J. Dairy Sci. 102:2539–2543
https://doi.org/10.3168/jds.2018-14979

Short communication: Occurrence and persistence of *Prototheca zopfii* in dairy herds of Korea

Ho-Sung Park, Dong Chan Moon, Bang-Hun Hyun, and Suk-Kyung Lim*
Bacterial Disease Division, Animal and Plant Quarantine Agency, 177 Hyeksin 8-ro, Gimcheon-si, Gyeongsangbuk-do 39660, Republic of Korea

ABSTRACT

Bovine mastitis caused by *Prototheca* has been reported globally, and its incidence is increasing in dairy herds. The present study aimed to investigate the occurrence of *Prototheca* and persistence of *Prototheca zopfii* strains in Korean dairy herds. A total of 187 (7.5%) *P. zopfii* strains were isolated from 2,508 quarter milk samples collected from 50 dairy farms throughout Korea from 2015 to 2017. *Prototheca zopfii* was isolated from one farm among the 50 farms over the 3-yr period. The *P. zopfii* isolates belonged to genotype 2. Overall, *Prototheca*-positive quarter milk samples showed high somatic cell counts with an average value of log 6.48 ± 6.54 cells/mL. *Prototheca zopfii* was found to be persistent in an infected farm over a 2-yr period. To the best of our knowledge, this is the first report on the presence and persistence of protothecal mastitis caused by *P. zopfii* genotype 2 in a Korean dairy herd. This disease leads to a significant increase in somatic cell counts in milk, which persists for more than 1 yr in the affected cow udder. These results suggest that *P. zopfii* could pose a serious risk to dairy herds. Thus, strict surveillance for protothecal mastitis is urgently needed and sanitary conditions regarding the environment and milk collection are essential because of the lack of effective treatment options.

Key words: *Prototheca zopfii*, genotype 2, somatic cell counts, persistence

Short Communication

Algae belonging to the genus *Prototheca* are ubiquitous in nature (Roesler et al., 2006). Three among the 5 *Prototheca* species—*P. zopfii* (Jánosi et al., 2001), *P. blaschkeae* (Ricchi et al., 2013), and *P. wickerhamii* (Marques et al., 2006)—can cause bovine mastitis (Roesler et al., 2006). Among them, *P. zopfii* is the most common organism responsible for protothecal mastitis, and the other 2 species are responsible for only sporadic cases (Ricchi et al., 2013).

Outbreaks of mastitis caused by *P. zopfii* have been described as a global problem (Ikeda et al., 1985; Buzzini et al., 2004; Jagielski et al., 2011). *Prototheca zopfii* induces chronic subclinical or clinical mastitis and leads to a reduction in milk production and elevation of SCC in milk (Ricchi et al., 2010; Sharma et al., 2011; Branko et al., 2017). Furthermore, the genus *Prototheca* does not respond to routine mastitis therapy and the only control measure developed to date involves the elimination of infected cows (Marques et al., 2006). Thus, timely identification of infected cows is extremely important for farm management.

In spite of a national mastitis-control program in Korea undertaken since the early 2000s (Nam et al., 2010), mastitis has remained a major problem in dairy herds. Until now, most studies on mastitis have focused on bacteria. No information about protothecal mastitis is available in Korea, despite worldwide distribution in dairy cattle. Thus, the present study aimed to investigate the presence of *Prototheca* and persistence of *P. zopfii* strains in dairy herds.

From January 2015 to June 2017, 2,508 quarter milk samples from 891 lactating cows on 50 dairy herds nationwide were examined in the Mastitis Diagnostic Laboratory of Animal and Plant Quarantine Agency in Korea. The sample herd size was around 0.9% of the total dairy herds (n = 5,450) in Korea. The milk samples used in our study were collected from lactating cows from herds with suspected mastitis as part of routine diagnostic samples submitted for determination of etiology of mastitis and susceptibility of isolates. Forty herds were studied; of these, 33 were visited only once in the 3-yr period of the study, whereas multiple visits were made for the remaining 7.

