



A survey on the incidence of *Prototheca* mastitis in dairy herds in Lublin province, Poland

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ABSTRACT

Prototheca mastitis has recently become an emerging disease; although its incidence is increasing steadily, its epidemiology remains largely understudied. The aim of this work was to investigate the prevalence of *Prototheca* spp. in dairy cows and their environment in Lublin province, covering most of southeastern Poland. Between December 2015 and July 2016, a total of 172 milking cows from 10 dairy farms were inspected for mastitis using clinical examination and the California Mastitis Test (CMT). Quarter milk samples (QMS, $n = 179$) and body site swabs ($n = 151$) from CMT-positive cows were collected for microbiological culture. In addition, we evaluated QMS and body site swabs from 23 healthy cows, along with 91 environmental samples. Of 100 CMT-positive cows, 71 had at least one QMS positive for microbial growth. In 8 (11.3%) of these cows, originating from 7 dairy farms, *Prototheca* spp. were cultured. The average somatic cell count of the *Prototheca*-containing milk was 4.02×10^6 cells/mL compared with 0.13×10^6 cells/mL of the *Prototheca*-free milk (collected from control animals). No significant differences were observed between mastitis and control cows with respect to counts of total white blood cells, lymphocytes, neutrophils, and eosinophils. Half of the cows with *Prototheca* spp. in their milk did not yield the algae from other anatomical sites. Eight cows were negative for the presence of *Prototheca* spp. in their milk but positive for the algae in swabs from anatomical sites. Among the environmental sources that were positive for *Prototheca* growth were watering

troughs, manure, feed, and mud. All (45) *Prototheca* isolates recovered in this study were subjected to species- and genotype-level molecular identification. All QMS and most of the animal swabs (90%) yielded *Prototheca zopfii* genotype (gen.) 2. Of the animal samples, *P. zopfii* gen. 1 and *Prototheca blaschkeae* were isolated only from feces and rectum. Environmental samples grew either *P. zopfii* gen. 2 (67%) or *P. zopfii* gen. 1 (33%). This study demonstrates that *P. zopfii* gen. 2 is the third most common pathogen of mastitis in cattle in southeast Poland, with an overall incidence of 4.6%. Finding *Prototheca* spp., including *P. zopfii* gen. 1 and 2 and *P. blaschkeae*, in stool and rectal swabs from healthy animals may suggest their role as nonpathogenic microflora of bovine gut.

Key words: *Prototheca* spp., dairy herd, (sub)clinical mastitis, Poland

INTRODUCTION

Bovine mastitis, defined as an inflammation of the mammary gland, is the most prevalent and devastating disease in dairy cattle worldwide. It is also the most expensive problem faced by dairy farm owners, with the annual costs of US\$2 billion in the United States (Viguier et al., 2009). Economic losses are incurred either directly, through reduced milk production and premature culling of affected animals, or indirectly, from treatment and veterinary care expenses. Mastitis exists in 2 forms—clinical and subclinical. Clinical mastitis (CM) is readily detectable due to the presence of visible changes in the udder (swelling, tenderness, hardness) and milk characteristics (wateriness, discoloration, clots, flakes), whereas subclinical mastitis (SCM) causes none or very few noticeable symptoms of inflammation and is detected by an elevated SCC in the produced milk. Subclinical mastitis is up to 40 times more frequent in dairy herds than CM (Seegers

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et al., 2003). The asymptomatic and latent course of SCM, with its tendency for chronicity, makes this form of mastitis much more epidemiologically hazardous and much less amenable to therapeutic intervention. Consequently, SCM imposes the greatest overall financial burden on the milk industry.

