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LEPTOSPIRA INTERROGANS ANTIBODIES IN FERAL PIGS FROM NEW SOUTH WALES

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ABSTRACT: The sera of 195 hunter-killed feral pigs (*Sus scrofa*), collected in New South Wales (Australia) from April to November 1995, were screened against a reference panel of 14 *Leptospira interrogans* serovars using a microscopic agglutination test (MAT). The panel represented those serovars previously isolated from wild and domestic mammals in mainland Australia. Anti-leptospiral agglutinins were detected in 20% of the sera tested and included nine *L. interrogans* serovars. The majority of serological reactors (63%) were to *L. interrogans* serovar *pomona*. Sera from 26% of immunoreactors cross reacted with antigens from one or more serovars. No differences were noted in the prevalence of *L. interrogans* antibodies between the sexes, or between pigs from areas of low and high rainfall. The implications of leptospirosis in feral pigs on the transmission of leptospires to wildlife, livestock, and humans are discussed.

Key words: Feral pigs, *Leptospira interrogans*, leptospirosis, reservoir, serosurvey, *Sus scrofa*.

INTRODUCTION

In Australia, the feral pig (*Sus scrofa*) is a potential reservoir for a range of pathogens including *Leptospira interrogans* (Keast et al., 1963), given the propensity of feral pigs to wallow in stagnant water, their catholic diet (Tisdell, 1982), and that they readily share pasture and water holes with other wildlife and domestic livestock. To date, all Australian studies of leptospirosis in feral pigs have concentrated on those serovars that are of perceived significance to the livestock industry, namely *L. interrogans* serovars *pomona, hardjo,* and *tarassovi*. This is despite the fact that feral pigs may act as a reservoir for other *L. interrogans* serovars which may pose a significant risk to the health of other free-living mammals and humans.

Immunoreactors to *L. interrogans* serovar *pomona* have been reported among feral pigs in New South Wales (Australia). Keast et al. (1963) noted the presence of *L. interrogans* serovar *pomona* in 48% of feral pig sera taken from central and western New South Wales, and concluded that feral pigs were a maintenance host important in leptospirosis in cattle where the two species co-occurred. In feral pig sera taken from three localities in central New South Wales Giles (1980) noted the presence of *L. interrogans* serovar *pomona* and "others". Saunders (1993) analysed the sera of 102 feral pigs from a subalpine environment in New South Wales for *L. interrogans* serovars *pomona, hardjo,* and *tarassovi,* but all sera were negative. In contrast, a serological survey in Queensland (Australia) identified immunoreactors to *L. interrogans* serovars *pomona, hardjo,* and *tarassovi* (Elder and Ward, 1978). Pavlov (1991) found between 2 and 23% of feral pigs from seven sites in northern Queensland were seropositive to *L. interrogans* serovar *pomona* and that prevalence increased from north to south.

A serological survey of 25 wildlife species undertaken in southeastern Australia detected the presence of antibodies to six leptospiral serovars (Milner et al., 1981). In that study the authors did not sample feral pigs, but were aware of this species' susceptibility to infection with *L. interrogans* serovar *pomona*.

Durfee and Presidente (1979) conducted a serosurvey in southeastern Australia for *L. interrogans* serovar *hardjo* in 10 wild-collected native marsupial species, and in three introduced cervid species and water buffalo (*Bubalus bubalis*) from a farm. Interstitial nephritis, a non-definitive symptom of leptospirosis, was associated with leptospiral infection in brushtail possums (*Trichosurus vulpecula*), and antibodies to *L. interrogans* serovar *hardjo* were detected in serum from rusa deer (*Cervus timorensis*) and wombats (*Vombatus ursinus*). *Leptospira interrogans* serovar *balcanica* was isolated from the kidney of one of the 352 brushtail possums tested. One of seven Bennett's wallabies (*Macropus rufogriseus rufogriseus*) and six of 25 wombats were seropositive to *L. interrogans* serovar *pomona* and interstitial nephritis was evident in both species. *Leptospira interrogans* serovars *robinsoni*, *zanoni*, and *grippotyphosa* also were detected in some of the wombats. Interstitial nephritis was found in the kidney samples from red kangaroos (*Macropus rufus*), black-striped wallabies (*Macropus dorsalis*), water rats (*Hydromys chrysogaster*) and farmed fallow deer (*Dama dama*), but sera were negative to *L. interrogans*. Interstitial nephritis and *L. interrogans* serovar *pomona* were detected in wombats from Tasmania by Munday and Corbould (1973). Past or present infection with leptospires in these wombats was associated with *L. interrogans* serovar *pomona* infection in cattle. The susceptibility of brushtail possums to *L. interrogans* serovar *balcanica* found by Durfee and Presidente (1979) has been investigated for the biological control of brushtail possums in New Zealand (Day et al., 1997).

