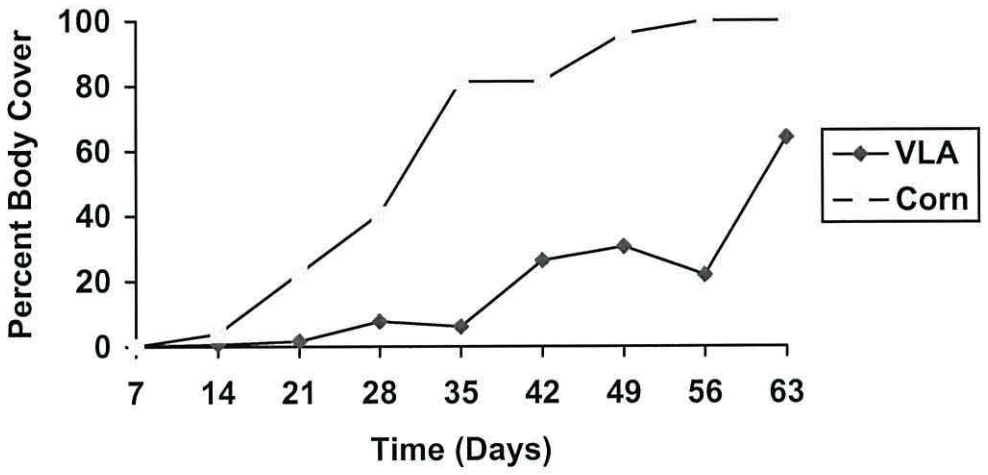

Figure 3.1.1: Mean lesion areas (percent body cover) with time (days) for full fleeced, yearling close wool sheep, challenged on the withers with 25 adult female *Psoroptes ovis*, VLA Reference or St.Brenard (Cornish) Field isolates. n = 24, 27, 14, 24, 12, 17, 22, 6 sheep challenged with the VLA Reference Isolate and 15, 14, 29, 44, 48, 39, 25, 24 and 2 sheep challenged with the St.Brenard Field Isolate, 7, 14, 21, 28, 35, 42, 49, 56 and 63 days, respectively.

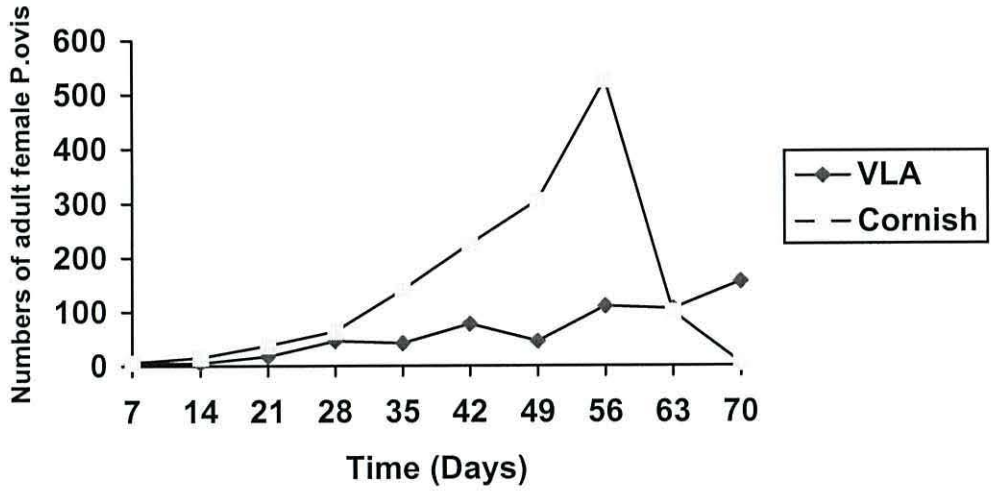

Figure 3.1.: Mean numbers of adult female *Psoroptes ovis* with time (days) for full fleeced, yearling close wool sheep, challenged on the withers with 25 adult female *Psoroptes ovis*, VLA Reference or St.Brenard (Cornish) Field isolates. n = 29, 29, 14, 24, 10, 19, 6, 7, 6 and 1 sheep challenged with the VLA Reference Isolate and 15, 24, 14, 30, 45, 47, 42, 26, 23 and 7 sheep challenged with the St.Brenard Field Isolate, 7, 14, 21, 28, 35, 42, 49, 56, 63 and 70 days, respectively.

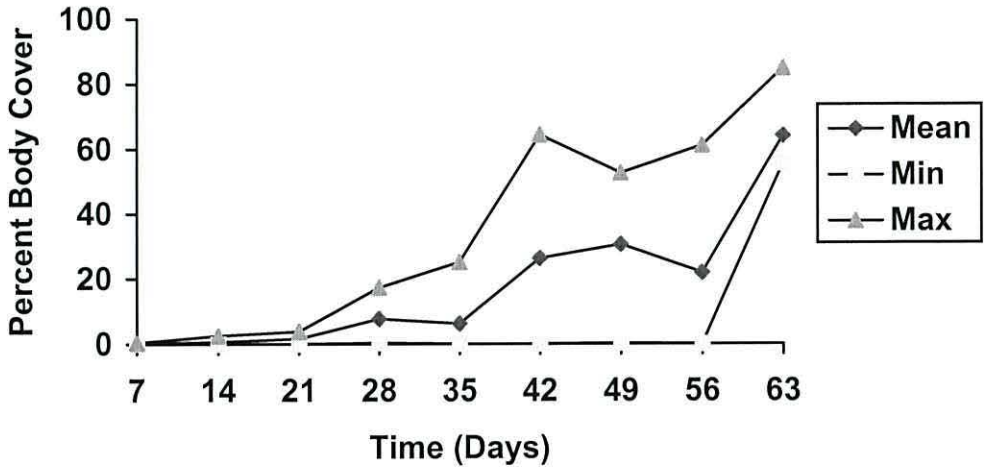

Figure 3.1.3: Mean, minimum and maximum lesion areas (percent body cover) with time (days) for full fleeced, yearling close wool sheep, challenged on the withers with 25 adult female *Psoroptes ovis*, VLA Reference Isolate. n = 24, 27, 14, 24, 12, 17, 22, 6 and 4 sheep, 7, 14, 21, 28, 35, 42, 49, 56, 63 days, respectively, after challenge.

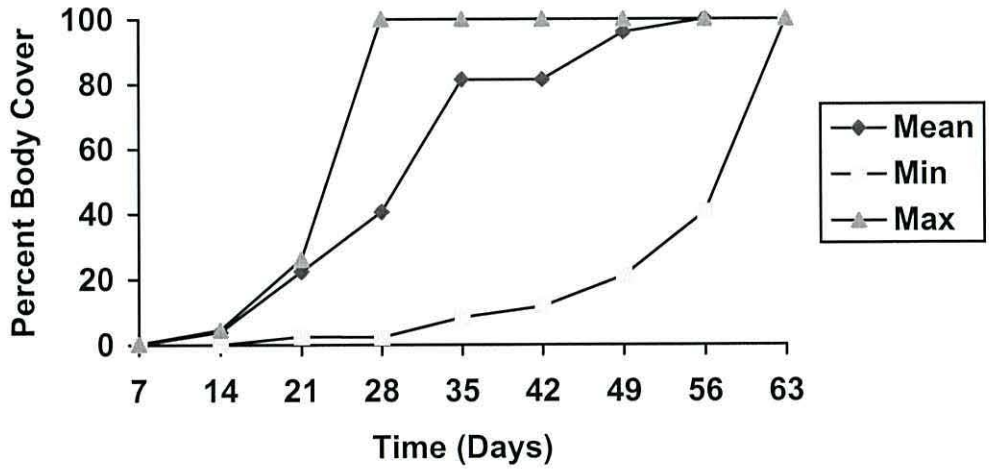

Figure 3.1.4: Mean, minimum and maximum lesion areas (percent body cover) with time (days) for full fleeced, yearling close wool sheep, challenged on the withers with 25 adult female *Psoroptes ovis*, St.Brenard (Cornish) Field Isolate. .n = 15, 14, 29, 44, 48, 39, 25, 24 and 2 sheep, 7, 14, 21, 28, 35, 42, 49, 56, 63 days, respectively, after challenge.

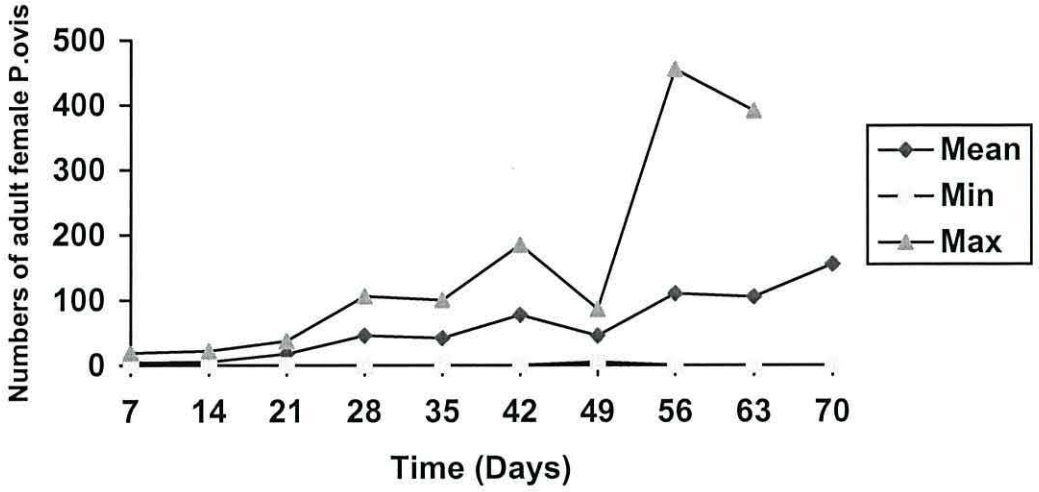

Figure 3.1.5: Mean, minimum and maximum numbers of adult female *Psoroptes ovis* with time (days) for full fleeced, yearling close wool sheep, challenged on the withers with 25 adult female *Psoroptes ovis*, VLA Reference. n = 29, 29, 14, 24, 10, 19, 6, 7, 6 and 1 sheep, 7, 14, 21, 28, 35, 42, 49, 56, 63 and 70 days post challenge, respectively.

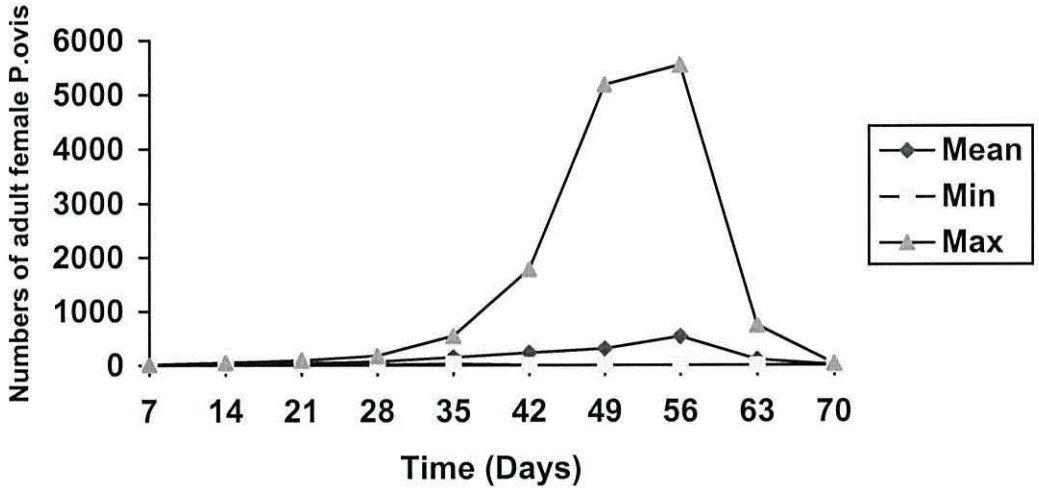

Figure 3.1.6: Mean, minimum and maximum numbers of adult female *Psoroptes ovis* with time (days) for full fleeced, yearling close wool sheep, challenged on the withers with 25 adult female *Psoroptes ovis*, St.Brenard (Cornish) Field isolates. n = 15, 24, 14, 30, 45, 47, 42, 26, 23 and 7 sheep, 7, 14, 21, 28, 35, 42, 49, 56, 63 and 70 days post challenge, respectively.

Chapter 3.2

Variations Within Geographical Isolates of the Sheep Scab Mite (*Psoroptes ovis*).

1.0 Summary

Workers in the USA in the 1970s demonstrated variations in virulence and acaricide susceptability between isolates of *P ovis*. The studies in this Chapter document similar variations between isolates of *P ovis* in Great Britain. Between 1987 and 1997 groups of between five and ten full fleeced, close wool breed, yearling sheep were challenged with 25 adult female *P ovis* originating from one of 16 geographical isolates. The relative virulence (rate of lesion and mite population growth with time) was compared to similar sheep challenged with the VLA Reference isolate. There was considerable variation in the relative virulence of the isolates. Some low virulence (chronic) isolates taking 8 to 10 weeks, or even longer, to cover sheep. Other high virulence (acute) isolates taking only 4 to 5 weeks to cover the sheep.

2.0. Introduction

Pathogenic and non pathogenic strains of the same organism are recognised by virologists, bacteriologists etc. Differences within the same species of insect are also well documented, particularly with reference to insecticide resistance. Roberts and Meleney (1971) investigating differences within the biology of *P. ovis* on sheep concluded that distinct strains of the mite existed in the USA. These differences being based upon a) the success of certain strains to withstand population reduction during the summer, b) these more "aggressive" strains survived contact with the organophosphate acaricide, coumaphos compared to less aggressive strains (where complete control was achieved), c) "aggressive" strains survived longer on sheep in individual isolation and d) "aggressive" strains of sheep origin were able to spread through herds of cattle more rapidly and produce more obvious clinical responses than less pathogenic strains. Hybrid vigour and/or selection of strains through the effects of sublethal drugs, breeds of sheep and or husbandry practices together with differences within and between flocks and geographical isolation, were responsible for the strain variation. Roberts and Meleney (1971) highlighted the importance of strain variation, postulating that the highly complex syndrome associated with scab has been a source of uncertainty, if not confusion. Much of this confusion could be dispelled with the application of the strain variation concept from the standpoint of drug resistance, oversummering and pathogenicity. Hybrid vigour or previous selection of vigorous strains throgh marginally effective drugs or sublethal concentrations of effective drugs could be responsible for these differences.

In order to investigate whether such variation existed within *P.ovis* populations in Great Britain, five separate studies were carried out between 1987 and 1997 comparing sixteen field isolates of *P. ovis,* originating from natural outbreaks of clinical sheep scab within the British Isles, against the VLA Reference isolate. Isolates were compared with respect to the increase mite populations, lesion area and touch hypersensitivity with time, with a critical comparison carried out 28 days after challenge.

3.0. Materials and Methods

3.1. Animals and Animal Maintenance

Groups of scab naive, full fleeced, Dorset cross or Suffolk cross yearling sheep (females or male castrates) were used in these studies. The sheep had never been exposed to acaricides/insecticides or anthelmintics as described in Chapter 3.1. The animals may have previously received a late May/June treatment of Vetrazine (6.0% cyromazine, Novartis Animal Health) as a pour-on for the prevention of blowfly strike (*Lucilia sericata*) and wormed, when necessary, with levamisol or benzamidazole based anthelmintics. Cyromazine, levamisol and benzamidazole have been demonstrated to have no effect on *P. ovis* (Bates, *unpublished data*). All sheep were certified "healthy" prior to infestation and were shown not to have any concomitant chewing louse (*Bovicola* (*Damalinia*) *ovis*) infestations.

During the course of the trial study groups of sheep challenged with their respective *P.ovis* isolate were housed in a covered yard and maintained on expanded metal flooring, in pens constructed of solid metal hurdles and breeze block walls, to prevent cross infestation. Sheep were fed a diet of unmedicated dried grass pellets (Torberry Feeds, Torberry Farm, Hurst, Petersfield, Hampshire, GU31 5RG), approximately one kilogram per sheep per day. Fresh mains water was available *ad lib* via floor troughs.

In order to estimate the percent body cover for each scab lesion the mean surface area for the "trunk" of an average shearling sheep was calculated from nine typical shearlings, directly after shearing. Body length was measured from the base of the neck to the tuba coxae (pin bones) and the girth was measured around the rib cage. The surface area was calculated by multiplying body length by the girth. The mean surface area for the nine shearlings measured was 4778.0 cm^2 (range 4240.0 cm^2 to 5185.0 cm^2).

With the exception of sheep in study 41/3 all other study groups comprised of sheep lambed from a standard source: 41/1 (Truro, Cornwall), 41/2 ((Truro, Cornwall), 41/3 (mixture of VLA, Weybridge and Compton), 41/4 (all VLA, Weybridge), 41/5 (all Etchinwell) and 41/6and 41/7 (all VLA, Weybridge).

3.2. Sources of Infestation

The *Psoroptes* isolates originated from either wool and skin scrapings taken in the field and submitted to the VLA, Weybridge for the statutory diagnosis of sheep scab, the live mites subsequently maintained on sheep at the VLA or heavily infested sheep were purchased from infested flocks and the *P. ovis* isolates subcultured on sheep at the VLA, Weybridge.

Field isolates were compared against to the VLA reference isolate in the following seven studies. Study 41/1 (May, 1989: Dorchester (Dorset)), 41/2 (February, 1990: St. Brenard (Cornwall)), Study 41/3 (May, 1992: Bacup (Lancashire), St. Brenard (Cornwall), Little Melton (Norfolk), Llanfach (Anglesey), Princetown (Devon) and St. Brenard (Cornwall)), Study 41/4 (November, 1995: Caithness I (Scotland) and Porlock (Somerset)), Study 41/5 (February, 1996: Alston (Cumbria), Arlington (Devon), Market Drayton (Shropshire) and St. Brenard (Cornwall)), Study 41/6 (February, 1996: Aberystwyth (Dyfed), Caithness III (Scotland) and Witney (Oxfordshire)) and Study 41/7 (February, 1997: Veterinary Research Laboratory (Dublin, Republic of Ireland) reference isolate).

In all studies *P. ovis* field isolates were compared against the VLA Reference isolate as regards the temporal increase in lesion area, mite populations and touch hypersensitivity response (THR) with time following challenge, with a critical comparison carried out 28 days after challenge. All studies were carried out on unshorn yearlings during the "sheep scab season" (September to early May) recognised in the UK.

3.3. Method of Infestation

In each comparative study live adult female *P. ovis* from each cultured field isolate were collected from the lesion periphery of donor sheep using a mounted needle and gently placed into a (30 x 8 mm) glass Durham Tube, the tube then sealed with non-absorbent cotton wool. Groups of between five or ten sheep for each field isolate were infested by placing 25 adult female *P. ovis* (gently removed from the Durham tube using a mounted needle) directly onto an area of skin plucked of wool on the withers of each of sheep. The area of challenge was marked using a stock marker ring (Agrimark, Pfizer).

3.4. Parasitological Assessments

Lesion areas (percent body cover), mite populations, the touch hypersensitivity response (THR) were recorded on days +7, +14, +21, +28, +35, +42 and +49 days after challenge, according to the methods described in Chapter 3.1.

4.0. Results

All the geographical isolates of *P.ovis* compared in this study produced the progressive lesion, characteristic of sheep scab (psoroptic mange) but the extent of the lesion produced with time varied considerably between isolates. The lesion area, mite burden, and touch hypersensitivity response between the *P. ovis* isolates were critically compared 28 days post challenge. Investigations in Chapter 3.1 demonstrated that comparing lesions 28 days post challenge would highlight those still in the subclinical phase of disease and those in the rapid growth phase.

4.1. Parasitological Assessments

Lesion area

Considerable differences in lesion area were observed between the geographical isolates of *P. ovis* 28 days post challenge. Some "low virulence" isolates produced relatively small lesions (mean lesion area 26.4 cm^2 / 0.6% body cover (Princetown)) compared to other "high virulence" isolates, producing more extensive lesions (mean lesion area 928.0 cm^2 / 19.4% body cover (Market Drayton)) over the same time period. The arithmetic means for the actual lesion areas (in cm^2) 28 days post challenge for each *P. ovis* isolate were ranked in descending order and are presented in Table 3.2.1. The mean percent areas of body covered by the lesion produced by each isolate, 28 days post challenge, are also shown in Table 3.2.1 and presented graphically in Figure 3.2.1.

Following this ranking of lesion area it was possible designate the *P. ovis* isolates as low, medium or highly virulent. Isolates were designated "medium virulent", if the mean lesion area for the study group fell within the range of the corresponding mean lesion areas recorded for the study groups challenged with the VLA reference isolate (ie: between Study 41/2: 151.0 cm^2 / 3.2% body cover and Study 41/5: 500.0 cm^2 / 10.5 % body cover). Consequently isolates were designated "low virulence" if the mean lesion area for the study group fell below this range and "highly virulent" if the mean lesion area for the study group was greater than this range.

All the isolates of *P.ovis* produced a progressive lesion, characteristic of sheep scab (psoroptic mange). The mean lesion areas for the 24 individual sheep challenged with the four low virulence *P.ovis* isolates recorded 7, 14, 21, 28, 35, 42 and 49 days post challenge were combined to give an overall mean lesion area for low virulence populations of *P. ovis* for these examination days. Similarly the overall mean lesion areas over the same time period were calculated for the seventy five sheep challenged with the twelve medium virulence isolates of *P. ovis* and the forty five sheep

challenged with the six high virulence isolates of *P. ovis*. These are represented graphically in Figure 3.2.3.

Figure 3.2.1. demonstrates considerable variation in the rate of lesion increase between low, medium and high virulence populations of *P. ovis*. Setting a threshold of 100 cm^2 for sub-clinical disease it can be seen that lesions produced by low virulent populations of *P. ovis* can theoretically remain subclinical for long periods (up to 35 days) following infestation. In the field this may be even longer when the infestive challenge and breed of sheep are taken into account (Bates, 1997). In medium and high virulence populations this sub-clinical period is reduced to only 14 days.

Calculation of the Rate of Lesion Increase.

. The relative rates of lesion increase (cm^2 / day) for low, medium and highly virulent populations of *P. ovis* over 42 days were calculated using the formula:-

$$\frac{(\Sigma\ (x_a/y) + (x_b/y) + (x_c/y) + (x_d/y) + (x_e/y) + (x_f/y)}{z}$$

Where x = mean lesion area (cm^2) 7 (x_a), 14 (x_b), 21 (x_c), 28 (x_d), 35 (x_e) or 42 (x_f) days post challenge: y = the time period between measurements (ie. 7 days) and z = total number of measuring days (ie.6).

Using this formula the relative rates of lesion increase for low, medium and highly virulent populations of *P. ovis* over 42 days were 23.8, 84.3 and 121.4 cm^2 / day, respectively. These are represented graphically in Figure 3.2.5.

Mite Numbers.

Considerable differences in mite burdens were also observed between the geographical isolates of *P. ovis* 28 days post challenge. The "low virulence" Princetown isolate presented a mean *P. ovis* burden of 12.8 mites per lesion (range 0.0 to 30.0 mites per lesion) compared to 139.3 mites per lesion (range 65.0 to 220.0 mites per lesion) for the "highly virulent" Market Drayton isolate. The arithmetic means for the mite burdens, 28 days post challenge for each *P. ovis* isolate, corresponding to their ranked lesion areas are presented in Table 3.2.1 and Figure 3.2.2.

The mite populations produced by all the isolates of *P.ovis* increased with time (days) in association with their increasing lesion. The mite populations for the 24 individual sheep challenged with the four low virulence *P.ovis* isolates recorded 7, 14, 21, 28, 35, 42 and 49 days post challenge were combined to give an overall mean mite population for the low virulence *P. ovis* for these examination days. Similarly the overall mean mite populations over the same time period were calculated for the seventy five sheep challenged with the twelve medium virulence isolates of *P. ovis* and the forty five sheep challenged with the six high virulence isolates of *P. ovis*. These are represented graphically in Figure 3.2.4.

In comparison to the rate of lesion increase Figure 3.2.4. demonstrates considerable variation in the rate of population increase between low, medium and high virulence isolates of *P. ovis*. Setting a threshold of 25 mites per lesion (equal to or below the original mite challenge) for sub-clinical disease, a similar pattern to that for the relative rates of lesion increase is observed. It can be seen that mite burdens produced by low virulence populations of *P. ovis* can theoretically remain subclinical for long periods (up to 35 days) following infestation. In medium and high virulence populations this sub-clinical period is reduced to 21 or 14 days respectively.

Calculation of the Rate of P.ovis Population Increase.

The relative rates of *P. ovis* population increase for low, medium and highly virulent populations of *P. ovis* over 42 days was also calculated from the formula used to calculate the rate of lesion increase.

Using this formula the relative rates of population increase for low, medium and highly virulent populations of *P. ovis* over 42 days were 4.1, 12.5 and 17.8 mites per day respectively, and are represented graphically in Figure 3.2.6.

Statistical Analysis.

Analysis of variance were carried out to compare the means for the VLA Reference isolate and among the fifteen field isolates. Because of the considerable variation in the standard deviations analyses were carried out on the logarithm to the base 10 of the areas and the square roots of the mite counts. The differences beween the years for VLA Reference isolate were not significant at the conventional 5.0% level ($p=0.069$ for mite numbers and $p = 0.060$ for lesion areas). In contrsat there were higly significant ($p < 0.001$) differences among the other isolates for both mite numbers and lesion areas. Pairwise differences between the means confirmed the conclusion that isolates in the higher virulence groups differ from those in the lower virulence groups.

The Touch (Hypersensitivity) Response

The mean scores for the touch (hypersensitvity) response (THR), 28 days post challenge for each *P. ovis* isolate, corresponding to their ranked lesion areas are presented in Table 3.2.1. Although differences in the (THR) were observed between the geographical isolates of *P. ovis* 28 days post challenge, they were not consistent with the virulence of the isolate. The mean THR score for the 24 individual sheep challenged with the four low virulence *P.ovis* isolates, 28 days after challenge was 0.97, compared to 1.66 for the seventy five sheep challenged with the twelve medium virulence isolates of *P. ovis* and 1.39 for the forty five sheep challenged with the six high virulence isolates of *P. ovis*. These mean THR scores corresponded with the numbers of animals suffering epileptiform seizures (4+ THR). Seizures were not

observed in any sheep infested with the low virulence isolates of *P. ovis*. However, seizures were presented by three out of the seventy five (4.0%) sheep infested with medium virulent and two out of the forty five (4.4%) sheep infested with the high virulence populations of *P. ovis*. Within the medium virulent population of *P. ovis* one sheep challenged with the VLA reference isolate (Study 41/1) manifested a fatal siezure 48 days after challenge, with a corresponding lesion area of 750 cm^2 (15.7% body cover). Another sheep challenged with the VLA reference isolate (Study 41/3) presented an epileptiform seizure 28 days after challenge, with a corresponding lesion area of 330 cm^2 (6.9% body cover). One sheep challenged with the Dorchester isolate (Study 41/1) manifested a fatal siezure 31 days after challenge, with a corresponding lesion area of 150 cm^2 (3.14 % body cover). Within the high virulence population of *P. ovis* one sheep challenged with the Market Drayton isolate presented an epileptiform seizure, 28 days after challenge, with a corresponding lesion area of 1232 cm^2 (25.78% body cover). Another sheep challenged with the Aberystywyth isolate (Study 41/6) presented a seizure (day after challenge not recorded) with a corresponding lesion area of 2046 cm^2 (42.82 % body cover).

Host Susceptability

As there were no significant differences regarding lesion area or mite burdens within the study groups challenged with the VLA, Reference Isolate, flock genetics appears to have minimal effect on the progress of clinical sheep scab.

P. ovis Isolate	Study	Number of Sheep	Lesion Area (cm^2) (± sd)		Lesion Area (% Cover)	Mite Burden (± sd)		THR
Princetown	41/3.	5	**26.4**	(± 17.6)	**0.6**	**12.8**	(± 10.7)	0.8
Bacup	41/3.	5	**49.2**	(± 59.7)	**1.0**	**12.4**	(± 8.0)	0.8
Witney	41/6.	7	**141.8**	(± 37.8)	**2.3**	**14.0**	(± 9.6)	nr
Caithness III	41/6.	7	**128.8**	(± 27.8)	**2.7**	**10.5**	(± 6.3)	1.3
VLA Reference	41/2.	5	**151.0**	(± 129.1)	**3.2**	**27.6**	(± 33.0)	nr
VLA Reference	41/1.	4	**227.0**	(± 139.4)	**4.8**	**26.0**	(± 16.0)	0.8
VLA Reference	41/4.	5	**245.8**	(± 128.8)	**5.1**	**20.6**	(± 9.9)	2.2
Dorchester	41/1.	4	**341.0**	(± 242.6)	**7.1**	**42.0**	(± 31.1)	1.8
Porlock	41/3.	5	**383.0**	(± 88.2)	**8.0**	**27.8**	(± 9.9)	2.0
VRL Reference	41/7.	10	**435.2**	(± 100.6)	**9.0**	**29.9**	(± 18.9)	1.4
St. Brenard	41/5.	10	**444.4**	(± 176.4)	**9.3**	**30.5**	(± 29.6)	1.4
St. Brenard	41/4.	5	**444.6**	(± 212.8)	**9.3**	**55.8**	(± 58.7)	1.5
VLA Reference	41/3.	5	**437.6**	(± 98.1)	**9.5**	**56.6**	(± 34.9)	1.6
Caithness I	41/4.	5	**458.6**	(± 90.8)	**9.6**	**55.8**	(± 10.2)	2.0
Llanfach	41/3.	7	**452.1**	(± 314.9)	**9.9**	**102.8**	(± 46.8)	1.7
VLA Reference	41/5.	10	**500.3**	(± 156.6)	**10.5**	**61.6**	(± 22.7)	1.6
Aberystywyth	41/6.	5	**566.0**	(± 289.9)	**11.8**	**81.2**	(± 46.2)	nr
St. Brenard	41/3.	5	**619.4**	(± 150.0)	**12.9**	**137.8**	(± 38.2)	0.8
Arlington	41/5.	10	**638.6**	(± 108.5)	**13.5**	**102.2**	(± 38.6)	1.6
Alston	41/5.	10	**649.4**	(± 315.7)	**13.6**	**86.8**	(± 62.6)	1.4
Little Melton	41/3.	5	**675.0**	(± 194.0)	**14.1**	**123.4**	(± 40.1)	1.2
Mkt Drayton	41/5.	10	**928.0**	(± 225.8)	**19.4**	**139.3**	(± 49.0)	1.5

Table 3.2.1.: All data collected 28 days after challenge with the exception of [1] = taken weekly from day 0 to day ? post challenge. 41/1, May 1989; 41/2, March, 1990; 41/3, May, 1995; 41/4, November, 1995; 41/5 and 41/6, both February, 1996; and 41/7, February 1997. nr = not recorded.

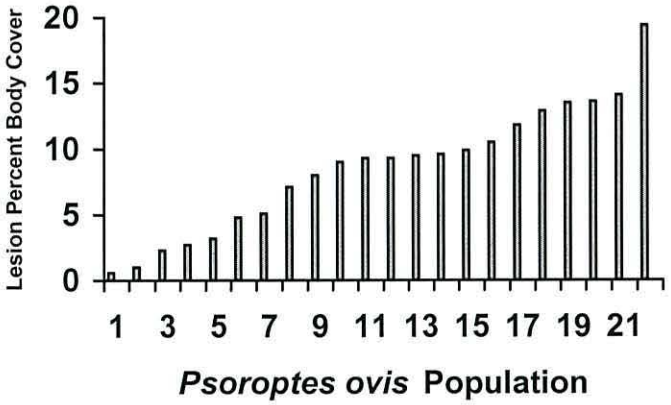

Figure 3.2.1: Graphical representation of data presented in Table 3.2.1. Lesion area (percent body cover), 28 days after challenge for groups of sheep challenged with one of twenty two populations of *P.ovis*. 1 = Princetown, 2 = Bacup, 3 = Witney, 4 = Caithness III, 5 = VLA (1990), 6 = VLA (1989), 7 = VLA (1995), 8 = Dorchester, 9 = Porlock, 10 = VRL (Dublin), 11 = St.Brenard (1996), 12 = St.Brenard (1995), 13 = VLA (1995), 14 = Caithness I, 15 = Llanfach, 16 = VLA (1996), 17 = Aberystwyth, 18 = St. Brenard (1995), 19 = Arlington, 20 = Alston, 21 = Little Melton and 22 = Market Drayton.

