Short Communication

A Survey of *Theileria sergenti* Infection, Tick and Horse Fly Infestation in 304 Herds of Grazing Holstein Heifers in Japan

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Summary

We conducted a questionnaire survey of 304 herds of grazing Holstein heifers in Japan. Using the results of the survey, we investigated *Theileria sergenti* infection, tick infestation and horse fly infestation in associated with potential risk factors such as environmental and management conditions. *Theileria sergenti* infection was more common in the southern part of Japan and herds with drug and acaricide usage were associated with *T. sergenti* infection. Herds with tick infestation was highly associated with *T. sergenti* infestation. Horse flies were more commonly observed in prefectures other than in Hokkaido. Proper preventive strategy for these parasite infestation is strongly required.

Keywords : *Theileria sergenti*, Ticks, Horse fly

Introduction

Seasonal grazing on grassland pasture between spring and autumn is a common practice for dairy heifers in Japan⁹. Private dairy farmers leave their heifers on public pasture land for grazing from spring to fall. During grazing time, heifers are exposed to a variety of infectious agents. Among these, tick and tick-borne infections such as *Theileria sergenti*, a tick-transmitted protozoan infection of cattle in Japan and other Asian countries^{2,6}, and horse fly infestation, which reported to be the vector of bovine leukosis¹ are big problems for grazing heifers^{5,7,9}.

Considering the above problems, reports on wide-scale field studies investigating on vector and vector-borne diseases were limited. Therefore, data of 304 herds used for grazing Holstein heifers were subtracted from the previous national survey of grazing herds (n = 701) and were utilized to investigate the prevalence and potential risk factors with *T. sergenti* infection, tick and horse fly infestations on grazing Holstein heifers.

Materials and methods

The previous study was conducted with the support of local

livestock animal hygiene centers throughout Japan⁹. A questionnaire that included questions on the status of *T. sergenti* infection, tick and horse fly infestation, environmental and management factors for grazing herds was sent to the livestock hygiene centers. Field veterinary officers at local livestock hygiene centers were requested to visit grazing herds and completed the questionnaire with the support of herd managers. From this database, we subtracted herds that met the criteria of having 30 or more Holstein-Friesian heifers and a grazing area of 10 ha or more. The final number of the herds was 304.

The following study outcomes (dependent variables) were investigated by questionnaire.

Theileria sergenti Infection (TS)

Yes : Herds reported by the manager as having problems associated with *T. sergenti* infection. The definition of problems with *T. sergenti* included whether heifers developing clinical signs of theileriosis, there are needs to prevent and treat infected animals during grazing season.

No : Herds reported by the manager as having no problems associated with *T. sergenti* infection.

This observation was done either by herd managers or veterinarians at the livestock hygiene centers.

Tick Infestation (TI)

During the high tick season, ticks were sometimes identified only a few (low infestation), some (medium infestation) to most of the animals (heavy infestation). In the same season, no ticks were identified (no infestation).

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Table 1 Continuous values for the independent and dependent variables

Variable	No. of herds*	Mean	95% LCI	95% HCI	Range
Area (ha)	298	200.7	170.3	230.9	10–2,186
Number of Animals (head)	304	299.0	248.4	349.6	30–3,473
Grazing Period (days)	287	165.9	162.9	168.9	76–274
Average Annual Temperature (°C)	238	12.2	11.7	12.6	3.7–21.3
Summer Time Temperature (°C)	242	22.6	22.1	23.1	16–34
Annual Rainfall (mm ³)	204	1,253.0	1,171.7	1,334.3	254–4,000
Facility Parameter	304	8.4	8.1	8.7	2–13
Average Daily Weight Gain (g/day)	143	614.5	588.7	640.3	250–1,070
Cumulative Conception Rate (%)	176	83.5	81.3	85.8	30–100
Cumulative Proportion of Heifers with any Disease or Treatment	184	0.16	0.14	0.18	0–1

* No of herds with available data

Table 2 Categorical variables for risk factors, productivity and disease status

Variable	No. of herds*	Yes	(%)	No	(%)
Located in Hokkaido	304	164	53.9	140	46.1
Vaccine Usage	299	264	88.3	35	11.7
Gastrointestinal Parasite Control	286	148	51.7	138	48.3
Acaricide Usage	282	210	74.5	72	25.5
Horse Fly Control	285	61	21.4	224	78.6
Drug Usage for <i>Theileria sergenti</i>	256	57	22.3	199	77.7
<i>Theileria sergenti</i> Infection (TS)	296	106	35.8	190	64.2
Tick Infestation (TI)	295	186	63.1	245	83.0
Horse Fly Infestation (HFI)	294	114	38.8	180	61.2

* No of herds with available data

Answers to this question were first categorized into four groups, which were then combined into two groups ($\geq$ = low infestation for Yes and no infestation for No) for statistical analysis.

