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A SEROLOGICAL STUDY OF SOME INFECTIOUS DISEASES OF TASMANIAN WILDLIFE

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Abstract: Twenty-six species of fauna were examined for the presence of antibodies in their sera. Reactors were found against *Erysipelothrix rhusiopathiae* (2/74 fallow deer and 3/27 forest ravens), *Leptospira pomona* (3/5 wombats and 1/2 Gunn's bandicoots), *Leptospira icterohaemorrhagiae* (2/13 Norway rats), *Leptospira hyos* (3/6 potoroos and 1/19 hares), *Leptospira grippotyphosa* (1/127 fallow deer), Q. fever (1/1 Bennett's wallaby), psittacosis - lymphogranuloma group (1/1 giant petrel and 1/18 short-tailed shearwaters), mucosal disease (2/44 Bennett's wallaby and 11/76 fallow deer), infectious bronchitis (1/84 short-tailed shearwaters), and *Toxoplasma gondii* (3/7 pademelons and 12/69 rabbits).

INTRODUCTION

Infectious diseases of wildlife are often difficult to study, because of problems associated with finding and/or identifying individual sick animals. Serological studies are one means whereby this problem can be circumvented to a degree; such studies may be on samples collected in "ad hoc" fashion or may be from sick animals. The sera examined in the series to be discussed were mainly "ad hoc" samples, but a few were from animals selected because they were obviously diseased.

MATERIALS AND METHODS

Bloods were collected from 26 species either post-mortem after shooting or by heart- or veni-puncture after trapping. The serum was removed as soon as practicable from each sample and stored at -20°C until tested.

The tests used were:—

1. Tube agglutination test for *Brucella abortus* (*B. abortus*) antibodies.² Minimum significant titre was taken as 25 international units per ml.
2. Complement fixation test for *Brucella ovis* (*B. ovis*) antibodies (Gorrie, C. J. R., pers. comm.) Minimum significant reaction was taken as 50% haemolysis at 1:10 serum dilution.
3. Complement fixation test for *Mycobacterium johnei* (*M. johnei*) antibodies (Gorrie, C. J. R., pers. comm.) Minimum significant reaction was taken as 50% haemolysis at 1:15 serum dilution.
4. Tube agglutination test for *Erysipelothrix rhusiopathiae* (*E. rhusiopathiae*) antibodies (Pullar, E. Murray, pers. comm.) Minimum significant reaction was taken as 50% agglutination at 1:100 serum dilution.
5. Microscopic agglutination test for leptospiral antibodies.^{3a} Minimum significant reaction was taken as 50% agglutination at 1:30 serum dilution.
6. Complement fixation test for *Coxiella burnetii* (Q. fever) antibodies.³ Minimum significant reaction was taken as 50% haemolysis at 1:10 serum dilution.
7. (a) Complement fixation test for psittacosis - lymphogranuloma group (P.L.G.) antibodies.¹⁰ Minimum significant reaction was taken as 50% haemolysis at 1:5 serum dilution.
(b) Complement fixation inhibition test for P.L.G. group.¹⁰ Minimum significant reaction was taken as complete inhibition of complement fixation at 1:8 serum dilution.

