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Effect of Chronic Hemoplasma Infection on Cattle Productivity

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ABSTRACT. The present study evaluated the effect of hemoplasmosis on cattle productivity. Prevalence of bovine hemoplasma was examined by polymerase chain reaction (PCR) using whole blood samples collected from 93 breeding cows and their 71 calves in Hokkaido, Japan. Monthly milk production records and other clinical data were compared between *Mycoplasma wenyonii* (Mw)-infected, "*Candidatus* Mycoplasma haemobos" (CMh)-infected, co-infected and PCR-negative groups. Blood chemical parameters were obtained from the 93 cows and 64 calves. PCR results showed that 89.2% (83/93) of cows and 14.1% (10/71) of calves were positive for bovine hemoplasma. Based on productivity data obtained from the 93 cows, Mw-infected, CMh-infected and co-infected cows had significantly lower monthly milk yield compared to PCR-negative cows. Furthermore, decline in milk yield was prolonged in CMh-infected and co-infected groups. No significant differences were found for other clinical findings among the four groups. Calf birth weight tended to be lower for Mw-infected, CMh-infected and co-infected groups compared to the PCR-negative group. There were no significant differences in all blood parameters of cows and calves among the four groups. In addition, no significant differences were found in any parameter between hemoplasma-infected and PCR-negative calves.

KEY WORDS: cattle, direct PCR, hemoplasma, productivity.

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Hemotropic mycoplasmas or hemoplasmas are Gram-negative, epierythrocytic bacteria that cause infectious anemia in several mammalian species [6, 11]. Originally known as *Haemobartonella* and *Eperythrozoon* species, these organisms have been reclassified as the genus *Mycoplasma* based on 16S rRNA sequences and morphologic similarities [8, 15]. Two distinct species have been identified that infect cattle: *Mycoplasma wenyonii* (Mw: formerly, *Eperythrozoon wenyonii*) [7] and a provisional species, "*Candidatus* Mycoplasma haemobos" (CMh: synonym, "*Candidatus* M. haemobovis") [2, 4, 13]. Although diagnosis of hemoplasma infection is usually based on cytological identification of the organism on blood smear, this method has low diagnostic sensitivity because the organism resembles Howell-Jolly bodies or background debris and nearly disappears during chronic infection [6]. Sensitive molecular biological techniques now represent the diagnostic method of choice for hemoplasma infection [2, 6, 13]. Clinical signs of hemoplasma infection in cattle include anemia, transient fever, depression, anorexia, lymphadenopathy and edema [6, 11]. However, chronic hemoplasma infection in animals, in which the organism disappears in peripheral blood smears, is subclinical [6].

Several reports have been published regarding the negative reproductive effect of hemoplasmosis in pigs. Subclinical hemoplasma infections have been associated with decreased reproductive efficiency in sows, including delayed estrus, early embryonic death and late-term abortion [16]. In an epidemiological study using antibody titers of hemoplasma in sows and gilts, the organism had an undesirable reproductive effect, including lower birth weight and greater number of stillbirths [17]. In cattle, decreased milk yield, abortion and delayed estrus have been reported during the acute phase of hemoplasma infection [9, 11, 12]. However, these studies were clinical case reports or studies on acute hemoplasmosis, and an epidemiological study focusing on the negative reproductive effect of chronic hemoplasmosis in cattle has not yet been performed. The objective of this study was to evaluate the effect of subclinical hemoplasmosis on productivity of breeding cattle.

MATERIALS AND METHODS

Animals: A total of 93 Holstein dairy cows which calved from August 2011 to August 2012 and their 80 calves, excluding 13 stillborn calves, were included in this study. These cattle were fed in a dairy herd using a tie stall barn in Tokachi District, Hokkaido, Japan. Ethylenediaminetetraacetic acid (EDTA)-anticoagulated blood samples were collected from

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Table 1. Comparison of monthly milk yield of breeding cows among *Mycoplasma wenyonii* (Mw)-infected, "*Candidatus* Mycoplasma haemobos" (CMh)-infected, co-infected and PCR-negative groups

Sampling time	Monthly milk yield (kg; Mean ± SD)			
	Mw-infected (n=33)	CMh-infected (n=18)	Co-infected (n=32)	PCR-negative (n=10)
1st	32.7 ± 12.4*	33.4 ± 8.6*	32.9 ± 11.7*	46.0 ± 10.4
2nd	37.9 ± 11.2	36.0 ± 10.1*	36.6 ± 11.3*	47.8 ± 8.0
3rd	36.7 ± 10.8	37.8 ± 8.7	35.4 ± 9.9	45.8 ± 9.5
4th	32.0 ± 9.9	33.0 ± 9.2	34.5 ± 9.57	35.3 ± 9.7