Horse Fly Infestation (HFI)

During the high horse fly season, horse flies were identified most of the time (heavy infestation), sometimes (medium infestation), or rarely (low infestation) in the environment. In the same season, horse flies were never identified (no infestation).

Answers to this question were first categorized into four groups, which were then re-combined into two groups ($\geq$ = medium infestation for Yes and $\leq$ = low infestation for No) for logistic regression analysis.

The descriptive statistics of independent continuous and categorical variables included in the analysis were summarized in Table 1 and 2. Facility parameter was the sum of facilities out of a maximum possible number of 13 facilities with which grazing herds may be equipped, including electricity supply, telephone, etc. A score of 1 was given for each item, giving a possible score

range of 0 to 13.

With regard to categorical variables, herd location was divided into two areas, namely Hokkaido and all other prefectures because of the big environmental and management differences between them (Table 2). Use of particular preventive or therapeutic strategies such as ectoparasite controls and drug usage for theileriosis were classified as Yes (= 1) or No (= 0).

For independent continuous variables, the available number of samples, means, 95% confidence interval and parameter range were calculated as in Table 1. For independent and dependent categorical variables, the available number of samples and proportion of herds belonging to each category were calculated as in Table 2.

Some dependent variables were also treated as independent variables to investigate their effects on other dependent variables. For example, to investigate the association of variables on TS, TI was used as independent variable.

Univariate relationships between independent and dependent

Table 3 Odds ratios of the variables associated with *Theileria sergenti* infection by a logistic regression model

Variable	Level	Odds ratio	95% LCI	95% HCI	p-value
Average Temperature	(°C)	1.23	1.05	1.43	0.09
Grazing Period	(days)	1.03	1.01	1.05	<0.001
Drug Usage for <i>Theileria sergenti</i>	No	1.00			
	Yes	29.3	7.46	114.9	<0.001
Acaricide Usage	No	1.00			
	Yes	32.1	3.12	331.50	0.003
Tick Infestation	No	1.00			
	Yes	4.50	1.47	12.65	0.022

Hosmer and Lemeshow statistic value for goodness of fit (9.66, $p = 0.289$; degree of freedom = 8)

variables were initially examined. Results were considered statistically significant at the $p < 0.05$ level. Herds that had missing data were excluded from analysis. For the second analysis, stepwise multivariable analysis was used to investigate the multiple effects of the independent variables on dependent variables. A stepwise logistic regression model was used for the dependent categorical variables, using $p < 0.1$ as inclusion criterion. The final model was checked for goodness-of-fit using Hosmer and Lemeshow statistics⁴⁾. All statistical analyses were conducted using SPSS version 16.0 (SPSS Inc, Chicago, IL, USA).

Results and Discussion

The targeted herds had high diversity in environmental conditions, productivity, and disease prevalence, as demonstrated by the large ranges of these parameters, but the large sample size meant that 95% confidence intervals for these continuous variables were small (Table 1). Vaccine and acaricide usage were more commonly practiced than horse fly control and drug usage for *T. sergenti* (Table 2). Proportions of herds with TI were relatively high compared to small proportion of herds had TS as shown in Table 2. Proportions of herds with HFI were medium, however, proportion of herds which utilize horse fly control were relatively small.

In the univariate analysis on TS, variables such as average temperature, summer time temperature, annual rainfall and grazing period were significantly high in herds with a 'Yes' answer for TS. Further, area and number of animals were significantly low in herds with a 'Yes' answer for TS. Among categorical variables, other prefecture, 'Yes' answers for acaricide usage and drug usage for *T. sergenti*, TI and HFI were significantly associated with a 'Yes' answer for TS. After stepwise logistic regression analysis, variables remaining in the model and their odds ratios are shown in Table 3.