For the follow-up study on a *Prototheca*-positive farm, 1,012 quarter milk samples (524, 171, and 317) from 265 cows (135, 45, and 85) on a *Prototheca*-positive farm were collected in 11 rounds (i.e., 4, 3, and 4 rounds in 2015, 2016, and 2017, respectively). The herd was composed of Holstein cows, with an average of 96

Received May 8, 2018.
Accepted November 6, 2018.
*Corresponding author: imsk0049@korea.kr

lactating cows/year during 2015 to 2017. Although we have no detailed information on each sample and cow, the main feature of this herd was an increase in SCC of bulk milk from around log 5.30 to log 5.60 cells/mL. In particular, injection of antibiotics and oregano oil into infected udder increased SCC; however, reduction of milk production, clinical signs of mastitis, and systemic changes were not clearly observed. Samples were collected from milk with high SCC and suspected *Prototheca* infection, such as those showing no response to antibiotics. Twenty-four to 290 quarter milk samples from 7 to 75 cattle were collected in each round. The samples were collected aseptically from individual mammary quarters by the herd owners or personnel from Animal and Plant Quarantine Agency. The milk samples were transported on ice to the laboratory immediately and stored in the refrigerator before microbiological examination within 24 h.

The SCC values of the 2,508 quarter milk samples were measured using the Fossmatic System 4000 (Foss Electric, Hillerød, Denmark) within 1 d of collection. The milk samples with log SCC >5.30 cells/mL or suspected *Prototheca* infection were streaked on Sabouraud dextrose agar plates (BD Biosciences, San Jose, CA) and the plates were incubated under aerobic conditions at 37°C for 48 to 72 h. Species identification was based on light microscopic observation of morphology and MALDI-TOF MS (Biomerieux, Marcy L'Etoile, France) using methods described previously (Pieper et al., 2012). The isolates were stored at −80°C until further use.

Extraction of genomic DNA was carried out using the Precellys Lysing kit (Bertin Technologies, Montigny-le-Bretonneux, France) per the manufacturer's instructions (https://www.bertin-instruments.com). Genotype-specific PCR assay was used to detect the genotypes of *P. zopfii* by the amplification of mitochondrial and chloroplast DNA using methods described by Capra et al. (2014). The PCR products were sequenced (Macrogen Inc., Seoul, Korea) and analyzed by comparison with known sequences (GenBank accession number: KM396177.1) using the BLAST programs available via the National Center for Biotechnology Information (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Prototheca was isolated from 187 of 2,508 quarter milk samples (7.5%) collected from 50 dairy farms from 2015 to 2017 (Table 1). The proportion of protothecal mastitis was 6.0 (3/50) and 10.4% (93/891) at the herd and cattle levels, respectively. The 3 positive herds were found on the same farm. The herd remained positive for *Prototheca* over the entire 2-yr period. Prevalence of *P. zopfii* was notably different between milk samples of low (0.4%, 5/1,363) and high (15.9%, 182/1,145) SCC. All 187 *P. zopfii* isolates were confirmed by morphological appearance and MALDI-TOF. *Prototheca*-specific amplicons (216 bp) and genotype 2-specific fragments (508 bp) obtained by PCR were detected in all 187 isolates, which were recovered from the stock. Other bacteria (CNS) were found in only 2 of the *Prototheca*-positive samples. The prevalence of *Prototheca* on a positive farm was 35.1 (93/265) and 18.5% (187/1012) at the cow and quarter milk levels during the study period, respectively (Table 2); however, prevalence at the cow level decreased from 43.7% (59/135) in 2015 to 20.0% (9/45) in 2016 and 29.4% (25/85) in 2017. Overall, *Prototheca*-positive quarter milk samples showed high SCC values (log) with an average of 6.48 ± 6.54 cells/mL (range = 4.72–7.43). Of the 63 cows that tested positive for *Prototheca*, 42 were re-examined more than 2 times for presence of *Prototheca* in their udder. Among them, 23 (54.8%) cows carried *P. zopfii* for 1 to 19 mo; 17 (40.5%) and 6 (14.3%) of the re-examined cows were persistently infected and showed intermittent shedding, respectively (Supplemental Table S1; https://doi.org/10.3168/jds.2018-14979). All 5 long-term (≥12 mo) persistent cows showed intermittent shedding, except 1 cow. Although 42 infected cows were culled (5, 23, and 14 in 2015, 2016, and 2017, respectively), most of the positive cows persisted on the dairy farm for 3 to 32 mo after being identified as positive.

