Serological surveillance of canine leptospirosis in Mongolia

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LEPTOSPIROSIS is a disease of worldwide distribution (Cullen and others 2002) caused by spirochaete bacteria of the genus *Leptospira*. Although dogs are the only companion animals in Mongolia, there is little information on the infectious diseases, including leptospirosis, in dogs in the country. Since dogs in Mongolia are not vaccinated against leptospirosis, seroconversion cannot be due to vaccination. This short communication describes serological surveillance carried out as a preliminary step in order to evaluate the prevalence of leptospirosis in dogs in Mongolia.

A total of 163 serum samples was collected randomly in July 2003 from owned dogs in two geographically distinct provincial centres: 72 samples from Choibalsan (the provincial centre of Dornod province) and 91 from Mandalgovi (the provincial centre of Dundgovi province). There are approximately 4000 owned dogs in Choibalsan and 3000 in Mandalgovi; they are used as guard dogs outdoors rather than being kept as pets, and 80 to 90 per cent of them are male.

The grassland steppe of Choibalsan, in the east of the country, is richer in soil humidity. It has an abattoir where various species of livestock from the eastern part of Mongolia are slaughtered; the animals for slaughter are driven to the abattoir on foot. Mandalgovi, in the centre of Mongolia, has a combination of dry steppe and desert, with inadequate water resources. There is no abattoir in this area. In both areas, the families who own dogs live in dwellings without a mains water supply; water pumped from a deep well is transported by vehicle to a water-supplying pool for distribution.

A commercial enzyme immunoassay (EIA) (Immunocomb; Biogal-Galed Labs) for the detection of canine immunoglobulin (Ig) G antibodies to leptospiral common antigens was used for screening the serum samples, following the manufacturer's instructions; the relationship between the CombScale value and the microscopic agglutination test (MAT) titre (CombScale value/MAT titre) were as follows: 2/1:100, 3/1:200, 4/1:400, 5/1:800 and 6/1:1600. Samples with a CombScale value of 2 or above were considered positive.

For an indirect ELISA to detect IgM antibodies to leptospiral common antigens, disrupted cells of strain Akiyami C, serovar Australis were used as the antigen (Kida and others 1982); the assay procedure was that described by Ausubel and others (1999), and was performed with slight modifications.

For the MAT, the antigen panel consisted of the following live leptospires of serovars (strains): Australis (Akiyami C), Autumnalis (Akiyami A), Ballum (Mus 127), Bataviae (Swart), Bratislava (Jez-Bratislava), Canicola (Hond Utrecht IV), Copenhageni (Shibaura #9), Cynopteri (3522 C), Grippotyphosa (Moskva V), Hardjo (Hardjoprajitno), Hebdomadis (Akiyami B), Icterohaemorrhagiae (RGA), Javanica (Veldrat Bataviae 46), Mangus (TRVL/CAREC/137774), Monjakov (Monjakov), Pyrogenes (Salinem), Ruparupae (M 3), Saxkoebing (Mus 24), Sentot (Sentot 90 C), Tarassovi (Perepelitsin), Weaveri (CZ 390) and Whitcombi (Whitcomb), grown in tryptose phosphate broth (TPB) containing 10 per cent normal rabbit serum. The dilution of serum sample that showed more than 50 per cent agglutination with the testing serovar when compared with a control culture diluted 1:2 in phosphate-buffered saline was considered positive (Faine 1982, Stallman 1984). The antigen that gave the highest titre was chosen as a serovar of the infective *Leptospira*.

TABLE 1: Detection of immunoglobulin G (IgG) antibodies to leptospiral common antigens in samples of serum from owned dogs in two areas of Mongolia

Provincial centre	Number of samples	CombScale value* ≤1	2	3
Choibalsan	72	71	1	0
Mandalgovi	91	63	22	6

* IgG antibodies were detected by using a commercial test (Immunocomb; Biogal-Galed Labs). Samples which reacted at the CombScale value of 2 or above were considered positive

In the EIA, one of 72 (1·4 per cent) of the serum samples from Choibalsan was positive, at the CombScale value of 2; in Mandalgovi 28 of 91 (30·8 per cent) of the samples were positive; of these, 22 (24·2 per cent) reacted at the CombScale value 2, and six (6·6 per cent) at value 3 (Table 1).

All the samples from both locations were negative in the ELISA for the detection of IgM antibodies to common leptospiral antigens at the dilution of 1:100 (data not shown). This indicates that all the dogs that were positive in the EIA had been exposed to leptospires in the past, and that none was in the acute stage of infection.

The leptospiral serovar-specific antibodies were detected by the MAT at a titre of 100 or more in three of 72 (4·2 per cent) of the samples from Choibalsan and 15 of 91 (16·5 per cent) of those from Mandalgovi. The distribution of serovars and titres is shown in Table 2. The positive serum samples reacted with different leptospiral serovars but predominantly with serovar Cynopteri. The highest antibody titre, of 400, was detected in one sample from Mandalgovi with serovar Autumnalis. These results suggest that transmission of leptospirosis between dogs was uncommon and that the positive dogs were probably infected accidentally from other sources.

Contrary to the high serological prevalence of leptospirosis in cattle in Dornod province, as determined by Sebek (1974) and recently by the authors (N. Odontsetseg, Y. Sakoda, H. Kida, unpublished observations), leptospirosis was not prevalent in dogs in Choibalsan. There was no reference information for the prevalence of leptospirosis in any animal species in Dundgovi province. The dry climate of Dundgovi did not affect the transmission of leptospires in dogs in Mandalgovi, which suggests that other factors must be present which support the survival of the organism in the desert conditions. Cynopteri was found to be the predominant serovar, and further studies are needed to determine the source of this infection. No animals were found to be seropositive to serovar Cynopteri in previous studies of domestic animals in Mongolia (Sebek 1974, Odontsetseg and others 2005; N. Odontsetseg, Y. Sakada, H. Kida, unpublished observations). The animal reservoir host of serovar Cynopteri is the

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TABLE 2: Antibody titres to different *Leptospira* serovars in the serum of owned dogs from two areas of Mongolia, assessed by a microscopic agglutination test

Provincial centre	Number of samples	Serovar	Titre 100	200	400
Choibalsan	72	Cynopteri	1	–	–
		Saxkoebing	1	–	–
		Copenhageni	1	–	–
Mandalgovi	91	Cynopteri	4	4	–
		Saxkoebing	2	2†	–
		Javanica	2	–	–
		Autumnalis	–	–	1
		Bratislava	1*	–	–
		Copenhageni	1*	–	–
		Pyrogenes	–	1	–

* Mixed equal reactor
† One of these was a mixed equal reactor

bat (Bharti and others 2003). Bats of the genera *Myotis*, *Plecotus*, *Pipistrellus*, *Eptesicus* and *Vespertilio* have been found to be widespread throughout Mongolia (Dulamtseren and others 1989). Despite a lack of information, it is speculated that bat populations are denser in Dundgovi province, which may contribute to the transmission of leptospires.

Although the dogs are fed by their owners, they may also fend for themselves, and thus come into increased contact with wild rodents; however, serovar Cynopteri is not considered to be maintained by rodent species. In a few surveillance studies in Europe, serovar Cynopteri was rarely found in cattle, sheep, pigs and horses (Ellis and Michno 1976, Rocha 1998, Ciceroni and others 2000).

The results of the present study indicate that a large-scale survey should be carried out to establish the current extent of infection of dogs with leptospires in Mongolia and to determine the risk factors, in order to develop and implement successful control measures.

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