There are an estimated 100,000 recreational hunters of feral pigs in Australia (Tisdell, 1982), and a number of hunters and processors are involved in the export of feral pig meat (Ramsay, 1994). A study in Queensland (Robson et al., 1993) has established that hunters and process workers who handle feral pigs are highly susceptible to infection with *Brucella suis*. It is unclear whether a similar link exists between human infection with *L. interrogans* and contact with feral pigs. However, there are numerous reported cases of human infection with *L. interrogans* as a result of contact with infected livestock (e.g., Kingscote, 1986) and wildlife (e.g., Looke, 1986). In this paper, we investigate the prevalence of different *L. interrogans* serovars in feral pigs from New South Wales and discuss the relative importance of feral pigs as hosts for *L. interrogans* serovars that infect wildlife, livestock and humans.

MATERIALS AND METHODS

Between March and April, 1995 an advertising program was implemented to encourage recreational hunters in New South Wales to collect serum samples from feral pigs. Hunters responding to the program were supplied with a sampling kit which comprised a 4 L polystyrene cooler containing: a freezer brick, latex gloves, record sheets, 10 mL blood clot tubes, 5 mL serum tubes, an air-courier voucher and an instruction sheet describing the procedure for the collection and dispatch of the sera. Each kit contained sufficient materials to collect serum samples from 10 pigs.

To collect sera from feral pigs, hunters punctured the heart of the pig immediately after death, drained about 7 mL of blood into a fresh, sterile 10 mL tube and allowed the tube to stand until the serum had separated. The serum was then decanted into a 5 mL sterile serum tube and kept in the cooler, but not frozen. The cooler containing the samples was dispatched to the laboratory by air courier. From April to November 1995, 212 serum samples were collected from numerous locations in New South Wales including all major vegetation types utilised by feral pigs (Fig. 1). These vegetation types include: subalpine forests and grasslands (Saunders, 1993), temperate forests (Saunders and Kay, 1991), and semi-arid rangelands (Choquenot, 1995).

The sera were screened against a reference panel of 14 *L. interrogans* serovars using a microscopic agglutination test (MAT). The panel represented those serovars previously isolated in mainland Australia. Each sample was diluted at 1:25 and then titrated to an end point (dilution showing 50% agglutination) using a series of doubling solutions (Stallman, 1984). The *L. interrogans* serovars used in the study were; *australis* (strain Ballico), *bulgarica* (Nikolaevo), *canicola* (Hond Utrecht IV), *celledoni* (Celle-