The etiological spectrum of bovine mastitis is wide and includes viruses; bacteria, with streptococci, staphylococci, and enterobacteria as the predominant pathogens; fungi, mostly yeasts; and achlorophyllous, yeast-like algae of the genus *Prototheca* (De Vliegher et al., 2012). The importance of the latter as causative agents of mastitis has long been underestimated, because of their misidentification as yeasts or dismissal as contaminating, saprophytic microflora. *Prototheca* algae were first implicated in bovine mastitis in 1952 (Lerche, 1952). Since then, protothecal mastitis has been recognized across the world, in countries such as the United States (Anderson and Walker, 1988), Canada (Pieper et al., 2012), Belgium (Lagneau, 1996), Denmark (Aalbaek et al., 1998), Germany (Möller et al., 2007), Italy (Buzzini et al., 2004), Brazil (Bueno et al., 2006), and Japan (Osumi et al., 2008). Over the last 2 decades, protothecal mastitis has become an emerging disease whose incidence is increasing steadily but whose epidemiology remains largely understudied. Additionally, given its inherent resistance to conventional antimicrobial therapies (Jagielski et al., 2012, 2017a), *Prototheca* infections pose a serious threat to the dairy sector.

Among the 8 *Prototheca* species currently recognized, 2 have been involved in mastitis events in cows: *Prototheca zopfii* and *Prototheca blaschkeae*, with the former being responsible for the vast majority of reported cases. Interestingly, of the 2 *P. zopfii* genotypes (1 and 2), only *P. zopfii* genotype (**gen.**) 2 has been identified as causing the disease (Möller et al., 2007; Marques et al., 2008; Osumi et al., 2008; Ricchi et al., 2010; Jagielski et al., 2011). Still, *P. zopfii* gen. 1 has pathogenic potential, because it was shown to induce subclinical mastitis in a cow upon experimental infection (Ito et al., 2011).

In Poland, the occurrence of mastitis due to *Prototheca* algae was demonstrated for the first time in the early 2000s (Malinowski et al., 2002). Since then, cases of protothecal mastitis have been reported in different parts of the country. However, a prospective, large-sample, cross-country or cross-regional study on the incidence of *Prototheca* mastitis has never been performed in Poland. Therefore, the aim of this work was to evaluate the prevalence of *Prototheca* spp. in dairy cows and their environment in Lublin province, covering most of the southeast of Poland.

MATERIALS AND METHODS

Dairy Farms and Herds

Ten dairy farms (I to X), located in the Lublin province, Poland, housing herds varying in size from 12 to 806 cows, were inspected and sampled between December 2015 and July 2016 (Figure 1). All farms had reported mastitis events in at least 2 mo before the survey, according to the local veterinary records. A 25-point questionnaire was developed and tested to collect basic information on the dairy farms and herds, in a standardized form (Supplemental Table S1; <https://doi.org/10.3168/jds.2018-15495>).

Sampling Procedures

Quarter milk samples (QMS) were collected from all lactating cows (if not being treated or during their pre- and postpartum periods) in herds up to 30 animals in total, or from 30 lactating cows, randomly selected, in herds whose size exceeded that number. Before milk collection, each cow was clinically examined, with special attention paid to the udder. A careful palpation of the udder was performed to detect any inflammatory swelling, fibrosis, induration, and other clinical signs. Premilking milk samples (~2 mL) from each udder fraction were evaluated visually for clotting, color changes, and consistency. This was done in Schalm paddle wells, just before performing the California Mastitis Test (CMT; Mastirapid, Vetoquinol Biowet, Poland). The CMT results were scored as negative (0), trace (T), weak positive (1+), distinctive positive (2+), or strong positive (3+), as proposed earlier (Quinn et al., 2002). A CMT-positive (score of T or above) quarter milk was sampled for culture. The CMT-positive QMS were also tested for SCC by using an automated cell counter (Fossomatic 5000; Foss Electric, Hillerød, Denmark), as described previously (Gonzalo et al., 1993).

A mastitic cow was defined as any cow with an SCC $>200 \times 10^3$ cells/mL (Hiitiö et al., 2017; Kirkpatrick and Olson, 2015). Clinical mastitis was determined based on apparent changes in milk (mild), signs of inflammation in the udder (moderate), and generalized clinical symptoms (severe). Subclinical mastitis was assessed using the CMT (Amer et al., 2018).

