A survey of leptosiral 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.

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Deer</th>
<th>Pig</th>
<th>Sheep</th>
<th>Cattle</th>
<th>Venison</th>
<th>Humans</th>
<th>Free water</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (n/#farms)</td>
<td>116</td>
<td>28</td>
<td>28</td>
<td>116</td>
<td>116</td>
<td>116</td>
<td>116</td>
</tr>
<tr>
<td>0% (%)</td>
<td>36.5</td>
<td>1/28</td>
<td>8/28</td>
<td>11/116</td>
<td>11/116</td>
<td>11/116</td>
<td>11/116</td>
</tr>
<tr>
<td>37.5% (%)</td>
<td>1/28</td>
<td>11/28</td>
<td>7/28</td>
<td>10/116</td>
<td>10/116</td>
<td>10/116</td>
<td>10/116</td>
</tr>
<tr>
<td>62.5% (%)</td>
<td>1/28</td>
<td>11/28</td>
<td>7/28</td>
<td>10/116</td>
<td>10/116</td>
<td>10/116</td>
<td>10/116</td>
</tr>
<tr>
<td>100% (%)</td>
<td>1/28</td>
<td>11/28</td>
<td>7/28</td>
<td>10/116</td>
<td>10/116</td>
<td>10/116</td>
<td>10/116</td>
</tr>
</tbody>
</table>

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).

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 (0.85.51). 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. (What was the reference range for the PCR test?) No significant associations were found between farms with at least one dog with positive urine PCR and the presence of sheep, deer, cattle, horses, pigs or waterways. All 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 previous study (dates? Reference)

Typing of amplified dna

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 serovor 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 venipuncture, 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 16S rRNA genes. PCR was not performed on urine samples from farms with a MAT titre >25, as dogs were likely to be serologically 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 "detected". Both "detected" and "weak detection" 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.

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 an important 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