To the best of our knowledge, the present study is the first to report protothecal mastitis by *P. zopfii* genotype 2 in Korean dairy herds and that *P. zopfii* persisted in the farm throughout the study period. The

Table 1. Farm-, cow-, and quarter-level prevalence [% isolation (no. positive/no. tested)] of *Prototheca zopfii* in Korea during 2015–2017

			Quarter milk		
Year	Farm	Cow	SCC <log 5.30 cells/mL	SCC ≥log 5.30 cells/mL	Total
2015	6.3 (1/16)	20.1 (59/293)	0.2 (1/625)	26.9 (138/513)	12.2 (139/1,138)
2016	7.1 (1/14)	2.6 (9/345)	0 (0/375)	3.3 (12/360)	1.6 (12/735)
2017	5.0 (1/20)	9.9 (25/253)	1.1 (4/363)	11.8 (32/272)	5.7 (36/635)
Total	6.0 (3[1]/50[2])	10.4 (93/891)	0.4 (5/1,363)	15.9 (182/1,145)	7.5 (187/2,508)

[1]Three positive herds were found on the same farm.

[2]Seven farms were sampled 2 or 3 times during study period.

Table 2. Cattle- and quarter-level prevalence in a farm and mean geometric log SCC of infected quarter milk for 2 yr

Sampling no.	Sampling date (yr.mo)	Percentage of isolation (no. positive/no. tested)		Average ± SD log SCC/mL of infected cattle (range)
		Cattle	Quarter milk	
I	2015.06	78.3 (18/23)	57.6 (53/92)	6.61 ± 6.48 (5.27–7.14)
II	2015.07	69.2 (9/13)	26.9 (14/52)	6.63 ± 6.52 (6.03–7.03)
III	2015.08	64.7 (11/17)	34.8 (23/66)	6.63 ± 6.47 (5.40–7.04)
IV	2015.10	24.0 (18/75)	15.2 (44/290)	6.44 ± 6.34 (5.64–6.95)
V	2015.12	42.9 (3/7)	20.8 (5/24)	5.87 ± 5.29 (5.58–5.97)
Subtotal		43.7 (59/135)	26.5 (139/524)	6.55 ± 6.46 (5.27–7.14)
VI	2016.08	15.2 (5/33)	4.6 (6/130)	6.09 ± 5.68 (5.63–6.26)
VII	2016.12	33.3 (4/12)	14.6 (6/41)	6.78 ± 6.97 (5.81–7.43)
Subtotal		20.0 (9/45)	7.0 (12/171)	6.56 ± 6.85 (5.63–7.43)
VIII	2017.01	29.4 (10/34)	9.4 (12/127)	6.48 ± 6.33 (5.66–6.85)
IX	2017.02	30.0 (3/10)	10.8 (4/37)	6.06 ± 5.68 (5.60–6.23)
X	2017.03	13.3 (4/30)	6.1 (7/114)	6.10 ± 6.22 (4.72–6.67)
XI	2017.06	72.7 (8/11)	33.3 (13/39)	6.65 ± 6.73 (5.20–7.32)
Subtotal		29.4 (25/85)	11.3 (36/317)	6.48 ± 6.58 (4.72–7.32)
Total		35.1 (93/265)	18.5 (187/1,012)	6.48 ± 6.54 (4.72–7.43)

prevalence of *P. zopfii* in cases of endemic protothecal mastitis ranged from 2 to 15% (Buzzini et al., 2004; Pieper et al., 2012; Shahid et al., 2016; Branko et al., 2017). The rate of infection in mastitic milk samples (15.9%) in our study was higher than those previously reported in China (13.5%; Shahid et al., 2016), Poland (12.6%; Wawron et al., 2013), Canada (5.1%; Pieper et al., 2012), and Serbia (1.8%; Branko et al., 2017). Our findings showed that, although an infected herd was present in only a single farm, the prevalence of *P. zopfii* in quarter milk samples was high. This result suggests that once the *Prototheca* species is introduced into the herd by infected cows or other factors it can induce endemic infection.

The persistent infection rate of *P. zopfii* in our study was lower than that reported previously. Around 75% of infected cows were found to be persistent in a longitudinal study in Germany (Roesler and Hensel, 2003), whereas 54.8% (23/42) of re-examined cows carried the *P. zopfii* in our study. It might be that the infected cattle of this herd, which produced the milk with high SCC, were continuously culled and their infected quarter udders were permanently dried off during the 2-yr study period. The prevalence rate of *P. zopfii* on the farms we studied decreased from 43.7% (59/135) in 2015 to 20.0% (9/45) 2016 and 29.4% (25/85) in 2017. Although the prevalence rate increased slightly again in 2017, a dramatic reduction in prevalence was found in 2016 after the infected cows (n = 23) were culled.