Diagnosis of mastitis required ≥ 1 QMS positive for microbial growth. If 2 or more QMS from the same cow yielded different pathogens, 2 (or more) distinct mastitis infections were considered. If more than one pathogen was isolated from a single QMS, a mixed etiology was considered, with both organisms causal. Cows without mastitis were referred to as control animals. From each dairy herd, 2 (herds II, IV–IX) or 3 (I, III, X) healthy

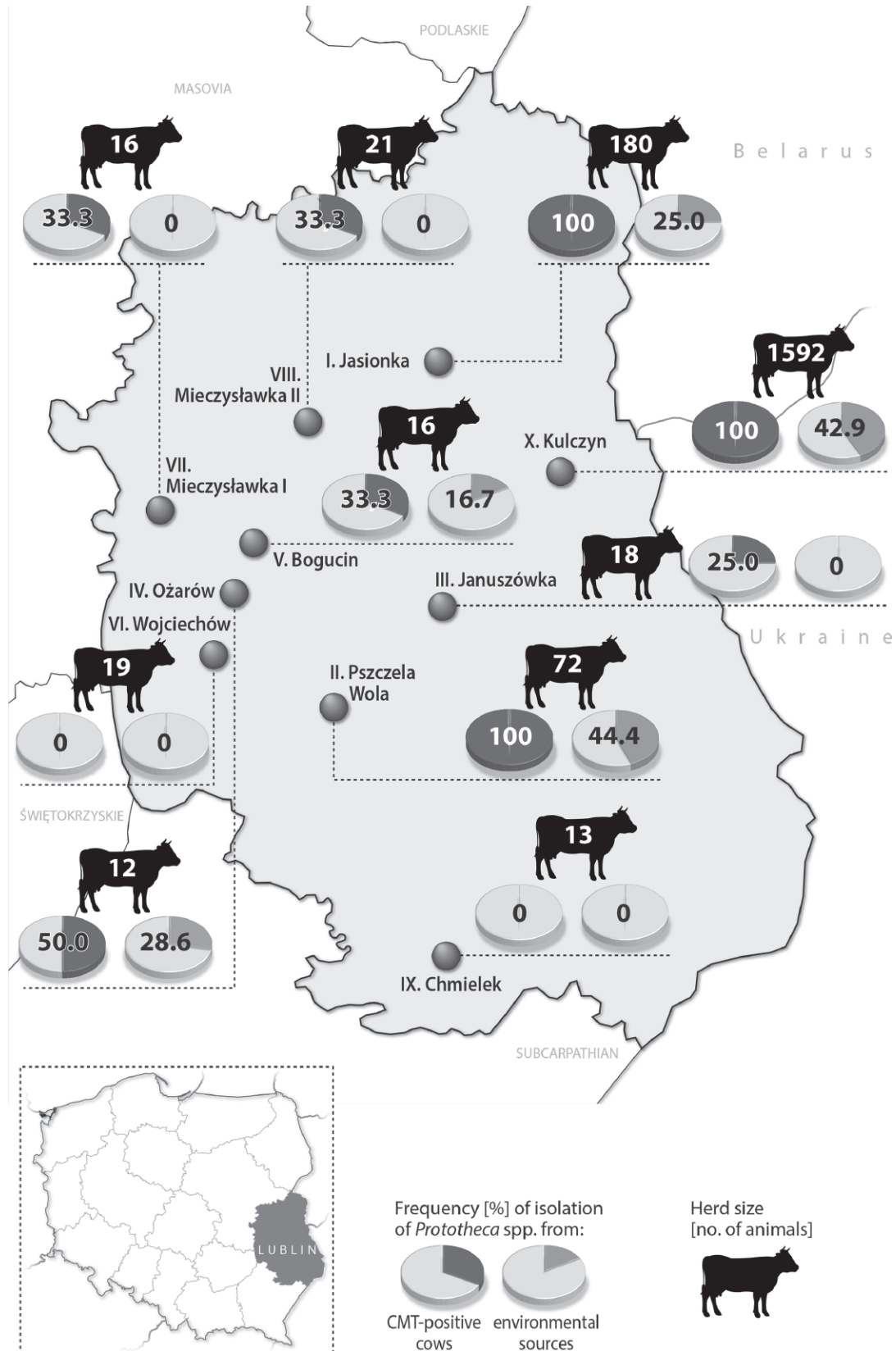


Figure 1. Prevalence of *Prototheca* spp. in California Mastitis Test (CMT)-positive cows and environmental samples collected from dairy farms (I to X) in Lublin province, Poland.