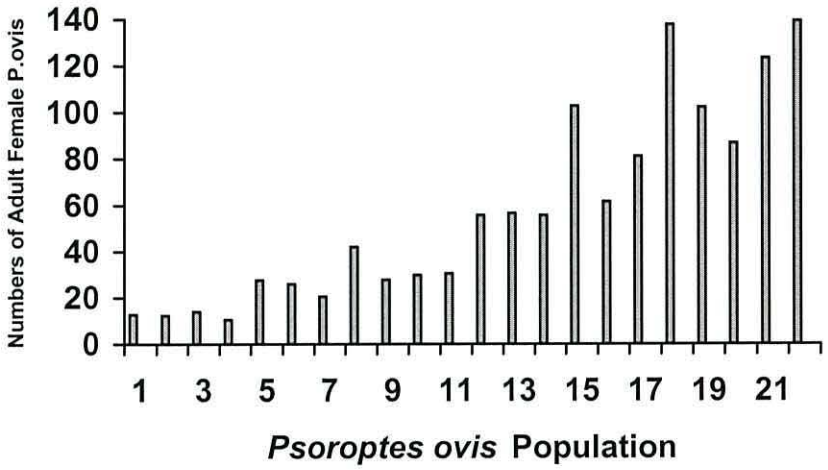

Figure 3.2.2: Graphical representation of data presented in Table 3.2.1. Numbers of adult female *P.ovis*, 28 days after challenge for groups of sheep challenged with one of twenty two populations of *P.ovis*. 1 = Princetown, 2 = Bacup, 3 = Witney, 4 = Caithness III, 5 = VLA (1990), 6 = VLA (1989), 7 = VLA (1995), 8 = Dorchester, 9 = Porlock, 10 = VRL (Dublin), 11 = St.Brenard (1996), 12 = St.Brenard (1995), 13 = VLA (1995), 14 = Caithness I, 15 = Llanfach, 16 = VLA (1996), 17 = Aberystwyth, 18 = St. Brenard (1995), 19 = Arlington, 20 = Alston, 21 = Little Melton and 22 = Market Drayton.

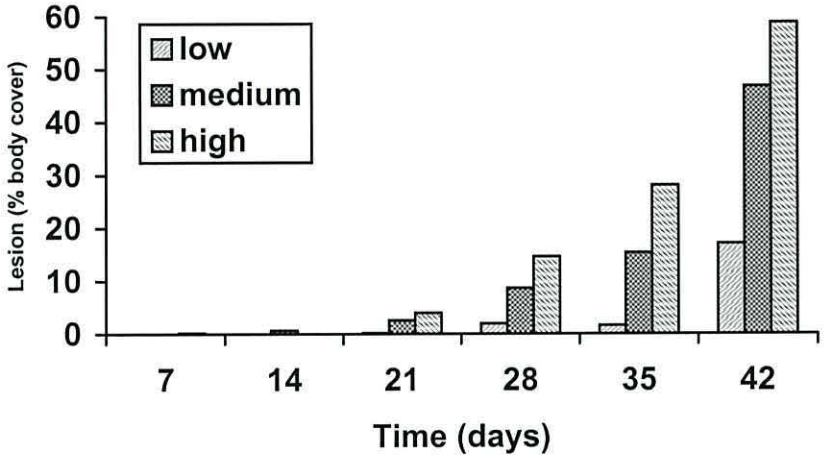

Figure 3.2.3: Lesion area (% body cover) with time (days) after challenge with 25 adult females from low, medium or high virulence populations *P.ovis*.

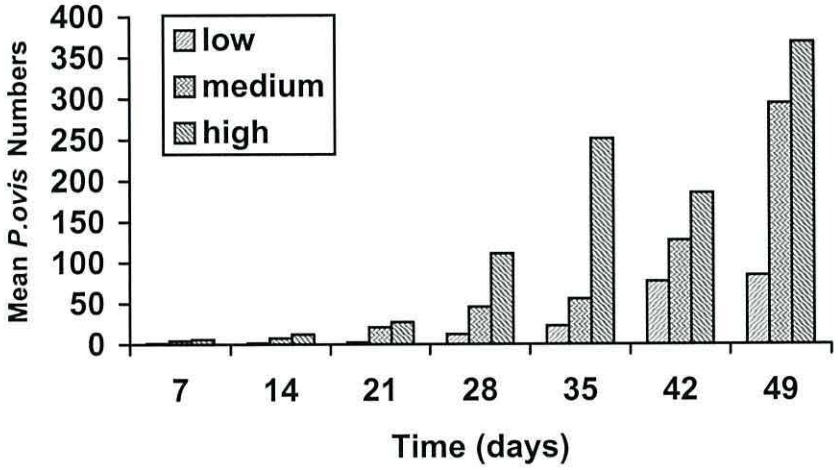

Figure 3.2.4: Numbers of adult female *P.ovis* with time (days) after challenge with 25 adult females from low, medium or high virulence populations *P.ovis*.

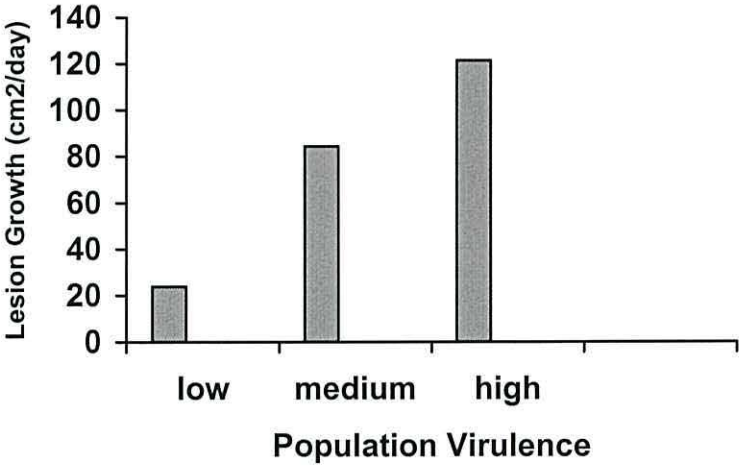

Figure 3.2.5: Rate of lesion growth (cm^2 per day) for low, medium and high populations of *P.ovis*.

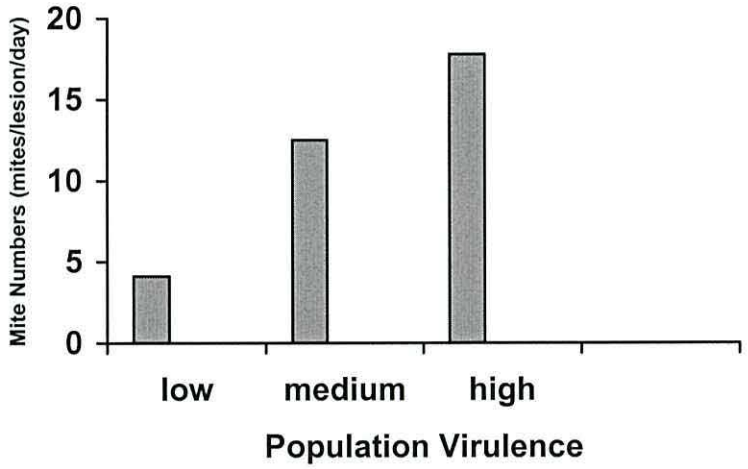

Figure 3.2.6: Rate of mite population increase (per day) for low, medium and high populations of *P.ovis*.

Chapter 3.3

Acquired Resistance in Sheep to the Scab Mite, *Psoroptes ovis*.

1.0. Summary

Little information is available on the effects of re-infesting sheep previously exposed to psoroptic mange (sheep scab). The studies in this Chapter investigated the effects of reinfesting sheep one year after eradication of a primary infestation. In March 1996 three yearling, full fleeced, close wool breed sheep were artificially infested with 25 adult female ("virulent isolate") *P ovis*. Lesion areas and mite burdens were monitored weekly up to six weeks post infestation. The sheep were then treated with ivermectin (s/c) and moved to mite free accommodation, where they received a second s/c injection of ivermectin. Scab was completely cured and five weeks after the last injection the sheep were shorn and introduced to a grass paddock. After 1 year the sheep were reinfested with the same isolate of *P ovis* and the lesion areas and mite burdens monitored as before. Primary lesions progressed normally with a 14 to 21 day sub-clinical period followed by a rapid growth phase, where the mean lesion area after 42 days was 3822.3 cm^2 (79.9% body cover) and the mite burden 240.6 mites per sheep. The progression of disease after the secondary challenge followed a different pattern with extremely low numbers of *P ovis* (range 5 to 16 mites per sheep) 49 days after secondary infestation. The mean lesion area 42 after the second challenge was only 187.6 cm^2 (3.93 % body cover). The scab lesions continued to progress up to 56 days post secondary challenge, but the mite burdens continued to remain extremely low.

2.0 Introduction

Little information is available on the effects of re-infesting sheep previously exposed to scab. Knowledge of acquired resistance to reinfestation is required in order to help understand the epidemiology of sheep scab on a flock and national basis, regarding lesion progress on previously infested sheep, compared to scab naive animals. This is especially important concerning the infestivity of *P.ovis* derived from the external auditory canal (as described in Chapters 2.6 and 3.1) to the original host sheep. Knowledge of an innate resistance is equally important in the development of potential immunological control techniques. Spence (1949) observed that treated sheep re-infested after a year were more resistant, where, despite more "exuberant exudation and encrustation", mite populations appeared to increase more slowly. Acquired resistance has been reported in cattle to *P. ovis* (Stromberg *et al.,* 1986; Stromberg and Fisher, 1986; Guillot and Stromberg, 1987 and Stromberg and Guillot, 1987) and in rabbits to the ear mite *P. cuniculi* (Urlir, 1991).

2.0 Materials and Methods

2.1. Sources of Infestation

The *P.ovis* isolate originated from an infested flock at Ollerton, Market Drayton, Shropshire in 1995 (Appendix One) and since maintained on sheep at the VLA, Weybridge. The isolate was designated "highly virulent"in Chapter 3.2.

2.2. Animals

Three scab naive, one year old, full fleeced Suffolk cross sheep (one ewe and two castrated males) were used in the study. The exposure of the sheep to acaricides/insecticides or anthelmintics was identical to that described in Chapter 3.1. The animals received a late May/June treatment of Vetrazine (6.0% cyromazine, Novartis Animal Health) as a pour-on for the prevention of blowfly strike (*Lucilia sericata*). Cyromazine has been demonstrated to have no effect on *P. ovis* (Bates, *unpublished data*). All sheep were certified "healthy" prior to infestation and were shown not to have any concomitant chewing louse (*Bovicola* (*Damalinia*) *ovis*) infestations.

2.3. Method of Infestation

P. ovis mites were collected from donor infested sheep and used to challenge the three shearlings on the withers, according to the methods described in Chapter 3.1. The primary challenge was carried out in March 1996 and the secondary challenge in April 1997.

2.4. Animal Housing and Feeding

For the duration of the primary and secondary challenges the sheep were housed and fed according to Chapter 3.1. The sheep were moved after the initial ivermectin treatment from the scab isolation unit to a biosecure open yard (not having housed infested sheep) where, 7 days after the first treatment, a second injection of ivermectin was administered. The sheep remained in this biosecure accomodation for five weeks. The sheep were shorn in July and introduced to a grass paddock, where they remained until April 1997. The sheep were re-introduced to the scab isolation unit on the 1st of April 1997 and on the 4th of April each sheep was re-infested on the withers as before.

2.5. Acaricidal Treatment

All three sheep were treated on day +42 post the primary challenge (19th of April 1996), with IVOMEC for Sheep™ (1.0% ivermectin, Merial). The mean sheep weight was 39.0 kg (range 37 to 40 kg) and ivermectin was administered according to the manufacturers instructions, with 1.0 ml injected subcutaneously into the upper neck of all sheep to give 200μg per kg body weight. The sheep were then moved from the scab isolation unit to a biosecure open yard (not having housed infested sheep) where, 7 days after the first treatment (26th of April 1996), a second injection of ivermectin was administered.

2.6. Parasitological Assessments

Lesion areas (percent body cover) and mite populations were recorded on days +7, +14, +21, +28, +35, +42 and +49 post challenge (PC), according to the methods described in Chapter 3.1.

3.0. Results

The primary challenge progressed normally on all sheep, with a 14 to 21 day sub-clinical phase, according to the criteria outlined in Chapters 3.1 and 3.2 (Figures 3.3.1 and 3.3.3). The sub-clinical phase was followed by a rapid growth phase, where the mean lesion area reached 79.9% body cover (range 71.1 to 89.8% body cover) and the mite burdens reached a mean of 240.6 mites per sheep (range 126.0 to 466.0 mites per sheep), prior to the first ivermectin injection (Day 42 PC). Seven days after the initial injection of ivermectin no live *P ovis* were observed on two sheep (SL 28 and SL 99) and only 7 adult female mites observed on the third sheep (SL 48). Mites were totally eradicated from all three animals after the second injection.

The ivermectin injection was effective in eradicating *P.ovis*. Prior to open grazing no live mites were observed and the scab lesion had resolved completely and had begun to lift away from the skin (by approximately 12.0 mm) with the growing wool. Making it easy to remove while shearing.

The progression of disease after re-infestation followed a different pattern. The sub-clinical phase was extended with numbers of adult female *P ovis* never exceeded the initial challenge for 49 days post challenge (Figure 3.3.2). Mite numbers increased on all sheep to a mean of 16.0 mites per animal (range 5.0 to 28.0 mites per sheep) on day 49 (Figure 3.3.4). The mean percent body cover of the lesion 42 days after the second challenge was 3.9% (range 1.2 to 7.8%) compared to 79.9% (range 71.1% to 89.8%) for the primary challenge.

The scab lesion continued to progress with a mean percent body cover of 13.1% (range 2.7% to 30.4%) 56 days after the second challenge. After this point the

animals were returned to stock where clinical scab eventually developed, but a very small mite burden was observed on all sheep.

The phases of scab described in Chapter 3.1 appear to be significantly altered during re-infestation. Acquired resistance after a year is therefore manifested by a lesion cover and a mite burden associated with an extended sub-clinical phase. Thus supporting the observations of Spence (1949). Colonies eventually establish and clinical sheep scab was observed on all test sheep.

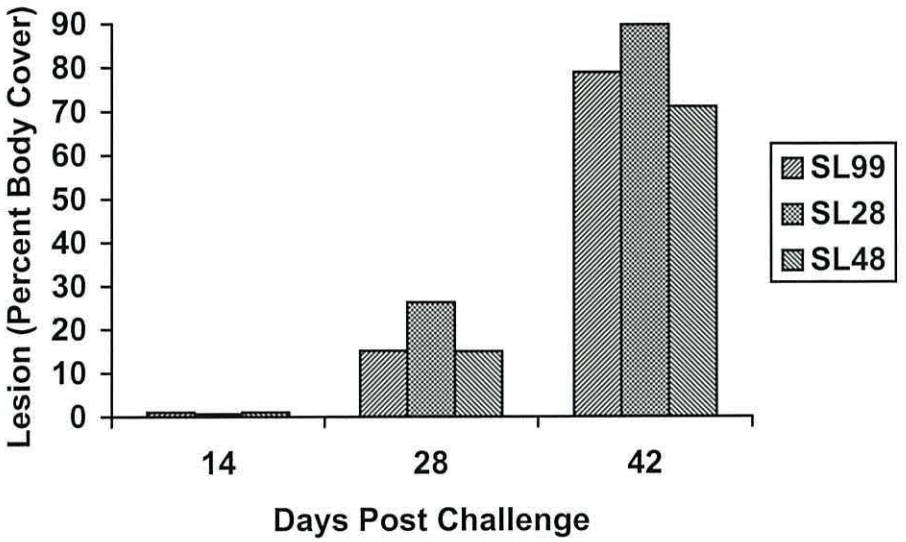

Figure 3.3.1: Area of lesion (% body cover) for sheep SL 28, SL 48 and SL 99, 14, 28 and 42 days after primary challenge with 25 adult female *P ovis* (Market Drayton Isolate).

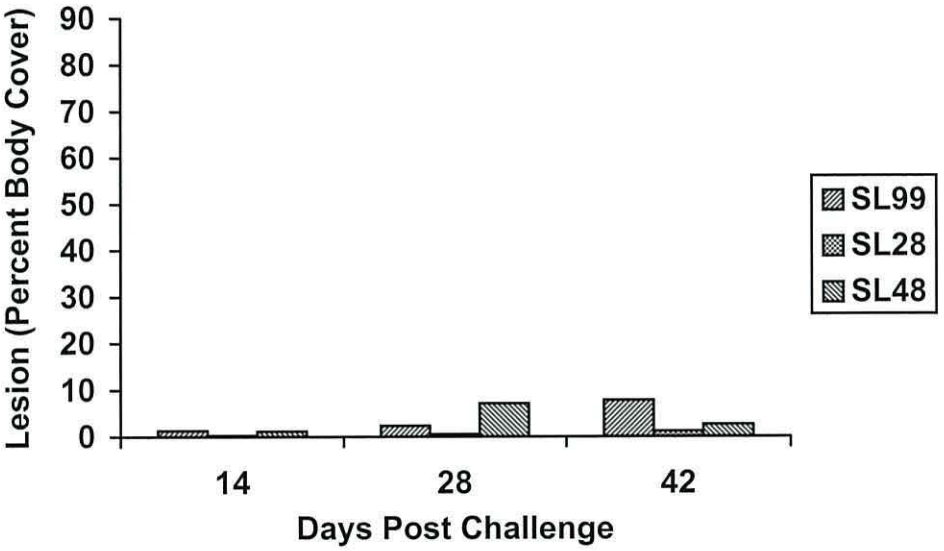

Figure 3.3.2: Area of lesion (% body cover) for sheep SL 28, SL 48 and SL 99, 14, 28 and 42 days after re-infestation with 25 adult female *P ovis* (Market Drayton Isolate), 384 days after ivermectin treatment of primary challenge.

Chapter 3.4

Investigations into Population Variation Within Geographical Isolates of the Sheep Scab Mite (*Psoroptes ovis*

Discussion

This chapter examined the temporal progression of psoroptic mange (regarding the rates of lesion growth and population increase of *P.ovis*), the prevalence and development of otoacariasis (*P.ovis*), the relative variation in pathogenicity between different populations of *P.ovis* originating from different geographical locations in the UK and the changes in clinical disease following re-infestation.

It is now almost certain that sheep scab is caused only partly, if at all, by the direct action of mite feeding. Scab is a form of allergic dermatitis initiated by mite antigens (Stromberg and Fisher, 1986), most probably in the faeces and possibly the cuticle (Bates, 1991a). The heat and humidity produced by the inflammation forms the micro-climate needed for mite survival and the leakage of serous exudate forms the basis of the mites nutrition (Bates, 1996). It is possible that the mite grazes the skin around the moist periphery of the lesion, taking in nutrients with the serous exudate, skin secretions and lipid. Mathieson (1995) confirmed experimentally that *P. ovis* ingests serum components likely to be present in the surface exudate associated with clinical sheep scab.

Variations due to Size of Challenge

It is possible that an outbreak of scab could be initiated by a single ovigerous female (Stockman and Berry, 1913) and thus the growth of the lesion may varying accordingly. Studies at Weybridge have demonstrated that a low challenge of only one attached *P.ovis* couple was insufficient in initiating an infestation and one sheep out of a pair of sheep challenged with four, eight, sixteen or twenty five attached couples also failed to develop infestations (Bates, *unpublished observations*). Data in this chapter describes the temporal phases of *P.ovis* infestation after a standard artificial challenge of 25 adult female *P. ovis*.

Population Virulence

All the geographical isolates of *P.ovis* compared to the VLA Reference isolate in Chapter 3.2. produced a progressive lesion, characteristic of sheep scab, but the extent of the lesion produced with time varied considerably between the isolates.

Considerable differences in lesion area were observed between the geographical isolates of *P. ovis* 28 days post challenge. Some "low virulence" isolates produced relatively small lesions (mean 0.6% body cover (Princetown)) compared to other "high virulence" isolates, producing more extensive lesions (19.4% body cover (Market Drayton)) over the same time period. Considerable differences in mite burdens were also observed. The "low virulence" Princetown isolate presented a mean *P. ovis* burden of 12.8 mites per lesion (range 0.0 to 30.0 mites per lesion) compared to 139.3 mites per lesion (range 65.0 to 220.0 mites per lesion) for the "highly virulent" Market Drayton isolate. In comparison to the rate of lesion increase, a considerable variation in the rate of population increase between low, medium and high virulence isolates of *P. ovis* was observed. Setting a threshold of 25 mites per lesion (equal to or below the original mite challenge) for sub-clinical disease, a similar pattern to that for the relative rates of lesion increase was observed. It can be seen that mite burdens produced by low virulence populations of *P. ovis* can theoretically remain subclinical for long periods (up to 35 days) following infestation. In medium and high virulence populations this sub-clinical period is reduced to 21 or 14 days respectively. The relative rates of population increase for low, medium and highly virulent populations of *P. ovis* over 42 days were 4.1, 12.5 and 17.8 mites per day respectively.

Phases of Infestation

In many host-parasite relationships there is a period of increase, followed by stability, then by decline (Matthyse *et al.,* 1974). In pigs, *Sarcoptes scabiei* populations increase initially following colonisation then decline with the onset of host's immune response (Mellanby, 1944 and Sheahan, 1974, 1975). Mites eventually check their own growth by exhausting their food source and contamination of environment. Five "ecological phases" of ectoparasite infestations have been documented in the progression of ectoparasitic infestations (Marshall, 1981): i) the sub-clinical (colonisation, lag, pre-patent, sensitisation, induction) phase; ii). the instability (late colonisation) phase; iii) increasing maturity (rapid growth) phase; iv) the maturity/climax (peak or plateau) phase and v) the death/decay phase. Most of these phases were visible within the temporal progress of sheep scab as populations of *P. ovis* adapt to the changing ecological conditions. Thus ovine psoroptic mange

(scab) is a dynamic process in a constant state of change. The general appearance and sequalae of infestation was common to all sheep challenged with the *P.ovis* isolates investigated in Chapter 3.0, only the temporal progression and severity of disease varied between sheep and the individual isolates.

The sub-clinical phase is characterised by low mite numbers (in these studies defined as below the original challenge of 25 adult female mites) and small lesions (below 5.0 % body cover). Observations of lesions in the sub-clinical phase were similar to those recorded by Downing (1936) and Spence (1949). Infesting mites feed within hours of sheep contact and within a minute produce an inflamed 'tumour', followed by vesication, rupture and exudation of serum. Infection of the vesicles by pyogenic bacteria causes them to become pustules of a greenish yellow colour, which increase in size before rupture and discharge. Later crusts are formed by the disintigration of the cuticular layers of the skin and the accumulation of serum and purulent matter derived from the ruptured vesicles and pustules. Pruett *et al.,* (1986) postulated that the resulting seepage of serous fluid could enhance the feeding environment of the mite.

No clinical signs were observed until 48 days after challenge, where a noticeable discolouration of the wool was observed over the site of challenge. Vishnyakov (1998) demonstrated that there was also a change in the immune status at the T-lymphocyte sub-population level within 30 to 40 days after artificial infestation. This coincides with the ELISA results demonstrating that circulating antibody was first detected after a mean of 40 days post challenge, when the mean lesion area covered only 1.27% of the body (Bates, *unpublished data*). Fisher (1983) also detected anti *P. ovis* antibody 14 to 21 days post challenge in the sera of infested stanchioned cattle. The sub-clinical phase can therefore last for 14 to 40 days, during which the mite adjusts to the new host and the host responds immunologically to the mite. If the sheep is unable to mount an allergic reponse, the mites will not colonise. If on the other hand the sheep is immunologically responsive (susceptible), an active lesion is produced. Stockman (1910) showed that experimental infestations remain undetectable until between 25 to 30 days post challenge, even with a challenge higher than expected in the field. The time period between contact to manifestation of disease

in the field, however, can be 2 to 8 months. Progression to the rapid growth phase may be slow on sheep of low scab susceptability but considerably shorter on sheep susceptable to scab.

It has long been recognised that all hosts of the same species are not always equally susceptible to colonisation. On one host, permenant ectoparasites may become established, produce a greater number of offspring and generally appear to be better adapted than on another host of the same species. Studies in Chapter 3.1 demonstrated a marked variation in the degree of individual susceptability to colonisation by *P.ovis*. Rafferty and Gray (1987) attributed similar observations on rabbits to individual resistance and possibly to particular strains or populations of mite. This was supported by observations in Chapters 3.1 and 3.2 where a number of sheep challenged with low or medium virulent isolates of *P. ovis* failed contract sheep scab, yet all sheep challenged with the highly virulent populations developed active disease with extensive lesions. The allergic nature of sheep scab could therefore account for the extreme variations observed in individual susceptability. Wright (1982) found that when infesting cattle with *P. cuniculi*, the density of mites within a given area of lesion was dependent on the susceptability of the individual animal to the mites and the size of the lesion was not indicative of mite density. In the current studies a strong positive correlation was recorded between lesion area and mite population for the VLA, Reference Isolate but correlation was not observed for the Cornish Field Isolate. Observations on cattle infested with *P. ovis* demonstrated that as the lesion progresses, specific serum antibody activity appears, when mite populations decline and the lesions resolve, specific serum antibody activity also declines (Fisher and Wilson, 1977). ELISA studies at the VLA (Weybridge) have demonstrated that animals that do not develop circulating IgG titres do not develop active disease. Thus individual susceptability to *P.ovis* may be due to the relative immunocompetence of the host and the "antigenicity" of the colonising population of mite. Roberts and Meleney (1971) observed that sheep could remain entirely free of a "low virulence", strain of *P. ovis* for over a year, yet succumbed within a month to a more 'virulent', strain. In the USA Roberts *et al.,* (1971) recorded that infestations could escape detection for over a year. Long periods of latency and a sudden increase in vigour and

pathogenicity of a mite strain could account for unexplained outbreaks of disease (Roberts and Meleney, 1971).

Chapter 3.2. demonstrated the considerable variation in the rate of lesion increase between low, medium and high virulence populations of *P. ovis*. Setting a threshold of 5.0 % body cover (≈250 cm^2) for sub-clinical disease it can be seen that lesions produced by low virulent populations of *P. ovis* can theoretically remain subclinical for long periods (up to 35 days) following infestation. In the field this may be even longer when the infestive challenge and breed of sheep are taken into account (Bates, 1997). In medium and high virulence populations this sub-clinical period is reduced to only 14 days.

Rapid Growth Phase

A marked increase in lesion growth and mite population increase was observed 56 days after challenge, as the lesions entered the "rapid growth phase", characterised by a rapid increase in mite numbers and lesion spread (together with an increase in circulating IgG (Bates, *unpublished data*)). The sequalae of events observed in Chapter 3.1 supported those of Spence (1949). In the initial stages mites were located (either as a single adult females or in feeding aggregations of 5.0 to over 100.0 mites of all instars) within a narrow zone of inflamed, moist skin (often pale green in appearance) at the periphery of the lesion, which began to spread outwards as the mite population increased. Lesions tended to spread downwards towards the axilla before spreading along the back towards the head or tail (although the "hairy" areas of the body are generally unfavourable to mite colonisation). Body heat dried the serous exudate at the zone of inflammation forming a scab composed of soft, moist crusts, becoming paler, harder and dryer towards the central challenge site. Telmophagic scab mites cannot feed on the hardened scab, and together with the hyperkeratonised skin and build up of toxic faecal material, are forced to continually aggregate around the moist, inflamed periphery of the expanding lesion.

Pruritus caused by histamine release induces the host to lick, scratch or rub (Cormic, 1952). Clinical signs (rubbing and head tossing) become excessive in the rapid growth phase, areas of wool loss may appear, together with open, bleeding

wounds. Sheep rapidly lose condition and epileptiform fitting (severe touch hypersensitivity) may be evident (Bygrave *et al.*, 1993). In these studies the highest THR scores for the VLA Reference Isolate occured 35 days post challenge when the mean lesion percent body cover was 6.1% (only just in the rapid growth phase). Bygrave *et al.*, (1993) postulated that the extent of the THR was relative to the virulence of the infesting mite population. In Chapter 3.2. seizures were not observed in any sheep infested with the low virulence isolates. However, seizures were presented by three out of the seventy five (4.0%) sheep infested with medium virulent and two out of the forty five (4.4%) sheep infested with the high virulence populations. Epileptiform seizures occured 28 to 120 days after challenge for the medium virulence isolates, with corresponding lesion areas of 1.2% to 58.3%, and after 28 days for the high virulence isolates, with corresponding lesion areas of 25.8% to 42.8%.

In advanced cases of ovine psoroptic mange mites may also (rarely) be found re-colonising the skin underneath the dry scabs which have begun to rise with the new wool growth (Downing, 1936 and Spence, 1949). These "flaker" sheep (analogous to 'crusted' or 'Norwegian' *S. scabiei* infestations of pigs and humans) are characterised by extensive wool loss, usually on the flanks and withers, and the denuded areas covered in a pavement of flakey ('cornflake'), scabs, revealing thousands of mites (Figure 3.4.1). Pruritus is nearly always mild, but mites are extremely abundant. This form of scab is extremely contagious, but recipients always develop typical infestations (Bates, *unpublished observations*). Pigs with crusted scabies do not typically scratch or rub (Martineau *et al.*, 1987) and some or all of the regulatory factors controlling mite numbers are inoperable and affected pigs may not react to mite antigen, leading to unrestricted mite population growth (Cargill and Dobson, 1979a, 1979b). In chapter 3.1 only two sheep infested with the St.Brenard isolate presented "flaking" lesions (both 56 days post challenge).

In the later stages of the rapid growth phase mites begin to migrate to the latent (cryptic) sites and the external auditory canal. In Chapter 3.1 *Psoroptes* mites were isolated from the ear canals of 25.9% of sheep infested with the VLA, Reference Isolate and 53.2% of sheep infested with the St.Brenard Isolate and were first recorded

28 and 35 days post challenge, when the leading edge of the lesion was 28.0 or 17.0 cm from the base of the ears, respectively. This was considerably earlier than recorded by Spence (1949), who observed that colonisation of the latent sites occured 45 to 60 days after the onset of the active (rapid growth) phase. Berriatua *et al.*, (1999) suggested that mite transmission was related to the stage (phase) of the lesion and the size of of the adult mite population. Thus mites are more likely to pass to other sheep, either by direct contact or via fomites during the rapid growth phase.

Overall lesions produced by the St.Brenard field isolate grew at a rate greater than those produced by the VLA Reference isolate, achieving 100% body cover on some sheep 28 days post challenge and on all sheep by 63 days post.

Decline

Eventually lesion growth can slow down or stopped completely and the mite population declined rapidly as the encrustation covered the whole body. Like the sub-clinical and rapid growth phases the sequalae of events supported those of Spence (1949). Wool becomes loosened and easily pulled out by the sheep and the general appearance of the lesion changes, the active moist edge becomes indistinct and scaly. Overall mite populations were greater on sheep infested with the St.Brenard isolate, peaking 28 to 70 days post challenge. But populations were also self limiting, reaching extinction, 7 to 28 days after peaking,

This rapid decrease in mite numbers is due to the lack of feeding sites and/or the effects of the circulating antibody. Studies at the VLA (Weybridge) have shown shown circulating IgG increase steadily until treatment, 103 days post challenge, when the mean lesion area covered 89.9% of the body (Bates, *unpublished data*). Watson *et al.*, (1992) demonstrated that immunoglobulin against the Australian blowfly (*Lucilia cuprina*) can be secreted onto the skin in concentrations comparable to circulating levels, and postulated that this may mediate immunological protection. Stromberg and Fisher (1986) investigating *P. ovis* infestations of cattle suggested that mite specific immunoglobulin and leucocytes attack the mid-gut cells of the mite (which also form the peritrophic membranes (PM) of *P ovis* faeces), inhibiting nutrient absorption and ultimately egg production. Immunoglobulins may also bind to

the PM directly, stearically hindering the passage of molecules through it, thereby reducing the efficacy of utilisation of ingested nutriants. Other potential modes of action of immune effectors against the PM include disruption of its structure, interference with its formation and inhibition of the functional molecules associated with it (Eisemann and Binnington, 1994). The gut is a site of digestive proteolysis, so ingested antibodies and other effector molecules or cells may also be broken down before (or while) they act on their target (Eisemann and Binnington, 1994).

During the decline phase mites continue to disperse at random over the whole body and continue to reach the latent sites (Spence, 1949). Aural haemotomae and secondary bacterial infections may also occur.

Cryptic Phase

After the decline phase it is possible for the mite population to die out completely and an animal to make a full natural recovery without treatment. New wool growth begins in previously denuded areas and the scab continues to lift away from the skin as the wool grows. Other sheep however "appear" to recover completely but may still be harbouring small populations of mites, under dry scabs or in the latent sites (Chapter 2.6), waiting to re-infest the sheep once normal skin conditions are restored ("pseudorecovery"). The latter seems unlikely, however, due to the development of an acquired resistance to re-infestation on sheep previously exposed to *P.ovis* infestations, as described in Chapter 3.3.