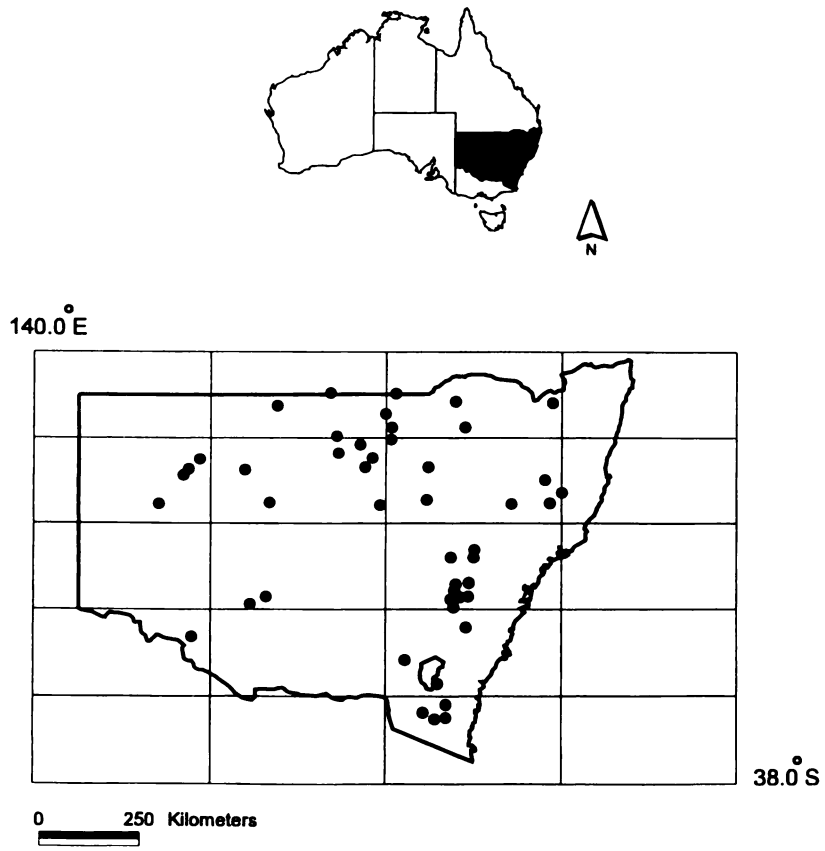

FIGURE 1. Blood sampling locations (●) of feral pigs in New South Wales, Australia.

doni), *copenhageni* (M20), *grippotyphosa* (Moskva V), *hardjo* (Hardjoprajitno), *kremastos* (Kremastos), *medanensis* (Hond HC), *pomona* (Pomona), *robinsoni* (Robinson), *szwajizak* (Szwajizak), *tarassovi* (Perepelicin) and *zanoni* (Zanoni). We did not test for *L. interrogans* serovar *icterohaemorrhagiae* which has been found in Norway rats, *Rattus norvegicus*, in Tasmania (Munday, 1972). Of the samples submitted, 17 (8%) were not suitable for analysis because they were too small (≤100 μL), haemolysed, or the serum had bacterial contamination.

Potential differences in the prevalence of anti-leptospiral agglutinins in feral pigs were examined between sexes using contingency tables (Snedecor and Cochran, 1967). Contingency tables also were used to compare the prevalence of anti-leptospiral agglutinins between samples taken from feral pigs in semi-arid rangelands (mean annual rainfall 200–500 mm) and those collected on the slopes and tablelands of the Great Dividing Range and coastal hinterland (mean annual rainfall >500 mm). Significance was determined for α at a level of $P \leq 0.05$.

RESULTS

Anti-leptospiral agglutinins with a titre of ≥1:50 were found in 20% of the 195 sera that were suitable for screening. Nine *L. interrogans* serovars were recorded, of which *L. interrogans* serovar *pomona* was most prevalent (Table 1). Although *L. interrogans* serovars *australis, canicola, copenhageni, grippotyphosa, szwajizak, hardjo,* and *tarassovi* also were detected, all had low prevalence (<2%).

Of the 39 immunoreactors, 10 demonstrated cross reactivity with the antigens of other serovars. In nine cases, *L. interrogans* serovar *pomona* cross reacted with one or more of the following *L. interrogans* serovars: *australis, canicola, copen-*

TABLE 1. Prevalence of anti-leptospiral agglutinins in feral pigs from New South Wales.

