MEAT & LIVESTOCK
AUSTRALIA

On farm

A Survey of Potential Wildlife Reservoirs for *Mycobacterium paratuberculosis*

Project number TR.054

Final Report prepared for MLA by:

Department of Natural Resources and Environment
June 2000

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Published by Meat & Livestock Australia Limited
March 2002

ISBN 1 74036 261 6

Animal Health & Welfare

TABLE OF CONTENTS

1. Objective

To examine potential wildlife reservoirs of Johne's disease in Victoria.

2. Introduction

In any disease control program eradication of disease depends upon the protection of disease free animals from potential sources of infection. Sylvatic hosts have the potential to thwart eradication campaigns Wild bovids and ungulates are a source of bovine tuberculosis for North American cattle herds. Two non ruminant wildlife reservoirs, possums and badgers in New Zealand and England respectively, have hampered eradication of bovine tuberculosis.

The presence of a common wildlife reservoir of Johne's disease could render the eradication of Johne's disease impossible or compromise current control measures. In Scotland, rabbits have been demonstrated to be infected with *M. paratuberculosis* (Grieg et al 1997). In order to assess the risk posed to Johne's disease control due to wildlife this survey examined rabbits and another herbivore commonly found on Australian pastoral land, eastern grey kangaroos.

3. European Rabbits

3.1 Aim

To survey European rabbits *(Oryctolagus cuniculus*) for the presence of *Mycobacterium paratuberculosis* in areas with endemic Johne's disease in sheep and cattle.

3.2 Method

In 3 areas where Johne's disease was present in livestock, rabbits were collected and examined for the presence of *Mycobacterium paratuberculosis*. Property A was a series of three contiguous farms with a long history of Johne's disease in sheep, Properties B (beef) and C (dryland dairy) were farms with history of Johne's disease in cattle. A minimum of 100 rabbits were examined from each area.

Rabbits were shot at night and necropsied within 12 hours of death. Weight, sex, and any gross pathological changes were recorded. A 20 cm section of ileum, ileo-caecal junction, ileum and mesenteric lymph nodes were taken and halved for histology and culture and faeces, when present, were kept for culture. Fresh tissue samples were pooled, stomached and processed as one sample. Samples were processed according to the relevant Australian Standard Diagnostic Technique and inoculated in BACTEC 12B (Becton Dickinson) media supplemented with Mycoabactin J (Allied Monitor), 10-17% eggyolk and PANTA plus (Becton Dickinson). Histological sections and faeces were stored for future reference.

3.3 Properties and Sampling

Farms were chosen on the basis of their high prevalence of Johne's disease in their livestock, either sheep or cattle, a high reported rabbit density in close contact with stock and the willingness of the manager to assist in the survey.

The sample size was determined by using Freecalc. A sample size of 100 animals will detect a disease prevalence of 3% or more at 95% confidence limits. Estimates were based on the assumption of the test having a perfect sensitivity and specificity. This gives a Type 1 error of 0.05.

3.3.1 Property A

A contiguous series of 3 farms in East Gippsland, with a purported long history of ovine Johne's disease (OJD) and significant mortalities due to clinical OJD. A study examining sheep from these farms, revealed Johne's disease prevalence of 21 - 37 % (Hope et al 2000). These farms were destocked in January 1997 and rabbits were sampled in March of that year.

3.3.2 Property B

A commercial beef operation of 200 breeders between Geelong and Ballarat, with a history of Johne's disease of at least three years. In 1996 this property had 9 clinical cases of Johne's disease. The farm also ran a commercial merino flock and had a small flock of 9-10 feral goats.

3.3.3 Property C

A commercial dairy farm of 131 milkers in the Peterborough region of South West Victoria. The first case of Johne's disease on the farm was diagnosed in 1996. A whole herd investigation in 1999 which was at the same time as the rabbit survey resulted in a faecal culture positive rate of 10%.

