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Relationship between genital carriage and udder infection with *Mycoplasma bovigenitalium* in dairy farms

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Abstract

We isolated *Mycoplasma bovigenitalium*(*M.bg*) from the vagina and the farm environment of the postpartum primiparous cows in two dairy farms where *M.bg* was previously detected in milk, and these strains were genotypically analyzed using pulsed-field gel electrophoresis. *M.bg* of various genotypes were detected at high rates in the vagina and the environment. Some of the *M.bg* detected from the vagina and environment had the same genotype as detected from previous positive milk. In these farms, there were cows carrying *M.bg* in their genital organs, and it was possible that *M.bg* could enter the udder through the environment from the lochia of postpartum cows. Our results indicate that it is important to manage postpartum cows to prevent udder infection with *M.bg*.

Key Words: genital organ, milk, *Mycoplasma bovigenitalium*

Recently in Japan, the prevalence of *Mycoplasma* mastitis is increasing[9)]. This type of mastitis results in significant losses as the eradication protocol recommends culling rather than treatment due to the severe symptoms and strong infectivity[5)]. Therefore, this infection is both an economic and spiritual burden on farmers. Bulk tank milk screening tests are currently being conducted for early detection. *Mycoplasma bovis* is the most common *Mycoplasma* detected in milk, and *Mycoplasma bovigenitalium* (*M.bg*) is found in 10–20%[3,6,9)]. *M.bg* is known to cause mastitis and is associated with pneumonia and infertility[4,11)]. It is detected in the milk, respiratory and genital organs[2,14,15)]. As with other *Mycoplasmas*, *M.bg* can be transmitted to the udder through an ascending infection that enters from the teat orifice, or a descending-infection

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Table 1. Number of *Mycoplasma bovigenitalium* positive cows and their detection on farm A and B.

	Farm A	Farm B
Bulk tank milk	+	+
Individual milk	3/400	3/70
Primiparous cows (within 5 days after parturition)		
Nasal swab	0/48	0/14
Vaginal swab	12/48	7/14
Milk	0/48	0/14
Environment	5/18	0/7

+ : M.bovigenitalium positive
positive samples / all samples

that moves from other parts of the cow, such as from the respiratory organs to the breast milk via the blood[1]. However, the actual transmission route has not yet been elucidated. This study investigated the carriage of *M.bg* in the genital organs of primiparous cows in two farms where infected milk was observed and then sought to determine the source of the udder infection.

A survey was conducted at farms A and B, where *M.bg* was detected using the bulk tank milk screening test. Farm A had 400 milking cows, divided into several groups for each lactation stage, and all cows were born on this farm. Farm B had 70 milking cows, divided into two groups, and recently introduced several multiparous cows from market. After *M.bg* positivity was confirmed in bulk tank milk at both farms, all milking cows were tested for *Mycoplasma* in their milk individually. *M.bg* was detected in three cows at each farm and was a single infection (Table 1). All the positive individuals showed no clinical symptoms but were promptly culled. These cows were within 2 weeks after parturition and housed with postpartum cows. In our regular surveys, *M.bg* has never been detected in the nasal swabs of calves and heifers at these farms.

One hundred microliters of *M.bg*-positive milk from individual cows were inoculated into 3 ml of modified DNA-supplemented Hayflick broth (*Mycoplasma* NK Medium; Miyarisan Pharmaceutical, Tokyo, Japan) and cultured at 37°C with 5% CO_2 for 3 days. Subsequently, 10 µl of this culture was streaked onto modified DNA-supplemented Hayflick agar plates (*Mycoplasma* NK Agar Medium, Miyarisan Pharmaceutical, Japan) and were incubated at 37°C and 5% CO_2 for 5–7 days. Colonies grown on the agar were cultured into 3 ml of modified DNA-supplemented Hayflick broth, incubated at 37°C with 5% CO_2 for 3 days, and then stored at -80°C.

After testing for *Mycoplasma* in the individual milk samples from the milking cows, at farms A and B, a sampling was performed for the next study. Nasal swabs, vaginal swabs, and the milk of primiparous cows within 5 days of parturition (farm A: n = 48, farm B: n = 14), and environmental (grooves of floor, rubber mats, waterers and under waterers) swabs housed these cows (farm A: n = 18, farm B: n = 7) were collected, respectively. The primiparous cows were in the same environment with the postpartum multiparous cows and antibiotic treated cows. The samples were inoculated into 3 ml of modified DNA-supplemented Hayflick broth and cultured at 37°C with 5% CO_2 for 3 days. DNA was extracted using a commercial kit (Insta Gene Matrix, Bio-Rad Laboratories, CA, USA), and *M.bg* was detected by species-specific PCR[8]. Subsequently, 10 µl of *M.bg* positive broth cultures were streaked onto modified DNA-supplemented Hayflick agar plates and were incubated at 37°C and 5% CO_2 for 5–7 days. Colonies were inoculated into 3 ml of modified DNA-supplemented Hayflick broth and were incubated at 37°C with 5% CO_2 for 3 days. The *M.bg* found in these samples and the individual milk samples were genotypically analyzed using pulsed-field gel electrophoresis (PFGE) with restriction enzyme BamH1[7]. All experiments were approved by the Ethics Committee of the Hokkaido Research Organization Animal Research Center.