Because of the lack of response of *Prototheca* spp. to most antibiotics, culling of infected cows is often recommended. However, 79.4% (50/63) of infected cows in our study remained on the farm for 3 to 32 mo. Furthermore, 22.2% (14/63) of positive cows persisted for over a year (Supplemental Table S1; https://doi.org/10.3168/jds.2018-14979), accounting for the persistence of the etiology on the farm. The farmer of this herd disrupted the infected udder instead of culling the infected cows. Because of the absence of serious clinical signs, except the high SCC of infected cows in the herd, we presumed that the farmer was reluctant to cull the infected cows.

Various risk factors for persistence were reported at the herd and cow level. Herd-level risk factors included the use of intramammary injection of antibiotics and drugs, use of dry cow teat sealant, and frequent treatment of displaced abomasum (Pieper et al., 2012). Cow-level risk factors included second or greater lactation and high SCC (Pieper et al., 2012). Although we have no detailed information on risk factors for the positive farm in our study, *Prototheca* first emerged in high-SCC milk samples, which were exposed to the use of antibiotics. Repeated use of antibiotics without laboratory examination, such as microbiological identification, might have led to an outbreak of protothecosis with high prevalence in the farm because unsanitary udder injections could introduce *Prototheca* spp. into the udders (Pieper et al., 2012). In addition, antibiotic treatment could promote protothecal infection by inhibiting the competitive natural udder flora (Baumgartner, 1997).

In our study, a few infected cows (15.8%, 10/63) tested negative for *P. zopfii* in the repeated examinations, consistent with a study in which 18% of infected cows could terminate the infection (Roesler and Hensel, 2003). However, 45.4% of these animals were found to be positive for the antibodies and the number of truly convalescent animals was low (Roesler and Hensel,

2003). We presumed that these animals might have shed intermediately during the disease process, as 7.9% (5/63) of infected cows in our study were culture positive for *P. zopfii* at the first and third or next examinations but negative at the second examination. Roesler and Hensel (2003) also showed that 4.9% of dairy cows were identified as intermittent shedders by examination 3 times for 12 mo.

In addition, the prevalence of *P. zopfii* in the positive farm varied from 13.3 to 78.3% and from 4.6 to 57.6%, at cow and quarter milk sample levels, respectively. Furthermore, 26.1% (6/23) of the cows persistently carrying infection were intermittently shedding the *P. zopfii* for more than 1 yr. It has been reported that this pathogen can induce a persistent infection with intermittent shedding owing to its ability to infect and survive in macrophages and to invade the udder tissue (Roesler and Hensel, 2003). In addition, *P. zopfii* strains from bovine subclinical mastitis are capable of biofilm production, which may contribute to their persistence in a milking and dairy environment (Gonçalves et al., 2015; Shahid et al., 2016). Further studies are needed to investigate the source or risk factor for persistence of *P. zopfii* in this farm.

Higher SCC was found in quarters infected by *P. zopfii* genotype 2 than in those infected by *Staphylococcus aureus*, which is the most common mastitis-causing agent worldwide. However, in the milk of some cows infected with *Prototheca*, low SCC were also detected. In the present study, the SCC in quarter milk samples of *P. zopfii*-infected cows was high, with an average log SCC 6.48 ± 6.54 cells/mL (ranging from 4.72 to 7.43). A similar observation was made by Malinowski et al. (2002), who reported that SCC fluctuated from around log SCC 5.77 to 7.36 cells/mL in subclinical and clinical mastitis caused by *Prototheca*. These findings suggest that eradication attempts should include whole-herd testing rather than testing only cow with high SCC.

