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Prevalence of Johne's disease in rabbits and kangaroos

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1.0 Acknowledgements

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2.0 Summary

Following reports from Scotland that rabbits on JD-infected farms in the Tayside region were infected with *M avium* subsp *paratuberculosis*, a study of rabbits and kangaroos on OJD-infected farms in NSW was commenced. Three hundred rabbits and 300 eastern grey kangaroos from 10 farms grazing OJD-affected sheep flocks were killed and examined for evidence of JD between late 1996 and late 2000.

Two hundred and fifty three rabbits were tested by radiometric culture of their faeces, while 47 were examined by smear and ZN stain of tissues combined with histopathology of the lower small intestine and regional lymph nodes. No evidence of JD or the causative organism was detected in any rabbit.

For kangaroos, 206 were examined primarily by faecal culture and 94 by smear and histopathology. Some animals were examined by faecal culture and histopathology. One kangaroo specimen produced evidence of low numbers of *M avium* subsp *paratuberculosis* in faeces but histopathological examination revealed no evidence of active infection, which might cause multiplication of the bacteria within the kangaroo. It was concluded that the bacteria identified in this animal were bacteria which had been ingested from pasture contaminated by OJD-infected sheep and which had survived passage through the gut.

Considering information available from studies of wildlife in Kangaroo Island and of rabbits in Scotland, we conclude that the prevalence of JD in rabbits and kangaroos on OJD-affected farms in Australia is very low (less than 1% of the adult population) or zero. Nevertheless, we recognise that there is a risk that adaptation of the organism to wildlife hosts could occur in future. There is evidence that the grazing pressures exerted by rabbits and kangaroos on sheep pastures in Australia is similar to that exerted by rabbits on beef cattle pastures in Scotland. We hypothesise that the S strains responsible for all or most of the OJD infection endemic in NSW sheep flocks are more host specific than C strains, particularly the C strains identified in cattle and rabbits in the Tayside region of Scotland.

The positive finding of *M avium* subsp *paratuberculosis* in the faeces of a kangaroo implies that there is a risk of physical transfer of organisms from one farm to another which may lead to transfer of infection from an infected flock to a neighbouring uninfected flock by kangaroos.

These results, together with the overseas findings, suggest that further research activities should be conducted into the host specificity of strains of *M avium* subsp *paratuberculosis* and the species studied should include the common domestic animals farmed in Australia and the rabbit and kangaroo. Further action on this recommendation should be postponed until the current study on KI is completed.

3.0 Introduction

Ovine Johne's disease (OJD) was first diagnosed in sheep in NSW in 1980. By 1993, 37 flocks in the central Tablelands district were known to be infected and, by late 1996, the disease was known to be present on 158 farms in NSW, 28 properties in Victoria and 6 farms on Flinders Island, Tasmania. In that year, a steering committee was established by NSW Farmers Association to develop a strategic plan to control and ultimately eradicate OJD from sheep in NSW. In response to the draft strategic plan, many concerns were expressed that the disease could not be eradicated with the resources likely to be mobilised

from industry and government. Amongst the concerns expressed was the view that wildlife could be a potential reservoir of infection with ovine strains of *Mycobacterium avium* subsp *paratuberculosis* (*M ptb*) and that any attempts at eradication would founder if this was the case. Further, wildlife could be an important source of transmission from an infected farm to a neighbouring, previously unaffected farm and, possibly, a source of lost efficiency of control measures in endemically infected flocks.

3.1 JD in other species

Natural infection of species other than domestic ruminants had been reported before 1996 (see, for example, Sharp et al 1996). Natural infection has also been reported in macaque monkeys (McClure et al 1987). Artificial infection of neo-natal rabbits was known to lead to clinical signs similar to those seen in ruminants and histopathological signs consistent with a JD-like granulomatous enteritis. Natural infection in rabbits had not been demonstrated equivocally, although Angus (1990) had reported on one rabbit in Scotland with histological lesions characteristic of JD. In 1994, Greig et al (1997) examined 33 rabbits from 4 farms in the Tayside region of Scotland. JD was known to occur in cattle on 3 of the farms. *M ptb* was cultured from 22 (67%) of the rabbits. All but 8 of these 22 had histopathological lesions of JD. Fourteen of the 33 rabbits had tissue smears positive for acid-fast bacteria.

