**Prevalence of antileptospiral serum antibodies in dogs in Ireland**

**Introduction**

Leptospirosis is an important worldwide zoonosis affecting most mammalian species including dogs (Bharti and others 2003). Dogs are susceptible to infection with a wide range of serovars of *Leptospira* and can develop a broad spectrum of clinical manifestations; these range from persistent urinary shedding in the absence of clinical signs to severe acute disease (Faine and others 1999; Rojas and others 2010). Based on the available seroprevalence data, the major serogroups to which dogs in Europe seroconvert to are Icterohaemorrhagiae, Grippotyphosa, Australis, Sejroe and Canicola (Ellis 2010). However, the level of exposure, as well as the prevailing serogroups can vary considerably between geographical regions. For example, exposure to serogroup Grippotyphosa appears to be less common in the UK, than in continental Europe (Ellis 2010). As currently available anti-leptospiral vaccines are primarily serovar specific, the recognition of leptospires commonly involved in canine exposure in specific areas is crucial in order to keep vaccination strategies up to date.

Data regarding the prevalence of anti-leptospiral antibodies in Irish dogs are lacking; the most recent publication dating back to 1974 (Timoney and others 1974). In this study, only exposure to serogroups Canicola and Icterohaemorrhagiae was examined in apparently healthy dogs of unknown vaccination status living in a dog pound; 17% of these dogs had reciprocal antibody titres >1:100 against serogroup Canicola and 15.5% against serogroup Icterohaemorrhagiae. More recently, detection of pathogenic leptospires in urine from dogs not suspected to have leptospirosis via quantitative RT-PCR showed that 7% of Irish dogs shed pathogenic leptospires in their urine (Rojas and others 2010). These findings suggest that a significant proportion of Irish dogs are exposed to pathogenic leptospires and that dogs are likely to contribute to the maintenance of pathogenic leptospires in the environment.

The aim of this present study was to determine the prevalence of anti-leptospiral antibodies to a panel of pathogenic *Leptospira* spp. in dogs in Ireland using the Microscopic Agglutination Test (MAT).

**Materials and Methods**

Ethical approval for this study was obtained from the Animal Research Ethics Committee of University College Dublin (AREC-P-12-70). Leftover canine serum samples submitted to the veterinary clinical pathology laboratory at University College Dublin for routine diagnostic testing were collected between December 2012 and April 2014 (cohort 1). In parallel, veterinary practitioners were contacted and offered free MAT testing of paired serum samples for any dogs with clinical signs consistent with acute leptospirosis (cohort 2).

Serum samples were frozen at -20ºC and batch wise shipped to the OIE reference laboratory for animal leptospirosis in Stormont (NI) for MAT testing. This laboratory operates under ISO 9001 and regularly participates in the International Leptospirosis Society MAT Proficiency Testing Scheme. Samples were tested for the presence of antibodies against a panel of 10 serovars of *Leptospira* belonging to 8 different serogroups (Table 1). The MAT was performed using standard protocols (Wolff 1954). Agglutination of ≥75% of leptospires by patient sera at a dilution of 1:10 and of ≥50% at all higher dilutions was reported as a positive result. Because of the high specificity of the MAT and in accordance with OIE guidelines, a reciprocal titre of ≥1:10 was defined as indicative of prior exposure (Benkirane and others 2014; OIE 2014). Seroprevalence was defined as percentage samples with positive MAT results. If a sample was positive for several serovars belonging to the same serogroup, only the serovar with the highest titre was reported. Because the vaccination status of the dogs was unknown, titres to serogroups Canicola and Icterohaemorrhagiae were not included in the analysis.

**Results**

MAT results for dogs not suspected to have leptospirosis (cohort 1) are reported in Table 2. Of the 464 dogs in this group, 210 dogs (45.3%) had antibody titres of 10 or higher to at least one serovar. However, the large majority of seropositive dogs (86.6 %) had low titres (≤100) against serovars Icterohaemorrhagiae and/or Canicola only, consistent with vaccination with a bi-valent anti-leptospiral vaccine. Thirty-one dogs (6 %) showed low antibody titres (≤100) against either serovars Ballum (n=13; 2.8 %), Bratislava (n=9, 1.9 %), Mozdok (n=1, 0.2%), Altodouro (n=4, 0.9 %) and Hardjo (n=2, 0.4%). One dog had equal titres (30) against serovars Bratislava and Ballum. None of the dogs had any titre to serogroups Grippotyphosa and Autumnalis. Of the 31 dogs with antibodies to serovars not included in the vaccine 22 dogs also had titres to vaccinal serogroups (Canicola and/or Icterohaemorrhagiae). Results of 13 sera from 11 dogs suspected to have leptospirosis (cohort 2) are shown in Table 3. Despite the fact that practitioners were encouraged to submit paired samples, convalescent samples were only collected in 2 cases. Based on the available data, a diagnosis of leptospirosis was considered likely in two dogs. One of them had only a low reciprocal titre (1:10) to serovar Bratislava but spirochaetes were found in urine via dark-field microscopy. The second dog showed a rising titre to serogroup Icterohaemorrhagiae. This dog had not been vaccinated against leptospirosis in several years.