Antibody titres may remain long after the rapid growth phase, as mite faeces are still bound to the dried scab, and will continue to elicit an immune response as long as the scab is in contact with the skin. Studies at Weybridge have demonstrated that circulating antibody titres decreased post treatment and were undetectable 195 days post challenge (94 days post treatment), when the lesion had lifted away from the skin with the growing wool by 4.0 cm^2 (Bates, *unpublished data*). Thus the exposure to specific *P. ovis* antigen does not stop with the end of parasite activity, *Psoroptes* antigens can still be bound within the matrix of the lesion.

All the data in these studies describe the phases of scab based upon one artificial challenge on the withers of each sheep. In the field a sheep may have several challenges with several discrete lesions or in the later stages many lesions coelescing. Certain areas of the sheep are unfavourable for initial colonisation by *P. ovis* (ie. the face, head, tailhead and belly) although these sites will be colonised as the lesion spreads. Areas favouring mite colonisation are the withers, flanks and brisket (Bates, *unpublished data*). The largest lesion may not therefore necessarily be the primary lesion.

Population Stability

The differences between the years for VLA Reference isolate were not significant ($p=0.069$ for mite numbers and $p = 0.060$ for lesion areas). In contrast there were highly significant ($p < 0.001$) differences among the other isolates for both mite numbers and lesion areas. In contrast to the VLA Reference isolate (laboratory attenuated for 13.2 to 22.9 years), highly significant differences in lesion area and mite numbers were observed between study groups infested with the St.Brenard field isolate, laboratory passaged for only 0.6 to 6.6 years. Hybrid vigour and/or population variation as a result host variation has therefore been lost in the VLA, Reference Isolate over the 22 years of artificial laboratory passage.

Acaricides

In South America the sheep scab mite developed resistance to plunge dips containing the organochlorine, lindane (HCH/BHC) in 1962 (Ault *et al.,* 1962) and the organophosphate, diazinon in 1970 (Rosa *et al.*, 1970). In Great Britain the mite has recently developed resistance to the synthetic pyrethroid, flumethrin (Synge *et al.*, 1995). Four resistant isolates have now been identified, including the Alston, Caithness I and Porlock isolates (investigated in Chapter 3.2) and one other isolate from Cumbria not investigated in this Chapter (Bates, 1998). All these isolates were extremely virulent with corresponding high mite populations, thus increasing the chances of selecting for resistant strains. Although the inappropriate use of a synthetic pyrethroid pour-on, not licensed for scab control, has been blamed for initiating resistance, the incidence of otoacariasis was recorded to be high in all isolates (Bates, 1998). Could the mites have been exposed to sub-lethal concentrations of acaricide whilst residing in the ear canal? In 1996 the first case of organophosphate (propetamphos) resistance was reported in the UK (Clark *et al.,* 1996). Further research has shown this to be increased tolerance to propetamphos and not true acaricide resistance (Bates, 1998).

The avermectin endectocide, ivermectin (22, 23 - dihydroavermectin B_1) has been shown to be effective in controlling *Psoroptes* spp infesting domesticated and non domesticated herbivores (Jackson, 1989). The efficacy of ivermectin has been assessed as either a single or double (7 days apart) subcutaneous injection (at a dose rate of 200 μg per kg sheep body weight) against the sheep scab mite (*P.ovis*) in a large number of countries: France (Euzeby *et al.*, 1983; Autef and Girard, 1987), Germany (von Graunke, 1984), India (Yousif *et al.*, 1989; Sharma *et al.*, 1990), Ireland (O'Brien 1993), Israel, (Yeruham *et al.*, 1982), Saudia Arabia (Wasfi and Hashim, 1986), South Africa (Soll *et al.*, 1992), Turkey (Alabay *et al.*, 1987), USA (Roncalli and Sutherland, 1986) and USSR (Ninkov and Savrin, 1986). All these authors highlight a major concern over the efficacy of a single injection of ivermectin. Efficacy may be relative to the climate, breed of sheep and strain of the infesting *P. ovis* in the relative countries of investigation.

Single injections of ivermectin at a dose rate of 200 μg kg^{-1} failed to eradicate artificial infestations of the VLA, Reference isolate of *P. ovis* (Bates and Groves, 1991). Mite burdens were reduced by 52% within 24 hours, 90% within 10 days and 96% within 20 days, compared to 87% within 9 days, and 100% control within 20 days for infested cattle treated with the same dose (Wright and Guillot, 1984). Bates and Groves (1991) also demonstrated that the numbers of mites surviving a single subcutaneous injection was related directly ($r = 0.96$) to the mite burden at the time of treatment. The reason for this is more than likely the effects of sub-populations of pharate mites not ingesting lethal concentrations of ivermectin. Potential for this evasive strategy is therefore increased the greater the mite population at the time of treatment. Differences in the efficacy of single injections of ivermectin with respect to mite virulence have also been observed. Chronic populations (Bacup, Llanfach and Princetown) can be almost eradicated after a single injection, but significant numbers of mites can survive within acute strains (Little Melton and St.Brenard) (Bates, 1994). Double injections of ivermectin, however will eradicate all strains of sheep scab mite.

Oral drenching with ivermectin (0.08% ivermectin, Oramec MSD Agvet) produced a 48% drop in mite numbers within 24 hours of treatment, but there was little further decline and no relationship between the initial mite burden and the extent of control was observed (Bates and Groves, 1991). However, infestations appeared more aggressive after exposure to oral ivermectin (Bates, *unpublished data*). The apparent inefficacy of oral ivermectin may have significant effects on the epidemiology of sheep scab in the UK by i.) Suppressing sub-clinical disease, ii.) selecting for resistance to doramectin, ivermectin or moxidectin based injections and iii.) selecting for more aggresive (endectocide resistant) sub-population of *P.ovis.*

The phases of scab appear to be significantly altered during re-infestation. Acquired resistance after a year is manifested by the lesion and mite burden remaining sub-clinical for over 50 days and thus supporting the observations of Spence (1949). Colonies eventually establish and clinical sheep scab was observed, although mite populations remained extremely low. Similar observations have been reported in other *Psoroptes* infestations. Stromberg *et al.*, (1986) and Stromberg and Guillot (1989) reported acquired resistance in Hereford cattle previously exposed to *P ovis*, was

characterised by a less extensive dermatitis and Urlir (1991) recorded that ear lesions in rabbits to *P. cuniculi* were considerably smaller on re-infestation. Comparisons can also be drawn from *S. scabiei* infestations of humans, where infestations begin after 4 weeks for primary infestations (with clinical symptoms mild or absent). On reinfestation more intense symptoms appear after only 24 hours and the mean number of mites is much smaller (Mellanby, 1943; 1944).

In Chapter 3.3. the time span (one year) between the two challenges may have also influenced the results of this study, through i.) the effects of residual acaricide, ii.) changes in the virulence of the *Psoroptes* isolate, iii.) changes in the host skin character and iv.) the increased age of sheep. It is unlikely that ivermectin would still be residual in the sheep after this period of time and, although no control animals were included in the second infestation, the Shropshire isolate of *P ovis* was still classified as "highly virulent" on contemporary stock donor animals. It has been reported that sheep scab can have a significant effect on the quality of processed leather (Pearson, 1996), suggesting significant changes in the character of sheep skin following *Psoroptes* infestation. These skin changes may also affect the feeding of mites following reinfestation. Studies at the VLA, Weybridge have suggested that sheep scab is more severe on hoggs compared to ewes (Bates, *unpublished observations*) the growth of the sheep between infestations may also have an influence on the apparent resistance to reinfestation.

Acquired resistance has a direct effect on the growth of sheep scab lesions, particularly if residual body mites or mites in the regressed or cryptic phase or colonising the external auditory canal are to re-infest their previously infested host. An immunological memory to *Psoroptes* infestations also indicates the possibility for developing a sheep scab vaccine (Pruett *et al.*, 1998; Stella *et al.*, 1997 and Taylor *et al.*, 1999).

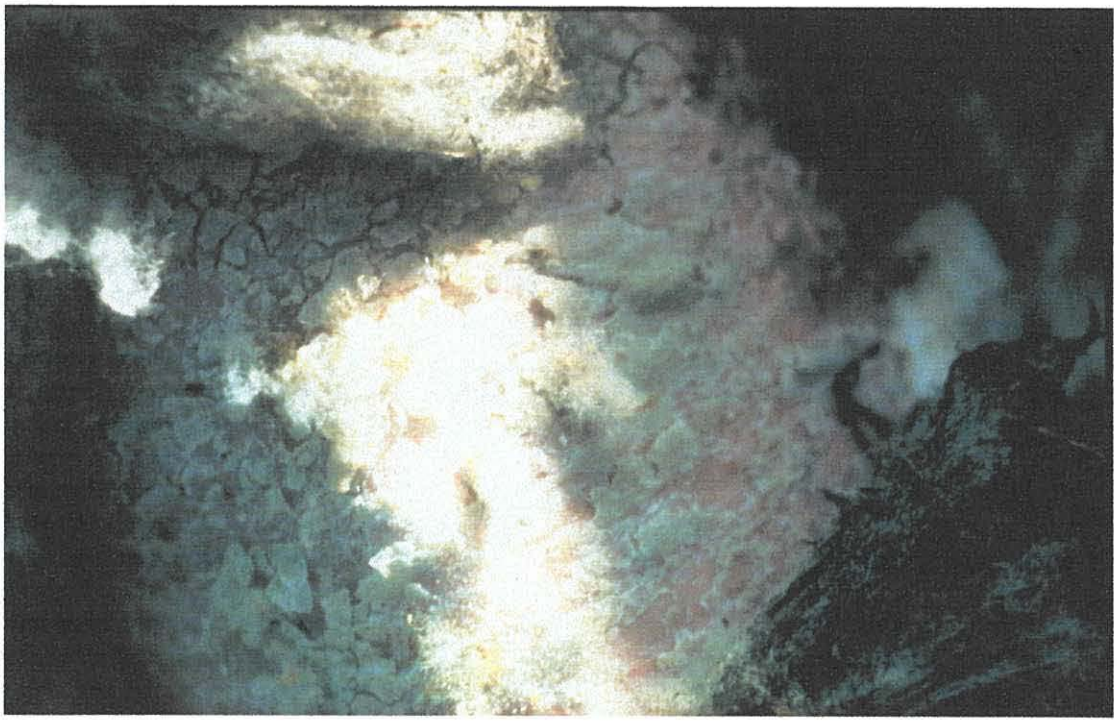

Figure 3.4.1: Lesion on the back of a "flaker" sheep in the later stages of the rapid growth phase.

Chapter 3.5

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Chapter 4.0

Investigations into the Speciation of *Psoroptes* Mites

Introduction

Chapter 2.0 investigated the prevalence and epidemiology of *Psoroptes* spp mites infesting sheep, goats, horses, rabbits and camelid hosts in Britain. Sweatman (1958) divided the genus *Psoroptes* into those that infest the body of the host (ie. producing mange. eg *P. ovis*, *P. equi* and *P. natalensis*) and those that infest the ears of their hosts (ie. producing otoacariasis. eg *P cuniculi* and *P cervinus*). In order to clarify the epidemiology of ear and body *Psoroptes* the nomenclature of Sweatman (1958) was used throughout Chapter 2.0. However, a variation occured in Chapter 2.6 where *Psoroptes* mites isolated from the ear canals of sheep with concommitant active body mange (sheep scab) were defined as *P.ovis*, as they were indisputably derived from the body mange populations. Chapter 3.0 investigated the inter specific variation within the sheep scab mite (*P. ovis*). This chapter investigates the host specificity and speciation of *Psoroptes* mites identified in Chapters 2.0 and 3.0.

Chapter 4.1

Host Specificity of *Psoroptes* spp:

Cross Transmission of *Psoroptes* Mites Derived from Domestic Livestock in Great Britain

1.0. Summary

Ten isolates of *Psoroptes* spp. originating from livestock hosts in Great Britain, including the ears of sheep, goats, alpaca and rabbits, together with rabbit "extra auricular" mites and sheep ear mites cultured in rabbits (all *P. cuniculi*) and sheep body (scab) mites, sheep body mites derived from the ear canals of scab infested sheep and sheep body mites cultured on restrained calves (all *P. ovis*), together with a field isolate of bovine psoroptic mange (*P.natalensis*?) all made available from studies in Chapters 2.0 and 3.0 for host specificity studies. A total of 120 cross transfer combinations were possible between these isolates and the bodies and ears of camelids, cattle, goats, rabbits, horses and sheep. Unfortunately due to Home Office regulations and resource availability only 28/120 (23.3%) of these cross transfers were able to be investigated in this study.

Sheep scab mites (*P.ovis*) taken from the active phase of infestation on donor sheep were infestive to the bodies of susceptable sheep (as described in Chapter 3.0), but were not infestive to the ears of sheep. Mites taken from the cryptic sites (eg. inguinum) while infestations were in the decline and cryptic phases of disease were not infestive to the bodies or ears of sheep. No animal presented mites in the external auditory canals. Sheep scab mites (*P.ovis*) removed from the ear canals of sheep in the decline/cryptic phases of sheep scab (Chapter 2.6) produced clinical scab, but did not produce otoacariasis if challenged directly into the ears.

Mites reach the cryptic sites as the lesion progresses (Chapter 3.1), mites adapting to the changing host environment. Mites removed from lesions in the active phase of disease may not have been pre-adapted to survive in the cryptic sites.

Ovine *P.cuniculi* (Chapters 2.1 and 2.2) failed to establish on the bodies of sheep, but on one occasion migrated from the site of challenge (the withers) to the ear canals, without initiating any allergic response on the skin. Ovine *P.cuniculi* cultured in rabbits were also non infestive to the ears and bodies of sheep.

The sheep scab mite (*P.ovis*) was not infestive to the bodies or ears of goats and goat ear mites (*P.cuniculi*) were not infestive to the bodies or ears of sheep. Mites derived from the ears of an alpaca (*P.cuniculi*?) failed to establish on the body or in the ears of sheep.

Ovine *P.cuniculi* established in the ears of rabbits, but the progress of disease was extremely slow, taking over a year to attain lesion score 4.0 (Guillot and Wright, 1981), compared to 88 days for *P.cuniculi* derived from rabbit ears. Subsequent passages were more rapid. Rabbit extra auricular *P.cuniculi* was infestive to the ears of rabbits, taking 109 days to attain a similar lesion score of 4.0. The sheep scab mite (*P.ovis*) will readily establish in the ears of rabbits.

Rabbit ear canker mites (*P.cuniculi*) failed to establish on the bodies of sheep but did migrate to the ear canal. However two sheep challenged with extra auricular rabbit *P.cuniculi* developed transient lesions with live mites but failed to establish permanent lesions. A single dead adult female mite was recovered from a heavy wax build up in the left inguinal pouch of another challenged sheep. Rabbit *P.cuniculi* were not infestive to the ears or bodies of goats.

The sheep scab mite (*P.ovis*) was infestive to the bodies of restrained cattle through five passages, during which period the mites were still infestive to sheep. Whereas mites derived from the last field outbreak of bovine psoroptic mange in Great Britain were not infestive to the bodies of sheep.

2.0. Introduction

Psoroptes spp have been isolated from the bodies and ears of a large number of domesticated and non-domesticated mammals. Chapter 2.0 investigated the prevalence of psoroptic mites in the ears of sheep, goats, horses and rabbits and the prevalence of psoroptic body mange on cattle and horses. Having ascertained the prevalence of *Psoroptes* spp infestations in Great Britain, it is necessary to investigate their potential to transfer between hosts.

Ten isolates of *Psoroptes* from camelids, cattle, goats, rabbits and sheep were made available from Chapters 2.0 and 3.0 for a host specificity studies. These isolates comprised of goat ear mites, rabbit ear canker mites, rabbit "extra auricular" mites, sheep ear mites and sheep ear mites cultured on rabbits (all *P. cuniculi*), ear mites from an alpaca and sheep body (scab) mites, sheep body mites derived from the ear canals of scab infested sheep and sheep body mites cultured on restrained calves (all *P. ovis*), together with a field isolate of bovine psoroptic mange. A total of 120 cross transfers combinations are possible between these isolates and the bodies and ears of camelids, cattle, goats, rabbits, horses and sheep. Unfortunately due to Home Office regulations (eg.involving horses), the availability of recipient hosts (eg. camelids), the synchronous availability of mites and recipient hosts and the availability of animal accomodation, only 28/120 (23.3%) of these cross transfers were able to be investigated in this study (Table. 4.1.1).

3.0. Materials and Methods

3.1. Sources of Mites

Sheep Body Mites (P.ovis)

Three isolates of the sheep scab mite (*P.ovis*) were used in these studies: the VLA, Reference Isolate and field isolates originating from St.Brenard (Cornwall) and Little Melton (Norfolk) (Appendix One). The isolates had been maintained by regular artificial passage using full fleeced, close wool breeds of sheep (Dorset Horn, Polled Dorset, Mule, Scottish Halfbred or Suffolk cross) that had never been exposed to insecticides/acaricides/endectocides, with the exception of Veterazine (6.0% cyromazine. Novartis Animal Health) for blowfly (*Lucilia sericata*) control in the summer. *P. ovis* were collected from active lesions, the pinnae, the inguinal fossae and the external auditory canal.

Sheep Ear Mites (P. cuniculi)

Seven isolates of ovine *P.cuniculi* were collected at post mortem from the EACs of naturally infested sheep. Three originating from Duns (Borders Region), Reedham (Norfolk) and Kirriemuir (Angus) (Field cases A, B and F, Chapter 2.1).

Four ovine ear mite isolates were also collected at post mortem from the EACs of lambs examined for psoroptic otacariasis from Slyfield Abattoir, Guildford (Chapter 2.2). Isolate One was collected on the 5th March 1990; Isolate Two was collected on the 31st October 1990 (from lambs originating from St Boswells, Borders Region); Isolate Three collected on the 30th November 1990 and Isolate Four collected on the 26th April 1990 (from lambs originating from Ross on Wye, Hereford and Worcester). Sheep heads may have been stored in a coldstore (+4°C) for up to five days prior to challenge. Mites were therefore incubated at +22.0°C for 30.0 minutes before challenge and examined for mite activity. Only active mites were used for challenge studies.

Goat Ear Mites (P. cuniculi).

Mites were harvested from laminated scab removed from the inner aspects of the pinnae of a goat presenting extra-auricular mange, originating from Corley, Coventry, West Midlands. The infestation involved the internal aspects of the pinna (Score 3+/4+ (Littlejohn, 1968) or severe to extensive (Munro and Munro, 1980).

Rabbit Ear Mites (P. cuniculi) Isolated From "Typical" Ear Canker

Twenty dead domestic rabbits, mainly New Zealand White (NZW), naturally infested with *P. cuniculi* were purchased from commercial rabbit breeders (Chapter 2.5). Lesions ranged from slight infestations at the base of the pinna (Score 2.0 to 3.0, Guillot and Wright (1981)) to severe scabbing over the entire inner pinna (Score 6.0, Guillot and Wright (1981)).

Rabbit Ear Mites (P. cuniculi) Isolated From Extra - Auricular Lesions

Two NZW rabbits (F 15 and F 18), from geographically isolated premises, presenting natural extra-auricular infestations were purchased as above. On the 15th of February 1990 mites were collected from Rabbit F 15 presenting extensive mange of the entire pinnae (Score 6.0, Guillot and Wright (1981)), the base of the ears, the cheeks, dewlap and face. Scab lesions involved both inner pinnae, with laminated lesions, approximately 1.0 cm thick. Extremely large numbers of dark red to black mites were present, both in the ear and under the body lesions. On the 23rd of May 1990 mites were collected from Rabbit F 18, also presenting severe mange involving the inner apects of both pinnae (Score 6.0, Guillot and Wright (1981)), and the base of both ears. Lesions and mites were also present between the digits of both hind feet covering the length of the digits and 1.0 cm over. The animal also suffered from malocclusion of all four incisors.

Camelid Ear Mites:

Mites were collected from the ear canals of an alpaca presenting psoroptic otoacariasis on a farm in Cumbria. Mites were submitted by the Veterinary Investigation Centre, Penrith, on the 6th of May 1999.

3.2 Recipient Animals

Full fleeced Dorset cross or Suffolk cross yearling sheep (ewes or male castrates) were used in the study as described in Chapter 3.1.

Goats consisted of young adult female British Saanens, originating from the National Institute for Research into Dairying (NIRD), Shinfield, Reading. The goats had never been exposed to acaricides/insecticides/endectocides and wormed, when necessary, with levamisol or benzamidazole based anthelmintics. All goats were certified "healthy" prior to challenge and were shown not to have any concomitant louse (*Bovicola* (*Damalinia*) *caprae*) infestations. Previous ear swabbing had demonstrated some experimental goats were naturally infested in the EAC with *P. cuniculi*. In order to eliminate these infestations all goats were treated with ivermectin (1.0 ml IVOMEC Pig Formulation. MSD Agvet) sub/cut in the side of the neck on the 22nd of February 1990. Monthly ear swabbing for four months failed to demonstrate *P. cuniculi* alive or dead.

Rabbits consisted of six month to one year old New Zealand Whites, with no history of psoroptic ear canker nor treatment with acaricides. Rabbits were not ear tagged due to the danger of bacterial infection, raising the ear temperature and thus affecting the *Psoroptes* infestation. Irritation may also induce accidental tearing of the ear at the site of tagging.

Recipient cattle consisted of six to eight week old, castrated Friesian bull calves with no history of ectoparasites and never been exposed to insecticide/acaricide either by spray, pour-on or impregnated ear tags nor been treated with systemic insecticides or endectocides (eprinomectin, ivermectin, doramectin, moxidectin or phosmet) by drench, pour-on, bolus or injection.

3.3 Animal Housing and Feeding

The investigations on sheep, goats and cattle were carried within the confines of the high security compounds at the VLA, Weybridge, designated to accommodate *Psoroptes* mites on sheep and cattle. During the course of the studies sheep and goats were housed in a covered yard and maintained on expanded metal flooring, in pens constructed of solid metal hurdles and breeze block walls, to prevent cross infestation. Throughout the studies sheep and goats were fed a diet of unmedicated concentrates (Torberry feeds, Torberry Farm, Hurst, Petersfield, Hampshire, GU31 5RG), approximately one kilogram per sheep, per day. Fresh mains water was available *ad lib* via floor troughs.

Calves were maintained in individual wooden calf rearing crates within a covered yard. Each rearing crate was fitted with a wooden slatted floor. The animals' heads were restrained in modified stocks to prevent self grooming. Throughout the investigations the calves were fed a diet comprising a proprietary unmedicated concentrate ration and rearing pellets. Feed and fresh water were available twice daily via buckets attached to each pen.

During the course of the study rabbits were housed in 70 cm (deep) x 75 cm (wide) x 50cm (high) metal cages (NKP Cages Ltd, 1 Bilton Road, Erith, Kent, DA8 2AN), within a constant temperature (22.0 °C) room at the VLA, Weybridge. Rabbits were fed a diet of unmedicated concentrates and fresh mains water *ad lib.*

3.4. Mite Harvesting

Sheep Body Mites:

Adult female *Psoroptes* mites were collected from the lesion periphery of donor sheep infested with the VLA, Reference, St.Brenard or Little Melton Isolates, using a mounted needle and gently placed into a (30 x 8 mm) Durham tube, the tube then sealed with non-absorbent cotton wool.

Sheep Ear Mites

The contents of external auditory canals (EACs) were removed and examined for *Psoroptes* mites according to the methods described in Chapter 2.2. Individual live mites were removed using a mounted needle and placed in glass Durham tubes, stoppered with non absorbent cotton wool.

Rabbit and Goat Ear Mites

Scab material and mites were removed from the infested ears by blunt ended forceps and a spatula and placed into honey jars. Honey jars were left overnight (approximately 19 hours) at 22.0°C (± 5.0 °C) and mites were harvested into 30 mm by 8 mm glass Durham Tubes the following morning, using an insecticide free camel hair brush. Durham Tubes were then stoppered with non absorbent cotton wool. In cases with extra-auricular lesions mites were taken separately from inside the ears, from the body and from interdigital lesions. Mites were harvested the following day and used immediately for sheep challenges.

Cattle Mites

Skin scrapings were taken from the lesions of infested cattle and contained in 4 inch diameter glass petri dishes. The petri dishes were stacked, with the uppermost lid weighted, above a glycerine mite trap and incubated at +22°C (± 5.0 °C). Active mites were collected at regular intervals from the petri dish lids using a short form glass pasteur pipette attached to a vacuum pump. The bore of the pipette was increased by filing off all but 1.0 cm of the pipette tip and the open end attached to the pump was plugged with cotton wool. Once 100 to 500 active mites had been collected the filed end of the pipette was immediately inserted into plastacine to seal the tube and prevent escape.

3.5 Challenge Methods

Body Challenges

Goats and sheep were infested with approximately 50 to 100 live adult female mites, removed from the Durham tube using a mounted needle and placed directly onto an area of skin, plucked of wool or hair, on the withers of each of sheep or goat. The area of challenge was marked using a stock marker ring (Agrimark, Pfizer). Restrained calves were challenged with approximately 100 to 500 adult female mites placed directly onto a plucked area of hair on the withers.

Aural Challenges

Sheep, goats and rabbits were infested directly in the ear by placing a "bolus" of 50 to 1000 harvested mites (all instars) directly above the opening of the ear canal and securing with a non absorbent cotton wool plug. Animals were prevented from dislodging the challenge by bandaging the ear and securing the ear with zinc oxide tape. The ears remained bandaged for 24 hours. All sheep were ear swabbed (See Chapter 2.1) prior to challenge.

3.6. Challenges

Sheep Body Mites (P. ovis) to the Bodies of Sheep.

Isolates of the sheep scab mite (*P. ovis*) have been maintained on sheep at the VLA, Weybridge, using the method described above for over 50 years. On two occasions in these studies sheep to sheep transfers were carried out as viability controls for other transfer studies.

On the 3rd of March 1989 four sheep (SC 39, SC 79, SC 247 and SC 410) were challenged on the withers with the VLA Reference isolate of *P. ovis.*

On the 25th of May 1990 two sheep (SD 170 and SD 865) were challenged on the withers with mites derived from the inguinal pouches of sheep SC 563 infested with the St.Brenard isolate of *P. ovis.*

Further studies investigating the transfer of sheep body mites (*P. ovis*) to the bodies of sheep have been well documented in Chapter 3.0.

Sheep Body Mites (P. ovis) to the Ears of Sheep.

Two studies were undertaken, aurally challenging sheep with *P. ovis* derived from the body. Study One with mites derived from an active lesion and Study Two with mites derived from a cryptic site (the inguinal pouches) of a sheep in the regressive phase of sheep scab.

On the 3rd of March 1989 three sheep (SC 311, SC 233 and SC 585) were challenged aurally with the VLA Reference isolate *P. ovis* and on the 25th of May 1990 two sheep (SD 728 and SD 753) were challenged in left ear with mites derived from the inguinal pouches of sheep SC 563 infested with the St.Brenard isolate of *P. ovis.*

Sheep Body Mites (P. ovis) to the Bodies of Goats.

On the 3rd of May 1990 two goats (GD 5 and GD 10) were artificially challenged on the withers with the Little Melton isolate of *P. ovis* derived from sheep. The animals were euthanased on the 22nd of August 1990 and the ear canals post mortemed

Sheep Body Mites (P. ovis) to the Ears of Goats.

On the 3rd of May 1990 two goats (GD 6 and GD 7) were artificially challenged in the left ear with the Little Melton isolate of *P. ovis* derived from sheep. The animals were euthanased on the 22nd of August 1990 and the ear canals post mortemed

Sheep Body Mites (P. ovis) to the Ears of Rabbits.

These investigations were not carried out as part of this study. The author has routinely cultured sheep body mites (*P. ovis*) in the ears of rabbits at the VLA, Weybridge (Kirkwood *pers comm*).

Aural Scab Mites (P. ovis) to the Bodies of Sheep

On three seperate studies sheep were challenged on the withers with *P. ovis* derived from the ear canals of heavily infested sheep.

Study One: Forty three VLA Reference Isolate *P. ovis* (eighteen adult females, one adult male, ten nymphs and fourteen larvae) were isolated from both ear canals of a heavily infested sheep (SB 600) at post mortem. Within three hours of isolation the mites were used to challenge two sheep (SE 367 and SE 385) on the withers.

Study Two: On the 25th of May 1990 sheep SD 845 was challenged on the withers with mites derived from the EAC of sheep SC 563 infested with the St.Brenard isolate of *P. ovis*.

Study Three: On the 30th of October 1991 sheep SB 693 was challenged on the withers with mites derived from the EAC of sheep SX 126 infested with the VLA Reference Isolate of *P. ovis*.

Aural Scab Mites (P. ovis) to the Ears of Sheep

On the 25th of May 1990 one sheep (SD 752) was challenged in the left ear with mites derived from the EAC of sheep SC 563 infested with the St.Brenard isolate of *P. ovis*.

Sheep Ear Mites (P. cuniculi) to the Bodies of Sheep

Three isolates of ovine *P. cuniculi* (Duns, Reedham and Slyfield, Case A and C Chapter 2.1 and Chapter 2.2)) derived from the ear canals of sheep were used to challenge the withers of recipient sheep. The Duns isolate was used to challenge one lowland (Dorset Horn) gimmer hogg. The Reedham Isolate was used to challenge a Suffolk cross gimmer hogg (SD 110). Ear mites collected from the EACs of lambs at Slyfield Abattoir were used to challenge two lowland (Suffolk cross) hoggs (SD 304 and SD 305).

Sheep Ear Mites (P. cuniculi) to the Ears of Rabbits.

Ear mites isolated from sheep at Kirriemuir (case F, Chapter 2.1) were used to challenge Rabbit 17. Mites isolated from Slyfield Abattoir (Chapter 2.2) were used to challenge Rabbit R2 (Isolate One), R1 (Isolate Two), R2 (re-infested with Isolate Three) and R16 (Isolate Four).

Ovine Ear Mites (P cuniculi) Cultured in Rabbits to the Bodies of Sheep.

On two occasions the Kirriemuir isolate of sheep ear mite (*P. cuniculi*) cultured in Rabbit R 17 was used to challenge sheep. On the 19th of May 1992 SF 230 and SD 11 were challenged on the withers 378 days after isolation. On 5th of July 1993 mites were used to challenge sheep SG 548 on the poll, SG597 on the withers and SG592 on the rump 759 days after isolation.

Sheep Ear Mites (P.cuniculi) Cultured on Rabbits to the Ears of Sheep.

On the 5th of July 1993 the Kirriemuir isolate of sheep ear mite (*P.cuniculi*) cultured in Rabbit R 17 was used to challenge the ears of sheep SG 591, 412 days after isolation.

Sheep Ear Mites (P cuniculi) Cultured in Rabbits to the Ears of Rabbits.

The Kirriemuir isolate of sheep ear mite (*P.cuniculi*) cultured in Rabbit R 17 was used to challenge the ears of two further rabbits (R 28 and R 30), 378 days after isolation.

Rabbit Ear Canker Mites (P. cuniculi) to the Bodies of Sheep.

On the 16th of February 1989 six sheep (SC 261, SC 389, SC 405, SC 449, SC 613 and SC 630) were challenged on the withers with *P. cuniculi*, originating from "typical" ear canker lesions in rabbits.

Rabbit Ear Canker Mites (P.cuniculi) to the Ears of Sheep.

On the 16th of February 1989 six full fleeced hoggs were challenged aurally with the "typical ear canker" *P. cuniculi* isolate. Three sheep were challenged in the left ear (SC 231, SC 574 and SC 407) and three sheep challenged in the right ear (SC 212, SC 664 and SC 450).

Rabbit Ear Canker Mites (P. cuniculi) to the Bodies of Goats.

On the 31st of May 1990 two goats (GD 3 and GD 8) were artificially challenged in the left ear with *P. cuniculi* derived from a naturally infestated rabbit. The animals were euthanased on the 22nd of August 1990 and the ear canals post mortemed

Rabbit Ear Canker Mites (P. cuniculi) to the Ears of Goats.

On the 31st of May 1990 two goats (GD 4 and GD 9) were artificially challenged in the left ear with *P. cuniculi* derived from a naturally infested rabbit. The animals were euthanased on the 22nd of August 1990 and the ear canals post mortemed

Rabbit Ear Canker Mites (P. cuniculi) to the Ears of Rabbits

Between the 2nd of February and the 27th of October 1990 six rabbits (R.1, R.2, R.7, R.9, R.10 and R.12) were infested in the right ear with 50 to 100 *P.cuniculi* derived from natural cases of rabbit ear canker.

Rabbit P. cuniculi ("Extra-Auricular") to the Bodies of Sheep

On three occasions sheep were challenged on the withers with *P. cuniculi* derived from rabbits presenting auricular and extra-auricular lesions.