8. Tissue - culture, virus neutralization test for infectious bovine rhinotracheitis (IBR) (V155 strain) antibodies.²¹ Initial testing was with undiluted sera.
9. Tissue - culture, virus neutralization test for mucosal disease (MD) (C24, Oregon strain) antibodies.²¹ Initial testing was with undiluted sera.
10. Tissue - culture, virus neutralization test for swine fever (SF) antibodies.²⁵ Initial testing was with 1:5 dilution of serum.
11. Tissue - culture, virus neutralization test for parainfluenza type 3 (PI₃) (bovine, PZL strain) antibodies.²² Initial testing was with 1:5 dilution of serum.
12. Tissue - culture, virus neutralization test for Newcastle disease (N.D.).³⁰ Initial testing was with undiluted sera.
13. Egg-culture, virus neutralization test for infectious-bronchitis (IB) antibodies.⁹ Initial testing was with 1:10 dilution of serum. Both the Connecticut 66579 and the New England strains of infectious bronchitis virus were used.
14. (a) Sabin - Feldman, dye - test for *Toxoplasma* antibodies.¹⁵ Minimum significant reaction was taken as 50% stained organisms at 1:16 serum dilution.
(b) Indirect, fluorescent - antibody test for *Toxoplasma* antibodies.³⁸ Testing was with 1:8 dilution of serum.
4. Tube agglutination test for *E. rhusiopathiae*; 2 Bennett's wallaby (*Macropus rufogriseus fruticus*), 3 ring-tail possum (*Pseudocheirus convolutor*), 1 native cat (*Dasyurus viverrinus*), 7 feral pig (*Sus scrofa*), 17 European hare, 1 rabbit (*Oryctolagus cuniculus*), 1 Cape - barren goose (*Cereopsis novae-hollandiae*).
5. Microscopic agglutination test for leptospirosis (7 serotypes); 2 echidna (*Tachyglossus setosus*), 7 forester kangaroo (*Macropus giganteus tasmaniensis*), 69 Bennett's wallaby, 19 pademelon (*Thylogale billardieri*), 8 brush possum (*Trichosurus vulpecula*), 6 ring-tail possum, 3 brown bandicoot (*Isodon obesulus*), 9 Tasmanian devil (*Sarcophilus harrisii*), 1 native cat, 5 velvet-furred rat (*Rattus lutreolus*), 7 feral pig, 49 rabbit, 6 native hen (*Tribonyx mortierii*), 1 giant petrel (*Macronectes giganteus*), 28 short-tailed shearwater (*Puffinus tenuirostris*), 1 spur - winged plover (*Lobibyx novae-hollandiae*), 1 Cape-barren goose, 5 black swan (*Cygnus atratus*), 2 black duck (*Anas superciliosa*), 27 forest raven (*Corvus tasmanicus*).
6. Complement fixation test for Q. fever; 2 pademelon, 1 wombat (*Vombatus ursinus*), 1 brush possum, 1 brown bandicoot.
7. (a) Complement fixation test for P.L.G. group; 1 Bennett's wallaby, 3 brush possum, 1 native cat, 4 fallow deer.
8. Virus neutralization test for IBR; 7 forester kangaroo, 44 Bennett's wallaby, 6 pademelon, 2 brush possum, 31 fallow deer.
9. Virus neutralization test for M.D.; 7 forester kangaroo, 10 pademelon, 2 potoroo, 3 brush possum, 1 brown bandicoot, 6 Tasmanian devil, 47 rabbit.
10. Virus neutralization test for S.F.; 4 feral pig.
11. Virus neutralization test for PI₃; 23 Bennett's wallaby, 1 pademelon, 10 fallow deer.

RESULTS

A. No detectable and/or significant titres were found using the following test systems and sera:

1. Tube test for *B. abortus*; 91 fallow deer (*Dama dama*), 15 European hare (*Lepus europaeus*).
2. Complement fixation test for *B. ovis*; 69 fallow deer.
3. Complement fixation test for *M. johnei*; 63 fallow deer.

12. Virus neutralization test for N.D.; 6 native hen, 1 giant petrel, 17 raven.
 13. Dye-test for *Toxoplasma*; 1 Bennett's wallaby, 3 ring-tail possum.

B. Those species and test systems in which significant titres were found are shown in Table 1.

Pertinent comments upon these results are:—

1. Erysipelas agglutination test.
Positive sera showed complete agglutination at serum dilution of 1:400, and in the case of the fallow deer one of the reacting animals was described as being "sluggish".
2. Leptospiral, microscopic agglutination test.

Titres in the various animals were: potoroos 1:30, 1:100 and 1:300 to *L. hyos*; Gunn's bandicoot 1:30 to *L. pomona*; wombats 1:3,000, 1:3,000 and > 1:10,000 to *L. pomona*; rats 1:100 and 1:300 to *L. icterohaemorrhagiae*; fallow deer 1:30 to *L. grippotyphosa*; and hare 1:30 to *L. hyos*. Leptospire were demonstrated by histological means (Levaditi's technique) in the wombats and rats and were also recovered from the rats' kidneys by culture in artificial media.