**Discussion**

The results of our study indicate that 6% of Irish dogs not suspected to have leptospirosis show evidence of prior exposure to leptospiral serovars belonging to the serogroups Ballum, Australis, Pomona and Sejroe.

For the purpose of this study a reciprocal titre of ≥1:10 for non-vaccinal serogroups was defined as indicative of exposure in dogs not suspected to have leptospirosis. This is in accordance with previous reports (Arent and others 2013; Renaud and others 2013). This low cut off was chosen because the MAT has high specificity in convalescent samples, in which anti-leptospiral IgG predominates (Chernukha and others 1976). Despite this fact we cannot completely exclude cross reactions of antibodies induced through natural exposure or vaccination with heterologous serogroups.

Serogroup Ballum was the most common non-vaccinal serogroup that canine sera reacted with. Exposure of dogs to this serogroup most likely results from contact with rats and mice, which are maintenance hosts for this serogroup (Collares-Pereira and others 2000). Seropositivity to serogroup Ballum is rarely reported in acutely infected dogs (Mayer-Scholl and others 2013), but this serogroup represents an increasingly important cause of human leptospirosis in many parts of the world (Rodriguez Gonzalez and others 2007; Storck and others 2008; Thornley and others 2002; Vieira and others 2006). Our data do not allow any conclusions as to whether dogs are chronic carriers and therefore a potential source of human infection. Nevertheless, dogs can serve as an indicator of the presence of this serogroup in the environment, which they share with humans. It would be very interesting to compare isolates associated with acute infection and apparently subclinical exposure. However, this requires isolation and culture of leptospires from infected dogs, which is notoriously difficult due to the fastidious nature of the organism.

Serogroup Australis was the second most common serogroup to which dogs had antibodies. Results of this study concur with MAT data showing that infection of dogs with serovars belonging to this serogroup is common across Europe (Ellis 2010; Major and others 2014). Serovar Bratislava has been isolated from a wide range of domestic and wild animals including hedgehogs, rats, sheep and horses in Ireland, with which dogs can come into contact or share environments (Ellis and others 1983; Hathaway and others 1983). Seropositivity to serogroup Australis can be associated with severe clinical disease in dogs (Major and others 2014; Mastrorilli and others 2007; Mayer-Scholl and others 2013). At the same time it has been postulated that dogs may also serve as a maintenance host for serovar Bratislava (Ellis 2010).

Antibodies against serogroup Pomona were present in 1.1% of dogs, the majority of which had antibodies against serovar Altodouro. This serovar has only recently been isolated from house mice (*Mus musculus*) and may be a more sensitive antigen for detection of exposure to serogroup Pomona in dogs than serovar Pomona (Paiva-Cardoso and others 2013). Serogroup Pomona was previously considered to be an uncommon cause of clinical leptospirosis in dogs in Europe (Ellis 2010). This serogroup is therefore not presently included in anti-leptospiral vaccines marketed in Europe. In a study from Germany, 14% of dogs with confirmed leptospirosis had antibody titres against serogroup Pomona (Mayer-Scholl and others 2013). Antibodies against serovar Altodouro were also significantly associated with the presence of clinical disease in a cohort of Greek dogs (Arent and others 2013). It has therefore been suggested that inclusion of serogroup Pomona into future vaccines should be considered (Arent and others 2013).

Exposure to serovar Hardjo, belonging to serogroup Sejroe was infrequently observed in this cohort and might indicate transmission from cattle, which are maintenance hosts for this serovar.

None of the dogs included in this study (cohorts 1 and 2) showed evidence of prior exposure to serogroup Grippotyphosa. This serogroup has been associated with clinical disease in some European countries including Germany (Mayer-Scholl and others 2013), and France (Renaud and others 2013), but exposure appears to be rare in the UK and Greece (Arent and others 2013; Ellis 2010). Regional differences are likely due to the distribution of known carrier rodents, such as the common vole (*Microtus arvalis*), the root vole (*Microtus oeconomus*), and the muskrat (*Ondatra zibethicus*) (Ellis 2010).

