



Herd-level risk factors associated with *Leptospira* Hardjo infection in dairy herds in the southern Tohoku, Japan



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ABSTRACT

A cross-sectional study was designed to generate information on the herd level prevalence and the risk factors for *Leptospira* serovar Hardjo (*L. Hardjo*) in Yamagata, the southern Tohoku, Japan. Bulk tank milk samples from 109 dairy herds were used to test the herd level sero-prevalence of *L. Hardjo* using a commercial ELISA kit, which detects both *L. interrogans* serovar Hardjo and *L. borgpetersenii* serovar Hardjo. A questionnaire survey was conducted at the sampled farms, and univariable and multivariable analyses were performed. Spatial clustering of *L. Hardjo* at the herd level was examined using spatial scan statistics. Seventy-one herds were found to be positive for *L. Hardjo*, and the apparent herd prevalence was 65.1% (95% CI: 56.2–74.1%). The risk factors for sero-positivity were larger herd size ($p = 0.004$) and cows with a history of staying in Hokkaido ($p < 0.001$). The spatial scan statistic detected a most likely cluster (relative risk = 1.87, log likelihood ratio = 9.93, radius = 13.70 km, $p < 0.01$) in the southern part of the study area where there are large herd sizes and farm density is high. This study revealed that *L. Hardjo* is prevalent throughout Yamagata, and large scale herd owners introducing cows from Hokkaido in particular should be aware of the risk of infection.

1. Introduction

Leptospirosis is one of the most prevalent zoonoses caused by bacteria of the genus *Leptospira* (Adler and de la Peña Moctezuma, 2010) and it has been reported world-wide in wild and domestic animals (Levett, 2001). Sixteen genomospecies and > 260 serovars of *Leptospira* have been identified (Levett, 2001). *Leptospira* are shed in the urine of infected animals which contaminates water or soil, and they can survive there for weeks to months (Ko et al., 2009). Infection with pathogenic strains of *Leptospira* commonly occurs through direct contact with infected animal urine or indirectly through contaminated water (Bharti et al., 2003; Levett, 2001). The bacteria can enter the body through the skin or mucous membranes (eyes, nose or mouth), especially if the skin is broken from a cut or scratch (Levett, 2001). Leptospirosis may present a wide variety of clinical manifestations in humans (Levett, 2001). Many human cases are mild, influenza-like illnesses lasting 3–5 days, which are difficult to distinguish from other infectious diseases (Evangelista and Coburn, 2010). The most severe disease form is Weil's syndrome, which is characterized by jaundice, acute renal failure and bleeding (Bharti et al., 2003; Evangelista and Coburn, 2010; Ko et al.,

2009; Levett, 2001).

The most common causal species of bovine leptospirosis is *Leptospira* belonging to serovar Hardjo (*L. Hardjo*), and cattle are the maintenance host (Grooms, 2006; Levett, 2001). A notification of cattle which contract the disease by *L. Hardjo* is obligated in Japan. Two types of *L. Hardjo* serovar have been identified: *Leptospira interrogans* serovar Hardjo (type Hardjoprajitno) and *L. borgpetersenii* serovar Hardjo (type Hardjo-bovis) (Grooms, 2006; Levett, 2001). A high prevalence of serovar Hardjo in dairy herds has been reported world-wide (Leonard et al., 2004; O'Doherty et al., 2013; Salgado et al., 2014). Although reported cases of *L. Hardjo* in Japan are limited (Kikuchi et al., 2013; Tsuchimoto et al., 1983), a cross sectional study throughout Japan found that the overall sero-prevalence of *L. Hardjo* was also high (Kikuchi et al., 2013). The risk factors for infection with *L. Hardjo* in dairy herds reported in previous studies in several parts of the world included herd size, geographical region, movement of cattle onto and off farms, co-grazing, professional visitors not wearing protective clothing, rearing calves naturally, adult cows in contact with calves, and not cleaning oral drenching equipment (Leonard et al., 2004; O'Doherty et al., 2014; Salgado et al., 2014; van Schaik et al., 2002).

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Bovine leptospirosis is economically important due to reproductive losses from abortions, stillbirths and infertility, as well as increased services per conception and prolonged calving intervals (Dhaliwal et al., 1996a, 1996b).

There is no reported case of human leptospirosis caused by *L. Hardjo* in Japan, and the most human cases caused by the other serovars are reported in Kyushu and Okinawa district (National Institute of Infectious Diseases, 2016) where a subtropical climate is prevalent. However, in New Zealand, it was the most common serovar in notified human cases between 2011 and 2014, and slaughterhouse workers and livestock farmers were the most frequently reported occupations among the cases (Sanhueza et al., 2015). Human leptospirosis cases in Tohoku which is located in the northern part of Japan and has a subarctic climate are rare, and there may be a possibility that leptospirosis cases caused by *L. Hardjo* relating to livestock occupations in Japan are neglected.

Thus, it is important to identify the prevalence and the risk factors for *L. Hardjo* infection on dairy herds to control it in the northern part of Japan on the aspects of both economic impacts on farming productivity and human public health. However, there is no risk factor analysis for *L. Hardjo* infection on dairy farms in Japan so far. The previous study (Kikuchi et al., 2013) showed the sero-prevalence in Tohoku, but the sample size was small. Therefore, Yamagata Prefecture Agricultural Mutual Aid Association initiated investigations into the situation including epidemiological analysis. The objectives of this study were to investigate the sero-prevalence and risk factors for *L. Hardjo* infection of dairy herds in Yamagata Prefecture, the southern Tohoku, Japan.