4.0 History of project

During early 1996 and following personal communication between Professor JR Egerton and Dr Alastair Greig of SAC, Perth, Scotland, Professor Egerton proposed that Australian wildlife should be examined to determine if they could have a role in the transmission of OJD between flocks. He suggested that there should be a study of the prevalence of JD in rabbits, because of their likely role in JD transmission in Scotland, and eastern grey kangaroos because of their high population densities in many sheep growing areas of the tablelands of NSW and the frequency of shared grazing between sheep and kangaroos.

Following discussions between Mr Bill Sykes and Professor Egerton in mid-1996, work on collection of specimens started on 2 September 1996. The protocol followed at that time required the collection and storage of sections of terminal ileum and mesenteric lymph nodes from killed animals, preparation of smears from tissues and, in some cases, culture of faecal samples. Serum was to be collected and frozen whenever possible.

An agreement was reached between MRC and Prof Egerton, specifying that 300 animals of each species were to be examined by gross histopathology and culture. The contract was signed by KA Abbott in July 1997. Funding was provided by MRC to meet the cost of 400 cultures at a cost of $25 each. No funding was provided for collection or storage costs, histopathology or costs of culture beyond $25. By this time, 104 kangaroos and 47 rabbits had been collected.

Outbreaks of myxomatosis and calicivirus reduced the availability of rabbits during 1997/98. Shortages of both staff and funding for shooters prevented the collection of animals during late 1997 and 1998. In 1999, attempts to encourage the supply of rabbits from OJD-affected farms in the central Tablelands produced 40 animals only.

The University of Sydney farm, Arthursleigh, had been diagnosed as OJD-affected in December 1997. Evidence at the time suggested that the prevalence of infection in the flock was low. By late 1999, however, there was serological evidence of a significant increase in the prevalence of OJD in the sheep flock, supported by the opinion of the farm manager that clinical cases of OJD were apparent. At this time, therefore, the focus of specimen collection switched to that property and, in particular, areas grazed by infected adult sheep.

5.0 Materials and methods

5.1 Selection of properties

The purpose of the study was to detect JD infection in rabbits or kangaroos if it was present at detectable levels, rather than to estimate the prevalence of infection in the wildlife species. Consequently, bias was exercised in the selection of farms, by collecting specimens from farms and areas where the prevalence of infection was believed to be moderate or high and where infection was believed to be well established, rather than recently introduced. The first farms selected were Walwa and two nearby infected farms (Merrill and Oakleigh) situated north of Gunning. Walwa had a history that suggested a well-established infection and a high sheep mortality from OJD, and the owners of Merrill and Oakleigh considered that kangaroo populations were mobile across all three farms as well as other neighbouring infected farms. To complement the sampling of flocks in the southern Tablelands, two farms in the central Tablelands were selected on the basis of established OJD infection, a significant kangaroo or rabbit population and losses of OJD which were considered to be high in one case (15% on Woodoona) and moderate (5% on Elourera) in the other. The collection of kangaroos on these farms were difficult and only 11 animals were killed.

The application of similar eligibility criteria led to the identification of Paling Yards, the farms around Ferndale and Pineview in the central Tablelands and Hillwood in the southern Tablelands.

Arthursleigh is a 7800 ha farm running over 15 000 adult Merino sheep in a self-replacing flock. At the time when wildlife were collected a property disease eradication plan was in force and the infected sheep were separated from the cattle herd and grazed on two thirds of the farm only. There are several large foci of rabbit populations in some areas of the farm and a very large population of eastern grey kangaroos, estimated to exceed 2000. Other information (see discussion) supported the view that kangaroos and rabbits were significantly exposed to JD infection on Arthursleigh. To further enhance the probability of detecting infection if present, collection of rabbits and kangaroos was restricted to paddocks grazed by infected sheep.

5.2 Collection of animals

Kangaroos were all collected by shooting at night with a rifle while held stationary in the beam of a spotlight. The activity was conducted under a Scientific Investigation Licence, issued by NPWS. Rabbits were collected in a similar way except for 40, on CT2 and CT3, which were collected after baiting with fluoroacetate.

5.3 Ageing of kangaroos

Most of the kangaroos shot after November 1999 were aged by the measurement of the length of that part of the hind leg including the tibia, from a point on the skin over the trochlea of the femur to the pad overlying the volar aspect of the fibular tarsal bone. Both the stifle and tarsal joints were flexed and the distance measured with a steel tape. The measured length of leg was then compared with a table produced by Poole et al (1982) and the age of each animal estimated.