Property	Enterprise	Location	Number of specimens	No of collections
A	Merino	Ensay Valley	100 rabbits, 1 hare.	3 nights, 18/3/97-20/3/97.
B	Beef Cattle	South Ballarat	104 rabbits, 1 hare 3 goats.	3 nights 5/3/99, 15/06/99, 4/06/99.
C	Dairy Cattle	Peterborough	106 rabbits, 2 hares.	3 nights 3/06/99, 21/06/99, 21/07/99.

3.4 Results

In total 310 rabbits, 4 hares and 3 feral goats were examined by culture.

No *M. paratuberculosis* was confirmed in any of the animals sampled. Eight of 314 samples demonstrated growth of organisms other than *M. paratuberculosis.*

3.5 Discussion

Several reviews have described the occurrence of Johne's disease in wildlife and zoological collections (Chiodini et al 1984, Morgan 1999). Wild populations of Bighorn Sheep, Rocky mountain goat, axis, fallow, white tailed, red, sika and roe deer have been reported as being infected with *M. paratuberculosis* (Williams et al 1979, Riemann et al 1979, Chiodini et al 1983, Hillermark 1966, Reid et al 1996).

Rabbits have been experimentally infected with Johne's disease. In two similar studies, 1 day old rabbits were orally dosed with ~10^8 organisms. Three of 16 (19%) and 9 of 21 (43%) had histological evidence of paratuberculosis at necropsy 9-12 months after inoculation (Mokresh et al 1989, Mokresh et al 1990). These studies demonstrated that rabbits are susceptible to *M.*

paratuberculosis but provide little information of the natural ecology of the disease due to the large dose and age of the animals infected.

There are several reports of Johne's disease in wild populations of rabbits in Scotland. A rabbit with intestinal lesions resembling Johne's disease was described on a Scottish deer farm with endemic JD in 1990 (Angus 1990). No cultural confirmation was performed but the lesions closely resembled those of infection in cattle. A total of 32 rabbits were examined on the farm with only one animal having any evidence of Johne's disease. The exposure of these rabbits was due to a "major outbreak" of JD in red deer (*Cervus elaphus*) on an experimental farm where rabbits were in plague proportions requiring regular culling.

The first confirmed report of wild rabbits being naturally infected with *M paratuberculosis* was in the summer of 1995 (Anon 1995). Researchers at the SAC Perth investigated rabbits on four farms in the Tayside region of Scotland. The rabbits were collected on 3 properties where Johne's disease was known and a fourth property where no clinical signs of the disease had been observed (Grieg et al 1997). A total of 33 were sampled with 23 of these being culture positive for *M. paratuberculosis* (one of which was faecal culture positive only), 19 had histological evidence of Johne's disease, 13 were both culturally and histologically positive.

A wider survey was carried out 1996 in Scotland (Grieg et al 1999). This survey involved shot samples from farms with and without a history of Johne's disease. It involved surveys in 7 counties and again included the Tayside region. The survey revealed little infection in areas outside the original survey area of Tayside. From the 169 rabbits, which were not from Tayside there was only one isolate confirmed as *M. paratuberculosis,* and this rabbit was from a farm with no known history of Johne's disease. A total of 87 rabbits from 10 farms with known Johne's disease from outside the Tayside region were examined without *M paratuberculosis* being isolated. Two of 87 rabbits from 2 of 10 farms had histological changes with acid fast bacilli (AFB) present. Eighty rabbits from 8 farms with no known history of JD were also tested with one culturally positive animal and 2 rabbits showing histological changes with the presence of AFB (it is not stated whether the animal is the same). RFLP strain typing revealed that all isolates tested except one were type B_C17 the predominant RFLP type in cattle in the UK. HPLC typing revealed some overlap of strains between species on one farm and none on the other tested.

It appears from the wider Scottish survey that Johne's disease in rabbits outside the Tayside region is uncommon. The Tayside County appears to be a hot spot of lagomorph Johne's disease. Evidence of JD has recently been detected in this area in stoats *(Mustela erminea)* (3 of 5 culture positive) and red fox (*Vulpes vulpes)* (8 of 9 culture positive) (Beard et al 1999). This is in the area of high prevalence of Johne's disease in rabbits, which is presumably the source of infection for the carnivores.