2. Materials and methods

2.1. Bulk milk sampling and diagnosis with *Leptospira Harjo*

A cross-sectional study was conducted with all of the 109 dairy herds belonging to the biggest dairy association in Yamagata, Yamagata Prefecture Dairy Farming Cooperative (YDFC), which covers the majority of the Okitama region (Yonezawa, Takahata, Nanyo, Kawanishi, Iide, Nagai and Shirataka) and the southern part of the Murayama region (Yamagata, Kaminoyama, Yamanobe and Asahi) (Fig. 1). This study population accounts for 33.6% (109/322 farms) of the target population in Yamagata Prefecture (Ministry of Agriculture, Forestry and Fisheries, 2014). None of the herds in this study were vaccinated against *L. Hardjo*.

Bulk tank milk samples were collected to test the herd level seroprevalence of *L. Hardjo* antibodies. Bulk milk sampling was conducted on 25th March 2014 at the normal time of milk collection. The Linnodee *Leptospira* ELISA Kit™ (Linnodee Animal Care, Ballyclare, Northern Ireland) was used (Yan et al., 1999) which detects an IgG antibody response to a lipopolysaccharide outer envelope epitope common to both *L. borgpetersenii* serovar *Hardjo* and *L. interrogans* serovar *Hardjo* (Yan et al., 1999). The test was assumed to have a sensitivity and specificity for individual serum samples of 94.1% and 94.8%, respectively (Ryan et al., 2012b).

2.2. Questionnaire survey

A questionnaire was designed to investigate the risk factors for herd level sero-positive responses to *L. Hardjo*. Table 1 shows the contents of the questionnaire. The questions were chosen based on the results of similar studies of dairy herds (Leonard et al., 2004; O'Doherty et al., 2013; Salgado et al., 2014; van Schaik et al., 2002). Open questions were used for the items of the farm information, and closed questions were used for the other items of the questionnaire. The survey was conducted between September 2014 and January 2015 by veterinary medical clinicians who regularly visit these herds, and the herd owners were interviewed. While the survey was conducted, no investigator

knew the result of the ELISA test. Pre-testing on a small sample from the study population was conducted and then the questionnaire was revised before applying it to the study population. All the investigators were explained how to conduct the survey before the administration. Data from the survey and the diagnostic tests were digitized using an Excel® spreadsheet (Microsoft® Office® 2013, USA).

2.3. Collection of annual milk yield data

To understand the economic impact of leptospirosis on milk yield, annual milk yield data of 109 farms in 2014 were obtained from YDFC.

2.4. Statistical analysis

The sensitivity and specificity for the ELISA kit were known as stated above. However, these parameters were calculated at the individual animal level, and cannot be applied to the herd level (Sargeant et al., 1997). Therefore, this study used the apparent prevalence, and the 95% confidence interval (CI) was calculated.

For the univariable risk factor analysis at the herd level, comparisons between the herd level sero-positivity on each factor of the questionnaire were analyzed. Wilcoxon rank sum test was performed for count and non-normally distributed continuous data, student's *t* test for normally distributed continuous data, Pearson's Chi-squared test with Yates' continuity for binary and categorical data, and Fisher's exact test for data when at least one cell included expected frequencies < 5.

The variables with a *p*-value < 0.2 (Dohoo et al., 2014) in univariable analyses were investigated further for collinearity, and variables whose correlation coefficient were < 0.9 with any of the other variables, and variables that had biological plausibility were fed into a multivariable model as explanatory variables. For the multivariable analysis, a logistic regression was performed using a GLM with binomial errors choosing the serological test results as the outcome variable. Step-wise model simplification was performed checking with a likelihood ratio test. Confounding was tested by monitoring the change of logit of a factor of interest by removing a suspected confounder from the model (Dohoo et al., 2014). Interaction terms among all the variables in the simplest model (Dohoo et al., 2014) were tested. To evaluate a model, goodness-of-fit tests by Hosmer-Lemeshow test and Akaike's Information Criteria (AIC) were used. All the statistics were performed using statistical software R version 3.1.0 (R Core Team, 2016).

To investigate for spatial clustering of *L. Hardjo* sero-positivity, spatial scan statistics were performed using SaTScan™ version 9.4.4 (Kulldorff, 1997). For the analysis, a purely spatial analysis for scanning the clusters with high rates using the Bernoulli model was used, and the default settings were used for the other features. The locations of participating farms were provided by YDFC.

For the economic impact, annual milk yield per cow, as total milk yield delivered from a farm in 2014 divided by the number of adult cows in the farm, was compared between sero-positive and negative herds using Wilcoxon Rank Sum test.

3. Results

3.1. Prevalence

Seventy-one of the 109 sampled herds were positive, and the herd-level apparent prevalence was 65.1% (95% CI: 56.2–74.1%).

3.2. Univariable analysis

Sero-positive herds (mean: 60.4, median: 39, range: 4–500) were significantly larger than sero-negative herds (mean: 30.1, median: 27, range: 4–160, *p* < 0.001). The average herd size of adult cows of the studied farms was 50.0, and was representative of dairy herds in Japan

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