5.4 Handling of specimens

Tissues were removed from kangaroos within 10 to 90 minutes of death and refrigerated or placed on ice. In most cases, the tissues removed were intestinal tract, with associated mesentery, and heart. Within 12 hours of collection, small sections of the terminal ileum, ileo-caecal valve area and one adjacent lymph node were placed in sterile tubes, for freezing and later culture, and in 60 ml containers of formal saline. A faecal pellet was collected from the rectum and placed in a sterile 5 ml tube. Blood was drawn from the heart where possible. Faeces and fresh tissues were frozen at -80EC until required for culture. Serum was separated from blood samples and stored at -20EC. Preserved tissues were held at room temperature until prepared for histopathology.

Rabbits were often refrigerated entire until necropsy was possible, usually within 12 hours of collection. Beyond that difference, tissues from rabbits were handled in the same way as described above for

kangaroos. The section of terminal ileum collected from rabbits included the *sacculus rotundus*.

5.5 Smear

Smears of fresh tissues were made by pressing a cut or freshly exposed surface onto a glass microscope slide, air-drying and staining with the Ziehl-Neelsen (ZN) method.

5.6 Culture

Radiometric culture was performed using the method described by Whittington et al (1998). Briefly, approximately 1.5g of faeces was mixed with saline in a 15 ml tube. The faeces is broken up and mixed with the saline with a sterile swab stick then allowed to settle for 30 minutes. Five ml was then removed from the top of the supernatant, avoiding floating debris, and transferred to a 35 ml tube containing 25 ml HPC/BCI. The tube was then placed at 37EC for approximately 24 hours before centrifugation at 900g for 30 minutes. The supernatant was discarded and 1 ml of VAN added to the pellet, which was resuspended. The tube was then placed at 37EC for 72 hours before 0.1 ml was removed to inoculate modified BACTEC 12B medium in a BACTEC vial. The growth index was measured weekly for 12 weeks.

5.7 Pooled culture

Samples from up to 10 animals were pooled as follows. The normal procedure for preparation of faecal samples was followed until the faeces mixed in saline had settled for 30 minutes. The 5 ml supernatant was withdrawn from each of the samples and added to a 50 ml falcon tube and mixed by repeated inversion. A 5 ml sample of the pooled supernatant was then withdrawn and added to the HPC/BCI and the procedure described above was continued.

5.8 Analysis by PCR

The identity of the mycobacterial isolates was confirmed by PCR directed at the IS*900* sequence of *M ptb*, using primers P90B and P91B (Millar et al 1995).

5.9 Histopathology

Fixed tissues from the ileum, ileo-caecal valve and mesenteric lymph node were embedded in paraffin wax. Sections were stained with haematoxylin and eosin and by the ZN method.

6.0 Results

Animals were collected from 10 OJD-infected sheep farms on the central and southern Tablelands of NSW between September 1996 and August 2000. All of the farms were situated in the high-risk areas for OJD, the central and southern Tablelands of NSW, and all had a history of OJD infection (Table 1). Estimates of the level of infection were made, relevant to the time of collection, from the farm operators' estimates of mortality of sheep due to OJD and, in the case of farms ST1, ST2 and ST3, from objective data as well. High losses on CT3 have been supported by independent evidence (Eppleston pers comm). At the time when animals were collected from farms ST4 and ST5 the prevalence of infection was probably low. Subsequently, losses on ST5 rose to very high levels by 1999. Farm CT1 is believed to have a low prevalence of infection but animals were collected from neighbouring farms, which had a moderate rate of sheep losses from OJD. Farm ST1 had a low seroprevalence of OJD in sheep when the study commenced but, by late 1999 when substantial collections commenced, the seroprevalence in the sheep flock was higher and there was clinical evidence of OJD. It could be classified as moderate for prevalence of OJD for the period during which wildlife were collected.

The putative levels of OJD on the source properties are summarised in Table 2. Only 46 rabbits (15%)

came from low prevalence farms. For kangaroos, none came from low prevalence farms and 44% came from highly infected properties. Three hundred wild rabbits (*Oryctolagus cuniculus*) and 300 eastern grey kangaroos (*Macropus giganteus*) were killed and submitted for post-mortem examination from the 10 farms.