3. Q. fever complement fixation test.
The Bennett's wallaby had a titre of 1:20.
4. Due to the degree of haemolysis in the samples, the end-points in the

TABLE 1. Proportion of reacting sera to total sera in those species and test systems in which significant titres were found.

Species		Test System							
Common Name	Scientific Name	1	2	3	4	5	6	7	8
Bennett's wallaby	<i>Macropus rufogrisea fruticus</i>			1/1		2/44			
Pademelon	<i>Thylogale billardieri</i>							3/7	
Potoroo	<i>Potorous tridactylus</i>		3/6						
Gunn's bandicoot	<i>Perameles gunnii</i>		1/2						
Wombat	<i>Vombatus ursinus</i>		3/5						
Norway rat	<i>Rattus norvegicus</i>		2/13						
Fallow deer	<i>Dama dama</i>	2/74	1/127			11/76			
European hare	<i>Lepus europaeus</i>		1/19						
Rabbit	<i>Oryctolagus cuniculus</i>								12/69
Giant petrel	<i>Macronectes giganteus</i>				1/1				
Short-tailed shearwater	<i>Puffinus tenuirostris</i>				1/18		1/84		
Forest raven	<i>Corvus tasmanicus</i>	3/27							

Legend for Table I

- Column 1 *E. rhusiopathiae* agglutination test.
 Column 2 Leptospiral, microscopic agglutination test.
 Column 3 Q. fever complement-fixation test.
 Column 4 P.L.G. complement-fixation inhibition test.
 Column 5 M.D. virus neutralization test.
 Column 6 I.B. virus neutralization test.
 Column 7 *Toxoplasma* dye-test.
 Column 8 *Toxoplasma*, indirect, fluorescent-antibody test.

complement fixation inhibition tests were not easily determined. However, it was considered that one short-tailed shearwater nestling, and the only giant petrel tested, gave reactions at a serum dilution of 1:32.

5. Mucosal disease, tissue-culture neutralization test. The reactions in the wallabies were at a dilution of less than 1:3, while those in eight of the deer were four at less than 1:3, one at 1:22, two at greater than 1:47 and one at 1:65. Titres were not determined for the other deer which reacted.
6. Infectious bronchitis, egg - culture neutralization test. One sample from a juvenile short - tailed shearwater neutralized 50 infective doses of Connecticut 66579 virus.
7. *Toxoplasma* dye-test. The padmelons, which reacted, had titres of 1:32, 1:1,024 and 1:2,048 and each was found to be infected with *Toxoplasma gondii* when brain homogenates were injected into white mice.

DISCUSSION

The results obtained suggest that fallow deer do not constitute a reservoir of infection for *B. abortus*, *B. ovis*, *M. johnei*, *Leptospira* spp. or infectious bovine rhinotracheitis in Tasmania. Similarly, sufficient Bennett's wallaby were sampled to indicate that they are not important in the perpetuation of leptospirosis, infectious bovine rhinotracheitis and parainfluenza 3.

It is probable that infections of deer with *B. abortus* and *M. johnei* are mainly incidental to these diseases in cattle.⁷ As bovine brucellosis has been eradicated from the Tasmanian mainland,¹⁴ and Johne's disease occurs mainly in the moist northeast and northwest of the state where deer are not found, the absence of reactions for these diseases in deer sera is not unexpected. The low level of reactors to *Leptospira* spp. (especially *L. pomona*) in deer is in contrast to some overseas studies,^{4,12} but similar to the position in New Zealand,⁷ and is almost

certainly a result of low deer density, together with infrequent occurrence of leptospirosis in domestic animals in the areas where deer occur.¹⁶

Although there was no opportunity to examine the animals with positive titres for erysipelas, it is likely that these were indicative of actual infection which could have been acquired by deer using sheep camps (resting areas) which are frequently contaminated with *E. rhusiopathiae*.⁵

