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 2064⁴), genotype 1 (SAG 2063⁵), and genotype 2 (SAG 2021¹) 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 recurrent and had investigated at cow-barn surroundings (drinking water, sewage, and feces) and milk samples from cattle with...
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 \textit{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 \textit{Prototheca} isolation medium (PIM) at 37°C for 48 hr. Next, 50 ml of liquid pre-culture was incubated on PIM at 37°C for 48 hr. The developed \textit{Prototheca} colonies were subcultured on PIM agar. The molecular typing procedures were performed as described in our previous studies [12].

Isolation of \textit{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). \textit{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, \textit{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 \textit{P. zopfii} genotype 2 in feces. \textit{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 with a herd with a history of protothecal mastitis tested positive for \textit{P. zopfii} genotype 2, whereas \textit{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 \textit{P. zopfii} genotype 2 might be fed \textit{Prototheca}-contaminated milk and carry \textit{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 \textit{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.

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**Table 1. Cows and calves in each herd**

| Herd | Cows | Calves | Area |
|------|------|--------|------|
| A<sup>a</sup> | 55   | 10     | Aichi|
| B<sup>a</sup> | 54   | 15     | Aichi|
| C<sup>a</sup> | 66   | 18     | Aichi|
| D<sup>a</sup> | 54   | 20     | Aichi|
| E<sup>a</sup> | 36   | 2      | Aichi|
| F<sup>a</sup> | 60   | 10     | Aichi|

<sup>a</sup> The herd has not a history of protothecal mastitis. <sup>b</sup> The herd has a history of protothecal mastitis.

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**Table 2. Isolation and identification from milk and fecal samples**

| Herd | Positive milk samples | Genotype in milk | Positive feces samples | Genotype in feces |
|------|-----------------------|------------------|------------------------|-------------------|
| A<sup>a</sup> | 0/55 | I (0)/II (0) | 1/55 | I (1)/II (0) |
| B<sup>a</sup> | 0/54 | I (0)/II (0) | 0/54 | I (0)/II (0) |
| C<sup>a</sup> | 0/66 | I (0)/II (0) | 1/66 | I (1)/II (0) |
| D<sup>a</sup> | 0/54 | I (0)/II (0) | 2/54 | I (1)/II (1) |
| E<sup>a</sup> | 0/36 | I (0)/II (0) | 0/36 | I (0)/II (0) |
| F<sup>a</sup> | 2/60 | I (0)/II (2) | 41/60 | I (18)/II (23) |

<sup>a</sup> The herd has not a history of protothecal mastitis. <sup>b</sup> The herd has a history of protothecal mastitis.
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