Most of the rabbits (250 or 83%) were collected on 4 farms in the southern Tablelands (Table 1) and most of these (209) were collected from one farm. Most of the kangaroos (205 or 68%) were collected from 3 farms in the southern Tablelands. Fifty kangaroos collected in one night from farm CT1 were in fact shot on the farms of several neighbours of CT1, all of which had OJD in their flocks.

Table 1. Farms from which rabbits and kangaroos were collected

Farm code[1]	Farm name	Time period for collections	Sheep losses from OJD	Rabbits collected	Kangaroos collected
ST1	Arthursleigh	Nov 99 - Aug 00	Mod	209	112
ST2	Walwa	Sep 96 - Apr 98	High	10	72
ST3	Hillwood	Feb 99	High	9	21
ST4	Oakleigh	Aug 96	Low	4	0
ST5	Merrill	Sep 96	Low	22	0
CT1	Ferndale	Mar 99	Mod	0	50
CT2	Pineview	June 99	Mod	20	0
CT3	Woodoona	Oct 96 & Jul 99	High	23	6
CT4	Elouera	Dec 96	Mod	3	5
CT5	Paling Yards	Apr & Jul 97	High	0	34
	TOTAL			**300**	**300**

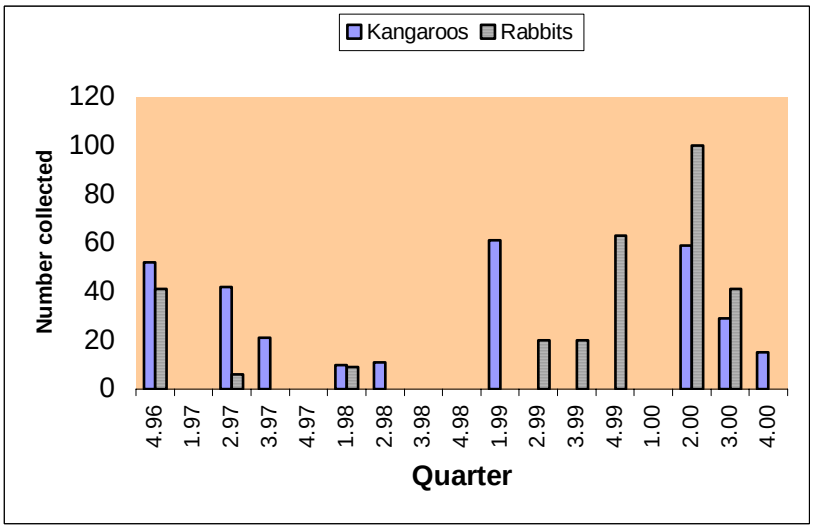
Table 2. Summary of source of specimens by perceived level of OJD losses

	Level of sheep losses			
	Low	**Moderate**	**High**	**Total**
Rabbits	46	212	42	300
Kangaroos	0	167	133	300

Figure 1. Time of collection of rabbits and kangaroos between the last quarter of

[1] ST and CT refer to southern Tablelands and central Tablelands respectively.

1996 and the end of 2000



Kangaroos were collected in late 1996 and mid-1997 principally from ST2 and CT5; in early 1999 from CT1 and ST3 and, during 2000, from ST1 (Figure 1). Rabbits were collected from 8 farms (but only 5 specimens from ST1) from late 1996 to mid-1999 but only from ST1 from late 1999 onwards.

Specimens were collected and processed on a total of 23 occasions. Individual animals were identified R1 to R300 for rabbits, K1 to K300 for kangaroos in the sequence in which they were collected.

6.1 Age of kangaroos

Ninety five kangaroos collected on ST1 were aged by the leg length method. Statistics of leg length are given in Table 3. Males with leg lengths over 523 mm and females over 463 mm are considered to be 36 months or more of age. By this method of estimation, 79 of the 95 kangaroos (83%) shot were aged 36 months or more (39 males and 40 females). Fourteen were aged under 30 months (9 males, 5 females). Interestingly, although males were larger than females, male kangaroos made up a greater proportion of the smallest animals collected. The one smallest female and the smallest 10% of males would be considered juveniles, with an average age of 15 to 18 months.

Table 3. Statistics of leg length of kangaroos collected on ST1

		Leg length, tibial region (mm)						
	Number	**Mean**	**Min**	**Max**	**Median**	**St dev**	**10 percent ile**	**90 percent ile**
Males	49	561	390	665	570	85	420	660
Females	46	507	380	570	520	40	450	550

On other farms where kangaroos were shot, aging was not carried out but the size of kangaroos covered a similar range to that on ST1. It is reasonable to believe that over 80% of kangaroos collected and examined from all farms were aged 3 years or more, and none was less than 12 months.