Study One: On the 16th of February 1990 mites were harvested from both the auricular and extra-auricular lesions of Rabbit F.15 and used to challenge sheep SD 487 (auricular) and sheep SD 408 (extra auricular). Each sheep was challenged both in the ears and on the withers.

Study Two: On the 26th of May 1990 mites were harvested from both the auricular and extra-auricular lesions of Rabbit F.18 and used to challenge sheep SD 736 (auricular) and sheep SD 715 (extra-auricular). Both sheep were challenged on the withers only.

Study Three: On the 25th of January 1994 mites were harvested from both the auricular and extra-auricular lesions of an infested rabbit and used to challenge sheep SH 211 (auricular) and sheep SH 555 (extra-auricular). Both sheep were challenged on the withers only.

Rabbit P. cuniculi ("Extra-Auricular") to the Ears of Sheep

On the 16th of February 1990 mites were harvested from Rabbit F.15 and used to challenge two sheep: Sheep SD 487 (auricular) and SD 408 (extra-auricular). Each sheep was challenged both in the ears and on the withers.

Rabbit "Extra-Auricular" Mites (P. cuniculi) to the Backs of Rabbits

One rabbit (Rabbit 11) was challenged on the 25th of May 1990 by placing mites originating from a case of rabbit psoroptic foot mange (Rabbit 18) directly onto the withers.

Rabbit "Extra-Auricular" Mites (P. cuniculi) to the Ears of Rabbits

Two rabbits (R 8 and R 11) were challenged on the 25th May1990 with an "extra-auricular" isolate of *P. cuniculi* originating from Rabbit F. 18. Rabbit 8 was challenged with mites originating from the ear and Rabbit 11 with mites originating from the inter-digital lesions.

Sheep Body Mites (P ovis) Passaged Between Sheep and Cattle.

The possibility that restrained cattle could be infested with the sheep scab mite (*P. ovis*) and that these mites could be passaged through calves and still be infestive to sheep was investigated. On the 27th of January 1987 a five month old friesian bull calf (CB 10) was challenged with > 1000 VLA Reference Isolate *P. ovis* (all instars). After approximately 6 weeks mites were harvested and > 1000 (all instars).used to challenge another Friesian calf (CB 52) and at one month intervals mites were passaged consecutively onto a further three restrained calves (CB 57, CB 89 and CB 110). At each calf challenge scab/acaricide naive sheep were also challenged with >50 mites from the same harvest (CB 10 = sheep ear tag not recorded, CB 52 = sheep 35, CB 57 = Sheep 1036 and CB 89 and CB 110 = sheep ear tag not recorded).

Goat Ear Mites (P. cuniculi) to the Bodies of Sheep

Goat ear mites used to challenge the withers of sheep SD 113.

Goat Ear Mites (P. cuniculi) to the Ears of Sheep

Goat ear mites were used to challenge sheep SD 113 in the ears.

Camelid Ear Mites to the Bodies of Sheep

Alpaca ear mites were used to challenge the withers of sheep SN 2166.

3.7. Clinical Examination

The bodies of sheep, goats and cattle were examined at the site of challenge and within an area radiating 25.0 cm from the site of challenge, for scab lesions and or live mites on days +7, +14, +28 and +56 post challenge (PC). Clinical examination included visual inspection of the inner and outer ear and an auroscopic examination of the external auditory canal (EAC). The broad body surfaces (including the "cryptic sites": the infra-orbital fossae, the inguinal pouches, the crutch and perineum) were examined for live mites and scab lesions and any touch hypersensitivity reflex recorded.

The inner and outer aspects of the challenged ears of sheep, goats and rabbits were examined directly and by auroscopic examination of the external auditory canal (EAC) on days +7, +14, +28 and +56 PC. Otoscopic examination was carried out using a 4.0 mm metal speculum attached to a Heine Microflex W2 opthalmoscope with a slit otoscope head, swivel loupe and 2.5v kryptogen bulb (Arnolds Veterinary Products, Shropshire). Aural lesions were scored according to the method of (Guillot and Wright, 1981), summarised in Table 4.1.2.

3.8. Post Mortem Examination.

All study sheep and goats were euthanased (by captive bolt and exsanguination) 56 days after initial challenge and the ears necropsied and examined for live *Psoroptes* mites according to the methods described in Chapter 2.2. The infra orbital fossae (IOF) of the sheep and the internal aspects of the pinnae of both sheep and goats were also examined for scab lesions.

4.0 Results

Pre - challenge ear swabs for all recipient sheep, goats or rabbit were negative for *Psoroptes* mites.

Sheep Body Mites (P. ovis) to the Bodies of Sheep.

All four sheep (SC 39, SC 79, SC 247 and SC 410) infested on the withers, acting as controls for simultaneous *P. ovis* aural challenges, developed active sheep scab. Fifty six days PC lesion areas had achieved 61.9% body cover, to the base of the neck (SC 39 and SC 247); 26.9% body cover, to the base of the neck (SC 410) and 25.0% body cover, to the mid back (SC 79). Live *Psoroptes* were recovered from the left and right EAC of SC 39. Mites were observed after KOH digestion of material removed from the left EAC of SC410. No mites were observed in the ear canals of the remaining two sheep (SC 79 and SC 247).

The two sheep (SD 170 and SD 865) challenged with the inguinal mites (ex SC 563) failed to develop active sheep scab. Sheep SD 865 presented a 0.5 x 0.5 lesion (no mites) and 2+ THR 14 days PC, but no lesion or mites were observed at subsequent examinations.

Sheep Body Mites (P. ovis) to the Ears of Sheep.

Fourteen days after challenge scarring, wounding and dried blood were observed at the level of the tragus in all sheep and live mites were observed on the lesion of sheep SC 585. Small amounts of scab were observed in the tragi of all sheep 21 days PC, but no mites were observed. No lesions or mites were observed at any further examination up to 56 days PC when the sheep were euthanased. No animal

was infested in either the pinnae or left or right ear canal at post mortem. Sheep SD 728 and SD 753 (aurally challenged with mites derived from the inguinal pouches of SC 563) also failed to develop psoroptic otoacariasis.

Sheep Body Mites (P. ovis) to the Bodies and Ears of Goats.

No goat developed active psoroptic mange anywhere on the body and no goat demonstrated psoroptic otoacariasis at post mortem.

Sheep Body Mites (P. ovis) to the Ears of Rabbits.

These investigations were not carried out as part of this study. The author has been involved in the routine culture in rabbits of *P.ovis* in rabbits at the VLA, Weybridge (Kirkwood *pers comm*).

Aural Sheep Scab Mites (P. ovis) to the Bodies of Sheep

Active sheep scab developed on sheep SE 385 (ex SB 600) with the lesion covering 37.2% of the body 56 days after challenge. The lesion was "flaking" (Chapter 3.0) and was 28.0 cm from the ears and extended to the venter of the sheep. The right pinna, crutch, both inguinal pouches and both EACs contained scab and live mites. Sheep SE 367 was slow to develop disease and had only grown to 3.1% body cover 77 days PC, but live *Psoroptes* were recovered from both ear canals. Sheep SD 845 (ex SC 563) and Sheep SB 693 (ex SX 126) failed to develop active sheep scab up to 56 days PC, although SB 693 demonstrated a strong THR 28 days PC.

Aural Sheep Scab Mites (P. ovis) to the Ears of Sheep

Sheep scab mites (*P. ovis*) derived from the ear canals of a heavily infested sheep (SC 563) failed to establish in the ear canal of sheep SD 752.

Sheep Ear Mites (P. cuniculi) to the Bodies of Sheep

On several occasions the sheep challenged with the Duns isolate (Case A, Chapter 2.1) demonstrated bouts of head shaking and ear irritation, but active sheep scab was not observed over the eight weeks of the study and all the cryptic sites were negative for live mites or lesions. However *Psoroptes* spp mites (two live protonymphs and one dead adult female) were isolated from the left EAC at post-mortem. Sheep (SD 110) infested with the Reedham isolate (Case C, Chapter 2.1) or Slyfield Isolates (SD 304 and SD 305) did not manifest any gross symptoms of psoroptic otacariasis or sheep scab over the 56 days days of the study respectively. All the cryptic sites were negative for live mites or lesions and post mortem examination demonstrated the EAC free of *Psoroptes* mites.

Sheep Ear Mites (P. cuniculi) to the Ears of Rabbits

The Kirriemuir isolate of sheep ear mite established in the ears of the recipient rabbit (Rabbit R 17), but the progress of disease was extremely slow, taking over a year (378 days) to cover half the pinna (lesion score 4.0 Guillot and Wright, 1981). Mites were harvested and used to challenge sheep SF 230 and SD 11.

Live dark red/black adult female mites were recorded in the right ear canal of the Rabbit R.1 challenged with Slyfield Isolate One, nine days after challenge. Sixteen days after challenge a scab lesion was recorded at the top of the canal and live mites were observed *in situ*. Twenty three days after challenge a pale yellow, exudative lesion was present, but no mites were observed. The lesion dried out thereafter. Rabbit R.16 challenged with Slyfield Isolate Three presented a slow progressive lesion, only reaching the top of the canal 399 days after challenge. Slyfield Isolate Two failed to establish in the ear of Rabbit R.2.

Sheep Ear Mites (P cuniculi) Cultured in Rabbits to the Bodies of Sheep

Of the two sheep (SD 11 and SF 230) challenged on the 22nd of May 1992 with mites collected from the first harvest of the Kirriemuir Isolate of sheep ear mites in Rabbit R.17, one sheep (SF 230) developed a 1.0 x 1.0 cm^2 lesion at the site of challenge after 7 days, developing to 2.0 x 2.0 cm^2 after a further 21 days (Day +28

PC). On both occasions the lesion was dry and lifting away from the skin with no mites, alive or dead, observed *in situ*. The lesion had broken up and was difficult to find at future examinations. No lesion was observed at any examination on the remaining sheep SD 11 throughout the period of study. Lesions did not develop on sheep SG 548 (challenged on the poll), SG 597 (withers), SG 592 (Rump) and the cryptic sites and ear canals were all uninfested at post-mortem examination 70 days after challenge.

Sheep Ear Mites (P.cuniculi) Cultured in Rabbits to the Ears of Sheep.

Ear mites isolated from Rabbit R.17 (Kirriemuir Isolate) failed to establish in the ears of sheep SG 591 and the cryptic sites and both ear canals were all uninfested at post mortem 70 days after challenge.

Sheep Ear Mites (P cuniculi) Cultured in Rabbits to the Ears of Rabbits.

Ear canker established in both rabbits challenged with the sheep *P.cuniculi.* Rabbit R 28 taking 279 days to score 4.0 (Guillot and Wright, 1981).

Rabbit Ear Canker Mites (P. cuniculi) to the Bodies of Sheep.

Rabbit ear canker mites failed to establish on all sheep (SC 261, SC 389, SC 405, SC 449, SC 613 and SC 630), seven days post challenge. Aggregations of dead mites were observed at the site of challenge. A number of sheep demonstrated discrete 1.0 cm^2 dry, black lesions at the site of challenge, eventually lifting away from the skin with the growing wool. All future examinations (up to +56 days PC) demonstrated no lesions or mites on any sheep challenged. One animal SC 630 recorded *Psoroptes* mites in the left ear canal after KOH digestion. Two sheep (SC 405 and SC 449) developed aural haematomae.

Rabbit Ear Canker Mites (P. cuniculi) to the Ears of Sheep.

No lesions or mites were observed on any sheep (SC 212, SC 231, SC 407, SC 450, SC 574 and SC 664) challenged in the ears with rabbit ear canker mites (*P. cuniculi*) 56 days PC. One sheep (SC 212) recorded live *Psoroptes* in the righ EAC at

post mortem and another (SC 231) recorded *Psoroptes* mites after KOH digestion of material extracted from the left EAC.

Rabbit Ear Canker Mites (P. cuniculi) to the Ears or Bodies of Goats.

No goat developed active psoroptic mange anywhere on the body and no goat demonstrated psoroptic otoacariasis at post mortem.

Rabbit Ear Canker Mites (P. cuniculi) to the Ears of Rabbits

All rabbits with the exception of Rabbit R.2, developed ear canker. Initially infestations were confined to the ear canal resulting in the eventual occlusion of the canal with scab and purulent material. Mites in this initial phase were pearly white, suggesting that they do not ingest red blood cells. The mean time taken for the infestation to occlude the auditory canal (Score 2.0 (Guillot and Wright, 1981)) was 39.6 days (range 7 to 98 days). Once the infestation spreads out of the ear the mites begin to ingest red blood cells and appear deep red or black. The time taken to reach 3+ (Score 4.0 (Guillot and Wright, 1981)) was 88 days. Considerable variation in the progress of ear canker was observed. Rabbit R.3 occluding the ear canal after 7 days and attaining 2+ (Score 3.0 (Guillot and Wright, 1981)) after 13 days. Rabbit R.9 occluded the ear canal (Score 2.0 (Guillot and Wright, 1981) after 98 days. It was also observed that mites can transfer between ears early in the disease (while the lesion was still confined to canal).

Rabbit P. cuniculi ("Extra-Auricular") to the Bodies of Sheep

Mange failed to establish on any sheep seven days post challenge. Aggregation of dead, black mites were observed on the skin or on the fleece at the site of challenge. The two sheep infested with "extra-auricular" mites derived from the body (SC 408) or the ears (SC 487) of rabbit R 15 presenting extra auricular psoroptic mange failed to develop active sheep scab or psoroptic otoacariasis 56 days PC. A heavy wax build up was noted in the left inguinal pouch of sheep SD 408. KOH digestion of the material revealed a single adult female *Psoroptes*. All other cryptic sites (including the EAC) on both animals were negative for scab or mites.

The two sheep infested with "extra-auricular" mites derived from the feet (SC 715) or the ears (SC 736) of rabbit F 18 presenting extra-auricular (interdigital) psoroptic mange failed to develop active sheep scab or psoroptic otoacariasis 70 days PC. Seven days PC aggregations of dead mites were observed at the site of challenge. All cryptic sites (including the EAC) on both animals were negative for scab or mites.

Of the two sheep (SH 211 and 555) challenged on the withers on 25th of January 1994, sheep SH 555 (challenged with body mites) presented a 1.0 cm^2 lesion with two live, pearly white mites 7 days PC and SH 211 (challenged with ear mites) presented a 0.25 cm^2 lesion with five, live, pearly white mites 7 days PC. No mites or lesions were observed at all subsequent examinations.

No aural scab lesions, haematomae, fibrosis, cauliflower ears or gross clinical symptoms of otoacariasis were observe on any sheep challenged.

Rabbit "Extra-Auricular" Mites (P. cuniculi) to the Bodies of Rabbits

Rabbit R.11 challenged on the withers with mites originating from a case rabbit psoroptic inter-digital mange failed to demonstrate any body lesions, 56 days after challenge.

Rabbit "Extra-Auricular" Mites (P. cuniculi) to the Ears of Rabbits

Both rabbits developed psoroptic ear canker. The mean time taken to occlude the ear canal (score 2.0 (Guillot and Wright, 1981)) being 20 days (range 13 to 27 days) and to attain + (score 2.0 to 3.0 (Guillot and Wright, 1981)) 59 days, ++ (score 3.0 (Guillot and Wright, 1981)) 110 days and +++ (score 4.0 (Guillot and Wright, 1981)) 108.5 days (range 69 to 148 days). Ear mites in extra-auricular cases were black, whereas auricular mites were pale brown or white. The mites from the interdigital lesions were slower to develop.

Sheep Body Mites (P. ovis) Passaged Between Sheep and Cattle.

Five sets of Friesian calves and sheep were challenged consecutively between January and June 1987. All sheep and restrained calves in the study developed active sheep scab or psoroptic mange. Thus the VLA Reference Isolate of *P. ovis* derived

from sheep is capable of infesting restrained calves and the isolate remained infestive to sheep despite five passages on cattle.

Goat Ear Mites (P. cuniculi) to the Ears and Bodies of Sheep.

Mites failed to establish on the body or in the ears of sheep SD 113 and all the cryptic sites (including the ear canals) were negative for *Psoroptes* or scab lesions.

Camelid Ear Mites to the Bodies of Sheep

Mites failed to establish on the body or in the ears of sheep SN 2166 and all the cryptic sites (including the ear canals) were negative for *Psoroptes* or scab lesions.

Table 4.1.1

Cross Transfer Studies Carried out in this Study

	Original Host/Location	*Psoroptes* spp	Recipient Host	Result
1.	Sheep body	*P.ovis*	Sheep body	+
2.	Sheep body	*P.ovis*	Sheep ears	-
3.	Sheep body	*P.ovis*	Goat body	-
4.	Sheep body	*P.ovis*	Goat ear	-
5.	Sheep body	*P.ovis*	Rabbit ear	+
6.	Sheep body	*P.ovis*	Cattle body	+
7.	Sheep ear	*P.ovis*	Sheep body	-
8.	Sheep ear	*P.ovis*	Sheep ear	-
9.	Sheep ear	*P.cuniculi*	Sheep body	-
10.	Sheep ear	*P.cuniculi*	Rabbit ear	+
11	Cattle body	*P.ovis*	Sheep body	+
12.	Sheep ear in rabbits.	*P.cuniculi*	Sheep body	-
13.	Sheep ear in rabbits.	*P.cuniculi*	Sheep ear	-
14.	Sheep ear in rabbits.	*P.cuniculi*	Rabbit ear	+
15.	Rabbit ear	*P.cuniculi*	Sheep body	-
16.	Rabbit ear	*P.cuniculi*	Sheep ears	-
17.	Rabbit ear	*P.cuniculi*	Goat body	-
18.	Rabbit ear	*P.cuniculi*	Goat ears	-
19.	Rabbit ear	*P.cuniculi*	Rabbit ears	+
20.	Rabbit Extra Auricular	*P.cuniculi*	Sheep body	-
21.	Rabbit Extra Auricular	*P.cuniculi*	Sheep ears	-
22.	Rabbit Extra Auricular	*P.cuniculi*	Rabbit body	-
23.	Rabbit Extra Auricular	*P.cuniculi*	Rabbit ears	+
24.	Goat ear	*P.cuniculi*	Sheep body	-
25.	Goat ear	*P.cuniculi*	Sheep ear	-
26.	Alpaca ear	*P.cuniculi?*	Sheep body	-
27.	Cattle body	*P.natalensis?*	Cattle body	+
28.	Cattle body	*P.natalensis?*	Sheep body	-

Table 4.1.2.: The Lesion Scoring Method of Guillot and Wright, (1981).

Lesion Score	Observation
0.0	No scab or mites
0.5	Irritation in canal, no mites seen
1.0	Small numbers of scabs in ear canal. Mites present.
2.0	Outer ear canal filled with scab. Mites present.
3.0	Scabs in outer ear canal and proximal quarter of pinna.
4.0	Half pinna affected. Mites present.
5.0	Three quarters pinna affected. Mites present.
6.0	Entire pinna affected. Mites present.

Chapter 4.2

Investigations into the Potential of Ivermectin to Select Mites Pathogenic to Sheep (*P.ovis*) from Populations of Non-Pathogenic *P.cuniculi*

1.0. Summary

Early work in the development of the systemic endectocide ivermectin, reported complete eradication of *P. cuniculi* from infested rabbits, but incomplete control of *P ovis* infesting the ears of rabbits. In this study four isolates of *P cuniculi* (two "typical ear canker" and two "extra auricular") were cultured in the ears of rabbits. Mites were harvested from the ears and used to challenge scab naive and acaricide free sheep (two sheep per isolate). The rabbits were then treated with ivermectin and the residual mites allowed to resurge. Resurged (ivermectin exposed) mites were harvested and used to challenge sheep as before. Mites from the original harvest (pre ivermectin exposure) did not produce clinical sheep scab. Ivermectin was 100% effective in eradicating the "typical ear canker" isolates but mites resurged in the rabbits infested with the "extra-auricular " isolates and these produced clinical sheep scab on challenging the bodies of sheep. One sheep presenting an extremely large lesion (covering 71.2% of the body) with live mites *in situ*, after only seven days post challenge. Some "extra auricular" populations of *Psoroptes* infesting rabbits may therefore contain sub-populations of *P cuniculi* (non infestive to sheep) and *P ovis* (infestive to sheep) and these populations can be selected for by the endectocide ivermectin. Sheep challenged with ivermectin selected populations of *P.ovis* presented acute and on occasions lethal, infestations.

2.0. Introduction

Wright *et al.,* (1983) observed that the behaviour of *Psoroptes* spp. on an unatural host was not normal, generally extra auricular and could often be fatal. They found that ear canker in rabbits infested with *P. cuniculi* took eight weeks to involve 1/4 to 3/4 of the ear, whereas rabbits infested with bovine *P. ovis* showed similar canker after only 4 weeks. Studies in Chapter 4.1 demonstrated that *P. cuniculi* derived from natural cases of psoroptic ear canker in rabbits failed to establish on the bodies of sheep. Chapter 4.1 also demonstrated that extra auricular rabbit *P.cuniculi* could produce transient lesions on the bodies of sheep. Shilston (1915) observed that adult *P. cuniculi* derived from rabbits could survive and oviposit for at least 17 days on sheep, but the second generation died out without reproducing.

Wright and Riner (1985) demonstrated that single subcutaneous or intramuscular injections of ivermectin (at 200 μg/kg body weight) were inadequate in eliminating either *P. cuniculi* or *P. ovis* from rabbits. Yet single injections at 400 μg/kg body weight ivermectin (either intramuscular or subcutaneous) eliminated *P. cuniculi* from all infested rabbits but eradicated *P. ovis* from only 50% of infested rabbits. The exact reason for this phenomena is still not understood since *P. ovis* infesting rabbits ingest nine times more red blood cells than *P. ovis* on cattle and single subcutaneous injections of ivermectin are 100% effective in eradicating *P. ovis* on cattle.

The objective of this study was to investigate whether populations of rabbit *P. cuniculi* exposed to ivermectin, could infest the bodies of sheep.

3.0. Materials and Methods

3.1.Study Outline.

P. cuniculi were harvested from naturally infested rabbits originating from a commercial colonies and used to challenge a further two or three rabbits. Lesions were allowed to progress to score 3.0 or 4.0 (Guillot and Wright, 1981) and mites were harvested and used to challenge two sheep. After harvesting the rabbits were treated with a dose of ivermectin sub-lethal to *P. cuniculi* and the residual mite populations allowed to resurge back to score 3.0 or 4.0. The ivermectin exposed mite populations were then harvested and used to challenge a further two sheep.

3.2. Rabbit *P. cuniculi* Isolates.

Thirteen domestic New Zealand White (NZW) rabbits artificially infested with five isolates of *P. cuniculi* derived from natural cases of "typical" psoroptic ear canker or "extra auricular" psoroptic mange were received from commercial rabbit colonies.

Isolate One: "Extra-Auricular Mange" (Ears)

P. cuniculi were harvested from a single rabbit (F 18) isolated on the 23rd of May 1990. The rabbit was suffering from extensive psoroptic "extra-auricular mange" involving the inner apects of both pinnae (lesion score 6.0) and the base of both ears. Lesions and mites were also present between the digits of both hind feet. Scab was taken from inside the ears and sub-cultured onto two rabbits (R 7 and R 8).

Isolate Two: "Typical" Ear Canker

P. cuniculi harvested from a single rabbit, naturally infested with "typical" ear canker (Lesion score 6.0 (Guillot and Wright, 1981) isolated on the 27th of April 1990 and sub-cultured onto two rabbits (R 9 and R 12).

Isolate Three: "Extra-Auricular Mange" (Interdigital)

P. cuniculi were harvested from rabbit (F 18) as for Isolate 2. Mites were isolated from the inter-digital lesions and sub-cultured onto two rabbits (R 10 and R 11).

Isolate Four: "Extra-Auricula Mange" (Body)

P. cuniculi harvested from a single rabbit isolated on the 25th of November 1991. The rabbit presented extensive psoroptic mange of the entire pinnae, the base of the ears, the cheeks, dewlap and face. Scab lesions involved both the left and right inner pinnae and lesion were laminated and approximately 1.0 cm thick (lesion score 6.0 (Guillot and Wright, 1981). Extremely large numbers of dark red to black *Psoroptes* mites were present both in the ear and under the body lesions. Scab was taken from these extra-auricular lesions and sub-cultured onto three rabbits (R 19, R 20 and R 21).

Isolate Five: "Typical" Ear Canker

P. cuniculi were harvested from six naturally infested rabbits on the 28th of November 1991. All rabbits presented typical psoroptic ear canker (Lesion scores between 4.0 and 6.0 (Guillot and Wright, 1981)). Mites were sub-cultured onto three rabbits (R 22, R 23 and R 24).

3.3. Harvesting *P. cuniculi.*

Mites were harvested according to the methods described in Chapter 4.1 and used within 24 hours of harvest.

3.4. Mite Challenges.

Suitable recipient rabbits were infested directly in the ear and maintained according to the methods described in Chapter 4.1. The external ear canals (EACs) of all rabbits were auroscopically examined prior to challenge and together with the internal and external aspects of the pinnae were examined for the presence of psoroptic ear canker on days +7, +14, +28 and +56 post challenge (PC).

Suitable recipient sheep were challenged on the withers according to the methods described in Chapter 4.1. Challenged sheep were examined for the presence of viable populations of mites on days +7, +14, +28 and +56 post challenge. Examination included the inner and outer ear and an auroscopic examination of the external auditory canal (EAC). The broad body surfaces (including the "cryptic sites" (the infra-orbital fossae, the inguinal pouches, the crutch and perineum) were examined for live mites. The identification of individual sheep with respect to the *Psoroptes* challenge pre- and post- ivermectin exposure are shown in Table 4.2.2. and Table 4.2.3.

3.5. Biosecurity of Sheep Challenges.

All challenged sheep were maintained in individual pens, consisting of open metal hurdles and wooden slatted floors. Pens may have held infested sheep prior to the test sheep, but were thoroughly cleaned using an industrial high pressure steam cleaner and left without sheep for at least three weeks prior to housing the test sheep.

3.6. Administration of Ivermectin to Rabbits.

After removal of mites and scab material for harvesting, the ears were disinfected with a 5.0% Savlon™ Solution ("Hibitane" 1.5% v/v chlorhexadine gluconate solution BP and "cetavlon" (cetrimide 3.0% w/v) incorporated as 7.5% v/v strong cetrimide solution). Rabbits infested with *P. cuniculi* Isolates One, Two and Three were treated with ivermectin (IVOMEC™ Pig Formulation. 1.0% ivermectin, MSD Agvet) administered as a subcutaneous injection in the "scruff" of the neck at a dose rate of 0.1 ml per rabbit, these animals were not weighed but an estimation of weight was deduced using a number of cull rabbits of similar age and condition. Cull rabbit body weights ranged from 4.25 kg to 5.75 kg, thus giving only an approximate dose of 200 μg per kg. Rabbits infested with *P. cuniculi* Isolates Four and Five were injected with ivermectin in a similar manner, but according to accurate rabbit body weights. Rabbits were weighed using a Salter Balance and the mean weight for the rabbits was 4.2 kg (range 4.0 to 4.5 kg). Individual rabbit weights and ivermectin doses are shown in Table 4.2.1.

4.0. Results

4.1 Pre - Ivermectin Exposure Sheep Challenges

The results of the pre-ivermectin exposure sheep challenges are shown in Table 4.2.2.

Isolate One

Canker failed to establish in the ears of Rabbit R 9 and mites harvested from Rabbit R 12 were reserved for sub-culturing onto Rabbit R 15 and not used to challenge sheep. One hundred and sixteen days after challenge, *P. cuniculi* from Rabbit R 15 failed to establish on sheep SC 135.

Isolate Two

Mites harvested from Rabbit R 8 were reserved for sub-culturing onto Rabbit R 7 and not used to challenge sheep. Sixty one days after challenge *P. cuniculi* harvested from Rabbit R 7 were used to challenge two sheep (SC 331 and SE 145). All *P. cuniculi* challenges from Rabbit R 7 failed to establish on recipient sheep.

Isolate Three

Mites from the original infested rabbit failed to establish on sheep SD 715 but established well in the ears of Rabbit R 11 (not used to challenge sheep). One hundred and fifty three days after infestation mites harvested from Rabbit R 10 failed to establish on sheep SD 715.

Isolate Four

P. cuniculi isolated from the original infestation failed to establish in the ears of Rabbit R 19. One hundred and eighteen days and eighty four days after infestation of Rabbits R 20 and R 21 repectively, mites were harvested and used to challenge sheep SF 541 and SF 362 (Rabbit R 20) and sheep SE 240 and SE 343 (Rabbit R 21). All *P.cuniculi* transferred from Rabbits R 20 and R 21 failed to establish on recipient sheep. Seven days after challenge Sheep SE 240 presented a 1.0 cm^2 crusty lesion, with no mites, at the site of challenge. Over the same time period sheep SE 343 presented 4.0 cm^2 crusty lesion, with one live adult female mite, at the site of challenge. Fifty six days after challenge the lesion on sheep SE 240 had progressed to 4.0 cm^2 but was dry and lifting away from the skin. No live mites were observed. On sheep SE 343 the lesion had progressed to 0.1% body cover (6.0 cm^2) at the site of challenge and was dry and lifting away from the skin. Another lesion, 3.2% body

cover (156.0 cm^2) with live mites was recorded on the right rump. This may be the result of a strain of rabbit *P. cuniculi* capable of infesting sheep but the position of the lesion suggests that it was more than likely the result of natural infestation from the environment.

Isolate Five

P. cuniculi isolated from the original infestation failed to establish in the ears of Rabbits R 22 and R 23. One hundred and sixty six days after infestation of Rabbit R 24 mites were harvested and used to challenge sheep SF 230 and SF 414. Both sheep presented 4.0 cm^2 lesions, without mites, at the site of challenge, but the lesions were undetectable at subsequent examinations.

4.2 Post Ivermectin Exposure Sheep Challenges

The results of the post-ivermectin exposure sheep challenges are shown in Table 4.2.3.

Isolate One

Rabbit 12 was harvested 140 days after the administration of ivermectin and mites used to challenge one sheep (SC 210). Seven days after challenge the sheep presented an extremley large scab lesion for the time period, covering 71.2% of the body, extending across the withers and back, with 150 cm^2 wool loss over the left shoulder. Few live mites were observed around the lesion periphery but live mites were isolated from both EACs. Fourteen days after challenge the animal was extremely irritated as the lesion extended over the entire body, again live mites were isolated from both EACs. Nineteen days after challenge the left EAC revealed one freshly dead nymph and the right EAC one freshly dead adult male. Thirty seven days after challenge the lesion continued to extend, including the legs, both inguinal pouches, the perineum and the head, but the internal aspects of pinnae and the infra-orbital fossae (IOF) remained uninfested. The lesion was grey and flakey and live mites were hard to find. The sheep remained extremely irritated and was euthansed on welfare grounds 42 days after challenge. Post mortem examination of the EAC revealed live *Psoroptes* in both canals. Mites were harvested from Rabbit R 15 101 days after administration of ivermectin and used to infested sheep SF 202. A small

lesion (4.0 cm^2), with no live mites, was recorded at the site of challenge after 28 days but was dry and lifting away from the skin by day 56 post challenge.

Isolate Two

Ear canker and mites failed to resurge in Rabbit R 7. Consequently no sheep were challenged.

Isolate Three

Rabbit 11 was harvested 126 days after administration of ivermectin and mites were used to infest sheep SC 319 and SC 365. Seven days after challenge SC 319 developed a 2.0 cm^2 lesion, with pearly white mites around the lesion. Both EACs were negative for *Psoroptes* prior to and seven days after challenge. Nine days after challenge (19th of December 1991) the lesion had grown to 4.0 cm^2 lesion with the leading edge 32.0 cm from head. Two "slightly pink" adult female mites were observed at the periphery of the lesion. Thirty five days after challenge the lesion was still 4.0 cm^2 but dry and lifting with no mites observed *in situ*. The sheep died 213 days after challenge with the lesion now covering over 15.0% of the body and extending to the crutch. No mites were present in the crutch, either infra orbital fossae or the inner aspects of the pinnae but live mites were observed in both inguinal pouches. Numerous live *Psoroptes* were also present in both ear canals.

Mites had not colonised either the body or the ears of SC 365 up to 35 days after challenge. Sixty seven days after challenge a scab lesion covering 32.8% of the body, with live mites, was recorded across the back, with the leading edge 6.0 cm from head. Ninety one days after challenge the lesion extended from the withers to base of ears with a 600 cm^2 area of wool loss around neck. Few mites were observed around the periphery of the lesion. One hundred and sixty three days after challenge the animal was covered in scab approximately 1.0 cm thick and suffering intense dehydration and euthanased on welfare grounds. No mites were present in both inguinals and few mites were found around the periphery of the lesion.