Genotype-specific PCR analysis can distinguish *P. zopfii* strains as genotype 1 or 2 (Möller et al., 2007). In the present study, all *P. zopfii* isolates belonged to *P. zopfii* genotype 2, consistent with previous reports (Salerno et al., 2010; Jagielski et al., 2011; Gao et al., 2012; Kalińska et al., 2017). Genotype 2 was found to be predominant in the etiology of bovine mastitis caused by *P. zopfii* in China (Gao et al., 2012), Brazil (Salerno et al., 2010), and Japan (Sobukawa et al., 2012), whereas genotype 1 has been isolated from environmental samples in Japan (Sobukawa et al., 2012). Because of the limitation of the current method, we could distinguish between only 2 genotypes. Identification of more subtypes within genotype 2 in future studies could help better evaluate the distribution and spread of this microalga in dairy herds. Recently, new techniques such as genetic markers for differentiating *Prototheca* spp. or genotyping on the basis of 18S small subunit rDNA and 28S small subunit rDNA were reported in Poland (Jagielski et al., 2018) and China (Shahid et al., 2016), respectively. In addition, different antibiotic susceptibilities were also observed in *P. zopfii* genotype 2 (Shahid et al., 2016). Thus, combination of genotypic and phenotypic characterization of *P. zopfii* genotype 2 could provide a better understanding of the epidemiology of this disease.

In conclusion, to the best of our knowledge, this is the first report of the incidence of protothecal mastitis caused by *P. zopfii* genotype 2. This genotype leads to a significant increase in SCC in milk and persists for more than 2 yr in the affected cow udder. These results suggest that *P. zopfii* could pose a serious risk for dairy herds in Korea. Thus, strict surveillance for protothecal mastitis is urgently needed to prevent the spread of this species. Furthermore, improved sanitary conditions and hygienic practices of milk collection are important owing to the lack of effective treatment options.

ACKNOWLEDGMENTS

This study was supported by a grant from the Animal and Plant Quarantine Agency, Ministry of Agriculture, Food, and Rural affairs, Republic of Korea (N-1543081-2017-36-0101).

REFERENCES

Baumgartner, B. 1997. Occurrence and control of bovine mastitis caused by *Prototheca* in the region of the Veterinary Institute Potsdam. Prakt. Tierarzt 78:406–414.

Branko, S., D. Vasilev, N. Karabasil, I. Vučurović, N. Čobanović, M. Babić, and V. Katić. 2017. Molecular identification of *Prototheca zopfii* genotype 2 mastitis isolates and their influence on the milk somatic cell count. Vet. Arh. 87:249–258.

Buzzini, P., B. Turchetti, R. Facelli, R. Baudino, F. Cavarero, L. Mattalia, P. Mosso, and A. Martini. 2004. First large-scale isolation of *Prototheca zopfii* from milk produced by dairy herds in Italy. Mycopathologia 158:427–430.

Capra, E., P. Cremonesi, C. Cortimiglia, G. Bignoli, M. Ricchi, P. Moroni, A. Pesce, M. Luini, and B. Castiglioni. 2014. Simultaneous identification by multiplex PCR of major *Prototheca* spp. isolated from bovine and buffalo intramammary infection and bulk tank. Lett. Appl. Microbiol. 59:642–647.

Gao, J., H. Zhang, J. He, Y. He, S. Li, R. Hou, Q. Wu, Y. Gao, and B. Han. 2012. Characterization of *Prototheca zopfii* associated with outbreak of bovine clinical mastitis in herd of Beijing, China. Mycopathologia 173:275–281.

Gonçalves, J. L., S. Hwa In Lee, E. de Paula Arruda, D. Pedroso Galles, V. Camargo Caetano, C. Fernandes de Oliveira, A. Fernandes, and M. Veiga dos Santos. 2015. Biofilm-producing ability and efficiency of sanitizing agents against *Prototheca zopfii* isolates from bovine subclinical mastitis. J. Dairy Sci. 98:3613–3621.

Ikeda, T., A. Kiuchi, K. Tabuchi, E. Hakogi, and Y. Okada. 1985. Bovine protothecal mastitis: A case caused by *Prototheca zopfii* in Japan. Japanese Journal of Medical Mycology 26:221–227.

Jagielski, T., J. Gawor, Z. Bakuła, P. Decewicz, K. Maciszewski, and A. Karnkowska. 2018. *cytb* as a new genetic 1 marker for differen-

tiation of *Prototheca* species. J. Clin. Microbiol. https://doi.org/10.1128/JCM.00584-18.

Jagielski, T., H. Lassa, J. Ahrholdt, E. Malinowski, and U. Roesler. 2011. Genotyping of bovine *Prototheca* mastitis isolates from Poland. Vet. Microbiol. 149:283–287.