Our data indicate that Irish dogs are exposed to a range of leptospiral serogroups. It is therefore important to test dogs suspected to have leptospirosis against a wide panel of serogroups including serogroups Ballum, Australis, Pomona and Sejroe along with serogroups Autumnalis, Canicola, Grippotyphosa, Icterohaemorrhagiae, as failure to include the infecting serogroup can lead to false negative MAT results (André-Fontaine 2006; Geisen and others 2007; Scanziani and others 2002).

Rising titres against serogroup Icterohaemorrhagiae in one dog suspected to have leptospirosis suggests that this serogroup is still a cause of infection in unvaccinated dogs in Ireland. As in the dog in this study, seroconversion may only occur days after the onset of clinical signs, leading to initially negative MAT titres. In dogs suspected to have leptospirosis it is therefore crucial to assess paired samples taken one or two weeks a part in order to increase the sensitivity of the MAT in the diagnosis of acute infection (Fraune and others 2013; Goris 2012).

In conclusion, results of this study indicate that a significant proportion of Irish dogs presented to veterinary practitioners for problems unrelated to leptospirosis have serum antibodies to serogroups Ballum, Australis, Pomona and Sejroe. While infection with serogroup Ballum is a rare cause of acute disease in dogs, canine exposure to this serogroup should be monitored as dogs can serve as sentinels for this serovar in the environment. There was no evidence for exposure to serogroup Grippotyphosa and Autumnalis in this population of Irish dogs. Given the potential of serogroup Australis to cause severe disease in dogs, vaccination with multivalent vaccines including this serogroup in addition to serogroups Icterohaemorrhagiae and Canicola is advisable.

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**Table 1: Genomospecies, serovar, strain and serogroup of *Leptospira* used as antigens for the Microscopic Agglutination Testing (MAT)**

NI: Northern Ireland

|  |  |  |  |
| --- | --- | --- | --- |
| **Species** | **Serovar** | **Strain** | **Serogroup** |
| *L. interrogans* | Canicola | Hond Utrecht IV | Canicola |
|  | Icterohaemorrhagiae | RGA | Icterohaemorrhagiae |
|  | Bratislava | S/82.0834 (NI isolate) | Australis |
|  | Autumnalis | Akyami A | Autumnalis |
|  | Pomona | Pomona | Pomona |
|  | Altodouro | Rim 139 | Pomona |
| *L. kirschneri* | Grippotyphosa | MoskvaV | Grippotyphosa |
|  | Mozdok | 5621 | Pomona |
| *L. borgpetersenii* | Hardjo | Hardjobovis (NI Isolate) | Sejroe |
|  | Ballum | Mus 127 | Ballum |

**Table 2: Percentages of positive MAT reactions and titres in dogs not suspected to have leptospirosis (cohort 1; n= 464))**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Serovar | Serogroup | Number of samples | % | MAT Titre |
|  Single titres |  |  |  | **10** | **30** | **100** |
| Ballum | Ballum | 13 | 2.8 | 4 | 7 | 2 |
| Bratislava | Australis | 9 | 1.9 | 2 | 7 |   |
| Altoduoro | Pomona | 4 | 0.9 | 2 | 2 |   |
| Hardjo | Sejroe | 2 | 0.4 | 1 | 1 |   |
| Mozdok | Pomona | 1 | 0.2 | 1 |   |   |
| Autumnalis | Autumnalis | 0 | 0 |   |   |   |
| Grippotyphosa | Grippotyphosa | 0 | 0 |   |   |   |
| Pomona | Pomona | 0 | 0 |  |   |   |
| Crossreactions |   |   |  |   |   |   |
| Altodouro/Mozdok |   | 1 | 0.2 |   |   | 1 |
| Equal titres |   |   |  |   |   |   |
| Ballum/Bratislava |   | 1 | 0.2 |   | 1 |   |
| Total |  | **31** | **6.7** | **10** | **18** | **3** |

**Table 3: MAT results in dogs suspected to have leptospirosis (cohort 2; n=11)**

|  |  |  |
| --- | --- | --- |
| **Dog #** | **Sample 1** | **Sample 2** |
| 1 | neg | - |
| 2 | 1:10 Bratislava\* | - |
| 3 | 1:30 Ballum | - |
| 4 | neg | neg |
| 5 | neg | - |
| 6 | neg | - |
| 7 | neg | - |
| 8 | 1:30 Bratislava1:100 Icterohaemorrhagiae | 1:10 Canicola1:10 Bratislava1:300 Icterohaemorrhagiae |
| 9 | neg | - |
| 10 | neg | - |
| 11 | neg | - |

\*Spirochaetes detected in urine via darkfield microscopy;
Leptospiral culture of urine negative.