

Epidemiologic Indicators Associated with Within-farm Spread of Johne's Disease in Dairy Farms in Japan

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ABSTRACT. Epidemiologic indicators associated with within-farm infection of Johne's disease in dairy farms in Japan were determined through a nationwide investigation of infected farms. We assumed that subsequent detection of the disease within one year after the first detection could represent the occurrence of within-farm spread occurring before the first detection. Of 594 infected farms, 158 farms (27%) had at least one additional detection. Logistic regression analysis using epidemiologic information obtained from infected farms at the time of the first detection revealed three epidemiologic indicators associated with subsequent detection. Farms at which the first cases included cattle with clinical signs were 3.8 (95% confidence interval: 2.2, 6.8) times more likely to have additional detections than those with cattle without clinical signs. Similarly, farms where two or more cattle were detected at the time of first detection and where cattle were held in a loose housing system were 2.8 (95% CI: 1.8, 4.5) and 2.0 (95% CI: 1.1, 3.6) times more likely to have additional detections than those where only one animal was detected and a tied-up housing system was used, respectively. These epidemiologic indicators are likely important determinants in the selection of farms requiring more intensive on-farm control measures.

KEY WORDS: epidemiology, Johne's disease, logistic regression model, retrospective cohort study.

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Johne's disease is a chronic intestinal disease of ruminants caused by *Mycobacterium avium subsp. paratuberculosis* which is found throughout the world [4, 12]. It affects dairy industries by reducing milk yield and slaughter values, and increasing culling rates [1, 3, 8, 10]. In the U.S.A., for example, annual economic losses due to Johne's disease have been estimated at US\$200–250 million [17].

Johne's disease is a notifiable disease in Japan, and by the mid-1990s, all cases were being reported to the Ministry of Agriculture, Forestry and Fisheries (MAFF). In addition to this passive surveillance, active surveillance is now also undertaken following an amendment of the Domestic Animal Infectious Disease Control Law in 1997. All targeted cattle designated by each prefecture undergo testing for Johne's disease at least once every five years. In 2004, these two surveillance methods together detected a total of 1,118 infected cattle and 608 farms, respectively [6].

Because of the nature of the Johne's disease it is difficult to detect all infected cattle by single testing only. In particular, detection of subclinically infected cattle is not easily achieved by current diagnostic methods owing to their strong dependence on the timing and proper use of diagnostic tests, as well as life stage or physiological condition of the cattle, including age or stress associated with pregnancy or calving [16, 20]. As a result, infected farms in Japan are placed under regulatory monitoring with more frequent testing for a certain period determined by the respective prefecture [13, 14]. All cattle in these farms are required to be confirmed negative in all repeated testings.

Control measures for this condition might be improved

when the degree of within-farm spread of the disease is grasped at the time of initial detection. We therefore considered it would be useful to identify possible indicators of spread within the farm at the time of first detection based on epidemiologic information. The availability of such predictors would assist local animal health administrations in achieving effective and efficient control of the disease.

Here, we report the results of a retrospective cohort study aimed at identifying epidemiologic indicators associated with within-farm spread of Johne's disease, defined on the basis of additional detection within one year after the first detection.

MATERIALS AND METHODS

Nationwide survey of farms with Johne's disease: In May 2005, a nationwide survey of farms infected with Johne's disease in Japan was conducted with the cooperation of MAFF. Computer-based questionnaires were prepared using commercial spreadsheet software (Excel, Microsoft Corp., Redmond, WA, U.S.A.), then sent electronically to local animal health authorities in all 47 prefectural governments. The questionnaires included two components, the first concerning the epidemiological information of infected farms and cattle including date of detection, number of detected animals in both of the first and subsequent detection, clinical signs, type of production, type of cattle housing system, origin of the detected animal(s), colostrum management, number of the adult cattle (hereafter, 'herd size') and culling after the first detection due to the reason other than Johne's disease (hereafter, 'culling'). The second part includes the questionnaire on the way of regulatory monitoring conducted in each prefecture. Responses were received by November 2005. Unclear or unspecific responses were

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clarified with the prefecture, and then all data were entered into a database (Excel).