$P<0.05$ vs. negative group (Steel-Dwass test). PCR, polymerase chain reaction

all breeding cows within seven days prior to delivery and their calves within seven days after birth for blood chemistry levels as described later. The remainder of the EDTA-anticoagulated blood samples was stored at −30°C prior to polymerase chain reaction (PCR) analysis for hemoplasma infections. All breeding cows were classified into four groups: Mw-infected, CMh-infected, co-infected and PCR-negative groups based on PCR results as described later. Their calves were classified into the four groups in two ways, based on PCR results of their mother and of themselves.

PCR assays: All samples were examined for bovine hemoplasma by a previously described screening PCR using whole blood [14]. The F2/R2 primer set amplifies the 16S rRNA genes of most hemoplasmas, including bovine organisms, and the amplicons of longer fragments (approximately 190 bp) and shorter fragments (approximately 170 bp) indicate Mw and CMh, respectively [13]. All amplicons were electrophoresed on a 2.0% agarose gel in tris-borate-EDTA (TBE) buffer and visualized under UV light.

Data collection: Monthly milk yield, obtained from dairy herd performance tests, was recorded for four months after calving. The following data, which can affect cattle productivity, were also collected from all breeding cows as background characteristics: age, parity, pregnancy period and incidence of postpartum disease. In addition, data for birth weight, number of stillbirths and gender were collected from all calves. Packed cell volume (PCV) was measured on all EDTA-anticoagulated blood samples using Celltac α (Nihon Kohden, Tokyo, Japan). Blood chemical parameters, including total protein (TP), albumin (Alb), globulin (Glob), glucose (Glu) and total cholesterol (T.Chol), were measured in all serum samples using a TBA-120FR (Toshiba Medical Systems, Ohtawara, Japan). The same blood parameters except for Glu and T.Chol were also obtained for calves. Blood chemical levels and other clinical data were obtained from medical records of the Animal Medical Center, Obihiro University of Agriculture and Veterinary Medicine.

Statistical analysis: Categorical variables, such as incidence of postpartum disease and number of stillborn calves, were analyzed by the chi-squared analysis. Continuous variables, such as monthly milk yield, calf birth weight and hematological parameters, were analyzed by the Mann-Whitney U-test (for 2 groups) and the Steel-Dwass test (for >2 groups). We considered differences to be statistically significant if $P<0.05$.

RESULTS

All 93 blood samples from breeding cows were analyzed by PCR, of which 83 (89.2%) samples were positive for bovine hemoplasma. Of these, 33 samples (35.5%) were only infected with Mw, 18 (19.4%) were only infected with CMh and 32 (34.4%) were infected with both species. Blood samples for PCR analysis were obtained for 71 of 80 calves, and blood chemistry studies were performed on 64 of the 71 samples. Of the 71 samples analyzed, 10 (14.1%) were positive for bovine hemoplasma. Of these, 5 samples (7.0%) were only infected with Mw, 2 (2.8%) were only infected with CMh and 3 (4.2%) were infected with both species. No hemoplasma-positive calf was born from a PCR-negative cow. In addition, there was no cattle which developed acute hemoplasma infection during observation period of this study.

Based on the data obtained from the 93 cows regarding milk yield, all hemoplasma-infected cows showed significantly lower milk yield than the PCR-negative cows at the first sampling. Furthermore, CMh-infected and co-infected groups showed significantly lower milk yield at the second sampling as well (Table 1). At the third sampling, low milk yield was noted in hemoplasma-infected groups, although the difference was not statistically significant. Similarly, no significant difference was found at the fourth sampling (Table 1). Background characteristics of breeding cows and calf birth weight, number of stillbirths and calf gender were compared among the four groups, and no significant difference was found for any parameter (Table 2). Calf birth weight was compared among Mw-infected, CMh-infected, co-infected and PCR-negative groups, and there was no significant difference among each group (Table 2). However, the calf birth weight of hemoplasma-infected group showed significantly lower than that of PCR-negative group (41.6 ± 7.2 vs 47.0 ± 5.4; $P=0.04$). Blood chemical parameters were obtained for 93 breeding cows and 64 calves. There was no significant difference in any blood parameter of cows and calves among the four groups (Table 3).