Isolate Four

Ear canker failed to establish in Rabbit 19 and mites failed to resurge after ivermectin treatment in Rabbit 20, consquently there were no further sheep

challenges. Rabbit 21 was harvested 102 days after the first harvest/treatment and mites were used to infest sheep SF 669 and SF 691. Both sheep failed to establish active sheep scab.

Isolate Five

Ear canker failed to establish in Rabbits 22 and 23 and mites failed to resurge after ivermectin treatment in Rabbit 24, consquently there were no further sheep challenges.

Table 4.2.1

Rabbit *Psoroptes cuniculi* Isolates and Ivermectin Treatment.

Rabbit	*P. cuniculi* Isolate	Type of Mange	Weight (kg)	Dose (ml)
R 7	One	EA (ears)	Estimated 5.0	0.1
R 8	One	EA (ears)	Estimated 5.0	0.1
R 9	Two	Typical	No take	No take
R 12	Two	Typical	Estimated 5.0	0.1
R 10	Three	EA (interdigital)	Estimated 5.0	0.1
R 11	Three	EA (interdigital)	Estimated 5.0	0.1
R 19	Four	EA (body)	No take	No take
R 20	Four	EA (body)	4.0	0.08
R 21	Four	EA (body)	4.1	0.08
R 22	Five	Typical	No take	No take
R 23	Five	Typical	No take	No take
R 24	Five	Typical	4.5	0.09

"No Take" = infestations failed to establish.
EA = Extra-Auricular

Table 4.2.2.

P. cuniculi Challenges to Sheep:
Prior to Administration of Ivermectin to the Donor Rabbit.

P. cuniculi Isolate	Donor Rabbit	Duration of Infestation (days)	Sheep Ear Tag	Clinical Sheep Scab	Recipient Rabbit
One	R 8	Not Recorded	-	No Challenge	R 7
	R 7	61	SC 331	Negative	None
			SE 145	Negative	None
Two	R 9	No take	-	-	-
	R 12	77	-	No challenge	R 15
	R 15	116	SC 135	Negative	None
Three	F.?	-	SD 715	Negative	R 11
	R 10	153	SD 496	Negative	None
	R 11	77	-	No Challenge	R 10
Four	R 19	NoTake	-	-	-
	R 20	118	SF 541	Negative	None
			SF 362	Negative	None
	R 21	84	SE 240	Negative	None
			SE 343	Negative	None
Five	R 22	No Take	-	-	-
	R 23	No Take	-	-	-
	R 24	166	SF 230	Negative	-
			SF 414	Negative	-

"No Challenge" = No sheep challenges carried out.
"No Take" = infestations failed to establish.

Table 4.2.3

P. cuniculi Challenges to Sheep:
Post Treatment of Donor Rabbits with Ivermectin.

P. cuniculi Isolate	Donor Rabbit	Duration of Infestation (days)	Sheep Ear Tag	Clinical Sheep Scab
One	R 7	No Resurgence	-	-
Two	R 12	109	SC 210	**Positive**
	R 15	101	SF 202	Negative
Three	R 10	Rabbit Euthanased	-	-
	R 11	126	SC 319	**Positive**
			SC 365	**Positive**
Four	R 19	Failed to take	-	-
	R 20	No Resurgence	-	-
	R 21	102	SF 669	Negative
			SF 691	Negative
Five	R 22	No Resurgence	-	-
	R 23	No Resurgence	-	-
	R 24	No Resurgence	-	-

Chapter 4.3.

Investigations into the Comparative Lengths of the Male L_4OOS: Inter- and Intra- Species Variation.

1.0. Summary

The relative lengths of the male L_4 outer opisthosomal seta (L_4OOS) from populations of *Psoroptes* spp. originating from the bodies of barbary sheep, cattle, goats, horses and domestic sheep or the ears of goats, rabbits and domestic sheep were compared, regarding their value as a taxonomic marker or as an indicatior of population virulence.

There was a considerable overlap in the comparative lengths of the male L_4 L_4OOS between populations of *P. ovis* and *P. cuniculi*, so much so that they could not be differentiated by this morphological criteria. Mites isolated from the ear canals of goats and sheep possessed the shortest setae (mean lengths 50.0 μm to 74.0 μm) and were predominantly "pure" *P.cuniculi* (ie. setal lengths below 74.0 μm) but mites isolated from the ears of rabbits varied considerably, and fell into two groups: short setal populations (mean lengths 54.8 μm to 79.2 μm) with corresponding medium to high frequencies of "pure" *P.cuniculi* (41.9 to 100%) and non-infestive to the bodies of sheep and long setal populations (mean lengths 98.5 μm to 100.3 μm). Long setal length populations tended to originate from extra-auricular lesions with the frequency of "pure" *P.cuniculi* relatively low (7.6 to 29.4%) and were not shown to be infestive to sheep until selected for by ivermectin (Chapter 4.2). A narrow band of setal lengths (79.5 μm to 88.7 μm), mainly from populations of bovine and ovine *P.ovis* divided the two variants of rabbit *P.cuniculi*.

Male L_4OOS were measured within 16 populations of *P.ovis* (originating from eleven geographically and temporally distinct isolates), producing active ovine psoroptic mange. The mean length of the male L_4OOS for *P.ovis* ranged between 66.8 and 100.9 μm. There appeared to be a relationship between the length of the male L_4OOS and population virulence.The mean setal length for the Witney and Penderyn isolates (S.2 and S.14), designated "low virulence" in Chapter 3.2, was 73.6 μm. Whereas the mean setal lengths for the "medium" (VLA Reference Isolate 1988 and 1995) and "high virulence" (Alston, Arlington, Little Melton and Market Drayton) populations of *P.ovis* were 80.2 and 90.5 μm, respectively. These differences were highly significant ($p = < 0.0001$). The percent "pure" *P.cuniculi* in these low virulence

isolates were also relatively high (42.0%) compared to the medium virulence VLA Reference population (31.0%) and the high virulence isolates (6.0%). Differences in the mean lengths of the male setae of mites recovered from the cryptic sites (inguinal pouches) were also significantly different from body mites.

Differences between the mean setal lengths of the VLA Reference Isolate of ovine *P.ovis* cultured on sheep or cattle (80.0 and 83.8 μm, respectively) were significant. The frequency of "pure" *P.cuniculi* was relatively low for all bovine isolates ranging between 7.1% and 29.4. Differences between the mean setal lengths for male *Psoroptes* from the Borders population were highly significant. compared to all the bovine *P.ovis* populations examined, with 84.0% of the population measuring above 258.0 μm, thus fitting the morphological criteria for *P.natalensis* and not *P.ovis* (Sweatman, 1958).

The equine population of *Psoroptes* presented long setae (mean 193.6 μm), significantly different from all the *P.ovis* and *P.cuniculi* populations examined. Setal length may not therefore be a marker to indicate the potential of a particular *Psoroptes* population to establish on the bodies of sheep.

Differences were highly significant between the mean setal lengths of male *Psoroptes* collected from the body and the ears of goats and also between goat body mites and other *Psoroptes* populations. Similarly the populations collected from the Barbary Sheep were highly significantly different from all other *Psoroptes* populations compared. Setal lengths from both the goat body and Barbary Sheep populations were predominantly (93.2 and 95.0%, respectively) within the overlap of *P.cuniculi/P.ovis*.

2.0. Introduction

Hirst (1922) examined large numbers of *Psoroptes* from various domestic hosts and, although he was the first to use the length of the male L_4 outer opisthsomal seta (L_4OOS) to differentiate species, he found little structural differences between them. He suggested that, with the exception of *P. natalensis* which are morphologically different (the L_3 and L_4 outer opisthsomal setae (L_3OOS and L_4OOS) of the male being distinctly flattened and blade like), the remaining mites in the genus should be regarded merely as races or slight varieties of a single species (*P. communis*). This hypothesis was ignored in later years and a plethora of new *Psoroptes* species appeared based upon dubious taxonomic values.

Sweatman (1958a) compared the lengths of the male L_4OOS and together with recording the host and location of the mite on the host, reduced the number of these specific names of *Psoroptes* to only five. Species were further divided into those infesting the body and those infesting the ears of their hosts (Tables 4.3.1 and 4.3.2.).

The use of the male L_4OOS as a taxonomic marker has been heavily criticised in recent years. There is considerable overlap and variation within the comparative setal lengths of *P. ovis* and *P. cuniculi*, so much so that they cannot be differentiated by morphological criteria. Wright *et al.,* (1983) suggested that this overlap was too wide, and the method was inadequate as a criterion for speciation. Furthermore Wright *et al.,* (1984) demonstrated that the length of the L_4 OOS varied with the isolate of *Psoroptes*. Boyce *et al.,* (1990) attempted to provide information on the phylogenic relationships between *Psoroptes* mites found on different hosts using discriminant analysis. They employed nine morphological characters and found that the length of the male L_4 OOS and the lateral margins of the opisthosomal knobs of the male were the two most important characters for grouping mites according to host species. Mites could clearly be separated within allopatric populations (populations within a species, which do not occur together but have mutually exclusive distributions), however differences were not detected between mites collected from sympatric populations of infested hosts, suggesting that these mites are not host specific and represent a single interbreeding population. Differences also were not

detected among mites collected from the ears and body of bighorn sheep and rabbits, demonstrating that the location of mites on a given host should not be used as a primary criterion in species identification. Morphometric classification is therefore influenced by spatial relationships between hosts (allopatry versus sympatry)

In this Chapter the relative lengths of the male L_4 outer opisthosomal seta (L_4OOS) from populations of *Psoroptes* spp. originating from the bodies of barbary sheep, cattle, goats, horses and domestic sheep or the ears of goats, rabbits and domestic sheep were compared, both as a taxonomic marker or as an indicatior of population virulence.

Table 4.3.1.

The Classification of the Genus *Psoroptes* (Sweatman, 1958).

a). Body Mites

Psoroptes species.	Synonym(s)	Male L_4OOS (μm)	Host(s)
Psoroptes ovis (Hering 1838).	*P. bovis* (Gerlach)	74.0 to 258.0	Domestic sheep. Bighorn sheep. Cattle. Horses.
Psoroptes equi. (Hering 1838)		L_4OOS = 333.0	Horses. Mules. Donkeys.
Psoroptes natalensis (Hirst 1919)		250.0 to 350.0	Cattle. Zebu cattle. Horses.

Table 4.3.2.

The Classification of the Genus *Psoroptes* (Sweatman, 1958).

b). Ear Mites

Psoroptes species.	Synonym(s)	Male L_4OOS (μm)	Host(s)
Psoroptes cuniculi (Delafond 1859)	*P. caprae* *P. hippotis*	64.0 to 164.0	Rabbits. Goats. Sheep. Horses.
Psoroptes cervinus (Ward 1915)		145.0 to 354.0	Bighorn sheep. Wapiti.

3.0. Materials and Methods

3.1. Preparation and Sources of *Psoroptes* Populations.

Male *Psoroptes* were derived from four sources:-

1. **Mounted reference material from the VLA (Weybridge) archives:** Mounted mites originated from 1960 onwards and were cleared and mounted in Berleses Fluid (Gum Chloral. BDH Chemicals).

2. **Preserved mites from the VLA (Weybridge) archives:** Mites were preserved in 75% ethanol plus 10% glycerine with material originating from 1960 onwards. Mites were gently removed from the preservative and placed in a 35.0 mm x 10 mm polystyrene petri dish (Lab Tek Division, Mile Laboratories Inc, Naperville, Illnoise, 66540) and scanned using a dissecting microscope with overhead lighting (American Optical x40) for male mites, which were then gently removed using a glass, short form, pasteur pippette under capillary action. Mites were gently deposited onto labelled glass microscope slides and once enough mites were deposited the slide was left at room temperature for at least 30 minutes to allow the alcohol to evaporate off. Two drops of Berleses Fluid, from a short form glass pasteur pipette, were then applied over the mites and a cover slip placed on top, avoiding the formation of air bubbles. Slides were left at room temperature for at least 7 days for the Berleses to set. Once set the coverslip was sealed with nail varnish and left for a further 24 hours before examination.

3. **Deep frozen mites:** Mites were harvested from either artificially infested sheep or directly from case material submitted to the VLA (Weybridge) for confirmation of sheep scab, and deep frozen at -20°C or -70°C. Isolates were allowed to defrost at room temperature for 3.0 hours prior to mounting. Mites were gently removed from the Durham tubes and placed in a polystyrene petri dish and mounted as above.

4. **Mites harvested directly from active lesions:** Mites were harvested from the ears of goats, rabbits and sheep, the extra auricular lesions of rabbits or the bodies of cattle and sheep according to the methods described in Chapter 4.1. Sheep ear

mites were removed from the external auditory canals of sheep, according to the method desribed in Chapter 2.2. Male mites were also mounted directly into Berleses Fluid from sheep, goat or rabbit aural swabs.

5. **Directly from diagnostic material submitted to the VLA (Weybridge):** Skin scrapings or wool samples were submitted to Weybridge for the statutory confirmation of sheep scab. Male mites were collected immediately according to the method described in Chapter 4.1 and the mites mounted directly into Berleses Fluid.

3.2. The *Psoroptes* Populations Examined.

Rabbit (Ear) Psoroptes Populations

Eight populations of rabbit *P.cuniculi* were compared, six from typical aural infestations, one pooled population including extra-auricular mites and one from extra auricular (interdigital) lesions of a rabbit simultaneously presenting aural infestations. Six populations were deep frozen and two were preserved in 75% ethanol plus 10% glycerine. All populations originated from geographically or temporally distinct sources.

Population R.1: Isolated from a natural outbreak of psoroptic ear canker in a commercial rabbit colony. Mites were harvested from 20 infested rabbits with lesion scores ranging between 4.0 and 6.0 (Guillot and Wright, 1981), a number rabbits also presented extra -auricular lesions. Mites were harvested on the 15th February 1989 and deep frozen (-20°C). Mites were also used to challenge sheep in Chapter 4.1 directly after harvesting.

Population R.2: Isolated from a natural outbreak of psoroptic ear canker in a commercial rabbit colony. Mites were harvested on 5th of February 1988 and deep frozen (-20°C).

Population R.3: Designated as *Psoroptes communis* var *cuniculi*. Mites were harvested from a rabbit ear in 1964 and preserved in 75% ethanol plus 10% glycerine.

Population R.4: Isolated from a natural outbreak of psoroptic ear canker in a commercial rabbit colony. Mites were harvested in March 1987 and deep frozen (-20°C).

Population R.5: On the 23rd of May 1990 mites were collected from a rabbit (F18) originating from a commercial colony and presenting severe psoroptic mange involving the inner apects of both pinnae (Score 6.0: Guillot and Wright (1981)), and the base of both ears. Lesions and mites were also present between the digits of both hind feet covering the length of the digits and 1.0 cm over. Population R 5 was harvested from the inter-digital lesions of rabbit F 18 and deep frozen (-20°C). This population was designated Isolate Three in Chapter 4.2. and was shown to be pathogenic to the bodies of sheep after exposure to ivermectin.

Population R.6: was harvested from the ears of rabbit F. 18.) (see Population R.5 above) and deep frozen (-20°C). This isolate was designated Isolate One in Chapter 4.2. and was shown not to be pathogenic to the bodies of sheep after exposure to ivermectin.

Population R 7: Was isolated from a natural outbreak of psoroptic ear canker in a commercial rabbit colony and subcultured onto Rabbit 9. Mites were harvested from rabbit R 9 in March 1987 and deep frozen (-20°C).

Population R 8: Harvested from a rabbit at Weybridge (Strain 11) on the 14th September 1980 and preserved in 75% ethanol and 10% glycerine.

Ovine (Body) Psoroptes Populations

Setal lengths of sixteen populations of sheep *P.ovis,* originating from body lesions in the rapid growth phase or from the cryptic sites during the late rapid growth phase or decline phases of disease, were compared: Twelve populations were deep frozen, two were preserved in 75% ethanol plus 10% glycerine and two populations of

mites were collected directly from diagnostic material. All populations originated from geographically or temporally distinct sources. Detailed information on the isolates can be found in Appendix One.

Population S 1 (VLA Reference Isolate, 1988): Mites originated from stock animals infested with the VLA Reference isolate maintained at Weybridge between 1953 and 1973 and originating from a pre eradication population of *P. ovis* augmented in January 1973 with mites from the first foci of infestation in Lancashire (Worsley and Dunsop Bridge) and since cultured *in vivo* at Weybridge. Mites were harvested and deep frozen at (-20°C) on 29th of November 1988.

Population S 2 (Penderyn, 1989): Originated from a pooled sample of mites isolated from inter-connected field cases in South Wales (Powys, Gwent and Mid Glamorgan) in November 1988. All infested premises were connected via sheep contact at Penderyn Market, Mid Glamorgan. Qualitative observations at Weybridge, on sheep heavily infested with this isolate demonstrated no obvious behavioural or clinical signs of active scab, despite a lesion covering over 80% of the body, extending over the back, flanks, sides and belly. The pooled isolate was cultured *in vivo* at Weybridge and harvested and deep frozen at (-20°C) on 14th of February 1989.

Population S 3 (Compton Dundon, 1989): Mites were mounted directly from diagnostic material submitted from a field case originating from Compton Dundon, Somerset on the 9th of February 1989.

Population S 4 (Calne, 1989): Mites were mounted directly from diagnostic material submitted from a field case originating in Calne, Wiltshire on the 23rd of March 1989. The infested flock consisted of 700 plus Welsh Mountain ewes under welfare investigations.

Population S 5 (VLA Reference Isolate, 1968): Mites originated from stock animals infested with the VLA Reference isolate prior to eradication and the augmentation with the mites from Worsley and Dunsop Bridge in 1973. Mites were preserved in 75% ethanol and 10% glycerine on the 17th of January 1968 and designated as *Psoroptes communis* var *ovis*.

Population S 6 (VLA Reference Isolate, 1958): Mites originated from stock animals infested with the VLA Reference isolate prior to eradication and augmentation with the mites from Worsley and Dunsop Bridge in 1973. Preserved in 75% ethanol and 10% glycerine on the 21st of November 1958. Designated as *Psoroptes communis* var *ovis*.

Population S 7 (Little Melton, 1991): Isolated 27th of March 1990 from diagnostic material submitted from sheep at Little Melton, Norfolk. Original recipient sheep died of a scab induced "epileptiform fit". Mites were harvested directly off the infested sheep and deep frozen at (-20°C).

Population S 8 (St.Brenard, 1991): Isolated on the 13th of August 1989 from diagnostic material submitted from sheep at St Brenard, Cornwall and since maintained at Weybridge. Mites were harvested directly off infested sheep and deep frozen at (-20°C).

Population S.9 (Caithness I): This is one of the original flumethrin (synthetic pyrethroid, SP) resistant isolates described by Synge *et al.,* (1995) isolated from a flock at Wick, Caithness, Scotland in October 1994. Mites were harvested directly off infested sheep and deep frozen at (-20°C).

Population S.10 (Market Drayton, 1995): Mites were isolated from sheep at Market Drayton, Shropshire on the 18th of December 1995. Mites were harvested directly from sheep challenged in Study 41/5 (Chapter 3.2) and deep frozen at (-20°C).

Population S.11 (Alston, 1995): Mites were isolated from an infested flock at Alston, Cumbria in November 1995. Controlled dippings at the VLA, Weybridge confirmed that the isolate was resistant to flumethrin. Mites were harvested directly from sheep challenged in Study 41/5 (Chapter 3.2) and deep frozen at (-20°C).

Population S.12 (VLA, Reference Isolate, 1995): Mites originated from stock animals infested with the VLA Reference isolate after the re-introduction of sheep scab to the UK and augmentation with mites from Worsley and Dunsop Bridge in 1973. Mites were harvested directly from sheep challenged in Study 41/5 (Chapter 3.2) and deep frozen at (-20°C).

Population S.13 (Little Melton, 1995): As for Population S.7. Mites were harvested directly from sheep challenged in Study 41/5 (Chapter 3.2) and deep frozen at (-20°C).

Population S.14 (Witney, 1995): Mites were isolated from infested sheep at Witney, Oxfordshire in July 1995. Mites were harvested directly from sheep challenged in Study 41/6 (Chapter 3.2) and deep frozen at (-20°C).

Population S.15 (Market Drayton, 1996): As for population S.10. Mites were harvested directly from infested sheep and deep frozen at (-20°C).

Population S.16 (Arlington, 1995): Mites were isolated from infested sheep at Arlington, North Devon in August 1995. Mites were harvested directly from sheep challenged in Study 41/5 (Chapter 3.2) and deep frozen at (-20°C).

Bovine (Body) Psoroptes Populations

Setal lengths of four populations of bovine *Psoroptes* originating from body were compared: Two populations were deep frozen and two preserved in 75% ethanol. All populations originated from geographically or temporally distinct sources.

Population SC 1 (VLA, Reference Isolate 1987): The VLA, Reference Isolate of ovine *P.ovis* was cultured on a restrained friesian calf (C 10) and harvested on the 20th of March 1987 and deep frozen (-20°C).

Population C.1 (Borders, 1987): The isolate originated from a field case of bovine psoroptic mange in the Borders of Scotland in 1984 (Linklater and Gillespie, 1984) and maintained on restrained calves at Weybridge. The population measured was harvested from a calf in January 1987 and deep frozen (-20°C).

Population C.2 (Liege, Belgium): Mites were collected from a Belgian Blue cow (No.8878) and submitted in 70% alcohol from infested cattle held at the University of Liege, Belgium (Losson, *pers comm.*).

Population C.3 (Liege, Belgium): Mites were collected from a Belgian Blue cow (No.8807) and submitted in 70% alcohol from infested cattle held at the University of Liege, Belgium (Losson, *pers comm.*).

Equine (Body) Psoroptes Populations

Setal lengths from only one population of equine *Psoroptes,* originating from body lesions were compared. The population (E.1) originated from a draught horse from Luton, Bedfordshire, England in 1946. Male mites (designated as *Psoroptes equi* var *equi*) were measured on mounted slides (prepared on the 2nd of January 1946).

Caprine (Body) Psoroptes Populations

Setal lengths of only one population of caprine *Psoroptes* originating from body lesions were compared. The population (G.1) originated from a goat at the VLA, Weybridge on the 17th of August 1962. Mites (designated as *Psoroptes communis* var *caprae*) were preserved in 75% ethanol plus 10% glycerine.

Miscellaneous (Body) Psoroptes Populations.

Setal lengths of of a population of *Psoroptes* (M.1) isolated from a Barbary sheep (*Ammotragus lervia*) at Flamingo Park Zoo, Manchester in 1974 and preserved in 75% ethanol plus 10% glycerine.

Ovine Psoroptes (Ear) Isolates

Setal lengths of four populations of ovine *Psoroptes* originating from the external ear canals of sheep were compared. All populations were mounted directly from diagnostic material presented in Chapters 2.1 and 2.2 and originated from geographically or temporally distinct sources.

Isolate ES 1 (Slyfield, 1989):): Mites were collected from the external ear canals of a group of lambs passing through Slyfield Abbatoir, Guildford, Surrey.

Isolate ES 2 (Llanelidan, 1989): Mites were collected from the external ear canals of a Suffolk ram at Llanelidan, Clwyd, North Wales (Case B, Chapter 2.1) in December, 1989.

Isolate ES 3 (Reedham, 1990): Mites were collected from the external ear canals of a Suffolk ram at Reedham, Norfolk, South East England (Case C, Chapter 2.1) in June 1990.

Isolate ES 4 (Duns 1989):): Mites were collected from the external ear canals of a Border Leicester ewe at Duns, Berwickshire, Southern Scotland (Case A, Chapter 2.1) in January 1989.

Caprine Psoroptes (Ear) Isolates

Setal lengths of two populations of ovine *Psoroptes* originating from the external ear canals of goats were compared. All populations were mounted directly from diagnostic material presented in Chapter 2.3 and originated from geographically or temporally distinct sources.

Isolate CS 1 (Shinfield, 1989): Mites were isolated from the ear canals of 178 dairy goats at the National Institute for the Research into Dairying (NIRD), Shinfield (Herd A, Chapter 2.3), in October 1989. Mites were mounted directly from aural swabs.

Isolate CS 2 (Ripley, 1991): Mites were isolated from the ear canals of 61 dairy goats at the VLA's Appstree Farm, Ripley, Surrey (pooled from Herds B, C and D, Chapter 2.3) in November 1991. Mites were mounted directly from aural swabs.

3.3. Setal Measurement

Male mites were examined under a compound microscope (x 250 using a Leitz Wetzlar Dialux) and where possible the lengths of both left and right L_4 OOS (Figures 4.4.1 and 4.4.2) were measured, either using an eypiece graticule (Graticules Ltd, Tonbridge, Kent. 100 x 0.01 = 1.0 mm) or a digitising tablet and microcomputer (Imagescan, Hewlett Packard) according to method of Boag (1981).

Inter and intra-specific differences in setal length were analysed statistically using analysis of data (ANOVAR). To compare *Psoroptes* species a linear mixed model with terms for mite species (fixed) and populations within species (random) was fitted to complete data using the REML procedure in the statistics package Genstat 5.

Sweatman (1958) demonstrated considerable overlap in the relative lengths of the male L_4OOS measurements for *P.ovis* and *P. cuniculi* (Tables 4.3.1 and 4.3.2). In these studies the relative frequencies of "pure" *P.cuniculi* (ie. L_4OOS lengths below 74.0 μm), "pure" *P.ovis* (ie. L_4OOS lengths beween 164.0 to 258.0 μm), overlapping

P.cuniculi/P.ovis (75.0 to 163.0 μm) and the *P.equi/P.natalensis* "complex" (ie. L_4OOS lengths above 258.0 μm) occuring within each *Psoroptes* population originating from different hosts and from different host locations were recorded.

4.0. Results

The ranges and mean lengths of the male L_4OOS setae lengths for the *Psoroptes* populations compared, together with the relative frequencies of "pure" *P.cuniculi*, "pure" *P.ovis*, overlapping *P.cuniculi/P.ovis* and *P.equi/P.natalensis* are shown in Table 4.3.3.

The REML procedure in the statistics package Genstat 5 indicated highly significant differences ($p < 0.001$) between *Psoroptes* species originating from the ears and bodies of barbary sheep, cattle, goats, horses, rabbits and sheep and within populations of the same species of *Psoroptes*.

The lengths of the male L_4OOS recorded for the two *Psoroptes* isolates recovered from the external ear canals of goats (mean 50.0 and 64.0 μm) were not significantly different ($p = 0.4034$) to the lengths recorded for the four isolates recovered from the ear canals of sheep (mean 49.1, 55.1, 62.2 and 74.0 μm). Goat ear mite populations consisted of 83.3 to 100% "pure *P.cuniculi*" (ie. setal lengths below 74.0 μm and not overlapping with *P.ovis*). Similarly three of the sheep isolates consisted of 88.8 to 100% "pure *P.cuniculi.*" The percentage of "pure" *P.cuniculi* within the remaining sheep ear population could not be calculated accurately due to the small sample size (n=2). None of these isolates were infestive to the bodies of sheep (Chapter 4.1).

Mites isolated from the ear canals of goats and sheep (*P.cuniculi*) possessed the shortest setae (mean lengths 49.0 μm to 74.0 μm), but setal lengths for *P.cuniculi* isolated from the ears of rabbits varied considerably, and fell into two distinct groups: short setal populations (mean lengths 54.8 μm to 79.2 μm) and long setal length populations (mean lengths 98.5 μm to 100.3 μm). Differences between these two groups were highly significant ($p < 0.0001$).

Short setal populations (Populations R.2, R.3 and R.7) possessed corresponding medium to high frequencies of "pure" *P.cuniculi* (41.9 to 100%) and were not infestive to the bodies of sheep. An exception was population 6 (mean length 79.2 μm), an aural population of *P.cuniculi* from a rabbit simultaneously presenting extra-auricular mange, where the frequency of "pure" *P.cuniculi* was relatively low (29.4%). A narrow band of setal lengths (79.5 μm to 92.6 μm), mainly from populations of bovine and ovine *P.ovis* divided the two groups of rabbit *P.cuniculi*.

The majority of long setal form rabbit *P.cuniculi* were from rabbits presenting extra-auricular lesions. The mean setal lengths for extra auricular Population 5 was 93.8 μm. The population was subsequently cultured in rabbits and not shown to be infestive to sheep until selected for by ivermectin (Chapter 4.2). The mean setal length for Population 1 harvested from a number of rabbits with lesion scores ranging between 3.0 (Guillot and Wright, 1981) and extra-auricular, was 98.5 μm. A pooled sample of this population did not establish on sheep (Chapter 4.1). Although Population 4 was isolated from a rabbit not presenting extra auricular mange, the lesion was still within the confines of the host pinnae, it may have presented extra auricular lesions with time. The infestivity of Population 4 (mean setal length 100.3 μm) to sheep was not tested. All these populations were low in the "pure" *P.cuniculi* sub-population (ranging between 7.6 to 29.4%), with all remaining male setal lengths overlapping (74.0 μm to 168.0 μm) with *P.ovis*.

Male L_4OOS were measured within 22 populations of *P.ovis* (originating from 16 geographically and temporally distinct isolates), producing active ovine psoroptic mange. The mean length of the male L_4OOS for *P.ovis* collected during the rapid growth phase of infestation ranged between 66.8 and 100.9 μm, with the actual setal lengths ranging between 40.0 and 128.0 μm. Seven populations were collected from sheep challenged in Chapter 3.2, comparing the relative virulence of *P.ovis* populations. There appeared to be a relationship between the length of the male L_4OOS and population virulence.

The Penderyn isolate (S.2), presenting low virulence infestations when qualitatively compared to the VLA Reference Isolate, also demonstrated an extremely short setal length (mean 66.8 μm). The mean setal length for the Witney isolate (isolate S.14), designated "low virulence" in Chapter 3.2, was 77.6 μm. The combined mean setal length for these two low virulence population was 73.6 μm. Whereas the mean setal lengths for the "medium" (VLA Reference Isolate 1988 and 1995) and "high virulence" (Alston, Arlington, Little Melton and Market Drayton) populations of *P.ovis* were 82.0 and 90.5 μm, respectively. Differences between setal lengths for the low, medium and high virulence populations were highly significant ($p = <0.0001$). The percent "pure" *P.cuniculi* in these low virulence isolates were also relatively high (34.0% for the Witney population and 50.0% for the Penderyn population) compared to the medium virulence VLA Reference population (11.1%) and the high virulence Alston (0.0%), Arlington (4.0%), Calne (22.9%), Caithness I (20.0%), Little Melton (6.3% to 16.7%) and Market Drayton (6.3 to 25.0%).

Evidence from Chapter 3.1 suggested that by the time mites are established in the cryptic sites the lesion is already in the late active /decline phase of disease. The mean lengths of the male setae recovered from the inguinal pouches from sheep infested with the Little Melton or Penderyn isolates ranged between 69.6 and 88.7 μm. Setal lengths of male *P.ovis* from both inguinal pouches of sheep infested with the Little Melton isolate varied considerably between sheep and between pouches (sheep 328 = 69.6 and 76.0 μm; sheep 914 = 71.0 and 85.6 μm). Setal lengths were also significantly different ($p = 0.0186$) between mites isolated from the body and the inguinal pouches. *P.ovis* taken from the body of sheep 328 infested with the Little Melton isolate was 83.7 μm, yet mites taken from the inguinal pouches of the same sheep presented shorter setae (69.6 and 76.0 μm). The mean setal length for mites taken from body lesions of a sheep infested with the Penderyn isolate, however, were considerably shorter (66.8 μm), compared to mites collected from an inguinal pouch (83.3 μm). The percent "pure" *P.cuniculi* within the inguinal populations ranged between 0.0 and 60.0%

The mean lengths of the male L_4OOS for *P.ovis* taken from the infra orbital fossa and the pinna of sheep 328 infested with the Little Melton isolate were 73.3 μm and 80.0 μm respectively, shorter than mites collected from the active body lesion of the same sheep. The percent "pure" *P.cuniculi* within the inguinal populations ranged between 0.0 and 60.0% and 33.5 and 50.0% for the infra-orbital fossae and the pinna respectively.

Differences between the mean setal lengths of the VLA Reference Isolate of ovine *P.ovis* cultured on sheep or cattle were significant (P = 0.0155), (80.0 and 83.8 μm respectively). Setal lengths of both Belgian bovine populations (85.8 and 107.0), indicated that they too were *P.ovis*, and presumably infestive to sheep. The frequency of "pure" *P.cuniculi* was relatively low for all bovine isolates ranging between 7.1% (C.2) and 29.4 (C.3). Differences in the mean setal lengths for male *Psoroptes* from the Borders population (C.1) were highly significant (p = <0.0001) compared to all the bovine *P.ovis* populations examined (SC.1, C.2 and C.3), with 84.0% of the population measuring above 258.0 μm, fitting the morphological criteria for *P.natalensis* (Sweatman, 1958).

