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REVIEW

Prevalence of leptospirosis in farm animals

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Leptospirosis is a worldwide zoonosis caused by infection with pathogenic spirochetes that belong to the genus *Leptospira*. *Leptospira* spp. colonize the proximal renal tubules of various mammals and are excreted in the urine of carrier animals. Transmission of leptospirosis in humans and animals occurs by exposure to water or soil contaminated by the urine of infected animals or by direct contact with infected animals [4]. The genus *Leptospira* consists of both pathogenic and saprophytic (non-pathogenic) species. *Leptospira* species are defined according to DNA relatedness. Twenty species, 8 pathogenic, 5 intermediate, and 7 non-pathogenic groups, have been described [2]. Leptospires are divided into numerous serovars. Approximately 250 serovars and over 60 serovars were recognized among the pathogenic *Leptospira* spp. and non-pathogenic leptospires, respectively [2,4]. Antigenically related serovars are grouped into serogroups, 26 of which have been described for pathogenic strains [2,7].

Farm animals such as cattle and swine are not only infected as incidental hosts but also are maintenance hosts of specific *Leptospira* serovar strains and serve as reservoir animals for the same animal species and/or humans. Incidental host infections of cattle with serovars such as Grippotyphosa, Icterohaemorrhagiae and Pomona cause acute, severe clinical disease [4]. Clinical signs of acute bovine leptospirosis include high fever, haemolytic anaemia, haemoglobiuria and jaundice. Infection in pregnant cattle can result in abortion. In lactating cows, infections are often associated with agalactia. Cattle also serve as a maintenance host of serovar Hardjo, which consists of two different genotypes, Hardjobovis and Hardjoprajitno. Antibodies against this serovar were detected in 25 to 65% of cattle in the US, Europe, South America and Australia and *L. borgpetersenii* serovar Hardjo type Hardjobovis (Lb Hardjobovis) is the most common in cattle worldwide [6]. Recent completion of genomic sequencing of two strains of Lb Hardjobovis revealed that Lb Hardjobovis has been evolving toward a host-adapted bacterium [1]. The Hardjobovis genome is decaying through a process of insertion sequence-mediated genome reduction. Loss of gene function is centered on impairment of environmental sensing and metabolite transport and utilization, which affects survival of Lb Hardjobovis in its environment and results in a strict host-to-host transmission cycle. Acute infection with Hardjobovis results in asymptomatic or mild cases, but chronic infection is associated with infertility and reproductive failures such as abortion, stillbirth and weak calves. Abortion and the other effects usually occur 1 to 6 weeks (serovar Pomona infection) or 4 to 12 weeks (serovar Hardjo infection) after the acute phase of infection [3]. With serovar Pomona infection,

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abortion usually occurs in the last trimester of gestation. With serovar Hardjo infection, abortion has been diagnosed at all stages and early embryonic death may occur [3]. Infertility has commonly been observed in Hardjo-infected herds [5]. The abortion rate after Lb Hardjobovis infection is 3 to 10% whereas the rate increases up to 30% for *L. interrogans* serovar Hardjo type Hardjoprajitno (Li Hardjoprajitno) infection [3,6]. Li Hardjoprajitno is often associated with acute infection in dairy cows leading to milk drop syndrome. Leptospirosis in cattle is a notifiable disease in Japan but there are almost no reports on the disease in recent years, and the actual prevalence of bovine leptospirosis remains unknown. Therefore, this study attempts to reveal the current situation of leptospiral infection and the relationship between abortion and leptospirosis in cattle in Japan.

We carried out testing for anti-leptospiral antibody among 343 healthy dairy cattle from 19 farms in Hokkaido by microscopic agglutination test (MAT). Anti-leptospiral antibody (reciprocal MAT titer $\geq$ 100) was detected from 44 cattle on 9 farms (12.8%, Table 1). The predominant reacting serogroup was Sejroe, to which serovar Hardjo belongs (42/44, 95.5%), followed by Hebdomadis (6/44, 13.6%) and Autumnalis (1/44, 2.3%). The leptospiral *flaB* gene was amplified by nested polymerase chain reaction (PCR), and then the nucleotide sequences of the amplicons were determined. Leptospiral *flaB* was detected in 2 of the 39 urine samples from 3 seropositive farms. Both PCR-positive cattle had histories of abortion. The nucleotide sequences of the two amplicons

Table 1. Prevalence of anti-leptospiral antibody in cattle in Hokkaido[a)]