In relation to the tests for antibodies against viruses in fallow deer sera, it is probable that the results for PI₃ were valid as there is considerable antigenic relationship between various PI₃ isolates;²⁸ indeed, using a human isolate as their test virus, Shah and Schaller²⁴ found antibodies against this virus in various deer sera collected in the U.S.A. and India. The absence of reactors in the series under consideration suggests that infection with PI₃ may not occur very frequently in Tasmanian fallow deer, even though there would be ample opportunity for contact with infected cattle (St. George²² found 77% of 156 Tasmanian cattle to have significant levels of antibody). This situation would appear to be very similar to that reported by Kahrs *et al.*,¹¹ in New York State, where 48% of cattle reacted in the haemagglutination-inhibition test, but no reactors were found amongst white-tailed deer.

St. George *et al.*²¹ suggested that deer in Tasmania would have little opportunity to become infected with IBR, because of the low incidence of this virus in cattle in the State (6% reactors) and therefore the absence of reactors in the deer population is not unexpected.

As deer can be naturally infected with MD virus²⁰ it is reasonable to expect that the titres observed were indicative of infection with this virus. Certainly, there is a tendency in Tasmania for deer to mix with cattle, and as the latter are frequently infected with MD (35%),²¹ the chances for transmission would be fairly high.

In the main, too few sera from species other than deer were examined for erysipelas to allow for interpretation, except

in the case of the forest raven, where the 11% reactor rate is in keeping with the isolation of *E. rhusiopathiae* from this species and the occurrence of an erysipeloïd-like syndrome in a laboratory worker pecked by one of these birds (Munday, B. L., unpublished data).

A fairly wide coverage was obtained in the survey for *Leptospira* spp. antibodies. The occurrence of *L. icterohaemorrhagiae* in Norway rats is consistent with the accepted role of this species in maintaining a reservoir of the organism.¹⁸ *L. hyos* infection has previously been recorded in bandicoots⁸ which are fairly closely related to the potoroo. The wombats and Gunn's bandicoot which had titres to *L. pomona* were captured on farms where leptospirosis had recently been diagnosed in domestic livestock and the wild fauna may have been incidentally infected; however the high rate of infection present in the wombats suggests that they could have been of some importance in perpetuating the disease.

Unfortunately, very few mammalian sera were tested for Q. fever but it is possible that the titre noted in a wallaby was indicative of infection. Indeed, Albiston¹ mentions marsupials as being carriers of *C. burneti*.

As infections with PLG organisms have been demonstrated in petrels,¹⁸ in short-tailed shearwaters in particular,¹⁹ the positive titres found in the sera of the giant petrel and short-tailed shearwater were probably valid, despite the technical difficulties encountered when testing these samples.

The susceptibility of marsupials to IBR, MD and PI₃ infections does not seem to have been investigated, and therefore it is difficult to interpret the results obtained with sera from these animals when tested for antibodies against the above viruses.

The IB antibody level found in the short-tailed shearwater was below that usually considered significant in domestic poultry, but further investigations are probably warranted in view of Cumming's⁹ observation that magpies (*Gymnorhina hypoleuca*) may be experimentally infected with this virus, and yet show antibody levels below the accepted positive level.

Isolation studies¹⁷ (Munday, B. L., unpublished data) have demonstrated that 43% of pademelons and 17% of rabbits harbour *Toxoplasma gondii* and hence, as indicated by the serological tests, these species are potential reservoirs of this infection.

In the main, it would appear that infections with the various agents investigated in this series were infrequent in the species from which sera were collected. Even where reactors were detected, it is possible that a proportion were directly or indirectly infected from domestic animals (e.g. infections with *E. rhusiopathiae*, MD). However, in the instances of infection with *L. icterohaemorrhagiae* in Norway rats, *L. hyos* in potoroos, *C. burneti* in wallabies and P.L.G. organisms in petrels and shearwaters, it is likely that the hosts were primarily involved, rather than incidentally to disease in domestic animals.

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