cows (i.e., clinically normal and CMT-negative) were sampled for quarter milk, whole blood, feces, and oral, nasal, rectal, and vaginal swabs.

Cows whose milk yielded *Prototheca* spp. in culture were revisited and sampled for the same specimens as the healthy animals. Milk samples were collected aseptically, according to the National Mastitis Council guidelines (National Mastitis Council, 2017). Briefly, the teats were washed, dried, and disinfected. After discarding the first 3 streams, the milk (2–4 mL) was collected into sterile tubes, with a separate tube for each udder fraction. All samples of the remaining types, including environmental samples, were collected into sterile vials or containers. All samples were transported, under refrigeration (4°C), to the laboratory for microbiological analyses. Blood samples were additionally subjected to standard hematological analysis.

Environmental sources sampled included water (tap water, drinking water from watering troughs, sewage, puddles, and any other watercourses on farms in their vicinity), surficial sources (topsoil, mud), plant sources (e.g., vegetation surrounding animal lots, plant compost), and other sources (animal feed/feeders/bedding, dung channels, milking machines).

Primary Isolation and Culture

All animal samples were cultivated in Columbia Agar with 5% sheep blood (Oxoid/Thermo Fisher, Waltham, MA), McConkey agar (Sigma Aldrich, St. Louis, MO), Sabouraud dextrose agar (SDA, Difco Laboratories, Detroit, MI), Edwards's Lab-Agar (Biocorp, Warsaw, Poland), and *Prototheca* Isolation Medium (PIM; Pore, 1973), under aerobic conditions at 37°C for up to 96 h. Environmental samples were cultured in PIM only. Liquid and semisolid specimens were spread on the medium surface either directly or after dilution in sterile water. Solid specimens were ground in a sterile porcelain vessel with sterile water, and then plated as above. Swabs from farm facilities and equipment were directly streaked on agar plates. To enhance the detection and recovery of algae, the samples were enriched with liquid PIM and cultured for 48 h at 37°C before plating.

All isolates used in the study are preserved (and available upon request) in the Culture Collection of the Department of Applied Microbiology, Faculty of Biology, University of Warsaw, Poland.

Species Identification

Any grown colonies were subjected to species identification, based on macro- and micromorphological criteria, using standard staining procedures. A milk

sample was classified as positive for *Staphylococcus aureus* or *Streptococcus agalactiae* when at least one characteristic colony was identified. Other microorganisms were considered as etiological agents of mastitis if more than 3 colonies were observed. Preliminary identification included colony morphology assessment, hemolytic profiling, and Gram staining (Color Gram 2 kit, bioMérieux, Marcy l'Étoile, France). For gram-positive cocci, a catalase test was carried out to differentiate catalase-negative streptococci and enterococci from catalase-positive staphylococci. Identification as *Staph. aureus* was based on conventional biochemical tests: catalase and coagulase reactivity, and clumping factor production using latex agglutination assay (Slidex Staph Kit, bioMérieux). A positive Christie, Atkins, Munch-Petersen (CAMP) test and a lack of esculin hydrolysis on Edward's medium allowed differentiation of *Strep. agalactiae* from other streptococci (Malinowski and Klossowska, 2002). Enterococci were initially identified with the use of bile esculin agar (Biocorp). Gram-negative bacilli were discriminated based on cultural and morphological characteristics, growth on MacConkey agar (Oxoid/Thermo Fisher), indole production, and oxidase test (bioMérieux). Coryneform bacteria (*Corynebacterium bovis*, *Trueperella pyogenes*) were primarily recognized by morphological criteria. Presumptive yeast colonies were subcultured on SDA and confirmed microscopically.