Jánosi, S., F. Ratz, G. Szigeti, M. Kulcsar, J. Kerényi, T. Laukó, F. Katona, and G. Huszenicza. 2001. Pathophysiology: Review of the microbiological, pathological, and clinical aspects of bovine mastitis caused by the algae *Prototheca zopfii*. Vet. Q. 23:58–61.

Kalińska, A., M. Gołębiewski, and A. Wójcik. 2017. Mastitis pathogens in dairy cattle—A review. World Sci. News 89:22–31.

Malinowski, E., H. Lassa, and A. Klossowska. 2002. Isolation of *Prototheca zopfii* from inflamed secretion of udders. Bull. Vet. Inst. Pulawy 46:295–299.

Marques, S., E. Silva, J. Carvalheira, and G. Thomson. 2006. Short communication: In vitro antimicrobial susceptibility of *Prototheca wickerhamii* and *Prototheca zopfii* isolated from bovine mastitis. J. Dairy Sci. 89:4202–4204.

Möller, A., U. Truyen, and U. Roesler. 2007. *Prototheca zopfii* genotype 2—The causative agent of bovine protothecal mastitis? Vet. Microbiol. 120:370–374.

Nam, H. M., S. K. Lim, J. M. Kim, H. M. Kang, J. S. Moon, G. C. Jang, J. M. Kim, S. H. Wee, Y. S. Joo, and S. C. Jung. 2010. Antimicrobial susceptibility of coagulase-negative staphylococci isolated from bovine mastitis between 2003 and 2008 in Korea. J. Microbiol. Biotechnol. 20:1446–1449.

Pieper, L., A. Godkin, U. Roesler, A. Polleichtner, D. Slavic, K. E. Leslie, and D. F. Kelton. 2012. Herd characteristics and cow-level factors associated with *Prototheca* mastitis on dairy farms in Ontario, Canada. J. Dairy Sci. 95:5635–5644.

Ricchi, M., C. De Cicco, P. Buzzini, G. Cammi, N. Arrigoni, M. Cammi, and C. Garbarino. 2013. First outbreak of bovine mastitis caused by *Prototheca blaschkeae*. Vet. Microbiol. 162:997–999.

Ricchi, M., M. Goretti, E. Branda, G. Cammi, C. Garbarino, B. Turchetti, P. Moroni, N. Arrigoni, and P. Buzzini. 2010. Molecular characterization of *Prototheca* strains isolated from Italian dairy herds. J. Dairy Sci. 93:4625–4631.

Roesler, U., A. Moller, A. Hensel, D. Baumannn, and U. Truyen. 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.

Roesler, U., and A. Hensel. 2003. Longitudinal analysis of *Prototheca zopfii*-specific immune responses; correlation with disease progression and carriage in dairy cows. J. Clin. Microbiol. 41:1181–1186.

Salerno, T., M. Ribeiro, H. Langoni, A. Siqueira, E. Costa, P. Melville, V. Bueno, A. Yamamura, U. Roesler, and A. Silva. 2010. In vitro algaecide effect of sodium hypochlorite and iodine based antiseptics on *Prototheca zopfii* strains isolated from bovine milk. Res. Vet. Sci. 88:211–213.

Shahid, M., T. Ali, L. Zhang, R. Hou, S. Zhang, L. Ding, D. Han, Z. Deng, A. Rahman, and B. Han. 2016. Characterization of *Prototheca zopfii* genotypes isolated from cases of bovine mastitis and cow barns in China. Mycopathologia 181:185–195.

Sharma, N., N. Singh, and M. Bhadwal. 2011. Relationship of somatic cell count and mastitis: An overview. Asian-Australas. J. Anim. Sci. 24:429–438.

Sobukawa, H., S. Yamaguchi, R. Kano, T. Ito, K. Suzuki, M. Onozaki, A. Hasegawa, and H. Kamata. 2012. Short communication: Molecular typing of *Prototheca zopfii* from bovine mastitis in Japan. J. Dairy Sci. 95:4442–4446.

Wawron, W., M. Bochniarz, T. Piech, W. Lopuszynski, and J. Wysocki. 2013. Outbreak of protothecal mastitis in a herd of dairy cows in Poland. Bull. Vet. Inst. Pulawy 57:335–339.