6.2 Rabbits

6.2.1 Examination by smear and ZN stain

R1 to R47 inclusive were examined by smears and ZN stain of ileal mucosa and mesenteric lymph nodes. All were negative for acid-fast bacteria.

6.2.2 Culture of faeces

R48 to R96 were cultured at EMAI under the supervision of Dr R Whittington. All cultures were negative for *M ptb*. R97 to R300 were cultured at The University of Sydney. Of these, 55 were cultured as individual samples and 149, all collected from farm ST1, were cultured in pools. All rabbit faecal cultures were negative.

6.2.3 Histopathology

Histological sections of ileum, sacculus rotundus and mesenteric lymph node from rabbits R1 to R47 inclusive were examined. No acid-fast bacteria or lesions suggestive of JD were seen.

Table 4. Summary of rabbit testing methods

R1 - R47	Smear, histopathology
R48 - R96	Radiometric culture, EMAI; individually
R97 - R300	R'metric culture; U of S; 55 individually 149 in pools of ~ 10

6.3 Kangaroos

6.3.1 Examination by smear and ZN stain

K1 to K136 were examined by smear and ZN stain of faeces or mesenteric lymph node. Of these, 24 had acid-fast (AF) material identified on smears although the material appeared fragmented rather than whole bacterial cells.

6.3.2 Culture of faeces

Faeces from those animals from which AF material had been detected were cultured. Fifteen of these, numbered up to and including K90, were cultured with non-radiometric methods so the negative findings were neither unexpected nor conclusive, given the insensitivity of the culture methods employed at the time. Faeces from 103 animals in the series K95 to K197 were submitted for radiometric culture at EMAI. One of these, from K113, was positive for *M ptb*. K113 was one of 9 animals for which AF material had been detected in smear. In BACTEC culture the sample took 9 weeks to reach a growth index of 999, suggesting that the number of organisms in the faecal sample was low. Faeces from all other kangaroos in the series K95 to K197 cultured at EMAI were negative. Faecal samples from the remaining 103 kangaroos, all collected from farm ST1, were cultured at The University of Sydney in pools of up to 10 animals per pool. All of these were negative.

6.3.3 Histopathology

Histopathological examination of sections of ileum and mesenteric lymph node from K113 revealed no evidence of Johne's disease or acid-fast bacteria. Examination of specimens K1 to K94 also revealed no evidence of JD infection.

Table 5. Summary of kangaroo testing methods

K1 - K94	Smear, histopathology
K95 - K197	Radiometric culture, EMAI; individually
K198 - K300	R'metric culture; U of S; in pools of ~ 10

7.0 Discussion

This report presents the results of a study of 300 rabbits and 300 kangaroos on 10 farms in NSW on which JD is endemic in the sheep flock. No evidence of active infection of kangaroos or rabbits was found despite evidence in one case that the causative bacterium was present in the faeces of a kangaroo from a farm on which the sheep flock was known to be heavily infected. The estimated prevalence of JD infection in kangaroos and rabbits on OJD-infected farms in NSW is 0%, with an upper 95% confidence limit of 1%.

An issue relating to the estimation of confidence limits is the prediction of the sensitivity of the tests applied. The estimate of the upper confidence interval (1%) is based on a predicted test sensitivity of 95%. While radiometric faecal culture is known to be a very sensitive test, able to detect fewer than 100 bacterial cells per gram of faeces (Whittington et al 1998), the ability of faecal culture to diagnose disease also depends on the relationship between infection and the continuity of shedding of bacteria in the faeces. For example, an animal may become infected with JD but not continually shed bacteria, or not shed them in sufficient quantities to be detected, particularly early in the course of the disease.

Radiometric culture was applied to about 200 kangaroos and about 250 rabbits (Tables 4 and 5). The remainder of the sample of 300 of each species were examined by histopathology which is believed, in sheep, to be less sensitive than faecal culture in detecting disease (Whittington et al 1998).

A second issue relates to the opportunity which rabbits and kangaroos have had on infected properties to develop OJD. On most of the farms surveyed, infection in sheep had probably only been detected for about 5 years before the kangaroos and rabbits were collected. ST2, the source of the one faecal-positive kangaroo, had been detected in 1993, 4 years before the kangaroo was killed. Undoubtedly, OJD had been present for some years before detection on most of the farms, but it is still likely that the environmental contamination was still rising towards a stable level at the time when the adult kangaroos and rabbits killed in this study were young and, perhaps, most susceptible to infection with *M ptb*.