Prototheca spp. Identification and Typing

Colonies suspected of being *Prototheca* spp. were subcultured on SDA or PIM plates and subjected to initial species identification. This included macro- and microscopic evaluation of colony morphology, and carbohydrate assimilation profiling, with the API 20C AUX system (bioMérieux). Phenotype-based identification was then confirmed with molecular methods. For these to be applied, DNA extraction was performed, essentially as reported elsewhere (Jagielski et al., 2017b). Species- and genotype-level identification was carried out using genotype-specific PCR assays, as described by Roesler et al. (2006) and with a newly described PCR-restriction enzyme analysis (PCR-REA) assay of partial *cytB* gene, as proposed by Jagielski et al. (2018).

All *Prototheca* isolates, under appropriate species (genotype) assignments were preserved in Viabank vials (Medical Wire & Equipment Co Ltd., Corsham, UK) and stored at –70°C.

Statistical Analysis

The Mann-Whitney U-test for independent means was used to evaluate differences between SCC and

blood cell counts of mastitis and healthy cows. Significance was defined at $P < 0.05$.

RESULTS

Farm Management

The dairy farms investigated in this study are shown in Figure 1 and their characteristics are given in Supplemental Table S1 (<https://doi.org/10.3168/jds.2018-15495>). They all housed Holstein-Friesian dairy cows, except 2 farms with Montbéliarde (I) and Polish White-Backed (VII) cattle. The mean herd size was 100 (range, 12–806). Most of the dairy farms had an open herd turnover (60%), tiestall barns (80%), with straw bedding (100%), traditional (80%) feeding, and drinking water supplied by municipal water distribution network (100%). The cows had temporary access to pasture and grazing grass (60%) and were under regular veterinary inspection (80%). The average 305-d milk production was 7,087 L per cow (range, 6,000–10,794 L), and the mean culling rate was 24.7% (range, 20–42%), with the predominant reasons for culling being infertility and mastitis.

The agricultural and zoohygienic (maintenance conditions affecting health and productivity of animals) aspects of the dairy cattle breeding in farms under the study are presented in Supplemental Table S1 (<https://doi.org/10.3168/jds.2018-15495>).

On all farms, milking was done mechanically in a double-row milking parlor (20%) or with a milking pipeline (80%), twice daily by experienced dairy farm milkers. On all farms, the milking staff used aprons and gloves on a regular basis. Premilking udder preparation involved washing teats by hand with water, drying teats with a paper towel, and disinfecting teats just before the machine attachment. A common (9/10 farms) post-milking hygiene practice was teat dipping in various iodine-based, commercially available products.

Identification of Udder Infection

Among all herds in the study, 100 dairy cows out of 172 (58.1%) tested positive by CMT. The proportion of CMT-positive cows in a sampled herd ranged from 37.5% (farm VI) to 85.7% (farm I), with a mean of 57.1% (Figure 2; Supplemental Table S2; <https://doi.org/10.3168/jds.2018-15495>).

Of the 179 QMS taken from CMT-positive cows, 114 (63.7%) QMS from 71 (71%) cows yielded microbial growth in culture. The relative culture-positive ratio differed between farms, ranging from 23.5% (farm VII) to 60% (farms III and X).

In all but 1 (VII) dairy herds, at least one culture of *Streptococcus* spp. was recovered from milk samples (50 cultures from 33 cows, in total). In all but 2 (VII and IX) dairy herds, at least one *Staphylococcus* culture was obtained from milk samples (45 cultures from 34 cows). Isolates of *Enterobacteriaceae* and *Pseudomonas* spp. were grown from single samples collected in 2 (V and X) and 1 (IX) herd, respectively. Yeast species were cultured from 1 (I and X), 2 (III), and 3 (V) samples, each representing individual cows. Overall, bacterial microflora was isolated from 110 (61.5%) QMS from 66 (66%) CMT-positive cows, resulting in a total incidence of 38.4%. Streptococci and staphylococci predominated, being almost equally represented [50 (27.9%) vs. 45 or (25.1%)].