Farm	No. of cattle tested	No. of positive cattle	Reacting serogroups (no. of positive cattle)
RK-1	5	0	
RK-2	5	1	Sejroe (1)
RK-3	5	0	
RK-4	10	5[b)]	Hebdomadis (2), Sejroe (5)
RK-5	5	0	
RK-6	5	0	
RK-7	10	4	Hebdomadis (1), Sejroe (3)
RK-8	5	0	
RK-9	5	0	
RK-10	10	3	Sejroe (3)
HU-1	22	0	
HU-2	22	5	Sejroe (5)
HU-3	22	0	
HU-4	22	0	
DY-1	36	15[b)]	Hebdomadis (2), Sejroe (15)
DY-2	44	2	Autumnalis (1), Sejroe (1)
DY-3	44	1	Sejroe (1)
DY-4	26	8[b)]	Hebdomadis (1), Sejroe (8)
DY-5	40	0	
19	343	44 (12.8 %)	Autumnalis (1), Hebdomadis (6), Sejroe (42)

a) Reciprocal MAT titer $\geq$ 100

b) There were 2 cattle, 2 cattle and 1 cow which reacted equally to both Hebdomadis and Sejroe in farms RK-4, DY-1 and DY-4, respectively.

Table 2. Prevalence of anti-leptospiral antibody in cattle with and cattle without histories of abortion[a)]

Farm	Seropositive rate (no. of positive cattle / no. of cattle tested)	Seropositive rate in cattle with histories of abortion (no. of positive cattle / no. of abortion-experienced cattle)	Seropositive rate in cattle without histories of abortion (no. of positive cattle / no. of abortion-naïve cattle)
DY-1	38.9% (14/36)	47.4% (9/19)	35.3% (6/17)
DY-2	4.5% (2/44)	9.1% (2/22)	0 % (0/22)
DY-3	2.3% (1/44)	0 % (0/22)	4.5% (1/22)
DY-4	30.7% (8/26)	23.1% (3/13)	38.5% (5/13)
DY-5	0 % (0/40)	0 % (0/20)	0 % (0/20)

a) Reciprocal MAT titer ≥ 100

were identical to each other and those of the Lb Hardjobovis strains mentioned above. *L. borgpetersenii* has been isolated from rodents and shrews in Hokkaido (Data not shown). However, the sequence obtained in this study was not identical to any of those obtained from small mammals in Hokkaido. These serological and DNA analyses strongly suggest wide spread of Lb Hardjobovis among cattle in Hokkaido, although attempts to isolate leptospires failed.

Next, we examined the relationship between history of abortion and presence of anti-leptospiral antibody on 5 farms where the history of abortion for each cow was recorded. The seroprevalence was high on 2 of the 5 farms (30.7% and 38.9%), but there was no difference between cattle with and cattle without histories of abortion (Table 2). On 3 other farms, low seroprevalence was detected in both abortion-experienced and abortion-naive cattle. On the other hand, comparison of the embryonic age at the time of abortion between seropositive and seronegative cattle revealed that abortion occurred earlier in seropositive cattle than in seronegative cattle (positive; 51.5 days (median, N = 8), negative; 82 days (N = 77), $p = 0.038$). The presence of anti-leptospiral antibody in abortion-experienced cattle and the differences in the fetal age at the time of abortion between seropositive and seronegative cattle suggested a positive correlation between abortion and leptospiral infection in Hokkaido, although further verification with a larger sample size is needed.

In this study, of two highly seroprevalent farms, one bought cattle from the other, and the other entrusted their cattle during the summer season to another operation, suggesting that contact with carrier cows may have caused infection. Thus, it is important to identify and eradicate carrier animals introduced from outside. Carrier animals can be identified using urine samples by isolation, fluorescent antibody staining or detection of leptospiral DNA using PCR [6,10]. However, it is difficult to carry out these methods in the field. More recently, a loop-mediated isothermal amplification (LAMP) method has been developed for detecting pathogenic leptospires [8,9]. Unlike PCR, the LAMP method amplifies a target DNA sequence under isothermal conditions for about an hour with high specificity and efficiency, and the results can be assessed with the naked eye, promising lower expenses for equipment. We have developed a LAMP method for the detection of leptospiral DNA in urine with a more simple procedure, which is applicable to point-of-care testing. We will report results of evaluation of this LAMP method using field animal urine samples.

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