Body weight and blood parameters of the calves were also compared. Because CMh-infected and co-infected groups included only two calves and one calf, respectively, statistical analysis was performed between the 7 hemoplasma-infected calves and 57 PCR-negative calves. No significant difference was found for any of the parameters (Table 4).

Table 2. Comparison of background characteristics, incidence of postpartum diseases and calf status of breeding cows and their calves among *Mycoplasma wenyonii* (Mw)-infected, "*Candidatus* Mycoplasma haemobos" (CMh)-infected, co-infected and PCR-negative groups

Parameters	PCR results (Mean ± SD)				
	Mw-infected (n=33)	CMh-infected (n=18)	Co-infected (n=32)	PCR-negative (n=10)	*P* value [a)]
Age (months)	44.1 ± 21.3	43.6 ± 20.1	41.2 ± 15.2	58.4 ± 32.2	–
Parity	1.39 ± 1.56	1.50 ± 1.38	1.19 ± 1.12	2.20 ± 1.87	–
Pregnancy period (days)	277.3 ± 7.6	277.8 ± 4.4	278.7 ± 3.6	282.3 ± 5.3	–
Number of postpartum disease cases	8	3	10	3	0.36
Calf birth weight (kg)	40.8 ± 6.7	41.2 ± 7.7	42.7 ± 7.6	47.0 ± 5.4	–
Number of stillbirths	6	2	4	1	0.85
Calf gender					
Male	13	4	15	6	0.28
Female	12	11	9	3	
Unknown	8	3	8	1	

* $P<0.05$ vs. negative group (Steel-Dwass test). a) : Comparison by χ^2 test. PCR, polymerase chain reaction.

Table 3. Comparison of blood parameters of breeding cows and their calves among *Mycoplasma wenyonii* (Mw)-infected, "*Candidatus* Mycoplasma haemobos" (CMh)-infected, co-infected and PCR-negative groups

Parameters	PCR results (Mean ± SD)			
	Mw-infected	CMh-infected	Co-infected	PCR-negative
Breeding cows	(n=33)	(n=18)	(n=32)	(n=10)
PCV (%)	32.4 ± 2.0	32.6 ± 3.0	32.0 ± 2.7	31.2 ± 4.1
TP (g/d*l*)	6.45 ± 0.62	6.68 ± 0.61	6.52 ± 0.73	6.83 ± 0.44
Alb (g/d*l*)	3.28 ± 0.20	3.32 ± 0.18	3.30 ± 0.21	3.28 ± 0.21
Glob (g/d*l*)	3.2 ± 0.6	3.4 ± 0.6	3.2 ± 0.7	3.6 ± 0.46
Glu (mg/d*l*)	63.3 ± 9.9	67.8 ± 13.7	64.4 ± 9.1	61.7 ± 6.3
T.Chol (mg/d*l*)	79.5 ± 16.8	82.0 ± 14.3	92.3 ± 41.9	68.4 ± 16.2
Calf	(n=20)	(n=15)	(n=21)	(n=8)
PCV (%)	33.0 ± 7.3	32.3 ± 6.1	31.4 ± 4.7	33.0 ± 6.0
TP (g/d*l*)	6.01 ± 1.06	5.60 ± 0.59	6.28 ± 1.12	5.84 ± 0.75
Alb (g/d*l*)	2.96 ± 0.17	2.98 ± 0.11	2.87 ± 0.18	3.00 ± 0.18
Glob (g/d*l*)	3.06 ± 1.17	2.62 ± 0.55	3.41 ± 1.17	2.85 ± 0.65

* $P<0.05$ vs. negative group (Steel-Dwass test). PCR, polymerase chain reaction; PCV, packed cell volume; TP, total protein; Alb, albumin; Glob, globulin; Glu, glucose; T.Chol, total cholesterol.

Table 4. Comparison of body weight and blood parameters of calves among *Mycoplasma wenyonii* (Mw)-infected, "*Candidatus* Mycoplasma haemobos" (CMh)-infected, co-infected and PCR-negative groups

Parameters	PCR results (Mean ± SD)			
	Mw-infected (n=4)	CMh-infected (n=2)	Co-infected (n=1)	PCR-negative (n=57)
Body weight (kg)	41.1 ± 5.7	49.0 ± 5.7	33.5	43.9 ± 7.2
PCV (%)	32.7 ± 6.6	35.2 ± 1.5	19.4	31.5 ± 7.2
TP (g/d*l*)	5.48 ± 0.79	6.10 ± 1.18	5.84	5.7 ± 0.8
Alb (g/d*l*)	3.05 ± 0.04	2.86 ± 0.08	2.66	2.9 ± 0.2
Glob (g/d*l*)	2.43 ± 0.82	3.20 ± 1.27	3.18	2.8 ± 0.9

PCR, polymerase chain reaction; PCV, packed cell volume; TP, total protein; Alb, albumin; Glob, globulin.