The equine population of *Psoroptes* (E.1) presented long setae, with a mean length of 193.6 μm, significantly different from all the *P.ovis* and *P.cuniculi* populations. Yet within the population 40.0% were overlapping *P.cuniculi/P.ovis*, 40.0% "pure" *P.ovis* and 20.0% above 258.0 μm.

Differences were highly significant (p = < 0.0001) between the mean setal lengths of male *Psoroptes* collected from body lesions (G.1 = 92.6μm) and the ears of goats (GE.1 = 64.0, GE.2 = 50.0) and also between goat body mites (G.1) and other *Psoroptes* populations. Similarly the populations collected from the Barbary Sheep (M.1) were highly significantly different from all other *Psoroptes* populations compared. Setal lengths from both the goat body and Barbary Sheep populations were predominantly (93.2 and 95.0%, respectively) within the overlap of *P.cuniculi/P.ovis*.

Setal length may not therefore be a marker to indicate the potential of a particular *Psoroptes* population to establish on the bodies of sheep: with *Psoroptes*

isolates, with extremely short setae, originating from the ears of sheep and goats (*P.cuniculi*) and isolates with extremely long setae, originating from the bodies of cattle *(P. natalensis)*, non infestive to sheep (Chapter 4.1). *P.ovis* isolates, on the other hand, have setal lengths mid-way between aural *P.cuniculi* and *P.natalensis*, and vary in their virulence as disease agents to sheep (Chapter 3.2).

Table. 4.3.3.

Comparative Lengths of Male L_4 Outer Opisthosomal Seta for Thirty Three *Psoroptes* Population.
Isolated from the bodies or Cryptic sites of Cattle, Goats, Horses, Rabbits and Sheep

Isolate	Location	L_4OOS (n)	Males (n)	Mean	Sd	Range	< 74 µm (%)	75 - 163 µm (%)	>164µm (%)	>258µm (%)
SE.1 Slyfield	Ear	8	8	49.0	?	40-80	?	?	0.0	0.0
GE 2 Ripley	Ear	6	6	50.0	11.9	40-75	83.3	16.6	0.0	0.0
R 7 Rabbit	Ear	7	7	54.8	8.4	36-64	100.0	0.0	0.0	0.0
SE 3. Llanelidan	Ear	9	9	55.1	11.7	40-80	11.2	0.0	0.0	0.0
SE 4. Reedham	Ear	9	9	62.2	9.3	44-76	100.0	0.0	0.0	0.0
GE 1 NIRD	Ear	2	2	64.0	4.0	60-68	100.0	0.0	0.0	0.0
S.2 Penderyn, 1989	Body	14	14	66.8	18.2	40-100	50.0	50.0	0.0	0.0
S 7 Ltl.Melton (328)	Inguinal	5	5	69.6	12.1	60-88	60.0	40.0	0.0	0.0
S 7 Ltl.Melton (914)	Inguinal	16	16	71.0	13.2	40-80	37.5	62.5	0.0	0.0
R 3 Rabbit	Ear	62	62	73.0	12.1	40-96	41.9	58.1	0.0	0.0
SR 1. Kirriemuir	Ear	15	15	72.8	12.9	48-88	33.3	66.7	0.0	0.0
S 7 Ltl.Melton (328)	IOF	3	3	73.3	9.4	60-80	33.3	66.6	0.0	0.0
SE 2 Duns	Ear	2	2	74.0	6.0	68-80	50.0	50.0	0.0	0.0
R 2 Rabbit	Ear	66	66	76.0	20.1	20-128	48.5	51.5	0.0	0.0
S 7 Ltl.Melton (328)	Inguinal	6	6	76.0	13.0	64-100	50.0	50.0	0.0	0.0
S 14.Witney	Body	26	26	77.6	8.8	60-92	34.6	65.4	0.0	0.0

C = cattle, E = horse, M = miscellaneous host species, R = rabbit, S = sheep, GE = goat ear, SC = sheep body mites cutured on cattle, SE = sheep ear, SR = sheep ear mites cultured in rabbit ears, (n) = sheep ear tag number,

Table. 4.3.3 (Continued).

Comparative Lengths of Male L_4 Outer Opisthosomal Seta for Thirty Three *Psoroptes* Populations.
Isolated from the bodies or Cryptic sites of Cattle, Goats, Horses, Rabbits and Sheep

Isolate	Location	L_4OOS (n)	Males (n)	Mean	Sd	Range	< 74 µm (%)	75 - 163 µm (%)	>164µm (%)	>258µm (%)
R 6 Rabbit	Ear	17	15	79.2	13.2	60-104	29.4	76.5	0.0	0.0
S 15. Mkt Drayton '96	Body	8	8	79.5	8.3	64-88	25.0	75.0	0.0	0.0
S 7 Ltl.Melton (328)	Pinna	2	2	80.0	20.0	60-100	50.0	50.0	0.0	0.0
S 7 Ltl Melton (328)	Rump	4	4	80.0	5.6	50-88	25.0	75.0	0.0	0.0
S.1 VLA Ref.1988.	Body	57	57	80.1	20.0	40-128	40.3	59.6	0.0	0.0
S.13 Ltl Melton '96	Body	16	16	81.0	12.9	72-100	6.3	93.7	0.0	0.0
S 7 Ltl.Melton	Body	8	8	82.0	6.6	72-96	16.7	83.3	0.0	0.0
S 7 Ltl.Melton (914)	Face	2	2	82.0	2.0	80-84	0	100	0.0	0.0
S.9. Caithness I	Body	5	5	82.4	8.2	68-92	20.0	80.0	0.0	0.0
S 8 St.Brenard	Body	16	14	82.5	9.5	60-96	18.8	81.3	0.0	0.0
S 2 Penderyn, 1989	Inguinal	12	12	83.3	14.7	60-112	25.0	75.0	0.0	0.0
S 7 Ltl.Melton (328)	Flank	14	14	83.7	7.2	72-96	7.1	92.9	0.0	0.0
SC 1 VLA Ref.	Body	136	136	83.8	13.2	54-120	20.6	79.4	0.0	0.0
S 4 Calne, 1989	Body	35	35	84.3	14.4	60-104	22.9	77.1	0.0	0.0
S 7 Ltl. Melton (914)	Inguinal	5	5	85.6	7.8	80-100	0	100	0.0	0.0
S 10. Mkt Drayton '95	Body	16	16	85.0	8.7	68-104	6.3	93.7	0.0	0.0

C = cattle, E = horse, M = miscellaneous host species, R = rabbit, S = sheep, GE = goat ear, SC = sheep body mites cutured on cattle, SE = sheep ear, SR = sheep ear mites cultured in rabbit ears, (n) = sheep ear tag number,

Table. 4.3.3 (Continued).

Comparative Lengths of Male L_4 Outer Opisthosomal Seta for Thirty Three *Psoroptes* Populations.
Isolated from the bodies or Cryptic sites of Cattle, Goats, Horses, Rabbits and Sheep

Isolate	Location	L_4OOS (n)	Males (n)	Mean	Sd	Range	< 74 µm (%)	75 - 163 µm (%)	>164µm (%)	>258µm (%)
S 3 Somerset, 1989	Body	29	29	85.7	8.3	80-116	3.4	96.5	0.0	0.0
C 3 Cattle (Liege 8807)	Body	17	15	85.8	28.5	48-120	29.4	70.6	0.0	0.0
S 2 Penderyn, 1989	Pizzle	18	18	86.2	16.3	52-120	16.6	83.3	0.0	0.0
S 11. Alston	Body	8	8	88.0	8.0	80-104	0.0	100.0	0.0	0.0
S 8 St.Brenard.	Inguinal	18	18	88.7	11.6	60-108	0	100	0.0	0.0
G 1 VLA 1968	Body	59	59	92.6	15.5	52-140	6.8	93.2	0.0	0.0
R 5 Rabbit	Ectopic	20	20	93.8	18.8	60-120	15.0	85.0	0.0	0.0
S 12. VLA Ref. '95	Body	9	9	95.1	19.0	60-128	11.1	88.9	0.0	0.0
S 5. VLA Ref '68	Body	19	19	98.4	13.9	76-120	0	100	0.0	0.0
R 1 Rabbit	Ear	46	46	98.5	21.0	44-140	10.9	89.1	0.0	0.0
R 4 Rabbit	Ear	66	66	100.3	17.6	60-140	7.6	92.4	0.0	0.0
S 6 VLA Ref '58	Body	37	37	100.4	16.6	68-140	2.7	97.3	0.0	0.0
S.16 Arlington '95	Body	25	25	100.9	14.5	72-128	4.0	96.0	0.0	0.0
C 2 Cattle (Liege 8878)	Body	14	11	107.1	24.7	48-140	7.1	92.9	0.0	0.0
M 1. Barbary 1974	Body	40	40	111.3	19.6	58-164	2.5	95.0	2.5	0.0
E 1 Luton, 1946.	Body	5	5	193.6	45.6	160-280	0	40.0	40.0	20.0
C 1 Borders, 1984	Body	154	154	304.5	57.9	157-584	0.64	1.29	11.0	84.4

C = cattle, E = horse, M = miscellaneous host species, R = rabbit, S = sheep, GE = goat ear, SC = sheep body mites cutured on cattle, SE = sheep ear, SR = sheep ear mites cultured in rabbit ears, (n) = sheep ear tag number,

Chapter 4.4

Investigations into the Speciation of *Psoroptes* Mites

Discussion

Host Specificity

Mites in the Genus *Psoroptes* are not considered to be host specific and readily transfer between hosts (Kemper and Peterson, 1953; Sweatman, 1958; Meleney, 1967). In assessing cross transfer studies it is important to consider five important factors (Marshall, 1981), namely:- i) physical isolation (ie. successful transfers under laboratory conditions may be impossible in the field, due to the physical isolation of the two hosts species. Hence sheep and rabbits may share the same grazing, but they are unlikely to interact); ii) variations in climate or microclimate (eg. a particular bacterial flora may be essential); iii) morphology (eg. structure of the mouthparts); iv) host behaviour (eg. grooming) and v) host physiology. Factors associated with host physiology were listed by Arlian *et al.*, (1987), as the physical properties of the skin (eg hair density), thermal and water properties, innate or acquired immunlogical resistance, nutritional condition of the host, stress, age, physical condition, pathogens, skin thickness, parasite density, parasite competition, hormone levels etc. Nelson *et al.,* (1977) stated that natural resistance may limit ectoparasite numbers, but Guillot and Wright (1981) observed that the level of *Psoroptes* infestations in rabbits was directly related to the degree of grooming and not with season, age or natural resistance. In sheep, there is a distinct lack of grooming behaviour and other factors may therefore be involved in parasite control.

The true host of a parasite is the natural host allowing for indefinitely continued reproduction and an accidental host is the result of chance (Marshall, 1981). Wright *et al.,* (1983) stated that the behaviour of *Psoroptes* spp. on an unatural host was not normal and could often be fatal for the host. Guillot and Wright (1981) demonstrated that it took 9 to 10 weeks for *P. cuniculi* to affect one half to one third of the rabbit pinna (lesion score 4.6 to 4.9). *P. ovis*, on the other hand, took only 4 weeks to affect the entire pinna, base of the ear and extensive involvement of the head and lesions developing on the back and shoulders (extra auricular disease). *P. cuniculi* infestations of rabbits seldom spread from the inner pinna but *P. ovis* infestations can be classified as extremely traumatic.

The results of many *Psoroptes* sp cross transmission studies are equivocal. In South Africa, Shilston (1915) was unsuccessful in transferring sheep scab mites (*P.*

ovis) to the ears of rabbits, yet *P. ovis* has been transferred to rabbits at the VLA, Weybridge, causing severe infestations, with mites passaged back to sheep with no loss of infestivity for three generations (Kirkwood, 1985). Meleney (1967) was also successful in rearing *P. ovis* derived from cattle in the ears of rabbits. Studies by O'Brien (1994) demonstrated that sheep scab mites (*P. ovis*) were not infestive to the ears or bodies of goats. Similar observations were made in Chapter 4.1, contradicting the results of Shilston (1915) who sucessfully transfered *P. communis* var *ovis* to the ears of goats.

Sweatman (1958) postulated that mites found in the ears of sheep can sometimes be found on the bodies of the host, but presented no evidence that body mites could be found in the ears of the host. Chapter 2.6 demonstrated that *P.ovis* could colonise the external ear canals of heavily infested sheep. Chapter 4.1 demonstrated that these mites can be infestive to scab naive sheep, yet fail to establish in the external ear canals (EACs) after artificial transfer. Ovine *P.cuniculi*, isolated from the EAC were not infestive to the bodies of sheep, but could migrate to the ear canal from the site of challenge, without initiating any lesion *en route*. A number of questions still remain to be answered. It is not known if *P.ovis* inhabiting the EAC remain infestive to the body, once they have adapted to their new defined habitat. This being so, how long after colonisation of the EAC do *P.ovis* remain infestive to the body? Will they eventually "evolve" into non-infestive *P.cuniculi* and more importantly can they ever revert back to *P.ovis*, capable of body colonisation?

Limited studies in Chapter 4.1 demonstrated that mites derived from the cryptic sites (eg. inguinal pouches or the internal aspects of the pinna) from sheep in the regressive phase of sheep scab failed to develop active scab and conversley *P. ovis* derived from sheep scab in the active phase were not infestive to the ears following artificial transfer. Mites survived for 14 days, presenting scarring and wounding of the ear base, but the infestations did not become established. Do mites need to pre adapt to these sites? These studies also demonstrated that *P.ovis* derived from the EAC could not colonise the ears, although this information was only based on one sheep transfer.

Sheep ear mites (*P. cuniculi*) can also establish in the ears rabbits, but the progress of disease is extremely slow, taking over a year to cover half the internal aspects of the pinnae (lesion score 4.0, Guillot and Wright, 1981), yet rabbits artificially infested in the ear in a similar manner with the rabbit ear canker mite or extra auricular mites (both also *P. cuniculi*) take only 88 or 108 days, respectively, to attain the same lesion score. Sheep ear mites (*P. cuniculi*) cultured in rabbits readily transfer between rabbits but, like the original sheep isolate, fail to establish on the bodies of sheep and remained uninfestive to sheep for several years (Chapter 4.1). In South Africa, Van der Merwe (1949) successfully infested the ears of goats with sheep ear mites (*P. cuniculi*), this was not repeated in the current study..

The infestivity of *Psoroptes* spp to cattle is also equivocal. Meleney (1967) failed to establish rabbit *P. cuniculi* on the bodies of cattle, but Roberts (1970) and Wright (1982) were successful. Wright (1982) successfully reared rabbit *P. cuniculi* on stanchioned cattle, for at least 20 mite generations, never losing their infestivity to rabbits. Roberts (1970) and Liebisch *et al.,* (1979) successfully transferred *Psoroptes* spp. mites from sheep to cattle, although Zielasko (1979) reported sheep mites survived on cattle for only a short time. Kemper and Peterson (1953) transferred *P. ovis* from sheep to cattle to sheep on a regular basis, but after three years the mite lost its virulence to sheep and totally adapted to cattle. In Chapter 4.1 the Weybridge ovine Reference Isolate of *P. ovis* was capable of infesting restrained calves and remained infestive to sheep despite five passages on cattle. The relative virulence of the mite strain may also be important. Roberts and Meleney (1971) demonstrated that 'aggressive' strains of the sheep scab mite (*P. ovis)* were able to spread through cattle herds more rapidly and produce more obvious clinical disease than less pathogenic strains.

Chapter 4.1 demonstrated that rabbit ear canker mites (*P. cuniculi*) can establish in the ear canals of sheep, corroborating the studies of Sweatman (1958). Chapter 4.1 also demonstrated that rabbit *P. cuniculi* fail to established in the ear canals or the bodies of goats, thus contradicting the studies of Sweatman (1958), who concluded from his observations that the auricular mites of rabbits ≡ auricular mites of goats (ie. *P. equi* var *caprae* and *P. cuniculi* were conspecific). Perucci *et al.,*

(1996) was successful in infesting rabbits with goat ear mites and goats with rabbit ear mites, and along with morphological studies concurred with Sweatman (1958) that goat ear mites and rabbit ear mites were the same species. Sweatman (1958) was also successful in transferring *P. cuniculi* from ears of rabbits to the ears of a donkey, concluding that *P hippotis* and *P. cuniculi* were also conspecific.

Faccini and Costa (1992) recorded that 40% of Brazilian sheep flocks were infested in the ears with *P. cuniculi* mites and observed that all infested flocks shared grazing with goats, postulating that transmission between sheep and goats was possible in the field. Shastri and Deshpond (1983) in India and Yeruham *et al.,* (1984/85) in Isreal both also reported otoacariasis in sheep when herded with goats. Shilston (1915) observed that mites transferred from the ears of goats (*P. communis* var *caprae*) to the ears of sheep were not infestive but Sweatman (1958) successfully infested the ears of sheep (both naturally and artificially) with goat ear mites. Unfortunately investigations in Chapter 4.1 and by subsequent authors have failed to transfer goat ear mites to either the body or ears of sheep either artificially (Meleney, 1967) or through natural exposure to infested goats (Williams and Williams, 1978 and Heath *et al.,* 1989). Shilston (1915) successfully transferred sheep body mites (*P. ovis*) to the ears of goats and Meleney (1967) was successful through natural exposure, although it was not stated whether the animals were negative for *P. cuniculi* prior to experimentation. Attempts in Chapter 4.1 failed to transfer *P.ovis* to the ears of goats.

The fact that rabbit *P. cuniculi* cannot cause sheep scab is well documented (Kirkwood, 1985 and Meleney, 1967), however Chapter 4.1 demonstrated that they can (on occasions) migrate away from the site of challenge to the ear canal. Shilston (1915) observed that adult *P. cuniculi* derived from rabbits could survive and oviposit for at least 17 days on sheep, but the second generation died out without reproducing. Similar observations were made in Chapter 4.1 with temporary lesions either with or without live mites, resolving after 14 days post challenge. This was particularly noted on two sheep challenged with mites derived from a case of rabbit extra auricular mange.

Caution should be attributed to interpreting the results of cross transmission studies. Negative results are not conclusive, the relative susceptability of the individual recipient host must be taken into account. It has long been recognised that all hosts of the same species are not always equally susceptible to colonisation by parasites of the same species. On one host, permenantly parasitic mites (eg *Psoroptes* spp) may become established, produce a greater number of offspring and generally appear to be better adapted than on another. Considerable variations in the susceptability of individual sheep to artificial challenges of *P ovis* were observed in Chapter 3.1. Unacceptably large numbers of recipient hosts may therefore be required in order to validate cross transmission studies, together with a large number mites (the size of the infesting challenge may also be a limiting factor).

Variations within the isolates of a given species may also be important. Colonisation by sheep body mites (*P.ovis*) may be a factor of virulence: ie. the low virulence isolates of *P.ovis* examined in Chapter 3.2 failed to establish on a number of recipient sheep, yet colonisation was guaranteed regarding the medium and high virulence populations.

Caution should also be taken regarding the nature of the study. Successful transfers under laboratory conditions may be impossible in the field due to the physical isolation of the two hosts. Sheep and rabbits may share the same grazing but they do not demonstrate "interactive behaviour." Sheep scab was eradicated from Australia and New Zealand by the end of the 19th Century yet *P.cuniculi* has been shown to infest the ears of 22 % of Australian domestic goat herds (Cook, 1981) and has been reported infesting the ears of feral goats that are distributed in groups over a large part of the continent (McKenzie *et al.,* 1979, and Hein and Cargill, 1981). In New Zealand feral goats are also considered to be an important source of *P. cuniculi* (Heath *et al.*, 1979). As previously mentioned Faccini and Costa (1992); Shastri and Deshpond, (1983) and Yeruham *et al.,* (1984/85) all recorded psoroptic otoacariasis in sheep flocks that shared grazing with infested goat herds, postulating that transmission between sheep and goats was possible. If *P.cuniculi* were transmissable to sheep, particularly in the form of mange, it would be a well reported serious problem for the Australian and New Zealand sheep industries.

Effects of Acaricides

The efficacy of injections of the endectocide ivermectin appear to vary with populations of rabbit *P. cuniculi*. Wilkins *et al.,* (1980) in Texas and Pandey (1989) in the UK reported that single injections of ivermectin, at a dose rate of 200 μg per kg body weight, cured psoroptic ear canker, but Wright and Riner (1985) required injections at a higher dose (400 μg/kg body weight) to achieve the same result. Yet Prosl and Kanout (1985) in Germany, failed to eradicate *P. cuniculi* at either 200 μg or 400 μg/kg body weight. In the preliminary development of ivermectin, studies in Texas by Wright and Riner (1985) demonstrated that single subcutaneous or intramuscular injections of ivermectin (at 200 μg/kg body weight) were inadequate in eliminating either *P. cuniculi* or *P. ovis* from the rabbits, yet single injections at 400 μg/kg body weight ivermectin eliminated *P. cuniculi* from all infested rabbits but *P. ovis* was eradicated from only 50% of infested rabbits. The exact reason for this variation in efficacy is not fully understood, since *P. ovis* infesting rabbits ingest nine times more red blood cells than *P. ovis* on cattle (Wright and Deloach, 1980) and single subcutaneous injections of ivermectin were 100% effective in eradicating *P. ovis* on cattle (Losson, 1996 and Meleney, 1982).

Studies in Chapter 4.2 modified the investigations of Wright and Riner (1985), in that relative pathogenicities of the pre- and post- ivermectin exposed populations of *P. cuniculi* to sheep were also investigated. Exposure of rabbit *P.cuniculi* to ivermectin, changed non-pathogenic "*P.cuniculi*" to pathogenic "*P.ovis*" on one occasion producing acute extensive virulent lesions, covering sheep by 71.0% over 7 days, but with low mite populations. Populations of *Psoroptes* naturally infesting rabbits can therefore contain sub-populations of both *P. cuniculi* (non-infestive to sheep) and *P. ovis* (infestive to sheep) selected for by ivermectin. These two sub-populations may represent the two populations of *P. cuniculi* ("K" and "L") identified relative to morphological and *in vitro* survival by Von Ribbeck and Gehrt (1974).

A detailed description of how *Psoroptes* mites feed is given Chapter 1. In this discussion it is important to emphasise some important points. There are no morphological differences between the gnathosoma of *P. cuniculi* or *P. ovis* (Rafferty

and Gray, 1987), both are adapted for both liquid and solid feeding. It is likely that *Psoroptes* spp are pool feeders (telmophages) feeding on lysed tissue rather than vessel feeders (solenophages) feeding directly on blood vessels or lymphatics.

P.ovis infesting cattle and laboratory rabbits are known to ingest blood serum and erythrocytes (Wright and Deloach, 1980, 1981), but this has not been observed in *P. ovis* infesting sheep. *P. ovis* feeding on cattle ingest erythrocytes, but at approximately one ninth the rate recorded while feeding in rabbits ears. DeLoach and Wright (1981) summarised that ingestion of whole blood was not specific to a particular *Psoroptes* spp. but may be common to all *Psoroptes* spp. when feeding on rabbits. Wright and DeLoach (1981) postulated that the thinness of the skin in the rabbits ear and the larger number of peripheral blood vessels allowed blood to be ingested more readily.

The efficacy of single injections of ivermectin (200 μg/kg body weight against sheep scab mites (*P. ovis*) is dependent on the relative virulence of the infesting population: low virulence populations can be eradicated with a single injection, but highly virulent isolates required two injections to achieve total eradication (Bates, 1994). Bates and Groves (1991) demonstrated that the efficacy of a single subcutaneous injection of ivermectin (at a dose rate of 200μg/kg body weight) was related to the mite burden at the time of treatment: ie. more mites survived treatment if the pre-treatment burden was high. This was attributed to a corresponding high 'sub-population' of non feeding pharate mites between moults, within the pre-treatment population and therefore not susceptible to ingested acaricides. Thus more mites survive a single injection of ivermectin in the 'virulent' isolates with characteristically high mite populations.

Morphological Differentiation

Hirst (1922) was the first to use the length of the male L_4 outer opisthsomal setae (L_4OOS) to differentiate species of *Psoroptes*, but apart from this feature, found little structural differences between them. He suggested that, with the exception of *P. natalensis,* mites in the genus *Psoroptes* should be regarded merely as races or slight varieties of a single species (*P. communis*). Sweatman (1958) observed that in *P.*

natalensis the L_4OOS and the inner seta (L_2OOS) were equal in length, whereas in *P. ovis* the outer seta is one third as long as the inner seta. If on the other hand both setae are equally long and threadlike then it is difficult to differentiate *P. natalensis* from *P. equi*.

Sweatman (1958) measured the setae from male *Psoroptes* mites from infested horses from two geographically isolated farms in England and recorded the setal lengths for the two isolates as 303.0 μm and 122.0 μm respectively. Sweatman postulated that only one isolate could definitely be speciated as *P. equi* (303 μm), the other, because of its shorter setae (122 μm) was arbitrarily designated as *P. equi.* Abu-Samra *et al.*, (1987) measured the setae of male mites isolated from infested donkeys in the Sudan and showed them to be 355.0 ± 5 μm. The mean setal lengths of another English isolate of *P. equi* measured in Chapter 4.2 was recorded as 193.6 ± 45.6 μm, midway between the two measurements described by Sweatman (1958) and Abu-Samra *et al.*, (1987). This suggests extreme variations within *Psoroptes* sp. infesting horses.

In Chapter 4.3 little difference was observed between the mean setal lengths of the VLA Reference Isolate of ovine *P.ovis* cultured on sheep or cattle, 80.0 and 83.8 μm respectively or between the two bovine populations of *P.ovis* compared. The frequency of "pure" *P.cuniculi* was relatively low for all bovine isolates ranging between 7.1% and 29.4. The mean setal lengths for male *Psoroptes* collected from the last outbreak of bovine psoroptic mange (Linklater and Gillespie, 1984) were significantly longer than all the bovine *P.ovis* populations examined, with 84.0% of the population measuring above 258.0 μm, thus fitting the morphological criteria for *P.natalensis* (Sweatman, 1958). This may account for their non-infestivity to the bodies of sheep.

Chapter 4.3, compared the relative lengths of the male L_4OOS, of bovine and equine *Psoroptes* and confirmed and modify the observations of Sweatman (1958) in that *P. natalensis* (mean L_4OOS = 304.5 ± 57.9 μm) and *P. equi* (mean L_4OOS = 193.6 ± 45.6 μm) are statistically different from each other and from all other

Psoroptes isolates examined. Wright *et al.*, (1984) also recorded highly significant differences between a Brazilian isolate of bovine *Psoroptes* (219.8 μm) compared with isolates of *P.ovis* and *P.cuniculi* from different hosts from around the World.

The VLA Reference isolate of *P. ovis* cultured on cattle and sheep (mean $L_4OOS = 83.8 \pm 13.2$ μm and 80.1 ± 20.0 μm, respectively), and *P. cuniculi* (Isolate R3 $L_4OOS = 73.0 \pm 12.1$ μm and Isolate R 4 $L_4OOS = 100.3 \pm 17.6$ μm) were not significantly different. There was a considerable overlap in the comparative lengths of the male L_4 L_4OOS between populations of *P. ovis* and *P. cuniculi*, so much so that they could not be differentiated by this morphological criteria. Mites isolated from the ear canals of goats and sheep possessed the shortest setae (mean lengths 50.0 μm to 74.0 μm) and were predominantly "pure" *P.cuniculi* (ie. setal lengths below 74.0 μm), but mites isolated from the ears of rabbits varied considerably, and fell into two groups: short setal populations (mean lengths 54.8 μm to 79.2 μm) with corresponding medium to high frequencies (41.9 to 100%) of "pure" *P.cuniculi* and non-infestive to the bodies of sheep and long setal populations (mean lengths 98.5 μm to 100.3 μm). Long setal length populations tended to originate from extra-auricular lesions with the frequency of "pure" *P.cuniculi* relatively low (7.6 to 29.4%) and were shown to be non-infestive to sheep until selected for by ivermectin (Chapter 4.2). A narrow band of setal lengths, mainly from populations of bovine and ovine *P.ovis* divided the two groups of rabbit *P.cuniculi*. Wright *et al.*, (1984) also demonstrated that the ear mites of mule deer in Colorado, a rabbit in New Mexico, a goat from Texas and a rabbit from the VLA Weybridge, all presented comparatively short setae (62.7 to 74.3 μm).

Male L_4OOS were measured within 16 populations of *P.ovis* (originating from eleven geographically and temporally distinct isolates), producing active ovine psoroptic mange. The mean length of the male L_4OOS for the ovine populations of *P.ovis* ranged between 66.8 and 100.9 μm. The population with a mean setal length of 66.8μm (Penderyn) was an exception, with the majority between 80.1 and 100.9 μm, shorter than the mean setal lengths for ovine *P.ovis* (86.5 to 118.0 μm) from around the World, recorded by Wright *et al.*, (1984). Wright *et al.*, (1984) compared eight

populations of ovine *P.ovis*, six from within the USA (Albuqueque (86.5μm), Mixed USA (86.9μm), ADP Strain (87.5 μm), Beltsville I (96.4μm), Beltsville II (97.5μm), and Mission Strain (118.0 μm), one from South Africa (100.5 μm) and one from the UK (VLA, Weybridge) (95.7 μm). Setal lengths for the Mission strain were significantly different from those of *P. ovis* from Albuquerque, England (VLA, Weybridge) and South Africa (86.5, 95.7 and 100.5 μm respectively). Wright *et al.*, (1984) thus postulated geographical differences in morphology within species of *Psoroptes*.

There appeared to be a relationship between the length of the male L_4OOS and population virulence. The Penderyn isolate presenting low virulence infestations when qualitatively compared to the VLA Reference Isolate, also demonstrated an extremely short setal length (mean 66.8 μm). The mean setal length for the Witney isolate (isolate S.14), designated "low virulence" in Chapter 3.2, was 77.6 μm. The combined mean setal length for these two low virulence population was 73.6 μm. Whereas the mean setal lengths for the "medium" (VLA Reference Isolate 1988 and 1995) and "high virulence" (Alston, Arlington, Little Melton and Market Drayton) populations of *P.ovis* were 82.0 and 90.5 μm, respectively. Differences between setal lengths for the low, medium and high virulence populations were highly significant ($p = <0.0001$). The percent "pure" *P.cuniculi* in these low virulence isolates were also relatively high (34.0% to 50.0%) compared to the medium virulence populations (11.1% to 40.3%) and the high virulence isolates (0.0% to 22.9%). The mean lengths of the male setae of mites recovered from the cryptic sites varied considerably from body mites.

Differences were highly significant between the mean setal lengths of male *Psoroptes* collected from the body and the ears of goats and also between goat body mites and other *Psoroptes* populations. Similarly the differences within populations collected from the Barbary Sheep were highly significant from all other *Psoroptes* populations compared and, like the mites collected from the body of an infested goat, were more than likely *P.ovis*. Setal lengths from both the goat body and Barbary

Sheep populations were predominantly (93.2 and 95.0%, respectively) within the overlap of *P.cuniculi/P.ovis*.

Setal length may not therefore be a marker to indicate the potential of a particular *Psoroptes* population to establish on the bodies of sheep. Wright *et al.,* (1983) suggested that the overlap in setal lengths was too wide, and the method was inadequate as a criterion for speciation. Furthermore Wright *et al.,* (1984) demonstrated that the length of the L_4 OOS varied with the isolate of *Psoroptes*. The reference isolate of *P. ovis* ("Mission strain"), a *Psoroptes* sp derived from bighorn sheep in Idaho and cattle mites from Brazil (setal lengths of 118.0, 140.3 and 219.3 μm respectively) were all significantly different from all other *Psoroptes* isolates compared. Rabbit *P. cuniculi* from Kerrville (Texas), England (VLA, Weybridge) and New Mexico were also all significantly different (87.4, 74.3 and 66.5 μm respectively). Cattle *Psoroptes* from USA (134.0 μm) were significantly different to cattle mites from Brazil (219.8 μm (*P. natalensis*?)). The "Mission strain" of *P. ovis* (118.0 μm) was significantly different from *P. ovis* from Albuquerque, England (VLA, Weybridge) and South Africa (86.5, 95.7 and 100.5 μm respectively). Thus demonstrating geographical differences in morphology within species of *Psoroptes*.

Boyce *et al.,* (1990) attempted to provide information on the phylogenic relationships between *Psoroptes* mites found on different hosts using discriminant analysis. They employed nine morphological characters and found that the length of the male L_4 OOS and the lateral margins of the opisthosomal knobs of the male were the two most important characters for grouping mites according to host species. Mites could clearly be separated within allopatric populations (populations within a species, which do not occur together but have mutually exclusive distributions) of bighorn sheep, rabbits and cattle into discrete groups. However differences were not detected between mites collected from sympatric populations of infested mule deer and bighorn sheep, suggesting that these mites are not host specific and represent a single interbreeding population. Differences also were not detected among mites collected from the ears and body of bighorn sheep and rabbits, demonstrating that the location of mites on a given host should not be used as a primary criterion in species

identification. Morphometric classification is therefore influenced by spatial relationships between hosts (allopatry versus sympatry).