In summary, this study has indicated that kangaroos and rabbits pose an insignificant risk as a source of re-infection with OJD on farms which are undertaking strategies to reduce infection by vaccination or pasture management in conjunction with cropping or grazing with alternate domestic species. Compared to the shedding of organisms which is likely to continue at a low level in a proportion of vaccinated sheep, and the survival of some organisms on contaminated pasture for several months during the practice of alternate grazing, the contribution likely to be made by infected kangaroos and rabbits will be insignificant.

This study does not allow us to conclude that infected wildlife will never be a significant reservoir of infection in cases where absolute freedom from disease is required, such as in eradication programs. On the other hand, we have found the prevalence of disease to be very low (<1%), so the risk of transmission of the disease from wildlife reservoirs is low. In cases where clean flocks are grazed close to infected flocks, the physical transfer of bacteria onto otherwise clean pastures by wildlife must be considered and steps should be taken to reduce the frequency of incursions of wildlife from infected farms onto clean farms as a risk-management practice.

The findings of this study contrast markedly with the studies in rabbits in UK. The results of further studies of the association between JD in rabbits and farmed ruminants in Scotland have been recently published (Greig et al 1999). In these studies, 210 rabbits from 22 farms in 7 regions of Scotland were examined.

Based on the presence of JD histopathology and/or positive cultures, JD-positive rabbits occurred on 57% of farms with JD-infected livestock and 25% of farms with no evidence of JD in their livestock. On farms with JD-infected livestock, 23% of rabbits were JD-positive, while, on the other farms, 7.5% of rabbits were positive. For farms outside the Tayside region, evidence of significant JD infection in rabbits was equivocal; only one rabbit was definitely culture positive from a farm in a region other than Tayside. For the two farms with JD-infected sheep but no cattle or goats, no rabbits were culture-positive although one rabbit had lesions typical of JD.

Strain typing of *M ptb isolates* using IS900 RFLP with the restriction endonuclease B*st* EII has divided isolates into two major categories; C strains, which are associated with cattle, and S strains, which occur principally in sheep (Collins et al 1990). Analysis of isolates of 11 rabbit isolates and 7 cattle isolates in the study of Greig et al (1999), discussed above, identified them as C strains, and all but one as the C17 strain, using the system of Pavlik (see http://www.vri.cz/labs/tbc/obrazky1.htm). The C17 strain is known to be a common type found in cattle and sheep in UK.

In sheep flocks in Australia, S strains are almost invariably the only ones associated with OJD and it is highly probable that, on the farms where rabbits and kangaroos were killed, only S strains of *M ptb* occurred (Cousins et al 2000). For farms ST2, ST3 and CT4, the presence of S strains and absence of C strains has been confirmed (Whittington pers comm). S strains have not been described in the UK and the techniques used to culture *M ptb* employed in the studies in UK discussed here are unlikely to culture them successfully, because of the notorious difficulty of growing S strains of *M ptb* on Middlebrook 7H11 slopes, the technique usually employed there.

The high prevalence of JD-positive rabbits in Scotland on some farms with infected domestic ruminants implies that, if transmission is occurring between the two or more host species, the strain or strains involved are well adapted to all hosts. It is, however, unclear why the simultaneous infection of rabbits and cattle is chiefly restricted to one region of the country and occurs at a much lower frequency on infected farms in other regions. There are a number of possibilities, including an increased JD-susceptibility of the rabbits in Tayside due to genetic or environmental factors, or other differences in the environment or husbandry between Tayside and other regions. A further possibility is that there has been adaptation of the infecting bacterial strain in that region, increasing its pathogenesis for rabbits.

It has been suggested that population densities of livestock and rabbits are significantly different from those which exist in Australia and that this factor could be a reason why wildlife have not been found with JD infection in this country to date. My own observations on one of the Tayside farms discussed in the report of Greig et al (1999), made in 1998, were that the stocking densities of cattle and grazing systems employed in the beef-sucker herds are, basically, similar to Australian grazing systems in regions with equivalent rainfall (650 mm to 700 mm annual rainfall), typical of the NSW tablelands. The rabbit populations, however, are very high compared to those on farms in Australia.
In a separate study on pastures grazed by sheep on Arthursleigh in 1999, Daniels and Abbott (unpubl) found that the mean numbers of wildlife faecal pellets on pastures were 0.63 (" 0.33) macropod, 0.59 (" 0.41) rabbit and 0.04 (" 0.04) wombat faeces per m^2, equivalent to a mean of 6300 macropod, 5900 rabbit and 400 wombat faeces per hectare.