The prevalence and occurrence of Johne's disease in rabbits in the Tayside region does appear to be unusual. The significance is yet to be determined and the reason this area has such a high prevalence of Johne's disease is unknown. The farms were originally surveyed because of the concordance of high levels of Johne's disease and the heavy density of rabbits. The association of lagomorph Johne's disease in this area may be due to a number of reasons; a close interaction between a large rabbit and infected ruminant populations with heavy exposure of rabbits to *M. paratuberculosis,* the age structure of the rabbit populations, a highly pathogenic strain of *M paratuberculosis,* a highly susceptible strain of rabbit in this area, or an unspecified environmental factor in this region which renders ruminants and rabbits highly susceptible to Johne's disease.

Major differences will exist between the ecology of wild populations of rabbits between different areas. In Australia, the proportion of animals that survive over 2 years of age is highly variable and can range from 7 - 17% with an annual survival of adults ranging from 40 - 60% (Williams et al 1995). Population dynamics will greatly affect the expression of a chronic disease such as JD.

Populations with high turnover and very few adults over 2 years of age are very unlikely to show expression of disease. No information about the ecology and population dynamics of the rabbits in Scotland was available.

There is no evidence that strains of *M paratuberculosis* differ in their pathogenicity. It is suggested that some strains, which are more commonly isolated from a particular species demonstrate a host preference for this species but this may be the result of an opportunity to disseminate within a species as opposed to a strain being more pathogenic to a particular host.

A similar argument exists for host susceptibility. Breed susceptibility has been suggested in the past but is difficult to separate from dissemination within a breed (Chiodini et al 1983).

3.6 Implications

The lack of any evidence of Johne's disease in the rabbits in the Victoria survey would suggest that, under the conditions examined, rabbits are unlikely reservoir hosts for Johne's disease. The survey was designed to examine a sufficiently large sample of rabbits to detect infection if it occurred in relatively low levels of 3% with confidence limits of 95%. To "prove" that a disease does not exist in a population the entire population must be sampled with a test of 100% sensitivity and specificity. This survey does not rule out that rabbits may occasionally or rarely become infected with JD but that if it occurs it is likely to occur in low enough numbers as to not be a significant risk to resident populations of farmed livestock.

Because of the limited range of farms and environments in the survey it is impossible to rule out the presence of a "focus" or "hotspot" of JD in rabbits as exists in Scotland.

3.7 Further Research

Large scale surveys for diseases that occur rarely are expensive and logistically difficult. A larger survey examining a wider range of environmental conditions over a larger number of farms with smaller numbers of rabbits per farm similar to the Scottish survey (Grieg et al 1995) would be worthwhile.

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4. Eastern Grey Kangaroo

4.1 Aim

To survey eastern grey kangaroos (*Macropus giganteus*) for the presence of *Mycobacterium paratuberculosis* in an area with endemic Johne's disease.

4.2 Method

Eastern grey kangaroos which were being culled for pest management purposes in central Victoria were necropsied and sampled for the presence of Johne's disease from April 1997 to November 1998.

The sampled farm was approximately 1000 hectares, in 2 portions, consisting of volcanic plains and sedimentary hills. Improved pastures and dams, for water, provided for the self replacing Merino flock and some breeding cattle. An initial diagnosis of ovine Johne's disease was by necropsy of sheep with clinical signs which had been reported by the owner. Following detection of infection on the sample property, six neighbouring properties were confirmed as infected, along with a number of close-by farms. One neighbouring farm had a Johne's disease in cattle confirmed in 1992 and was then destocked of cattle until 1997. On another neighbouring property, clinical cases in sheep were common, more than 18% of ewes and 10% of wethers showed clinical signs and intestinal lesions characteristic of Johne's disease at slaughter.