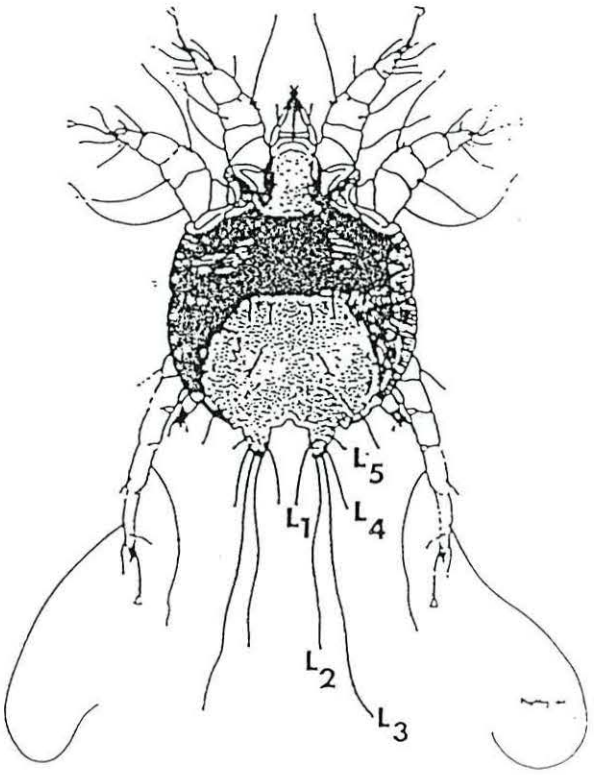

Figure 4.4.1: Diagramatic representation of a male *Psoroptes* mite indicating the position of the inner (L_1, L_2 and L_3) and outer (L_4 and L_5) opisthosomal setae.

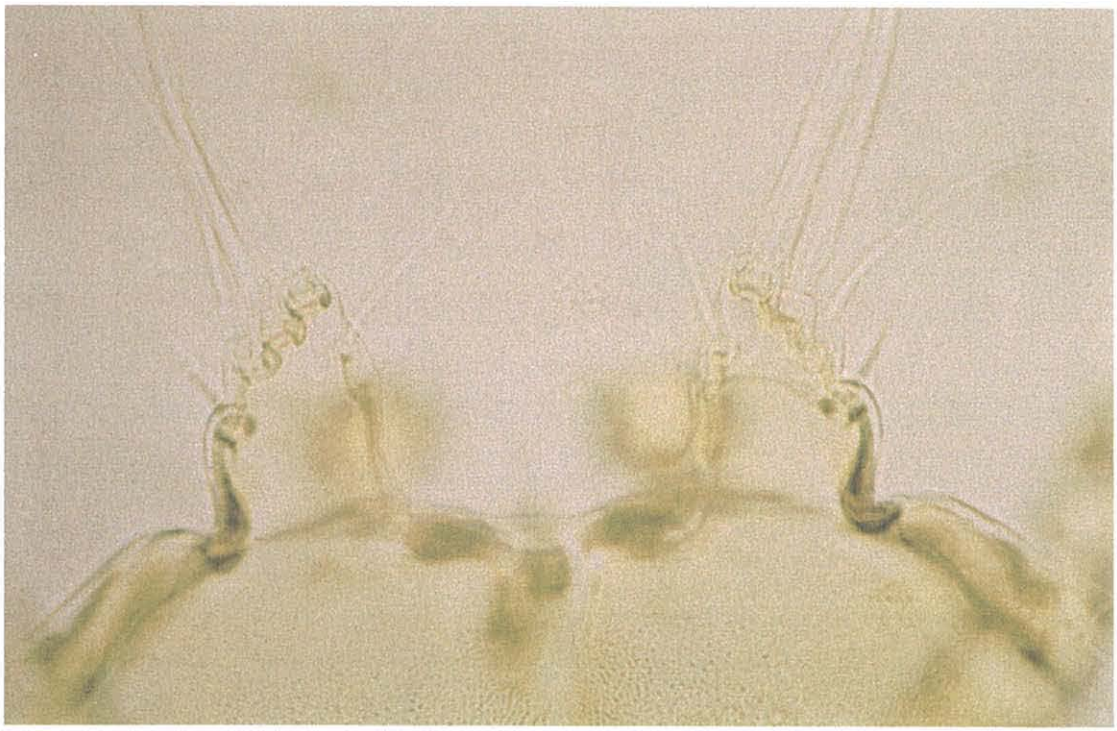

Figure 4.2: Detail of the opisthosomal lobes of a male *Psoroptes natalensis* (Scottish Borders 1984), indicating L_1, L_2 and L_3 inner opisthosomal setae and the L_4 and L_5 outer opisthosomal setae. Magnification x250.

Chapter 4.5

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Chapter 5.0

Overall Discussion and Conclusions

1. Introduction

Astigmatid mites in the family Psoroptidae are non burrowing mammalian ectoparasites. Four genera, all present in Great Britain, are currently recognised: *Psoroptes* parasitising the ears and bodies of herbivores (Sweatman, 1958a), *Chorioptes* parasitising the bodies (and occasionally the ears) of herbivores (Sweatman, 1957), *Otodectes* parasitising the ears (and occasionally the body) of carnivores (Sweatman, 1958b) and *Caparinia* spp, represented in the UK by *C. tripilis* parasitising the European hedgehog (*Erinaceous europaeus*) (Fain and Till, 1987).

2. Classification

The first recorded classification of *Psoroptes* was by Megnin (1877), describing the body mites of sheep, horses and cattle and the ear mites of rabbits as variants of the one species, *Psoroptes longirostris* (ie. *P. longirostris* var *ovis*). Raillet (1893) also regarded all *Psoroptes* mites to be variants of a single re-defined species, *Psoroptes communis* (eg *P. communis* var *ovis*). Neveau-Lemaire (1938) yet again regarded *Psoroptes* as variants of one species, but it was re-defined as *Psoroptes equi* (eg *P. equi* var *ovis*). Hirst (1922) examined large numbers of *Psoroptes* from various domestic hosts and, although he was the first to use the length of the male L_4 outer opisthsomal setae (L_4OOS) to differentiate species, he found little structural differences between them. He suggested that, with the exception of *P. natalensis,* which are morphologically different (the L_3 and L_4 outer opisthsomal setae (L_3OOS and L_4OOS) of the male being distinctly flattened and blade like), the remaining mites in the genus should be regarded merely as races or slight varieties of a single species (*P. communis*)

The extensive morphological studies by Sweatman (1958a) reduced the number of specific names to only five, recognised by the comparative lengths of the male L_4OOS and by host and location of the mite on the host; species were divided further into those infesting the body and those infesting the ears of their hosts. The classification devised by Sweatman (1958a) was outlined in Chapter 4.0 and has been ammended in Tables 5.1 and 5.2 to include hosts described by subsequent authors.

Table 5.1.

The Classification of the Genus *Psoroptes* (Sweatman, 1958).

a). Body Mites

Psoroptes species.	Synonym(s)	Male L_4OOS (μm)	Host(s)
Psoroptes ovis (Hering 1838).	*P. bovis* (Gerlach)	74.0 to 258.0	Domestic sheep[1]. Bighorn sheep[1]. Cattle[1]. Horses[1]. Giraffe[2] Dromedary[3]
Psoroptes equi. (Hering 1838)		L_4OOS = 333.0	Horses[1]. Mules[1]. Donkeys[1]
Psoroptes natalensis (Hirst 1919)		250.0 to 350.0	Cattle[1]. Zebu cattle[1,4]. Horses[1].

1= Sweatman (1958), 2 = Burr (1984), 3 = Gabaj *et al.*, (1992), 4 = Shastri and Ghafoor (1974).

Table 5.2.

The Classification of the Genus *Psoroptes* (Sweatman, 1958).

b). Ear Mites

Psoroptes species.	Synonym(s)	Male L_4OOS (μm)	Host(s)
Psoroptes cuniculi (Delafond 1859)	*P. caprae* *P. hippotis*	64.0 to 164.0	Rabbits[1]. Goats[1]. Sheep[1]. Horses[1]. Bighorn sheep[2,3] blackbuck antelope[4] impala[5] mountain goat[6] mule deer[7] stone sheep[8] water buffalo[9] white tailed deer[10,11] yaez[12]
Psoroptes cervinus (Ward 1915)		145.0 to 354.0	Bighorn sheep[13]. Wapiti[14].

1 = Sweatman (1958), 2 = Lange *et al.,* (1980), 3 = Kinzer *et al.,* (1983), 4 = Wright and Glaze (1988), 5 = Rauchbach cited by Yeruham *et al.*, (1985), 6 = Yeruham *et al.*, (1985), 7 = Roberts *et al.*, (1970), 8 = Foreyt (1997), 9 = Shastri and Ghafoor (1974), 10 = Strickland *et al.,* (1970), 11 = Schmitt *et al.,* (1982), 12 = Yeruham *et al.,* (1978), 13 = Ward (1915) and 14 = Hepworth and Thomas (1962).

Undefined Species

A *Psoroptes* mite, tentatively described as *P. equi* var *leporis* was isolated from the European hare (*Lepus capensis* (*L. europaeus*)) by Vanek and Novakova (1959). *Psoroptes auchinae* (*P. communis* var *auchinae*) has been identified from the ears of alpacas (*Llama pacos*) in South America (Chavez and Guerrero, 1965; Fowler, 1989) and ears and bodies of llamas (*Llama glama*) (Alverado, 1966; Foreyt *et al.*, 1992; Guerrero and La Rosa, 1962). Fain (1970) isolated a *Psoroptes* mite from the head of African Buffalo (*Syncerus caffer*) and named it *P. pienaari*. All three mites have not been subjected to modern re-analysis and their actual status is still unknown (Strong and Halliday, 1992).

The classification of the Genus *Psoroptes* described by Sweatman (1958), indicted that many domestic host species can be infested with more than one species of *Psoroptes* (even simultaneously), eg. *P cuniculi* in the ears (goats, horses rabbits and sheep) and the body mites *P ovis* (sheep, cattle), *P equi* (horses) and *P natalensis* (horses, cattle).

The Report of the Sheep Scab Working Party (1988) reported that 399 flocks were shown to be infested with the sheep scab mite (*P. ovis*) in Great Britain, between 1983 and (May) 1988, during the period of two annual compulsory plunge dips. Although the origins of the outbreaks were fully explained in over 73% of cases, the origins of infestation remained obscure in 18.5% of cases and the disease recrudesced in 0.7% of flocks. The development of acaricide resistant strains of *P. ovis,* during this period, was not suspected in the recrudescence of disease and the high incidence of outbreaks of obscure origin suggested a method or reservoir of infestation unknown to the Veterinary Field Service. Throughout the history of sheep scab many authors have attributed the failure to eradicate or control the disease to an unknown facet of *Psoroptes* biology, ie. i) the survivability of the mite off the host, ii) the presence of a resting stage (deuteronymph or hypopus) able to survive for long periods, either on or off the host, without causing any pathology, iii) the presence of a latent or cryptic phase of disease and iv) other hosts, (cattle, wild rabbits, deer, and domestic and feral goats, horses and ponies) as reservoirs of disease.

O'Brien *et al.,* (1994a) conclusively proved that *P.ovis* can only remain infestive to sheep for 15 to 16 days off the host. The presence of a hypopal resting stage is extremely unlikely as the life cycle of *P.ovis* (and *P.cuniculi*) has been thoroughly researched, with no evidence of such developmental stage. Sheep scab is primarily a winter disease, but a significant number of cases do occur during the summer (Bates, 1991). Oversummering was once explained by the phenomenon of "latent phase" or "suppressed scab" (described by Downing (1936) and Spence (1949)), where mites were thought to intentionally migrate to the "cryptic sites" (ie the ears, infra-orbital fossae (IOF), the inguinal fossae, the crutch and perineum) in order to survive the rigours of summer. This theory has been disproved, primarily by the observations of Kirkwood (1985), but also by Roberts *et al.,* (1971), who recorded infestations in one or more cryptic site during the summer in only 7% of sheep examined, compared to mites oversummering on the broad body surfaces of 32% of sheep examined.

Knowledge of the natural reservoirs of *Psoroptes*, in both domesticated and non domesticated herbivores, is essential for sheep scab control strategies to be effective. In the USA *Psoroptes* mites have been recorded infesting a number of wild herbivores (Tables 5.1 and 5.2). Lange (1988) postulated that outbreaks of psoroptic mange (*P. cuniculi*) in Bighorn sheep in the USA, could have been derived from domestic cattle or sheep and that concurrent infestations of *P. ovis* and *P. cuniculi* could occur. The role of cattle, rabbits, goats, horses and camelids as reservoirs of disease was investigated in Chapters 2.0 and 4.0.

3. Mite Host Relationships

3.1 Equine Psoroptic Mange and Otoacariasis

Horses, donkeys and mules can be infested in the ears and on the body. Henry (1920: cited by Sweatman, 1958a) described mites from the ears of horses (otoacariasis) as *P. hippotis* (≡ *P. cuniculi*) and those causing body mange as *P. equi*, an observation supported by Sweatman (1958a). Psoroptic ear mites of horses (equine psoroptic otoacariasis) is a common infestation worldwide (Montali, 1976), with 20% of horses

in Queensland, Australia, shown to be infested (Arundel, 1978). Although a case was recorded in Great Britain in 1984 (Gerring and Thomsett, 1984), the limited survey described in Chapter 2.4 failed to demonstrate further infestations. Equids are often grazed along with sheep and nine breeds of semi-wild native ponies share upland common grazings with sheep, particularly in Dartmoor, Exmoor, the Pennines and the Welsh mountains, all known foci of sheep scab. Unfortunately access was not available to sufficient equine material from these areas during the course of this study. Psoroptic mange (*P.equi*) was once a notifiable disease in Great Britain but has since been eradicated. Sweatman (1958a) considered the body mite, *P. equi,* to be unique to England but the disease has subsequently been recorded in Germany (Dietz and Wiesner, 1984), Libya (Gabaj *et al.,* 1992), South Africa (Zumpt, 1961) and in the Sudan (Abu Samra *et al.*, 1981, 1987).

3.2 Caprine Psoroptic Mange and Otoacariasis

Caprine psoroptic otoacariasis was first described by Gedoelst in 1909 and has since been reported throughout the world (Cook, 1981; Faccini and Costa, 1992; Littlejohn, 1968; Williams and Williams, 1978). Psoroptic otoacariasis appears to be a common, yet unnoticed infestation of domestic goats in Great Britain. Chapter 2.3 recorded psoroptic otoacariasis in 80.0% of goat herds examined, with 30.0% of goats infested. All lesions were confined to the external ear canal and no gross clinical symptoms were observed in any herd, other than the occasional individual exhibiting ear scratching with the hind feet. The species involved is usually described as *P. cuniculi*. True psoroptic mange is frequently recorded on the bodies of goats, although it is rare in Great Britain. A mite, identified as *P.ovis* was recorded as a cause of psoroptic mange at the VLA, Weybridge in 1962 (Chapter 4.3). In meat and dairy goats infestations are usually confined to the ears but true psoroptic mange is frequently recorded on the bodies of old or debilitated animals, younger goats showing milder symptoms (Heath *et al.,* 1983; Littlejohn, 1968; Munro and Munro, 1980). The ears may act as reservoirs of mites, goats transferring mites to accessable parts of the body using their hind feet, where they may not colonise completely, but a continual mite challenge may maintain an apparent infestation. However extensive body mange can be presented in "wooly" angora goats (Graham and Hourigan, 1977).

There are no truly wild herds of goats in Great Britain. Feral herds share hill grazing with sheep in the remote parts of the Scottish Highlands, the Hebrides, Lundy Island, Holy Island (Iona), the Cheviot Hills, and North, Central and South Wales, although they usually inhabit high, exposed rocky zones, inaccessible to sheep (Lawrence and Brown, 1979). Faccini and Costa (1992) recorded that 40% of Brazilian sheep flocks were infested in the ears with *P. cuniculi* mites and observed that all infested flocks shared grazing with goats, postulating that transmission between sheep and goats was possible in the field. Shastri and Deshpand (1983) in India and Yeruham *et al.,* (1984/85) in Isreal both also reported otoacariasis in sheep when herded with goats. Shilston (1915) observed that mites transferred from the ears of goats (*P. communis* var *caprae*) to the ears of sheep were not infestive but Sweatman (1958a) sucessfully infested the ears of sheep (both naturally and artificially) with goat ear mites. Subsequent investigations in Chapter 4.1 and by other authors have failed to transfer goat ear mites to either the body or ears of sheep either artificially (Meleney, 1967) or through natural exposure to infested goats (Williams and Williams, 1978 and Heath *et al.,* 1989).

Sheep scab was eradicated from Australia and New Zealand by the end of the 19th Century yet *P.cuniculi* has been shown to infest the ears of Australian feral and domestic goats (Cook, 1981; McKenzie *et al.,* 1979, and Hein and Cargill, 1981). In New Zealand feral goats are also considered to be an important source of *P. cuniculi* (Heath *et al.*, 1979). As previously mentioned Faccini and Costa (1992); Shastri and Deshpand, (1983) and Yeruham *et al.,* (1984/85) all recorded psoroptic otoacariasis in sheep flocks that shared grazing with infested goat herds, postulating that transmission between sheep and goats was possible. If *P.cuniculi* were transmissable to sheep, particularly in the form of mange, it would be a well reported serious problem for the Australian and New Zealand sheep industries.

3.3 Bovine Psoroptic Mange

Hirst (1922) described two species of *Psoroptes* infesting the bodies of cattle: *P. communis* var *bovis* (Furstenberg, 1861) and *P. natalensis* (Hirst, 1919) based upon the relative morphology of the male L_3OOS and L_4OOS. Sweatman (1958a) defined *P. ovis* (Hering) as a body mite of domestic sheep, bighorn sheep, cattle and horses

and considered the body mite of sheep (*P. ovis*) to be synonymous with the body mite of cattle, previously known as *P. bovis* (Gerlach) or *P. communis* var *bovis* (Furstenberg 1861). *Psoroptes* infestations of cattle have been recorded in Europe, Asia and north and south America (Nuñez, 1989; Losson, 1996; Gill *et al.,* 1989; Graham and Hourrigan, 1977).

Sweatman (1958a) defined *P. natalensis* as a body mite of domestic cattle, zebu cattle, Indian water buffalo and horses. It ws originally described in South Africa but has since been recorded in many areas of the world (Rocha *et al,* 1952 and Sweatman, 1958a). Shastri and Ghafoor (1974) reported the incidence of *P. natalensis* on the horn base of water buffaloes (*B. bubalus*) to be quite high and speculated that horn infestations act as reservoirs that spread to other parts of the body and that *P. natalensis* were essentially body mites, driven to thrive on the uppermost part of the body such as the withers, back, croup and horns, due to the host habit of wallowing in water.

In Great Britain bovine psoroptic mange was considered to be host specific and unable to transfer to sheep (Anon, 1949). Kettle (1992) stated that in Britain the two populations appear to be distinct, due to the observations of Kirkwood (1985) and that cattle *Psoroptes* do not survive on sheep. This separation finds support from a survey of other countries of which 12 reported *P ovis* on sheep but not on cattle and 16 countries reported the reverse, with *P ovis* on cattle but not sheep (Jensen *et al.*, 1979). Linklater and Gillespie (1984) on investigating the last outbreak of bovine psoroptic mange in Great Britain found no clinical scab on contact sheep and Evans and Kirkwood (1984) failed to establish (under laboratory conditions) cross transmission of these mites to sheep and concluded that psoroptic mange in cattle and sheep (sheep scab) in Great Britain were epidemiologically distinct. Re-examination of the mites from this case (Chapter 4.3) however, demonstrated that the infesting mites were in fact *P natalensis* (L_4OOS = 304.5.1 μm ± 57.9) as opposed to (L_4OOS = 84.3 μm ± 14.4) for *P ovis* cultured on cattle, and were not infestive to sheep.

At the beginning of the First World War *Psoroptes* spp. were considered the main causes of bovine mange in Great Britain (Anon, 1949). The results presented in

Chapter 2.8 demonstrated that between 1962 and 1997, only 8.6% of cases of bovine mange were due to isolated outbreaks of *Psoroptes* spp, imported from mainland Europe. Bovine psoroptic mange is not therefore currently endemic to the UK. The single European market and the relaxation of EU import regulations in 1993 may result in a subsequent importation of the disease into Great Britain.

The infestivity of *Psoroptes* spp to cattle under laboratory was investigated by Roberts (1970) and Liebisch *et al.,* (1979) who successfully transferred *Psoroptes* spp. from sheep to cattle, although Zielasko (1979) reported sheep mites survived on cattle for only a short time. Kemper and Peterson (1953) transferred *P. ovis* from sheep to cattle to sheep on a regular basis, but after three years the mite lost its virulence to sheep and totally adapted to cattle. In Chapter 4.1 the Weybridge ovine Reference Isolate of *P. ovis* was capable of infesting restrained calves and remained infestive to sheep despite five passages on cattle. The relative virulence of the mite strain may be important. Roberts and Meleney (1971) demonstrated that 'aggressive' strains of the sheep scab mite *(P. ovis)* were able to spread through cattle herds more rapidly and produce more obvious clinical disease than less pathogenic strains.

3.4 Psoroptic Ear Canker in Rabbits

Two forms of *P. cuniculi* infestations are recognised in domestic rabbits: typical psoroptic "ear canker", confined to the ear canal and the pinnae and "extra auricular" mange, spreading over the body of the rabbit. Psoroptic "ear canker" was first described in Paris in 1858 by Delafond (cited by Sweatman, 1958a) and has been recorded worldwide. Infestations start deep in the external auditory canal (EAC) and may progress for long periods before being noticed. Thus symptoms can vary from a slight crusting deep in the ear canal to extensive crusty scabs and excoriations in the pinnae (Timm, 1988).

Zurn (1875) described cases of 'extra auricular mange' dispelling the theory that the mite exclusively inhabits the rabbit ear. The condition has been extensively described by Von Ribbeck and Ilchmann (1969) and Guilhon (1990). Extra-auricular mange has been demonstrated in 5.0% of infested rabbits submitted to the VLA, Weybridge for examination, with rabbits presenting laminated pinneal lesions, approximately 1.0 cm

thick, with hyperkeratotic crusting of the skin extending to the base of the ears, the cheeks, dewlap, face and between the digits of both hind feet (Chapter 2.5).

In Australia psoroptic infestations appear confined to domestic rabbits (Mykytowycz, 1957 and Williams, 1972) and similar observations were recorded in England (Chapter 2.5) although Guilhon (1990) observed one or more pairs of psoroptic mites present in the external auditory canals of wild rabbits in France. The wild rabbit (*Orctylagus cuniculi*) is not native to Great Britain, but was introduced by the Normans in the 12th century. The animal was once extremely common over the whole of Britain, especially in the Western Counties. In 1954 rabbit myxoma virus (myxomatosis) was introduced "by accident" from France and the disease spread rapidly via blood sucking vectors, such as mosquitoes and the rabbit flea (*Spilopsyllus cuniculi*), eradicating over 60 million wild rabbits by 1956. It is interesting to note that the near total eradication of the wild rabbit coincided with the apparent eradication of sheep scab in 1952! If *Psoroptes* spp infestations exist in wild British rabbits they are not integral to the epidemiology of *Psoroptes* spp. in Great Britain. Hywell Dda documented his scab control strategy in 949, long before the introduction of the rabbit to the UK.

Investigations in Chapter 4.1 demonstrated that rabbit ear canker mites (*P. cuniculi*) can (under artificial passage) establish in the ear canals of sheep, corroborating the studies of Sweatman (1958a). Chapter 4.1 also demonstrated that rabbit *P. cuniculi* fail to established in the ear canals or the bodies of goats, thus contradicting the studies of Sweatman (1958a), who concluded from his observations that the auricular mites of rabbits ≡ auricular mites of goats (ie. *P. equi* var *caprae* and *P. cuniculi* were conspecific). Perucci *et al.,* (1996) was successful in infesting rabbits with goat ear mites and goats with rabbit ear mites, and along with morphological studies concurred with Sweatman (1958a) that goat ear mites and rabbit ear mites were the same species. Sweatman (1958a) was also successful in transferring *P. cuniculi* from ears of rabbits to the ears of a donkey, concluding that *P hippotis* and *P. cuniculi* were also conspecific. The infestivity of rabbit *P.cuniculi* to cattle is also equivocal. Meleney (1967) failed to establish rabbit mites on the bodies of (unstantioned) cattle, but Roberts *et al.,* (1970) and Wright (1982) successfully reared

rabbit *P. cuniculi* on stanchioned cattle, for at least 20 mite generations and never losing their infestivity to rabbits.

The fact that rabbit *P. cuniculi* cannot cause sheep scab is well documented (Kirkwood, 1985 and Meleney, 1967), however Chapter 4.1 demonstrated that they can (on occasions) migrate away from the site of challenge to the ear canal. Shilston (1915) observed that adult *P. cuniculi* derived from rabbits could survive and oviposit for at least 17 days on sheep, but the second generation died out without reproducing. Similar observations were made in Chapter 4.1 with temporary lesions either with or without live mites, resolving after 14 days post challenge. This was particularly noted on two sheep challenged with mites derived from a case of rabbit extra auricular mange.

Two species of native deer (the red deer (*Cervus elephas*) and the roe deer (*Capreolus capreolus*)) and four species of introduced deer (Chinese muntjac (*Muntiacus reevsi*), Chinese Water Deer (*Hydropotes inermis*), Fallow deer (*Dama dama*) and Sika deer (*Cervus nippon*)) are present in Great Britain (Lawrence and Brown, 1979). Axis or chetal deer (*Axis axis*) and White Tailed Deer (*Odocoileus virginianus*) established temporarily from escaped stock in 1940's to 1950's (Lawrence and Brown 1979). White tailed deer are known reservoirs of *P. cuniculi* (Strickland, *et al.,* 1970 and Schmitt, *et al.,* 1982). Although *Psoroptes* mites (*P. cervinus)* have been isolated in North America from the ears of the Wapiti (*Cervus elephas canadensis*), a genetic variant of the British red deer (*Cervus elephas*) (Sweatman 1958a), there is no published information on the prevelence of *Psoroptes* spp in British deer. In Ireland, Sleeman (1983) examined red, fallow and sika deer and red/sika hybrids for ectoparasites, but did not examine the ears. Deer ectoparasite surveys have been carried out in England (Jackson, 1975) and throughout Great Britain (McDiarmid, 1975) but neither author specifically examined the ears. Unfortunately, like horses, access was not available to deer material during the course of this study.

3.5 Psoroptic Otoacariasis and Mange in Sheep

It has been argued by some authors that *Psoroptes* mites may exist in the ears of sheep, without presenting clinical signs of body disease (Henry, 1917 (cited by Sweatman, 1958a); Van der Merwe, 1949; Bates, 1991a and Faccini and Costa, 1992). Accordingly Sweatman (1958a) defined two species of *Psoroptes* infesting sheep: *P. cuniculi* in the ear canal and *P. ovis* on the body. This differentiation is misleading as the sheep scab mite *P. ovis* has also been recorded infesting the ear canals of infested sheep (Zurn, 1877; Imes, 1916; Miller, 1925; Verney, 1926 and Spence, 1949) and studies in Chapter 2.6 recorded live *P. ovis* in the external ear canals of 38.6% of artificially infested sheep. It was also observed that 60.4% of these sheep could become infested in the EAC during the early stages of active disease, when the lesion covered only 11.0% and 44% of the body and the leading edge was as far away as the mid back (20.0 to 55.0 cm from the ears). The remaining sheep were only infested in the ear canal once the lesion had reached the ears and the pinnae themselves were infested. Chapters 3.1 demonstrated live *P. ovis* in the EACs of sheep challenged with the VLA Reference or St.Brenard isolates, as early as 28 or 35 days post challenge, with the leading lesion edge 28.0 or 17.0 cm from the ears, respectively.

Natural *P. cuniculi* infestations in the ears of goats and rabbits have been shown to be able to present extra auricular infestations (Munro and Munro, 1980; Von Ribbeck and Ilchmann, 1969; Guilhon 1990). This may occur in sheep, but the results presented in Chapter 3.3 recorded that treated sheep reinfested after a year were more resistant to subsequent infestation. This resistance was characterised by a slower rate of increase in the mite populations. It has been reported that sheep scab can have a significant effect on the quality of processed leather (Pearson, 1996), suggesting significant changes in the character of sheep skin following infestation. These skin changes may also influence the feeding of mites following reinfestation. Ear mites may not therefore be able to re-infest the same host but they may be able to infest scab naive sheep.

Reliability of cross transmission studies.

The results of many *Psoroptes* sp cross transmission studies are equivocal and the interpretion of results should be carried out with caution, negative results may not be conclusive. Marshall (1981) described five major factors influencing host specificity: a) Physical isolation. Extreme caution should be taken regarding the interpretation of laboratory results, successful transfers under laboratory conditions may be impossible in the field due to the physical isolation of the two hosts. Sheep, horses, goats, cattle and rabbits may share the same grazing but they may not demonstrate "interactive behaviour" allowing for the easy transfer of mites between hosts; b) Variations in climate or microclimate (eg. a particular bacterial flora may be essential); c) Morphology (eg. structure of the mouthparts); d) Host behaviour. Host grooming is a significant behavioural restraint on the establishment of disease. Cattle need to be restrained from grooming in order to develop extensive lesions. But this is of low significance regarding sheep as they exhibit little grooming behaviour and e) host physiology.

Host physiological factors were listed by Arlian *et al*, (1987) as the physical properties of the skin (eg hair density), thermal and water properties, innate or acquired immunlogical resistance, nutritional condition of the host, stress, age, physical condition, pathogens, skin thickness, parasite density, parasite competition, hormone levels etc. The relative susceptability of the individual recipient host to *Psoroptes* infestation must be considered. Considerable variations in the susceptability of individual sheep to artificial challenges of *P ovis* were observed in Chapter 3.1. Variations within the relative "virulence" of the isolates may also be important. eg. the low virulence isolates of *P.ovis* examined in Chapter 3.2 failed to establish on a number of recipient sheep, yet colonisation was guaranteed regarding the medium and high virulence populations.

Psoroptic ear mites can be considered as mild parasites, where there is no benefit to the host. Therefore they are not symbiotic or mutualistic as described by Nutting (1985) but somewhere between commensalistic (gaining nutrient and protection with little or no damage to the host other than casual abrasion) and parasitic

(gaining nutriants, substrate and protection but cause cellular destruction through toxins or pathology). It is interesting to note that in non-domesticated herbivores *Psoroptes* mites are confined to the ear (but can on occasions cause quite traumatic body mange) yet on domesticated animals psoroptic mange constitutes a serious economic and welfare problem. Permanent ectoparasites are highly dependent on their individual hosts, have lower mobility and a lower tolerance to starvation and cannot escape a dead host and are therefore more likely to evolve towards decreased virulence (Lehmann, 1993). Wright *et al.,* (1983) concluded that *P. cuniculi* (and *P. cervinus*) are essentially ear mites that sometimes infests the body, where they cause a more severe pathology than when confined to the ears. The true host of a parasite is the natural host allowing for indefinitely continued reproduction and an accidental host is the result of chance (Marshall, 1981). Wright *et al.,* (1983) stated that the behaviour of *Psoroptes* spp. on an unatural host was not normal and could often be fatal for the host. Guillot and Wright (1981) demonstrated that it took 9 to 10 weeks for *P. cuniculi* to affect one half to one third of the rabbit pinna (lesion score 4.6 to 4.9). *P. ovis*, on the other hand, took only 4 weeks to affect the entire pinna, base of the ear and extensive involvement of the head and lesions developing on the back and shoulders (extra auricular disease). *P. cuniculi* infestations of rabbits seldom spread from the inner pinna but *P. ovis* infestations can be classified as extremely traumatic.

Nutting (1985) stated that there is a possability that the degree of pathogenesis may be useful as an index of the time span of such parasitic associations. It is well documented in protozoan symbionts that those most recently adapted to a host tend to produce the most damage. The mite species least pathogenic to its mammalian symbiont species is the most co-evolved with it's host. Conversely the greater the pathogenesis and host reaction the more recent the association.