Compared to results obtained for rabbits from JD-infected properties in Scotland, contamination levels with rabbit faeces were much lower on Arthursleigh: a 'standing crop' contamination of 5900 compared to a range of 22 000 - 81 000 pellets per ha in Scotland (Daniels et al, in press). It is likely that the lower figure for rabbits reflects a lower rabbit population density as the faecal deposition rate reported here was also much lower than for infected properties in Scotland (457 as opposed to 7357 faeces per hectare per day.

In contrast to Scotland, where rabbits are the only significant wildlife species grazing livestock pasture on infected properties, these Australian pastures were contaminated by rabbit, macropod and wombat faeces, the combined level of which (12 600 faeces per ha) is of a similar order of magnitude to the figures from Scotland, particularly when the larger mass of each macropod faecal pellet, compared to rabbit pellets, is

considered.

These findings support the view that the most likely explanation for the failure to detect similar levels of JD prevalence in Australian wildlife to the prevalence found in Tayside, Scotland, is that the strains of *M ptb* are different and the S strains which occur in Australian host have not developed the adaptations necessary to infect rabbits or kangaroos under natural conditions. Theoretically one can predict that this will ultimately occur, and it will occur faster if the population densities of wildlife co-grazing pastures with infected sheep flocks are high, rather than low.

In mid-1999, a report from Kangaroo Island (Lehmann, pers comm) stated that, in a survey of 10 kangaroos, 34 Tammar wallabies and 46 brushtail possums were collected from a known OJD- infected farm on the island and samples of intestine, lymph node and faeces were examined. One culture of intestinal tissue pooled from two wallabies was positive for *M ptb*. RFLP typing showed it to be an S strain, as expected. Histology on intestinal and lymph node tissue from both of these animals did not detect infection or disease. Faeces has not yet been cultured

Tammar wallabies are reported to occur at very high densities on the pastures in KI and to share the pastures with sheep and cattle. It seems, therefore, not unlikely that *M ptb* bacteria deposited on pastures by infected sheep will be ingested by other grazing animals and travel through the gut and be passed in the faeces. The opportunity of detection by bacterial culture of gut tissues or faeces obviously exists. This is also likely to be the explanation of the positive faecal culture from K113 reported here.

Two observations can be made, based on these findings. First, the opportunity for the occurrence of an infected macropod or rabbit with an S-strain of *M ptb* is real and significant, given that it has been shown that the animals ingest the bacteria when grazing with infected sheep. Assuming that there is age and genetic variation in susceptibility within these potential host species, as there apparently is in cattle and sheep, it is predictable that infected animals will occur. Adaptation of the bacterium for the host is then an inevitable consequence. Second, macropods have been shown to carry the bacterium in their guts and, in one case, to excrete it in their faeces. This clearly implies that physical spread of the organism can occur from infected pastures to other pastures where the wildlife graze. If those other pastures are on a previously uninfected farm transmission of organisms could lead to the transmission of disease and establishment of infection in a previously uninfected flock.

The potential role of other wildlife species in Australia has not been explored. There are a number of candidates beyond rabbits and macropods. Brush tail possums are involved with the epidemiology of tuberculosis in NZ and may be susceptible, under natural conditions, to JD in Australia. Foxes, shown to be infected in Scotland (Beard et al 1999), occur commonly on Australian farms and are important scavengers of sheep carcases here. As well as these two species for which there is evidence of involvement in the epidemiology of mycobacterial disease, there is of course a long list of animals which may be considered a risk on theoretical grounds.

Further studies on the role of wildlife are currently underway on KI and the findings there will be important in influencing the direction of further research in this area. Given another negative finding of evidence of active infection with S-strain *M ptb*, the imperative for research should shift away from exploration of wildlife reservoirs and towards a clearer understanding of the relative susceptibilities of sheep, cattle and rabbits for S-strains and Australian C-strains. These studies must, however, be performed under conditions which mimic natural exposure, as it has been well demonstrated that artificial infection can be readily achieved in a wide variety of host species.

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