The sample farm, adjoining farms and roadsides had heavily wooded areas providing ideal habitat for large mobs of kangaroos that readily moved between adjoining properties. The shot kangaroos were adults and sub-adults. Juvenile animals under 1 year of age were excluded for the purpose of this survey.

Sex, location, estimation of age, and any gross pathological changes were recorded. Sections of ileum, ileo-caecal valve, proximal colon, caecum and mesenteric lymph nodes were taken for histology and fresh sections and faeces for culture. Initially all samples were cultured individually but for the last 35 animals sampled, lymph nodes and gut sections were pooled and processed. The culture technique used was in accordance with the Australian Standard Diagnostic Technique, recommendations (Tennant et al 1996).

A total of 100 animals were examined. The sample size was selected to detect a 3% infection level with 95% confidence limits.

4.3 Results

Thirty-seven female and sixty-three males were sampled. Five faecal culture samples had some growth, which was shown to be contaminants, all other faecal samples and tissue samples were found to be negative.

There was no evidence of *M. paratuberculosis* in the kangaroos examined.

4.4 Discussion

Paratuberculosis has been recorded in a wide range of species; no confirmed cases have been recorded in marsupials.

There have been recorded cases of marsupials infected with bacteria from the *M avium* complex in zoological collections and *M bovis* in brush tailed possums (*Trichosurus vulpecula*) in New Zealand (M Lynch pers com, Davidson 1991). The eastern grey kangaroo is predominantly a grazing animal with specific food preferences restricted to grasses and forbes. Their dietary habits overlap those of sheep and they are often in close association with sheep on grazing land.

Eastern greys tends to range from 4 - 5km for family groups and up to 20km for solitary males. All the animals sampled would have had some exposure to pastures grazed by OJD infected animals.

The sampling regime was based on shot samples. Shot samples of kangaroos can often be biased towards males and younger animals. Shot samples often favour males because females tend to associate in family groups with one dominant male, and tend to disperse when a member of the group is shot. This studied favoured males. To avoid a bias towards younger animals, which would have been less likely to have shown evidence of Johne's disease, young animals were deliberately excluded from the study.

The sample size was determined by using Freecalc. A sample size of 100 animals will detect a disease prevalence of 3% or more at 95% confidence limits. Estimates were based on the assumption of the test having a perfect sensitivity and specificity. This gives a Type 1 error of 0.05 or in other words a 5% chance that the survey would misclassify a population as free if there was in fact disease present at 3% or more.

The other potential Type 1 error is with the assumption of prevalence. Surveys should be designed to detect a minimum expected prevalence. The level of JD within an infected cattle herd or sheep flock is likely to be from one animal within a herd to 50% depending on the time the herd has been infected and the opportunity for infection to spread. To declare populations free in this situation where the minimum expected prevalence may be very low is very difficult without doing a complete census of the population with a test that has perfect accuracy.

The probability of detecting diseased animals from an 'infinite' population when sampling 100 animals with an expected prevalence of 2 and 1 is 87 and 63% respectively. Surveys looking for a low prevalence of disease would require large numbers of animals to be sampled (143 for 2% or 277 for 1% at a 95% confidence limit).

4.5 Implications

Although no animals were found infected with JD in this survey it does not rule out that eastern grey kangaroos could become infected with paratuberculosis. It does however demonstrate that infection if it occurs is unlikely to occur at a significant levels and eastern grey are unlikely to act as significant reservoirs of infection of Johne's disease.

4.6 References

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Dr Michael Lynch Senior Zoo Veterinarian Melbourne Zoo Parkville.

Acknowledgments
The authors wish to acknowledge; Animal Health Staff of the Department of Natural Resources and Environment Victoria, Meat and Livestock Australia, for their assistance and financial contributions. Dr Robin Condron, Wendy McDonald, Dr John Galvin and Dr Bill Sykes for their editorial contributions. Paul Tulk, Marios Carajias, Simoun Hakim for their excellent laboratory support.

The cooperating farming families on whose property the kangaroos and rabbits were sampled. Ken O'Meara, his hunting skills and his assistance with sample collection over the 20 month period were essential to the success of this project.