Protothecal Mastitis

Cows with protothecal mastitis were from 7 dairy farms (II–V, VII, VIII, and X). Clinical and subclinical disease was recognized on 2 (II and VII) and 5 (III–V, VIII, and X) farms, respectively. These were all single cases (1 case per herd), except farm IV, where 2 cows were affected. The prevalence of protothecal mastitis in individual herds ranged from 0% (farms I, VI, and IX) to 25% (IV) with a mean of 4.6%.

Prototheca spp. were detected in 13 (5.6%) QMS collected from 8 cows housed in 7 dairy farms (II–V, VII, VIII, and X). Two of these cows experienced clinical changes in their udders. In milk of 5 (62.5%) cows, *Prototheca* spp. comprised the only microflora cultured. In 3 (37.5%) SCM cows, *Prototheca* spp. grew along with, but never from the same QMS, staphylococci (2 cows from herds IV and X) or staphylococci and yeasts (a cow from herd V).

The average SCC of the *Prototheca*-containing milk was 4.02×10^6 cells/mL compared with 0.13×10^6 cells/mL in the *Prototheca*-free milk collected from control animals ($P < 0.00001$). No significant differences between SCC of milk samples from CM and SCM cows were observed (mean counts 4.59×10^6 and 3.45×10^6 cells/mL). Likewise, we detected no significant differences between mastitis and control cows with respect to counts of total white blood cells, lymphocytes, neutrophils, and eosinophils (Table 1).

Prototheca spp. in Stool and Body Site Swabs

Overall, 151 body site swabs and stool samples were collected from 23 healthy cows and 8 cows with protothecal mastitis. Among 19 *Prototheca* spp. cultures, 14 were isolated from 8 healthy cows, whereas the remaining 5 were isolated from cows with SCM (4) and CM

Table 1. Milk SCC and blood cell counts (±SD) in cows with clinical and subclinical *Prototheca* mastitis and in control cows

Item	Clinical mastitis (n = 5 QMS ¹)	Subclinical mastitis (n = 8 QMS)	Control (n = 92 QMS)
Average milk SCC, ×10 ⁶ /mL	4.59 ± 3.61	3.45 ± 2.03	0.13 ± 0.01
White blood cells, ² ×10 ⁹ /L	7.75 ± 2.55	6.10 ± 0.70	7.13 ± 1.40
Lymphocytes, ² ×10 ⁹ /L	3.55 ± 1.45	3.02 ± 0.56	3.77 ± 1.03
Neutrophils, ² ×10 ⁹ /L	2.65 ± 1.45	2.05 ± 0.53	2.33 ± 1.03
Eosinophils, ² ×10 ⁹ /L	1.25 ± 0.55	0.72 ± 0.18	0.74 ± 0.55
Cows, no. (%) ³	2 (1.11)	6 (3.34)	23 (12.85)
Herds, no. (%)	2 (20)	5 (50)	10 (100)

¹Quarter milk sample.
²Average counts for all animals from each group.
³Percentage of cows of different groups given in parentheses.

(1). *Prototheca* spp. collected from the affected animals were found in stool (4 isolates; 1 from CM and 3 from SCM cows) and vagina (1 isolate from SCM cow). A wider distribution occurred among isolates cultured

from control animals. Here, *Prototheca* spp. were isolated from mouth (3), nose (3), vagina (1), rectum (3), and feces (4) (Figure 2; Supplemental Table S2; <https://doi.org/10.3168/jds.2018-15495>).