Term definitions and study population: In this study, 'cases' means individual animals confirmed by any of the following officially approved criteria for Johne's disease, namely 1) identification of the agent by direct microscopic inspection of samples from cattle showing clinical signs, 2) isolation of the agent by culture, 3) a positive reaction on both ELISA and in the Johnin intradermal hypersensitivity test, 4) a positive reaction on both the Johnin intradermal hypersensitivity test and complement fixation test and 5) two sequential ELISAs performed within a certain period, usually two weeks. 'First case(s)' indicates the initially confirmed case(s) in a farm and 'detection' means the confirmation of any cases in general. The first cases detected in a farm were defined as first detection, and any additional detection as 'subsequent detections'.

Given these definitions, we focused on dairy farms having at least 10 adult cows and data on subsequent detections that could be traced for more than one year after the first detections.

Longitudinal observation and statistical analyses of subsequent detections: This study was designed as a retrospective cohort study in which the study unit was the individual infected farm. Since all responding prefectures conducted intensive testing at least for one year after the detection, all infected farms were followed to identify any subsequent cases occurring within one year of the first detection, on the basis that cases subsequently detected in this period were missed by the initial test. Farm with subsequent detection were regarded as those in which the disease had already spread by the time the first case was detected.

Statistic analyses were then conducted to identify epidemiologic indicators associated with the presence or absence of subsequent detections. First, associations between each item from the questionnaire survey and subsequent detections were screened in univariable analysis. The chi-square test or Fisher's exact test was used for the categorical vari-

able. Numeric variables were categorized into several levels according to the range of each variable, then applied to the same procedures for screening. Second, a multivariable binominal logistic regression analysis was performed using variables with $p < 0.25$ in the univariable analysis. As the objective of this analysis was to determine the indicators associated with the presence or absence of subsequent detections, items that could be potential confounders were always forced into the model. The type of testing used during the relevant period (ELISA alone or combined use of ELISA and fecal culture) and its frequency (\leq twice/year or $>$ twice/year) were regarded as potential confounders because they may influence the efficacy of the detection in the monitoring period. These two variables were amalgamated into one factor with four levels and forced into the model. It was also assumed that herd size was related to the subsequent detections, and was also included in the model as a categorical variable with four levels based on the 25th, 50th and 75th percentile of the number of adult cattle in each farm.

For the multivariable logistic regression model, variables were selected using a stepwise backward elimination approach, using $p < 0.15$ for entry and $p < 0.05$ for retention. All possible two-way interactions were then assessed, and retained in the model if the associated p -value was < 0.05 . The final model was checked for goodness-of-fit using Hosmer and Lemeshow statistics [9]. All statistical analyses were conducted using SPSS version 15.0 (SPSS Inc., Chicago, IL, U.S.A.).

RESULTS

A total of 1,105 farms having a first case detected after 1997 were reported from 46 of the 47 prefectures in Japan. Of these, 594 farms met conditions for our further analysis.

Of these 594 farms, 158 (27%) had cases of subsequent detection within one year. Results of univariable analysis between subsequent detection and items of the survey are

Table 1. Results of univariable analyses to identify potential epidemiologic indicators associated with subsequent detection within one year after the first detection of bovine Johne's disease

Variable	Level	Subsequent detection within one year after the first		P
		Yes	No	
Total		158	436	
Number of first cases	One	91	372	<0.001
	Two or more	67	64	
Clinical signs in first cases	Absent	112	399	<0.001
	Present	46	37	
Origin of the first cases	Home-bred	100	260	0.42
	Introduced	58	176	
Type of cattle housing	Tied-up system	85	342	<0.001
	Loose system	73	94	
Colostrum management	No	54	129	0.7
	Yes	28	60	
Culling after the first detection	No	85	350	<0.001
	Yes	73	86	

Table 2. Final multivariable logistic regression model of epidemiologic indicators associated with subsequent detections within one year after the first detection of bovine Johne's disease adjusted by 'herd size' and 'test used and its frequency within one year after the first detection'

Variable	Level	B ^{a)}	SE ^{b)} (B)	p	OR ^{c)} (95%CI ^{d)})
Clinical signs in first cases	Absent				1
	Present	1.3	0.3	<0.001	3.8 (2.2, 6.8)
Type of cattle housing	Tied-up system				1
	Loose system	0.7	0.3	0.01	2.0 (1.1, 3.6)
Number of first cases	One				1
	Two or more	1	0.2	<0.001	2.8 (1.8, 4.5)
Constant		-4.8	0.7	<0.001	

a) Logistic regression coefficient, b) Standard error, c) Odds ratio, d) Confidence interval, Hosmer-Lemeshow goodness-of-fit chi-square score = 5.42 (d.f.=8, p=0.71)

summarized in Table 1. In this analysis variables with $p < 0.15$ were the number of cattle detected in the first detection, presence of cattle with clinical signs consistent with Johne's disease in the first detection, type of cattle housing, and culling.