5.0 Variations Within Populations of P. ovis Infesting Sheep

Roberts and Meleney (1971) investigating differences within the biology of *P. ovis* on sheep concluded that distinct "strains" of the mite existed in the USA. These differences being based upon a) the ability of strains to withstand population reduction during the summer; b) the observation that more "aggressive" strains were less susceptable to an organophosphate acaricide (coumaphos) compared to less

aggressive strains, c) "aggressive" strains survived longer on individual sheep in isolation and d) "aggressive" strains of sheep origin were able to spread through herds of cattle more rapidly and produce more obvious clinical responses than less pathogenic strains. Roberts and Meleney (1971) highlighted the importance of strain variation and postulated that the highly complex pathology and epidemiology associated with scab could be clarified by the application of the strain variation concept. Differences in drug resistance, survival over summer and pathogenicity may be attributed to the selection of vigorous pathogenic strains through ineffective drugs or sublethal concentrations of effective drugs. Chapters 3.1 and 3.2 investigated variations in virulence regarding the rates of lesion growth and population increase.

Investigations in Chapter 3.1 compared the VLA Reference isolate of *P.ovis* and a field isolate from St.Brenard in Cornwall. Results demonstrated three distinct phases in the clinical progression of sheep. eg the "subclinical", "rapid growth" and "decline" phases. The subclinical phase (ie. lesion areas below 5.0% body cover), lasted 14 to 28 days post challenge for the St.Brenard isolate and 21 to 28 days for the VLA Reference isolate. The subclinical phase also varied with the individual sheep. Up to 63 days post challenge for some sheep challenged with the VLA Reference isolate and 98 days for some sheep challenged with St.Brenard isolate. In the field this may be even longer when the infestive challenge and breed of sheep are taken into account (Bates, 1997c). In contrast to the VLA Reference isolate (where mites failed to establish on a number of sheep), clinical scab developed on all sheep challenged with the St.Brenard isolate.

After the subclinical phase lesion areas rapidly increased with time, "the rapid growth phase." Lesions produced by the St.Brenard isolate grew at a greater rate than those produced by the VLA Reference isolate. The mean percent body cover for the VLA Reference Isolate did not achieve 100% on any sheep challenged over the 63 days of the study. The St.Brenard isolate, however, achieved 100% body cover on some sheep 28 days post challenge and on all sheep by 63 days post challenge. Overall the mean percent body cover of the lesion produced post challenge by the St.Brenard isolate was in the magnitude of 30.5 % after 42 days and 100.0 % after 63 days PC, compared to only 7.6% and 60.9 % for the VLA, Reference Isolate over the

same periods. Differences in the mean lesion area with time for sheep challenged with the VLA Reference isolate were not significant (P=0.083). In contrast the variations in lesion area with time between study groups challenged with the St.Brenard isolate were highly significant (P<0.001).

Mite burdens for both the VLA Reference isolate and St.Brenard isolates varied considerably between individual sheep as well as between the two isolates. In comparison to lesion area, the differences in mite burden with time within groups of sheep infested with the VLA Reference isolate were not significant at the 5% level (P=0.200) but differences in the mite burdens with time between groups of sheep infested with the St.Brenard isolate were highly significant (P=0.003).

Subclinical lesions presented low mite burdens (ie. mite numbers per sheep below the 25 adult females) for 14 to 35 days in sheep infested with the VLA Reference isolate. From then on mite numbers gradually increased, although climax populations were not achieved during the time period of the individual studies. A long subclinical phase of 21 to 35 days was observed in two studies and a shorter subclinical phase of 14 to 21 days was observed in the remaining three studies. The duration of the subclinical phase also varied with individual sheep, ranging between 35 to 49 days. An overall subclinical phase of 21 to 28 days was observed in sheep challenged with the St.Brenard isolate. Mean and maximum mite burdens climaxed (with exceptionally high numbers compared to the VLA Reference isolate) 49 to 63 days PC, then declined rapidly, often to extinction.

There was a strong positive correlation between lesion area and mite burden for the VLA Reference isolate (r = 0.7783), but no correlation was observed between lesion area and mite burden for the St.Brenard isolate (r = 0.3628). The populations of the VLA Reference isolate are therefore more stable than the field isolate regarding their pathogenicity.

A further sixteen geographical field isolates of *P. ovis* from the British Isles were compared with the then 25 year old Weybridge reference isolate, with respect to their speed of lesion production, rate of mite population increase, touch

hypersensitivity response. Populations of the sheep scab mite can vary in virulence. Analysis of variance revealed highly significant ($p<0.001$) differences between the isolates for both mite burden and lesion area.

Considerable differences in lesion area were observed between the geographical isolates of *P. ovis* 28 days post challenge. Some "low virulence" isolates produced relatively small lesions (mean lesion area 26.4 cm^2 / 0.6% body cover (Princetown)) compared to other "high virulence" isolates, producing more extensive lesions (mean lesion area 928.0 cm^2 / 19.4% body cover (Market Drayton)) over the same time period. Considerable differences in mite burdens were also observed between the geographical isolates of *P. ovis* 28 days post challenge. The "low virulence" Princetown isolate presented a mean *P. ovis* burden of 12.8 mites per lesion (range 0.0 to 30.0 mites per lesion) compared to 139.3 mites per lesion (range 65.0 to 220.0 mites per lesion) for the "highly virulent" Market Drayton isolate. The differences between the years for VLA Reference isolate were not significant ($p=0.069$ for mite numbers and $p = 0.060$ for lesion areas). In contrsat there were highly significant ($p < 0.001$) differences among the other isolates for both mite numbers and lesion areas. *P. ovis* isolates were designated "medium virulent", if the mean lesion area for the study group fell within the range of the corresponding mean lesion areas recorded for the study groups challenged with the VLA reference isolate (ie: between Study 41/2: 151.0 cm^2 / 3.2% body cover and Study 41/5: 500.0 cm^2 / 10.5 % body cover). Consequently isolates were designated "low virulence" if the mean lesion area for the study group fell below this range and "highly virulent" if the mean lesion area for the study group was greater than this range.

Up to the turn of the century there was free movement of live sheep between Britain and Australia, Europe, New Zealand north America, South Africa and south America, a large proportion of consignments infested with scab (Page, 1969), allowing for a continual influx of new genetic material. Since the apparent eradication of sheep scab from Britain and its reintroduction in 1973 theoretically all populations of *P.ovis* originated from this one Irish population imported into Britain. The Sheep Scab Order (1992) defined sheep scab as an infestation of *Psoroptes* mites, regardless of trhe species or location of infestation. Thus aural *P.ovis* and *P.cuniculi* were

defined as scab and consequently we may never have eradicated ovine *Psoroptes* and therefore "technical sheep scab" from Britain. An important question is whether sheep with pre-existing aural *P.cuniculi* infestations (Chapters 2.1 and 2.2) can be colonised simultaneously by aural *P.ovis* (Chapter 2.6). If this is so could they inter-breed and thus add to the genetic pool.

In the USA Roberts *et al.,* (1971) recorded that infestations could escape detection for over a year. Long periods of latency and a sudden increase in vigour and pathogenicity of a mite strain could account for unexplained outbreaks of disease (Roberts and Meleney, 1971).

6.0 Morphological Differentiation

Hirst (1922) was the first to use the length of the male L_4 outer opisthsomal setae (L_4OOS) to differentiate species of *Psoroptes*, but apart from this feature, found little structural differences between them. He suggested that, with the exception of *P. natalensis,* in which the L_3OOS and L_4OOS of the male are distinctly flattened and blade like, mites in the genus *Psoroptes* should be regarded merely as races or slight varieties of a single species (*P. communis*). However Sweatman (1958a) observed that in *P. natalensis* the L_4OOS and the inner seta (L_2OOS) were equal in length, whereas in *P. ovis* the outer seta is one third as long as the inner seta. If on the other hand both setae are equally long and threadlike then it is difficult to differentiate *P. natalensis* from *P. equi*.

Chapter 4.3, compared the relative lengths of the male L_4OOS, confirm and modify the observations of Sweatman (1958) in that *P. natalensis* (mean L_4OOS = 304.5 ± 57.9 μm) and *P. equi* (mean L_4OOS = 193.6 ± 45.6 μm) are significantly different ($p<0.001$) from each other and from *Psoroptes* isolates originating from sheep ears and body, cattle, rabbits and goats. Chapter 4.3 reported a considerable overlap in the comparative lengths of the male L_4OOS between populations of *P. ovis* and *P. cuniculi*, so much so that they could not be differentiated by this morphological criteria, thus supporting similar observations by Wright *et al.,* (1983).

Mites isolated from the ear canals of goats and sheep possessed the shortest setae (mean lengths 50.0 μm to 74.0 μm) and were predominantly "pure" *P.cuniculi* (ie. with setal lengths below 74.0 μm), but mites isolated from the ears of rabbits varied considerably, and fell into two highly significany ($p = 0.0001$) groups: short setal populations (mean lengths 54.8 μm to 79.2 μm) with corresponding medium to high frequencies of "pure" *P.cuniculi* (41.9 to 100%) and non-infestive to the bodies of sheep and long setal populations (mean lengths 98.5 μm to 100.3 μm). Long setal length populations tended to originate from extra-auricular lesions with the frequency of "pure" *P.cuniculi* relatively low (7.6 to 29.4%) and were not shown to be infestive to sheep until selected for by ivermectin (Chapter 4.2). A narrow band of setal lengths (80.0 μm to 92.6 μm), mainly from populations of bovine and ovine *P.ovis* divided the two groups of rabbit *P.cuniculi*.

Male L_4OOS were measured within 22 populations of *P.ovis* (originating from 16 geographically and temporally distinct isolates), producing active ovine psoroptic mange. The mean length of the male L_4OOS for *P.ovis* ranged between 66.8 and 100.9 μm.

There appeared to be a relationship between the length of the male L_4OOS and population virulence. The Penderyn isolate, presenting low virulence infestations when qualitatively compared to the VLA Reference Isolate, also demonstrated an extremely short setal length (mean 66.8 μm). The mean setal length for the Witney isolate, designated "low virulence" in Chapter 3.2, was 77.6 μm. The combined mean setal length for these two low virulence population was 73.6 μm. Whereas the mean setal lengths for the "medium" (VLA Reference Isolate 1988 and 1995) and "high virulence" (Alston, Arlington, Little Melton and Market Drayton) populations of *P.ovis* were 82.0 and 90.5 μm, respectively. Differences between setal lengths for the low, medium and high virulence populations were highly significant ($p = <0.0001$). The percent "pure" *P.cuniculi* in these low virulence isolates were also relatively high (34.0% for the Witney population and 50.0% for the Penderyn population) compared to the medium virulence VLA Reference population (11.1%) and the high virulence isolates (0.0% to 22.9%).

The mean lengths of the male setae of mites recovered from the cryptic sites (inguinal pouches) varied significantly from body mites (p = 0.0186). Wright *et al* (1984) demonstrated that the length of the L_4 OOS varied with the isolate of *Psoroptes*. Their "mission strain" of *P ovis*, a *Psoroptes* sp derived from bighorn sheep in Idaho and cattle mites from Brazil (setal lengths of 118.0, 140.3 and 219.3 μm respectively) were all significantly different from all other *Psoroptes* isolates; Rabbit *P cuniculi* from Kerrville (Texas), England (CVL) and New Mexico were all significantly different (87.4, 74.3 and 66.5 μm respectively); cattle *Psoroptes* from USA (134.0 μm) were significantly different to cattle mites from Brazil (219.8 μm (*P natalensis*?)) and the "Mission strain" of *P ovis* (118.0 μm) was significantly different from *P ovis* from Albuquerque, England (CVL) and South Africa (86.5, 95.7 and 100.5 μm respectively). Thus demonstrating geographical differences in morphology within species of *Psoroptes*.

Differences in setal length were significant (p = 0.0155) for the VLA Reference Isolate of ovine *P.ovis* cultured on sheep or cattle, 80.0 and 83.8 μm respectively. The mean setal lengths for male *Psoroptes* from the Borders population were significantly longer than all the bovine *P.ovis* populations examined, with 84.0% of the population measuring above 258.0 μm, thus fitting the morphological criteria for *P.natalensis* (Sweatman, 1958). The equine population of *Psoroptes* also presented long setae (mean 193.6 μm), significantly different from all the *P.ovis* and *P.cuniculi* populations examined. Setal length may not therefore be a marker to indicate the potential of a particular *Psoroptes* population to establish on the bodies of sheep.

Differences were highly significant (p = < 0.0001) between the mean setal lengths of male *Psoroptes* collected from the body and the ears of goats and also between goat body mites and other *Psoroptes* populations. Similarly the populations collected from the Barbary Sheep were highly significantly different from all other *Psoroptes* populations compared. Setal lengths from both the goat body and Barbary Sheep populations were predominantly (93.2 and 95.0%, respectively) within the overlap of *P.cuniculi/P.ovis*.

Boyce *et al.,* (1990) attempted to provide information on the phylogenic relationships between *Psoroptes* mites found on different hosts using discriminant analysis employing nine morphological characters and found that the length of the male L_4 OOS and the lateral margins of the opisthosomal knobs of the male were the two most important characters for grouping mites according to host species. Mites could clearly be separated within allopatric populations (populations within a species, which do not occur together but have mutually exclusive distributions) of bighorn sheep, rabbits and cattle into discrete groups. However differences were not detected between mites collected from sympatric populations of infested mule deer and bighorn sheep, suggesting that these mites are not host specific and represent a single interbreeding population. Differences also were not detected among mites collected from the ears and body of bighorn sheep and rabbits, demonstrating that the location of mites on a given host should not be used as a primary criterion in species identification. Morphometric classification is therefore influenced by spatial relationships between hosts (allopatry versus sympatry)

7. Molecular Typing

Taxonomic relationships among mites should not be solely based on phenotypic typing (morphology), genotypic typing may be more useful (Strong and Halliday, 1992). Zahler *et al* (1998) investigated the heterogeneity of the ITS2 (second internal transcibed spacer) region in *Psoroptes* and demonstrated little or no sequence divergence between 'species' of *Psoroptes* previously differentiated on morphological grounds by Sweatman (1958a). Goddard *et al.,* (1998) compared mitochondrial DNA sequences of individual mites collected from a variety of hosts and geographical locations throughout the British Isles and revealed low sequence divergence within the isolates of the sheep scab mite (*P ovis*) although distinct differences were observed among different 'species' of *Psoroptes*: ie between *P ovis* (sheep), *P cuniculi* (rabbit ears) and *P cuniculi* (goat ears), although these differences were not significant at 'species' level, but may indicate 'varieties' within a species.

8. Effects of Acaricides

Variations also exist in the susceptability of isolates to acaricides. Roberts and Meleney (1971) observed that more "aggressive" strains of *P. ovis* were less

susceptable to the organophosphate acaricide, coumaphos compared to less aggressive strains, where complete control was achieved. In South America the sheep scab mite (*P. ovis*) developed resistance to plunge dips containing the organochlorine, lindane (γ HCH/ γ BHC) in 1962 (Ault *et al.,* 1962) and to the organophosphate, diazinon in 1965/66 (Rosa and Lukovich (1970). In Britain *P. ovis* has recently developed resistance to the synthetic pyrethroid, flumethrin (Synge *et al.*, 1995) and the organophosphate, propetamphos (Clark *et al.,* 1996). All isolates demonstrating resistance to synthetic pyrethroid plunge dips were all classified as 'virulent', with associated high mite burdens (Bates, 1997d).

The efficacy of injections of the endectocide ivermectin appears to vary with populations of rabbit *P. cuniculi*. Wilkins *et al.,* (1980) in Texas, USA and Pandey (1989) in the UK reported that single injections of ivermectin, at a dose rate of 200 μg per kg body weight, cured psoroptic ear canker, but Wright and Riner (1985) required injections at a higher dose (400 μg/kg body weight) to achieve the same result. Prosl and Kanout (1985) in Germany failed to eradicate *P. cuniculi* at either 200 μg or 400 μg/kg body weight.

In the preliminary development of ivermectin, studies in Texas by Wright and Riner (1985) demonstrated that single subcutaneous or intramuscular injections of ivermectin (at 200 μg/kg body weight) were inadequate in eliminating either *P. cuniculi* or *P. ovis* from rabbits, yet single injections at 400 μg/kg body weight ivermectin (either intramuscular or subcutaneous) eliminated *P. cuniculi* from all infested rabbits but *P. ovis* was eradicated from only 50% of infested rabbits. The exact reason for this phenomena is still not understood since *P. ovis* infesting rabbits ingest nine times more red blood cells than *P. ovis* on cattle (Wright and Deloach, 1980, 1981) and single subcutaneous injections of ivermectin were 100% effective in eradicating *P. ovis* on cattle (Losson, 1996 and Meleney, 1982).

Similar studies were repeated in Chapter 4.2, but in addition the relative pathogenicities of the pre- and post- ivermectin exposed populations of *P. cuniculi* to sheep were also investigated. *P. cuniculi,* originating from commercial colonies in Britain, were assessed for their infestivity to sheep. No sheep developed clinical

mange when challenged with any of the original *P. cuniculi* isolates but clinical scab developed on sheep infested with mites originating from the extra auricular lesions and one out of two sheep infested with mites originating from the post-ivermectin population. Populations of *Psoroptes* infesting rabbits can therefore contain sub-populations of both *P. cuniculi* (non-infestive to sheep) and *P. ovis* (infestive to sheep) and these sub-populations can be selected for by ivermectin injections. These two sub-populations may represent the two populations of *P. cuniculi* ("K" and "L") identified relative to morphological and *in vitro* survival by Von Ribbeck and Gehrt (1974).

Single injections of ivermectin (200 μg/kg body weight) are effecive against *P. cuniculi* infesting the ear canals of sheep but the efficacy of single ivermectin injections against sheep scab mites (*P. ovis*) is dependent on the relative virulence of the infesting population: low virulence populations can be eradicated with a single injection, but highly virulent isolates required two injections to achieve total eradication (Bates, 1994). Bates and Groves (1991) demonstrated that the efficacy of a single subcutaneous injection of ivermectin (at a dose rate of 200μg/kg body weight) was related to the mite burden at the time of treatment: ie. more mites survived treatment if the pre-treatment burden was high. This was attributed to a corresponding high 'sub-population' of non feeding pharate mites between moults, within the pre-treatment population and therefore not susceptible to ingested acaricides. Thus more mites survive a single injection of ivermectin in the 'virulent' isolates with characteristic high mite populations. Variations in the relative efficacy of single injections of ivermectin against *P.ovis* around the World may be the result of local population variation.

9. Cross Mating and Cross Antigenicity

Wright *et al.,* (1983) sucessfully cross mated (bovine) *P. ovis* with *P. cuniculi* from a rabbit, the reciprocal crosses were infestive to both cattle and rabbits and suggested that *P. ovis* and *P. cuniculi* were not reproductively isolated, but distinct races of the same mite, differentiated by host preferences.

P. ovis and *P. cuniculi* also share common antigens, but also have antigens unique to each other (Fisher, 1972; Fisher and Wilson, 1977; Rafferty and Gray, 1987). In fact antigen made from *P. cuniculi* mites are commonly used in ELISA techniques serodiagnosing *P. ovis* infestations (Boyce *et al.*, 1991). ELISA and immunoelectrophoresis reactivity patterns of infested rabbit sera suggested that extracts of *P. ovis* and *P. cuniculi* were antigenically similar (Fisher and Wilson, 1977). Both *P. ovis* and *P. cuniculi* produced 8 cross reacting antigens.

Boyce and Brown (1991) using immunoblotting techniques with defined antigen and antisera demonstrated extensive and nearly complete antigen cross reactivity between *Psoroptes* isolated from bighorn sheep, mule deer, cattle and rabbits.

10. Conclusions

P ovis and *P cuniculi* are therefore synoxenous, occuring sympatrically on the same host (sheep) and may be syntopic (sharing the same habitat, ie the ear canal) and are expressions of different phenotypes within a single species. Studies at Weybridge have demonstrated that artificial challenges of the sheep scab mite (*P ovis*) will readily colonise the withers, flanks and the brisket of recipient sheep, but would not colonise the head, rump or ventral surfaces until reached there by the progressing lesion itself (Bates, *unpublished observations*). Mite populations must therefore adapt to the changing skin environment (ie. changes in humidity and temperature caused by inflammation or wool loss, hyperkeratinisation of the skin leading to reduced feeding areas and the increased immune response of the sheep as the lesion progresses). Do populations of *Psoroptes* on sheep pre-adapt to these constraints by constantly changing the proportions of the pathogenic *P ovis* and relatively non-pathogenic *P cuniculi* 'variants'? Populations high in the '*P cuniculi*' variant being less virulent than those high in the '*P ovis*' variant. It is suggested in this thesis that they are probably expressions of different phenotypes within a single species.

Analogies to the problems in speciating *Psoroptes* can be drawn from research carried out on the scabies mite, *Sarcoptes scabiei*. Prior to 1978 there were 30 species and 15 varieties of *S. scabiei*, based upon morphological characteristics without

taxonomic value, such as the extent and size of the cuticular scales and the size and shape of the antero-dorsal shield. Fain (1978) examined populations of *Sarcoptes* from different hosts and geographical locations and deduced that there was only one variable type species, *Sarcoptes scabiei*. Fain (1978) defined three sources for this variability within *Sarcoptes*: variation within isolates obtained from two different hosts (host variability), variation within a single isolate infesting a single host (individual variability) and variation with hosts from different locations (geographical variabilty). Each isolate of *Sarcoptes* was a combination of 'variants' and that isolates infesting various hosts could be distinguished only by the different proportions of these variants. Each host group possesses a finite combination of variants, morphologically adapted to these hosts and each isolate of *S. scabiei* is able to infest any host group simply by modifying the proportion of these variants. This may require a second generation and in most cases the mite populations are rejected before adaptation occurs. However colonisation may be successful if a predisposing condition is present in the new host, such as decreased immunity.

In conclusion it is suggested here that *Psoroptes* infesting sheep (ie. *P. ovis* and *P. cuniculi*) are not reproductively or ecologically isolated but are phenotypic variants of the same species. Populations vary in the relative proportions of the '*P. cuniculi*' and '*P. ovis*' variants. Thus populations high in *P. cuniculi* act almost as commensals of the ear canal and populations high in *P. ovis* act as highly pathogenic agents of mange. It is suggested here that the type species *Psoroptes communis* should be re-instated, with two variants infesting sheep, *P. communis* var *ovis* and *P. communis* var *cuniculi*.

Chapter 5.2

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Appendix One

Sheep *Psoroptes ovis* Isolates

1. **Aberystwyth (1995):** Mites were isolated from an infested flock at Aberystwyth, Dyfed in October 1995 from material submitted by Carmarthen Regional Centre (Veterinary Investigation Centre), from a flock infested with suspect synthetic pyrethroid resistance mites. Controlled dippings at the VLA, Weybridge demonstrated that the population was susceptible to flumethrin. Mites were cultured *in vivo* at the VLA, Weybridge.

2. **Alston (1995):** Mites were isolated from an infested flock at Alston, Cumbria in November 1995. Swaledale lambs were prophylactically dipped in Bayticol Scab and Tick Dip (6.0% flumethrin, Bayer UK Ltd), at the sheep scab strength (44 ppm) on the 11th of September 1995. Dipping did not reduce clinical symptoms and the lambs were again dipped in Bayticol on the 19th of September 1995 (at a "higher than normal strength") on the 21st and 30th of September and the 24th and 31st of October. Draft ewes, also present on the home premises, showed similar clinical symptoms and had been dipped twice in flumethrin dipwash. On the 20th of November 1995 all lambs were well grown but in poor body condition. Intense itching was evident, with many lambs scratching, nibbling or shaking their heads. Wool loss was evident, although in some cases the wool had begun to regrow. Live *Psoroptes ovis* were still present and cultured *in vivo* at the VLA, Weybridge. Controlled dippings at the VLA confirmed that the isolate was resistant to flumethrin.

3. **Arlington (1995):** Mites were isolated from infested sheep at Cleave Farm, Arlington, Barnstaple, Devon in July 1995. The flock was last dipped at least 6 months previous to examination in an organophosphate based acaricide (make not recorded). Ivomec Injection for Sheep™ (1.0% ivermectin. MSD Agvet) was administered to infested sheep in the spring. Ivomec instructions were not followed to the full, with only the sheep showing obvious clinical symptoms receiving a single injection and then being immediately returned to the untreated flock. The infested flock was involved in a commercial acaricide field efficacy study and mites were cultured *in vivo* at the VLA, Weybridge.

4. **Bacup (1991):** Mites were isolated on the 9th of October 1991 from skin scrapings submitted to the VLA, Weybridge for the confirmation of sheep scab. Approximately 100 sheep out of 387 were presenting clinical signs of sheep scab at Mitchell Field, Nook Farm, Stackstead, Bacup, Lancashire and mites were cultured *in vivo* at the VLA, Weybridge.

5. **Caithness I (1994):** This was one of the original flumethrin (synthetic pyrethroid, SP) resistant isolates described by Synge *et al.,* (1995) isolated from a flock at Wick, Caithness, Scotland in October 1994. The flock was plunge dipped twice in Bayticol Scab and Tick Dip (6.0% flumethrin, Bayer UK Ltd) at the higher tick strength of 66 ppm flumethrin, the second time supervised by staff from Bayer UK Ltd on the 27th of September 1994. Clinical sheep scab was not resolved after either treatment. SP pour-ons were not used on the flock. Live *Psoroptes ovis* and skin scrapings were received from by SAC Veterinary Services Thurso and used to challenge two sheep at the VLA, Weybridge on the 7th of October 1994 and two infested sheep were purchased from the infested flock and maintained at the VLA. Controlled dippings at the VLA confirmed that the isolate was resistant to flumethrin.

6. **Caithness III (1995):** Mites were isolated from a flock in Caithness, Scotland, contiguous to were the Caithness I (1994) isolate originated. The flock was dipped in Flyte 1250 (40% propetamphos. Robert Youngs) on the 18th of October 1995, but live *Psoroptes ovis* were observed the following month. Scab and live mites were submitted by SAC Veterinary Services, Thurso on the 29th of November 1995 and cultured on sheep at the VLA, Weybridge. Controlled dippings at the VLA confirmed that the isolate was resistant to propetamphos

7. **Calne (1989):** Mites were isolated from a field case originating in Calne, Wiltshire on the 23rd of March 1989. The infested flock consisted of 700 plus Welsh Mountain ewes under welfare investigations. The population was not cultured at the VLA, Weybridge.

8. **Compton Dundon (1989):** Mites were isolated from a field case originating in Compton Dundon, Somerset on the 9th of February 1989. The population was not cultured at the VLA, Weybridge.

9. **Dorchester (1988):** Mites were isolated on the 18th of February 1988 from skin scrapings submitted to the VLA, Weybridge for the confirmation of sheep scab. Scrapings were taken from infested sheep at Old Henley Farm, Buckland Newton, Dorchester, Dorset and since maintained on sheep at the VLA, Weybridge.

10. **Llanfach (1991):** Mites were isolated on the 29th of January 1991 1988 from skin scrapings submitted to the VLA, weybridge for the confirmation of sheep scab. Scrapings were taken from infested sheep Rhos-y-Gaer, Llanfach, Ynys Mon, Gwynedd. The focus of infestation centered around Holyhead, Lladdewrant, Bodedern (Ynys Mon, Gwynedd). Only one sheep out of seventeen was visibly affected with wool loss and scab formation over a 30.0 cm diameter area over the lower abdomen. Mites were subsequently maintained on sheep at the VLA, Weybridge.

11. **Little Melton (1990):** Mites were isolated on the 27th of March 1990 from skin scrapings submitted to the VLA, Weybridge for the confirmation of sheep scab. Scrapings were taken from infested sheep at Beckhithe Farm, Little Melton, Norwich, Norfolk (infested premises Holly Tree Farm, Little Melton, Norwich, Norfolk) where 25% of 450 sheep were visibly affected. Mites were subsequently maintained on sheep at the VLA, Weybridge although the original recipient sheep died of a fit.

12. **Market Drayton (1995):** Mites were isolated from sheep at Beeches Farm, Ollerton, Market Drayton, Shropshire. Scab was identified in a 120 head lamb flock but only one animal in the flock of 240 ewes was shown to be infested with scab. The Clun in-lamb ewe, with haematomae of both ears, was seen repeatedly rubbing it's ears against it's flanks (which were blood stained from the burst haematoma). The flock was dipped on the 14th of December 1995. The infested ewe was purchased by VLA, Weybridge on the 18th of December 1995 and mites

were subsequently maintained on sheep at the VLA, Weybridge. Source Chris Lewis, Shrewsbury Regional (Veterinary Investigation) Centre.

13. **Penderyn (1988):** Originated from a pooled sample of mites isolated from inter-connected field cases in South Wales (Powys, Gwent and Mid Glamorgan) in November 1988. All infested premises were connected via sheep contact at Penderyn Market, Mid Glamorgan. Qualitative observations at Weybridge, on sheep heavily infested with this isolate demonstrated no obvious behavioural or clinical signs of active scab, despite a lesion covering over 80% of the body, extending over the back, flanks, sides and belly. The population were subsequently maintained on sheep at the VLA, Weybridge.

14. **Porlock (1994):** Mites were isolated from sheep at Westcott Farm, Porlock, Somerset in November 1994. Sheep scab was contracted via agisted sheep at Crediton, Devon, returning to the home property sheep were seen rubbing and scratching, and suspecting chewing lice (*Bovicola ovis*) were treated with a synthetic pyrethroid pour on (2.5% cypermethrin) with little effect. The flock was consequently plunge dipped, three consecutive times in Coopers Green Label Scab Approved Dip (6.0% flumethrin), the first two at sheep scab strength (44 ppm flumethrin), again with little effect. The last dipping was at tick strength (66ppm), taking place on the 26th and 27th of July 1994. Synthetic pyrethroid pour-ons had been used routinely for tick control in the spring and the autumn and all ewes were treated on turnout onto common grazing, after July. On the 7th October 1994, 50 store lambs were examined, with approximately 30% shown to have active sheep scab. Oramec (0.8% ivermectin) drench was not used on the premises. Mites within the flock were suspected of being resistant to synthetic pyrethroid (SP) dips, subsequent *in vivo* investigations at the VLA, Weybridge confirmed SP resistance. This was the second SP resistant isolated described by Synge *et al* (1995). The population were subsequently maintained on sheep at the VLA, Weybridge.

15. **Princetown (1991):** Mite were isolated on the 18th of September 1991 from skin scrapings submitted to the VLA, Weybridge for the confirmation of sheep scab. Scrapings were taken from infested sheep at Beardown Farm, Two Bridges,

Princetown, Lydford, Devon (Dartmoor). The population were subsequently maintained on sheep at the VLA, Weybridge.

16. **St.Brenard (1989):** Mites were isolated on the 13th of August 1989 from skin scrapings submitted to the VLA, Weybridge for the confirmation of sheep scab. Scrapings were taken from infested sheep at Whiteheads Farm, St Brenard, Cornwall and since maintained at the VLA, Weybridge.

17. **VLA Reference Isolate:** The original isolate was derived from a field outbreak before eradication and was maintained at the VLA (CVL) Weybridge in the period between 1953 and 1973, when sheep scab was eradicated from Great Britain. The original isolate was augmented in 1973 with mites from the first foci of infestation in Lancashire on re-introduction of sheep scab in 1973. Mix cultures of mites isolated from Plattfold Farm, Worsley, Lancashire on the 1st of January 1973 and Thorne, Holme and Wood End Farms, Dunsop Bridge, Clitheroe, Lancashire on 4th of January 1973. The isolate has since been maintained *in vivo* at the VLA, Weybridge, and has been shown to be susceptable to the organochlorine (OC) γ HCH (γ hexachlorcyclohexane, γ benzenehexachloride (γ BHC), lindane), the organophosphates (OPs), diazinon and propetamphos, the synthetic pyrethroids (SPs), flumethrin and high cis cypermethrin (HCC) by plunge dipping and the endectocides ivermectin and moxidectin by subcutaneous injection at a dose rate of 200 μg per kg sheep body weight 7 or 10 days apart respectively and doramectin by a single intramuscular injection at 300 μg per kg sheep body weight.

18. **VRL Dublin (1995):** The original isolate was received on the 1st of December 1995 from the reference strain maintained at the Department of Agriculture, Food and Forestry, Veterinary Research Laboratory (VRL), Abbotstown, Dublin, Republic of Ireland. The isolate was supplied by Dr D J O'Brien, VRL, and since maintained at the VLA, Weybridge.

19. **Witney (1996):** Mites were isolated from infested sheep at New Foundout Farm, Witney, Oxfordshire in July 1996. The flock of 800 ewes and lambs were treated with ivermectin (Ivomec for Sheep. 1.0% ivermectin. Merial) on the 26th of October 1995 and dipped on the 4th of December in Flyte 1250 (40% propetamphos. Youngs Animal Health). The isolate was suspected to be organophosphate resistant, but was shown to be susceptable through controlled field and laboratory investigations. Live mites were cultured *in vivo* at the VLA, Weybridge. Source John Dallyn, Contract Dipper.