At farm A, *M.bg* was detected in 12 of the 48 vagina samples of the primiparous cows and 5 of the 18 environmental samples of these cows. *M.bg* was not detected in the milk or nasal swabs of these cows (Table 1). In the environment, *M.bg*

Table 2. Classification based on BamH1 pulsed-field gel electrophoresis (PFGE) patterns of *Mycoplasma bovigenitalium* at farm A

%Simillary 40 60 80 100 / PFGE patterns	Number of samples		
	Positive milk*	Vagina**	Environment
i	1	3	0
ii	1	0	2
iii	0	0	1
iv	0	1	0
v	0	1	0
vi	0	2	0
vii	0	2	0
viii	0	2	0
ix	0	0	1
x	0	1	0
xi	0	0	1
xii	1	0	0

* : already culled cows
**: primiparous cows within 5 days after parturition

Table 3. Classification based on BamH1 pulsed-field gel electrophoresis (PFGE) patterns of *Mycoplasma bovigenitalium* at farm B

%Simillary 40 60 80 100 / PFGE patterns	Number of samples		
	Positive milk*	Vagina**	Environment
i	2	3	0
ii	1	2	0
iii	0	1	0
iv	0	1	0

* : already culled cows
**: primiparous cows within 5 days after parturition

was detected in grooves of floor, rubber mats and under waterers, but not in the waterers. The PFGE pattern of the *M.bg* isolated from farm A revealed 12 genotypes. The similarity of each strain was less than 80%. Three patterns of genotypes were detected from the previous positive milk, seven patterns from the vagina of the primiparous cows, and four patterns from the environment. The one pattern detected from the vagina and other one pattern detected from the environment were the same genotypes as that of the positive milk, respectively (Table 2, i, ii). At farm B, *M.bg* was detected in 7 of 14 vagina samples from the primiparous cows. *M.bg* was not detected in the milk, nasal swabs, and the environment of these cows (Table 1). The PFGE pattern of *M.bg* isolated from farm B revealed 4 genotypes. Three of the 4 strains were showed more than 80% similarity; however, one strain was different. Two patterns were detected in the previous positive milk samples and four patterns were found in the vagina of the primiparous cows. The two patterns detected from the vagina were the same genotype as that of the positive milk (Table 3, i, ii).

In the present study, primiparous cows were selected for the survey. Since heifers were housed separately from milking cows, the primiparous cows were not in contact with milk from the previous culling of the *M.bg* positive cows; thus,

an ascending infection due to milking operations did not occur in these cows. In addition, *M.bg* was not detect in the nasal swabs and waterers at both farms. Therefore, it was considered that infection from nasal discharge to the udder was unlikely to occur. At both farms, various genotypes of *M.bg* were detected in the vagina of primiparous cows. The presence of variable PFGE patterns suggests the introduction of carrier animals[10] or long-term carriage[12]. Although these infections may have occurred during the birth canal passage or artificial insemination etc., it is currently unclear how *M.bg* had entered the vagina of the primiparous cows. However, *M.bg* was considered to remain in the genital organs of cows for an extended period. Even though high rates of *M.bg* were detected in the vagina of the primiparous cows, it was not detected in the milk of these cows. These results indicated that the risk of a descending-infection from the genitals to the udder through blood flow was low. The same genotype of *M.bg* detected in the environment of the postpartum cows was also found in the milk of previously culled cows. According to an interview with the owner of farm A, when the milk infection of *M.bg* occurred at farm A, the groups of postpartum cows were overcrowded and cows were laying in the pathway of the free-stall housing. This situation suggests that *M.bg* was discharged into the environment from the vagina of the postpartum cows through the lochia and then the bacteria would have entered other cows via the udder. On the other hand, according to an interview with the owner of farm B, when the milk infection of *M.bg* occurred at farm B, the cows were calving in the milking herds and the automatic manure scraper system was not operated. At the time of environmental sampling, the calving pen was in use and the manure scraper system was operating correctly. Therefore, it was considered that *M.bg* was not detected in the environment at farm B.

In conclusion, it is important to recognize that cows might carry *M.bg* in their genitals and that vaginal discharge from these cows could be a source of udder infection. Therefore, to prevent the spread of *M.bg* mastitis on each farm, control measures should be enacted that prevent *M.bg* from entering the udder through the environment from the lochia of postpartum cows. Cows should give birth in the calving pens, should be prevented from lying in the free-stall housing pathways, and the cow bed should be kept clean.

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