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NOTE

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Is bovine protothecal mastitis related to persistent infection in intestine?

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ABSTRACT. *Prototheca zopfii* is associated with bovine mastitis, which causes a reduction in milk production and secretion of thin, watery milk with white flakes. However, the source of infection and an infection route of mastitis have not been clarified. In this study, we evaluated the prevalence of *P. zopfii* genotype 2 in fecal samples from Japanese dairies with or without a history of protothecal mastitis in 2017. *P. zopfii* genotype 2 was detected in 23 of 60 (38%) fecal samples in only the herd with a history of protothecal mastitis. These results suggest that occurrence of bovine protothecal mastitis is related to persistent infection in intestine and the source of infection is feces.

KEY WORDS: bovine protothecal mastitis, genotype, intestinal flora, *Prototheca zopfii*

The genus *Prototheca* consists of achlorophyllic algae that are ubiquitous among cow-barn surroundings. *Prototheca zopfii* is associated with bovine mastitis, which causes a reduction in milk production and secretion of thin, watery milk with white flakes. Most cases of bovine protothecal mastitis in Japan are chronic and subclinical. Bovine mastitis due to *Prototheca* is a chronic infection with no effective treatments. Therefore, affected cows should be clearly identified and milked last in the milking order until they can be culled. Typically, the identification of *P. zopfii* as the causative agent of mastitis has been depend on a positive result in culture tests or via molecular analysis of milk samples.

Epidemiologic studies of environmental sources in a *P. zopfii* outbreak of bovine mastitis have been conducted [1, 3]. The investigators isolated *P. zopfii* from dairy environments (e.g., cattle drinking water; a feed trough; mud, dirt, and excreted feces from a dirt lounging area; water, sludge, mud, and vegetation from a creek in the lounging area; and the floor of a freestall barn) and concluded that *P. zopfii* seemed to be widespread throughout the dairy herd environment [1, 3].

P. zopfii has been biochemically and serologically divided into at least two genotypes, *P. zopfii* genotypes 1 and 2 [9, 10]. Isolates from bovine mastitis in Germany, Italy, Japan, Portugal, and Poland were nearly all identified as *P. zopfii* genotype 2, suggesting that it is the main causative agent of bovine protothecal mastitis [2, 4, 6–11].

We molecularly characterized Japanese isolates of *P. zopfii* from cattle with bovine mastitis and cow-barn surroundings to clarify routes of infection for bovine protothecal mastitis. We isolated *Prototheca* from cow-barn surroundings (drinking water, sewage, and feces) and milk samples from cattle with bovine mastitis [5, 8, 12]. All mastitis isolates were identified as *P. zopfii* genotype 2. Conversely, nearly all isolates from cow-barn surroundings were identified as *P. zopfii* genotype 1, with only three isolates (drinking water, sewage, and feces) identified as genotype 2 [5, 8, 12]. Given these results, both *P. zopfii* genotypes could exist in cow-barn surroundings, but no sites were identified as frequent sources of *P. zopfii* genotype 2.

The source of infection and an infection route of bovine protothecal mastitis have not been clarified. Therefore, in this study, we evaluated the prevalence of *P. zopfii* genotype 2 in fecal samples from Japanese dairies with or without a history of protothecal mastitis in 2017.

P. zopfii type strains *P. zopfii* var. 1 (SAG 2064^T), genotype 1 (SAG 2063^T), and genotype 2 (SAG 2021^T) were used in this study. In addition to the type strains, *P. zopfii* clinical strains were isolated from milk samples of two cases (Holstein cows) of protothecal mastitis and normal Holstein cows in dairies without a history of protothecal mastitis in Aichi Prefecture, Japan (Table 1). Each milk sample was collected from milking of four teats together. The protothecal mastitis positive dairy is typically recurred and had investigated at cow-barn surroundings (drinking water, sewage, and feces) and milk samples from cattle with

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Table 1. Cows and calves in each herd

Herd	Cows	Calves	Area
A[a)]	55	10	Aichi
B[a)]	54	19	Aichi
C[a)]	66	18	Aichi
D[a)]	54	20	Aichi
E[a)]	36	2	Aichi
F[b)]	60	10	Aichi

a) The herd has not a history of protothecal mastitis. b) The herd has a history of protothecal mastitis.

Table 2. Isolation and identification from milk and fecal samples

Herd	Positive milk samples	Genotype in milk	Positive feces samples	Genotypein feces
A[a)]	0/55	I (0)/II (0)	1/55	I (1)/II (0)
B[a)]	0/54	I (0)/II (0)	0/54	I (0)/II (0)
C[a)]	0/66	I (0)/II (0)	1/66	I (1)/II (0)
D[a)]	0/54	I (0)/II (0)	2/54	I (1)/II (1)
E[a)]	0/36	I (0)/II (0)	0/36	I (0)/II (0)
F[b)]	2/60	I (0)/II (2)	41/60	I (18)/II (23)

a) The herd has not a history of protothecal mastitis. b) The herd has a history of protothecal mastitis.

bovine mastitis and normal cows [5, 8, 12]. However, the control for protothecal mastitis has not been succeed by culling the prototheca positive cows.

All cases of bovine protothecal mastitis in this study were chronic and subclinical and confirmed by positive cultures and somatic cell count of milk samples [2, 8].

Forty-five strains of *P. zopfii* were isolated from feces from the same cows (Table 1). Isolation procedures were performed as modified our previous studies [8]. Briefly, samples were pre-cultured in liquid *Prototheca* isolation medium (PIM) at 37°C for 48 hr. Next, 50 *ml* of liquid pre-culture was incubated onto PIM at 37°C for 48 hr. The developed *Ptototheca* colonies were subcultured on PIM agar. The molecular typing procedures were performed as described in our previous studies [12].