DISCUSSION

The present study is the first to report the effect of subclinical hemoplasmosis on productivity of breeding cattle by using a molecular diagnostic method. Previous reports have described reproductive effects of hemoplasmosis only in swine. These organisms have been known to cause reproductive failures in sows in the perinatal period and also affect newborn pigs [3, 17]. The effect of acute hemoplasma infection on cattle productivity, including decreased milk production, was observed in some clinical case reports [9, 12]. Effects of chronic hemoplasma infection on cattle pro-

ductivity have not been investigated.

To evaluate the effect of hemoplasmosis on cattle productivity, monthly milk yield was compared between hemoplasma-infected and PCR-negative groups. We observed significantly lower milk yield in all hemoplasma-infected groups compared to the PCR-negative group at the first sampling time. In general, milk yield is affected by many factors, including genetics, nutrition, age and disease [1]. Although background characteristics of animals that might affect milk yield, including age and parity, were also compared, there was no significant difference in age and parity. The effects of those characteristics on milk yield were unclear, as postpartum diseases, such as ketosis, milk fever and abomasal displacement, also affect milk production [1]. However, the incidence of those diseases was not significantly different among the four groups. It was thought that at least milk yield was not affected by those characteristics. Furthermore, only CMh-infected and co-infected groups showed significantly lower milk yield at the second sampling. According to a past study using hematological examinations, "*Candidatus* M. haemobos" appears to be more pathogenic [14]. This hypothesis might be supported by the low milk yields observed in CMh-infected and co-infected groups at the second sampling. It was reported that acute hemoplasma infection developed a sudden drop in milk yield in cattle [9, 11, 12]. Most affected cattle also showed some severe clinical signs, including high fever, anemia, malaise and edema of the hind limbs, and a large number of organisms were seen on blood smears [9, 11, 12]. In addition, abortion, infertility and delayed estrus have been reported in some affected heifers [11]. However, no cattle showed such a severe acute hemoplasma infection during observation period of this study. Thus, chronic hemoplasma infection in cattle may induce decline in milk yield without a clinical sign associated with hemoplasma infection.

We observed no significant differences in any of the hematological parameters measured in cattle in all four groups. Hematological parameters of calves were also compared, and there were no significant differences among calves which were classified based on PCR results of their mother cows. A past study revealed that cattle with chronic hemoplasmosis exhibit slight anemia [14]. However, mean blood levels were within reference range, and the data overlapped significantly with normal cattle [14]. In addition, very few studies have examined the blood chemistry characteristics of chronic hemoplasma infection. Further examination is necessary to evaluate the effect of hemoplasma infection on hematological parameters in cows and their calves.

High titers of swine hemoplasma have been associated with low birth weight in pigs [17]. Although there was no significant difference in calf birth weight in all four groups, hemoplasma-infected group showed significantly lower calf birth weight compared with PCR-negative group. Several factors, including genetics, sex and dam height, are associated with calf birth weight [10]. Pregnancy period and calf gender were included in this study as background characteristics which might affect calf birth weight. No significant differences were found in the background characteristics among the four groups. Thus, it was thought that calf birth weight was not affected by those characteristics. These results may suggest that hemoplasma infection in cow can reduce birth weight of the calf as well as chronic hemoplasma infection in pig [17].

In this study, ten calves were found to be positive for bovine hemoplasma. Routes of infection remain uncharacterized, although mechanical and transplacental transmission routes have been suggested [5]. Since blood sampling was not carried out immediately after delivery, the route of hemoplasma infection for these calves is unknown. No significant differences were found in body weight and blood parameters between hemoplasma-negative and -positive calves, suggesting that hemoplasma infection might not have any appreciable effect on the calf.

In conclusion, we found lower milk yield and lower calf birth weight in cattle infected with bovine hemoplasma. Chronic hemoplasmosis has negative effect on cattle production, and it is necessary to consider the effect of bovine hemoplasma infection which has been overlooked. However, the precise mechanism responsible for this effect is unknown, and many factors affect milk yield and calf birth weight, potentially confounding these results. Further investigation to control these confounding factors is needed to clarify the effect of hemoplasma infection on cattle productivity.

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