Serovar	Negative	Sero-positive*	Titre range	Prevalence (%)
australis	193	2	400–800	1.0
bulgarica	195	0	—	—
canicola	192	3	50–100	1.5
celledoni	195	0	—	—
copenhageni	194	2	50–200	1.0
grippotyphosa	192	3	100–800	1.5
hardjo	193	2	50–100	1.0
kremastos	195	0	—	—
medanensis	195	0	—	—
pomona	168	27	50–>6400	13.9
robinsoni	195	0	—	—
szwajizak	194	1	50	0.5
tarassovi	194	1	50	0.5
zanoni	193	2	100	1.0

* Does not include cross-reactions between antigens.

hageni, grippotyphosa, robinsoni, szwajizak, and *zanoni.* One of the immunoreactors to *L. interrogans* serovar *grippotyphosa* cross reacted with the antigen of *L. interrogans* serovar *celledoni* and another *L. interrogans* serovar *grippotyphosa* reacted to antigens of *L. interrogans* serovars *australis, bulgarica,* and *copenhageni*; all had an end point titre of 1:800. We are uncertain whether this is a result of multiple infections (past or present) or a result of cross reactions between serovars. There was no significant difference in the prevalence of anti-leptospiral agglutinins between sexes ($\chi^2 = 0.06$, $P = 0.81$, df = 1) or between feral pigs taken in areas of high and low mean annual rainfall ($\chi^2 = 0.80$, $P = 0.37$, df = 1).

DISCUSSION

Leptospira interrogans serovar *pomona* is the predominate serovar associated with leptospiral antibodies in feral pigs of New South Wales. The high titres recorded from several of the samples indicated current or recent infection with *L. interrogans* serovar *pomona.* Other Australian studies also have demonstrated the relatively high local prevalence of *L. interrogans* serovar *pomona* in feral pig populations (Keast et al., 1963; Elder and Ward, 1978; Giles, 1980; Pavlov, 1991). Leptospirosis is common among feral pigs from different habitats throughout eastern Australia and transmission could either be through environmental transmission or contact. Although our study resulted in the collection of eight *L. interrogans* serovars other than serovar *pomona,* their low prevalence suggests that feral pigs are incidental hosts of these serovars in New South Wales.

Milner et al. (1981) found anti-leptospiral agglutinins in seven of the 25 wildlife species they sampled and tested for 12 *L. interrogans* serovars. Antibodies to *L. interrogans* serovar *hardjo* was most prevalent and antibodies to *L. interrogans* serovar *pomona* were found only in two of 419 animals. However, only a small number of animals (2%) showed evidence of past or present infection with leptospires and titres of all serovars were generally low (Milner et al., 1981). Both Dufee and Presidente (1979) and Milner et al. (1981) found *L. interrogans* serovar *hardjo* prevalent in brushtail possums and farmed rusa deer. However, given the low prevalence of *L. interrogans* serovar *hardjo* and more common prevalence of *L. interrogans* serovar *pomona* that we found in feral pigs, it is unlikely that feral pigs are important

in the maintenance of leptospiral infections in native wildlife or rusa deer. Exceptions may occur where the densities of feral pigs and susceptible wildlife species, such as brushtail possums and wombats, are locally high and where stagnant water is communally used by these species.

Hunters of feral pigs are potentially susceptible to infection with leptospirosis through direct contact with the tissue, blood, or urine of infected animals. Hunters and other outdoor recreation groups also may be susceptible to infection from leptospires shed by infected feral pigs in water and mud. Similarly, domestic livestock may be exposed to *L. interrogans*, particularly serovar *pomona*, at foci such as watering points. The risk of infection is probably greatest in the semi-arid rangelands where ephemeral water bodies and high local densities of feral pigs may lead to concentration of leptospires in moist areas favoured by feral pigs for wallowing and drinking. These areas are favoured by hunters because of the higher local densities of feral pigs found there (Tisdell, 1982).

Leptospira interrogans serovars have been grouped into serogroups based on their degree of cross reactivity (Stallman, 1984). Our study showed broad cross reaction between the leptospiral serovars found in feral pigs and cross reactions between serogroups were also found. Further serological and isolation studies of *L. interrogans* serovars in feral pig populations in Australia are required to understand the role, if any, of feral pigs in the transmission of leptospirosis.

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