The reason that average temperature and grazing period

remained in the model is probably due to the fact that theileriosis is more common in the southern part of Japan, which has higher temperatures and longer grazing periods. In an experimental study, hatching rates for *H. longicornis* eggs were highest between 28–32°C³⁾, which may support our hypothesis. A high odds ratio of 29.3 was observed for drug usage for *T. sergenti*, which was reasonable given that herds with TS likely require drug treatment. In addition, a high odds ratio of 4.5 was observed for TI which was biologically understandable considering ticks are the main vector of TS²⁾. Even though HFI was associated with TS in the univariate analysis, this factor was excluded from the model probably due to the multicollinearity of effects with TI. High temperature may be acting as confounding effects between TI and HFI. Alternatively, HFI may be associated with TS, which is partly consistent with a report that one type of horse fly, *Tabanus nipponicus*, is able to transmit *T. sergenti*⁸⁾.

In the univariate analysis for TI, facility parameters and number of animals were significantly decreased in herds with a 'Yes' answer for TI. Among categorical variables, a 'Yes' answer for gastrointestinal parasite control, drug usage for theileriosis, acaricide usage, TS and HFI was significantly associated with herds that answered 'Yes' for TI. After stepwise logistic regression analysis, variables remaining in the model were only facility parameter and TS infection (Table 4). The deleted variables in the logistic regression analysis may be due to multicollinearity effects of these factors on TS. The strong association observed between TS and TI may be a reverse association because ticks are sources of *T. sergenti* infection. The slight association between facility parameter and TI is not well explainable.

In the univariate analysis for HFI, the continuous variables such as area and number of grazing heifers were significantly decreased in herds with a 'Yes' answer. For the categorical variables, herds in other prefectures, and those with a 'Yes' answer for gastrointestinal parasite control, drug usage for theileriosis,

Table 4 Odds ratios of the variables associated with tick infestation by a logistic regression model

Variable	Level	Odds ratio	95 % LCI	95 % HCI	p-value
Facility Parameter		1.16	1.02	1.31	0.025
<i>Theileria sergenti</i> infection	No	1.00			
	Yes	3.86	1.74	8.55	0.001
Hosmer and Lemeshow statistic value for goodness of fit (9.73, p = 0.285 ; degree of freedom = 8)					

acaricide usage, TS and TI were significantly associated with herds with a 'Yes' answer for HFI. After stepwise logistic regression analysis, variables remaining in the model was only location of the herds. The HFI was 2.86 (95% C.I. ; 1.48–5.52) more common in other prefectures than Hokkaido probably because of their higher temperature and rainfall, which are beneficial to the survival of horse flies. Although many factors such as drug usage for theileriosis, acaricide usage, TS and TI were significant with HFI on univariate analysis, only location of the herds remained significant on multivariable analysis, probably due to the multicollinearity effects of other factors with location of the herds.

Overall, TS, TI and HFI were observed in 36%, 63%, and 39% of the herds which resulted in acaricide usage (75%), horse fly control (21%) and drug usage for TS (22%) in Japanese grazing herds. These medical cost in addition with management efforts may be a big burden for people involving in the grassland for grazing heifers. Therefore, proper preventive strategies with reasonable prices for tick, horse flies and associated with their vector-borne infections continued to be important in pasture used for grazing heifers in Japan.

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短 報

ホルスタイン未経産牛の放牧場 304 カ所における小型ピロプラズマ病, ダニ, アブの汚染に関する全国調査

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要旨

日本のホルスタイン未経産牛を飼育する放牧場 304 カ所を対象に、アンケートを用いた調査を行った。調査結果を用いて、放牧場の環境や管理方法などの各種因子の小型ピロプラズマ病、ダニおよびアブ汚染への影響について解析を行った。小型ピロプラズマ病は南の地域で発生が多く、放牧場における小型ピロプラズマ病治療薬の使用や殺ダニ剤の使用と小型ピロプラズマ病汚染との間に関連が認められた。また放牧場のダニ汚染と小型ピロプラズマ病汚染と

の間に強い関係が認められた。アブは北海道以外の地域においてより頻繁に認められた。今回の調査の結果、小型ピロプラズマ病、ダニおよびアブ汚染に対する適切な予防対策が、わが国の放牧場において今後も求められる事が明らかになった。

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