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A survey of leptospiral antibodies and urinary shedding of leptospires in farm working dogs in the South Island of New Zealand

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Introduction

- Antibodies to *Leptospira* serovars Copenhageni, Pomona, Hardjo and Ballum have been identified in New Zealand dogs and in notified human cases.
- Infections of dogs with Copenhageni have been reported more commonly in the North Island.
- A recent serological study found farm working breeds, and dogs from the North and South Islands to have an increased risk exposure to Hardjo.
- Recent veterinary practitioner observations have attributed clinical disease to serovar Pomona.
- Working dogs are exposed to livestock with a high prevalence of antibody to Hardjo and Pomona.
- Licensed leptospiral vaccines for dogs available in New Zealand only provide protection against serovar Copenhageni.
- No data are available on urinary shedding of leptospires by farm dogs in NZ, or association with leptospiral serovars in livestock.

Materials and Methods

A cross-sectional survey was conducted to determine the prevalence of titres to serovars Copenhageni, Pomona, and Hardjo and the prevalence of urinary shedding in unvaccinated South Island farm dogs. The sampling frame included South Island farms where spot tests of 20 animals per herd/flock (beef cattle, sheep, or deer) had also been previously tested for Hardjo and Pomona. If more than 1 livestock tested on each farm was positive (microscopic agglutination test (MAT) titre ≥25) for serovar Pomona or more than 6 tested positive for Hardjo, the livestock prevalence on that farm was classified as 'high'. Otherwise the prevalence was classified as 'low'. A survey was completed by the farmer at the time of sample collection, detailing the signalement, vaccination and health status of each dog, and the presence on each farm of livestock species, horses, vermin and free standing water (Table 1).

Blood was collected from dogs by venipucnture, and urine by free catch. Serum was tested for serovar-specific antibodies using the MAT. Urine was subjected to PCR, using primers designed to amplify a conserved region of the ..... MATs of >25 were considered positive. PCR results with a threshold cycle (Ct) >45 were considered negative, those with a Ct <45 and >37 were considered "weak detection", and those with a Ct <37 were considered as 'detected". Both "detected" and "weak detected" results were regarded as PCR positive for this study. Associations between dog MAT titres and urinary shedding with the serological prevalence in livestock was investigated, with the presence of deer, pigs and natural waterways on the farm included as variables.

Results

Urine (n=85) and serum samples (n=116) were collected from 117 dogs (58 male, 49 female, 10 unrecorded) on 28 farms. Four dogs had been unwell in the prior 12 months (one gastric dilation volvulus, one foot laceration, one pyometra, one unspecified illness). The dog ranged in age from 10 months to 14 years.

Based on previous livestock MAT surveys, 10 farms had a high livestock leptospiral prevalence, 11 farms had a low prevalence, and MAT results were not available for 5 farms.

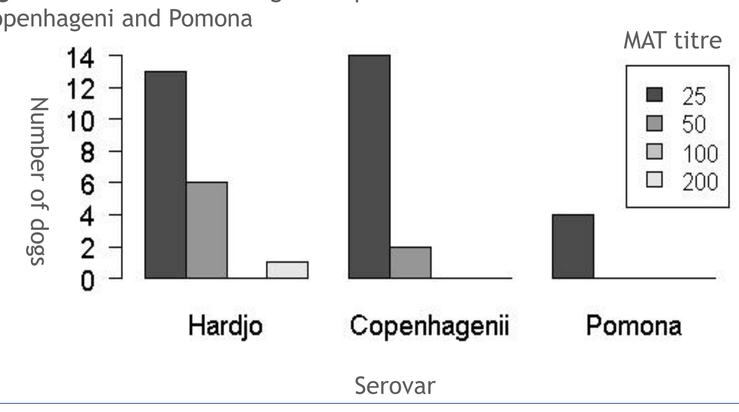
30/116 dogs had positive MAT titres ≥25 (Figure 1). Only one dog had a MAT titre greater than 50 (MAT titre of 200 to Hardjo, from a high livestock prevalence farm). The farm adjusted prevalence of dogs with MAT >50 for any of the three serovars was...

Table 1 Animal and environmental variables present on South Island farms surveyed for working dog leptospiral MATs and urinary shedding

Variable	Deer	Pigs	Sheep	Cattle	Vermin	Horses	Free water
% (n/#farms)	89.3% (25/28)	32.1% (9/28)	96.4% (27/28)	100% (28/28)	96.4% (27/28)	28.6% (8/28)	96.4% (27/28)

Leptospiral DNA was detected by PCR in the urine of 14 dogs from 9 farms, yielding a prevalence, adjusted for the effect of clustering, of 15.9 (8.0-28.0)%. Seven dogs had a positive urine PCR test with negative serology for all three serovars. Four dogs had a positive urine PCR and a positive MAT to one serovar (Titres of 25 to Hardjo in three dogs, and a titre of 25 to Copenhageni in one dog). Three dogs had a positive urine PCR and positive MATs to two serovars (Titres of 25 to Hardjo and Pomona for one dog, and titres of 25 to Hardjo and Copenhageni in two dogs).

Figure 1 Number of 116 dogs with positive MAT titres to serovars Hardjo, Copenhageni and Pomona



The prevalence ratio of urinary shedding by dogs on high *vs.* low livestock seroprevalence farms was 4.2 (0.92-19.35). The prevalence ratio of urinary shedding by male *vs.* female dogs was 2.3 (0.7-7.7), and for dogs on farms where pigs were present was 2.1 (0.8-5.51). The prevalence ratio of urinary shedding was not significantly associated with the presence/absence of deer, horses, cattle, vermin or waterways.

When the PCR results were grouped at a farm level, the farms with a high prevalence of titres in livestock were 2.25 (0.55-9.17) time as likely to have at least one dog with a positive urine PCR test. (##check using PR term correctly). No significant associations were found between farms with at least one dog with positive urine PCR and the presence of sheep, cattle, deer, horses, pigs or waterways. ## male dogs run code Association between livestock status and serological status of other dogs on farm Description of serology of dogs with positive PCRs

Discussion

Data still being collected – results may reach statistical significance with a larger data size
Seasonal and annual variation in leptospiral exposure
Inter – Intra lab variability
Comparison to stock seroprevalence on the same farms in prevous study (dates? Reference)
Typing of amplified dna

Conclusions

- South Island working dogs shed leptospiral DNA in their urine at a higher rate than previously reported in dogs (Rojas *et al*).
- Urinary shedding of leptospires in New Zealand dogs is a new finding, and may have a significant role in the epidemiology of leptospirosis on farms.
- Urinary shedding can occur in the presence of low or negative MATs
- Urinary shedding may be associated with high livestock seroprevalence farms, although statistical significance has not been reached using the data collected to date.
- Urinary shedding does not appear to be associated with male dogs, or the presence of pigs or natural waterways
- The prevalence of positive MAT titres in South Island farm dogs is lower than suggested by previous serosurveys (Harland *et al*) .
- The MAT may be a poor predictor of urinary shedding of leptospires in dogs, in contrast to sheep (Benschop *et al* 2013)

References

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