Among these items, culling was not used for further analysis since this item was not related to the degree of within-farm spread at the time the first case(s) was detected. The multivariable binominal logistic regression analysis was therefore performed using three candidates, 'the number of first cases (one, or two or more)', 'whether the first case(s) included cattle with clinical signs' and 'type of cattle housing (free stall or tie stall)', and two potential confounders, 'herd size' and 'test(s) used in the monitoring period and frequency within one year after detection of the first case(s)'.

As a result of the logistic regression analysis, all variables initially included in the model were retained ($p < 0.05$) and no significant two way interactions were observed ($p > 0.06$) among the variables. We therefore established the final model with three variables controlled by two confounders, as shown in Table 2. That is, farms with first cases showing clinical signs consistent with Johne's disease were 3.8 times more likely (95% confidence interval: 2.2, 6.8) to have subsequent detections than farms without those with clinical signs ($p < 0.001$). Similarly, farms where cattle were held in loose housing and had two or more cattle detected as first cases were 2.0 (95% CI: 1.1, 3.6, $p = 0.01$) and 2.8 (95% CI: 1.8, 4.5, $p < 0.001$) times more likely to have subsequent detections, respectively. The Hosmer and Lemeshow statistics value for goodness-of-fit of the final model was 5.42 ($P = 0.71$; degree of freedom=8), which was considered to be acceptable.

DISCUSSION

Control programs against bovine Johne's disease vary by country, mainly due to the difference in prevalence [11]. Most of these programs adopt necessary control measures in accordance with the degree of infection of each farm. Given the nature of the disease, however, diagnostic methods for Johne's disease do not always provide results with sufficient reliability to allow the estimation of farm prevalence [16,

20]. Nevertheless, estimation of the degree of within-farm infection should assist animal health administrators to determine appropriate measures at an early stage. The present retrospective cohort study took a longitudinal approach to identify epidemiologic indicators associated with subsequent detection, which represents the occurrence of within-farm spread, and found three significant indicators as follows.

First, farms with a first case showing clinical signs consistent with Johne's disease were more likely to have subsequent detections than those without clinical signs ($p < 0.001$). In general, clinical signs of Johne's disease are observed in the middle or late stage of the infection, although the infection itself is supposed to occur during infancy and subclinically infected cattle can excrete the agent intermittently before showing clinical signs [2, 5]; in such farms the agent might be transmitted to other herd mates before the first cases are detected.

Second, another indicator for subsequent detection is loose as opposed to tied-up housing ($p = 0.01$). Large amounts of agent are contained in the feces of infected cattle in the excretion stage [4]. In the loose housing system, including free stalls and free barns where infected cattle can move freely, susceptible cattle directly contact contaminated feces from infected cattle [18].

Third, the final indicator significantly associated with subsequent detection was the number of cattle detected as first cases ($p < 0.001$). This implies that Johne's disease in such farms had already become prevalent before the first detection compared to those with only one infected cow, making subsequent detection in the monitoring period more likely.

We did not find that the origin of the first cases was a significant indicator for subsequent detections (Table 1). In general, earlier studies have reported that introduction of infected cattle is the critical point in the introduction route of Johne's disease [12]. If the first detections were the infected importees, this might suggest that early detection was achieved and within-farm spread was prevented. In the present study, however, no epidemiological evidence for such an association was seen. This difference with previous findings may be related to the diagnostic difficulty of Johne's disease [15].

Given that the main purpose of the present study was to analyze the current epidemiological features of Johne's disease in Japan, cases were defined as those cattle satisfying at least one of the diagnostic criteria defined by the law in Japan. Case definitions in studies from other countries, however, have varied widely, such as on the basis of combinations of single or combined test results, and epidemiological characteristics of cattle or farms [7, 10, 17, 19]. These differences should be kept in mind in any comparison of the present and previous studies.

Finally, the indicators identified in this study were expected to determine farms for the degree of within-farm spread of the disease and intensiveness of the control measures to be taken. These indicators are actually reflected in the official guidelines for Johne's disease control and prevention issued by MAFF in November 2006 for adopting measures by the degree of infection on farms. Based on the present study, further research to clarify the risk factors related to between- and within-farm transmission will contribute to the improvement of control measures for Johne's disease in Japan.

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