Isolation of *P. zopfii* genotype 2 was performed on individual milk and fecal samples in Japanese dairies with or without a history of protothecal mastitis (Table 2). *P. zopfii* genotype 2 was detected in 2 of 60 (3.3%) milk samples and 23 of 60 (38%) fecal samples from the herd with a history of protothecal mastitis herds (Table 2). Conversely, *P. zopfii* genotype 2 was isolated from only one fecal sample among herds without a history of protothecal mastitis (Table 2). This result indicates that there is a possibility for occurrence of protothecal mastitis in herds without a history of protothecal mastitis.

This study was performed to clarify the source of protothecal mastitis and excretion of *P. zopfii* genotype 2 in feces. *P. zopfii* genotype 2 was detected in fecal samples in only the herd with a history of protothecal mastitis. Moreover, all fecal samples (n=10) from calves from the herd with a history of protothecal mastitis tested positive for *P. zopfii* genotype 2, whereas *P. zopfii* genotype 2 was not detected in 0/69 fecal samples from calves from herds without a history of protothecal mastitis (data not shown). Calves positive for *P. zopfii* genotype 2 might be fed *Prototheca*-contaminated milk and carry *P. zopfii* genotype 2 as intestinal flora.

These results suggest that occurrence of bovine protothecal mastitis might be related to persistent infection in intestine and the source of infection is feces that might contaminate in dairy environments. The drinking water contamination employed in breeding farms as a possible source of bovine infection. By the way, the results in this study indicated that it has also possibility of microbial translocation, which *P. zopfii* might be disseminated from intestine to mammary grand or the other organ.

One major limitation associated with this study is that we investigated only one herd with a history of protothecal mastitis. It is difficult to investigate the other many protothecal mastitis positive herds, because of this mastitis in not known well and understudied. Therefore, further studies of other protothecal mastitis herds are necessary in order to identify preventative measures for bovine protothecal mastitis.

CONFLICTS OF INTEREST. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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REFERENCES

1. Anderson, K. L. and Walker, R. L. 1988. Sources of *Prototheca* spp in a dairy herd environment. *J. Am. Vet. Med. Assoc.* **193**: 553–556. [Medline]
2. Bozzo, G., Bonerba, E., Di Pinto, A., Bolzoni, G., Ceci, E., Mottola, A., Tantillo, G. and Terio, V. 2014. Occurrence of *Prototheca* spp. in cow milk samples. *New Microbiol.* **37**: 459–464. [Medline]
3. Da Costa, E. O., Ribeiro, A. R., Watanabe, E. T., Pardo, R. B., Silva, J. B. and Sanches, R. B. 1996. An increased incidence of mastitis caused by *Prototheca* species and *Nocardia* species on a farm in São Paulo, Brazil. *Vet. Res. Commun.* **20**: 237–241. [Medline] [CrossRef]
4. Jagielski, T., Lassa, H., Ahrholdt, J., Malinowski, E. and Roesler, U. 2011. Genotyping of bovine Prototheca mastitis isolates from Poland. *Vet. Microbiol.* **149**: 283–287. [Medline] [CrossRef]
5. Kishimoto, Y., Kano, R., Maruyama, H., Onozaki, M., Makimura, K., Ito, T., Matsubara, K., Hasegawa, A. and Kamata, H. 2010. 26S rDNA-based phylogenetic investigation of Japanese cattle-associated *Prototheca zopfii* isolates. *J. Vet. Med. Sci.* **72**: 123–126. [Medline] [CrossRef]
6. Marques, S., Silva, E., Kraft, C., Carvalheira, J., Videira, A., Huss, V. A. and Thompson, G. 2008. Bovine mastitis associated with *Prototheca blaschkeae*. *J. Clin. Microbiol.* **46**: 1941–1945. [Medline] [CrossRef]
7. Möller, A., Truyen, U. and Roesler, U. 2007. *Prototheca zopfii* genotype 2: the causative agent of bovine protothecal mastitis? *Vet. Microbiol.* **120**:

370–374. [Medline] [CrossRef]
8. Osumi, T., Kishimoto, Y., Kano, R., Maruyama, H., Onozaki, M., Makimura, K., Ito, T., Matsubara, K. and Hasegawa, A. 2008. *Prototheca zopfii* genotypes isolated from cow barns and bovine mastitis in Japan. *Vet. Microbiol.* **131**: 419–423. [Medline] [CrossRef]
9. Roesler, U., Scholz, H. and Hensel, A. 2003. Emended phenotypic characterization of *Prototheca zopfii*: a proposal for three biotypes and standards for their identification. *Int. J. Syst. Evol. Microbiol.* **53**: 1195–1199. [Medline] [CrossRef]
10. Roesler, U., Möller, A., Hensel, A., Baumann, D. and Truyen, U. 2006. Diversity within the current algal species *Prototheca zopfii*: a proposal for two *Prototheca zopfii* genotypes and description of a novel species, *Prototheca blaschkeae* sp. nov. *Int. J. Syst. Evol. Microbiol.* **56**: 1419–1425. [Medline] [CrossRef]
11. Ricchi, M., Goretti, M., Branda, E., Cammi, G., Garbarino, C. A., Turchetti, B., Moroni, P., Arrigoni, N. and Buzzini, P. 2010. Molecular characterization of *Prototheca* strains isolated from Italian dairy herds. *J. Dairy Sci.* **93**: 4625–4631. [Medline] [CrossRef]
12. Sobukawa, H., Yamaguchi, S., Kano, R., Ito, T., Suzuki, K., Onozaki, M., Hasegawa, A. and Kamata, H. 2012. Short communication: Molecular typing of *Prototheca zopfii* from bovine mastitis in Japan. *J. Dairy Sci.* **95**: 4442–4446. [Medline] [CrossRef]