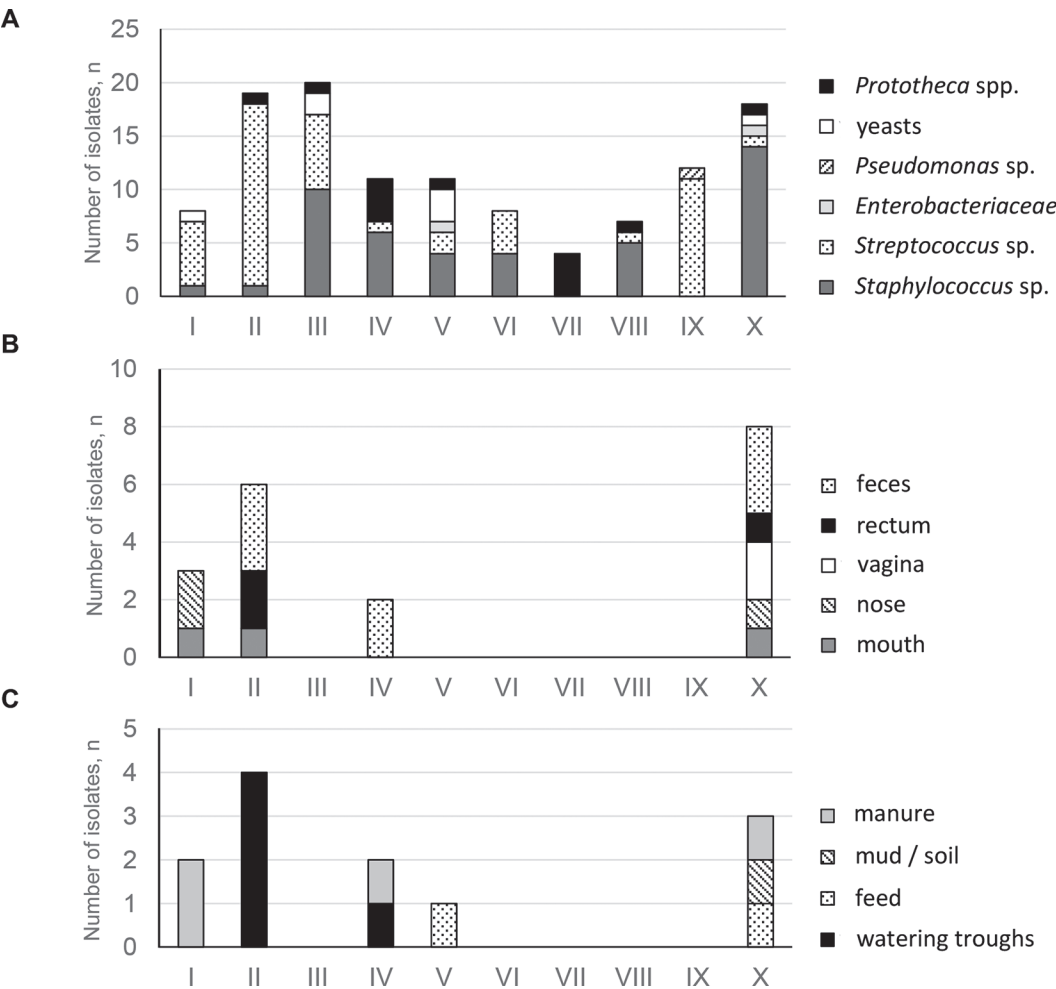


Figure 2. Distribution of mastitis pathogens in milk samples (A) and *Prototheca* spp. in body swabs (B) and environmental sources (C) in dairy farms (I to X) in Lublin province, Poland.

Prototheca spp. in Environmental Samples

A total of 91 environmental samples were collected (Supplemental Table S2; <https://doi.org/10.3168/jds.2018-15495>). Of these, 12 (13.2%) were positive for *Prototheca* spp. These samples originated from 5 dairy farms (I, II, IV, V, and X), including 1 (I) where no *Prototheca* spp. were isolated from milk samples. Among the environmental sites that yielded *Prototheca* growth were watering trough (5 positive samples), manure (4), feed (2), and mud (1).

Prototheca spp. Genotyping

All (44) *Prototheca* isolates recovered in this study were subjected to species- and genotype-level molecular identification (Figure 3; Supplemental Table S3; <https://doi.org/10.3168/jds.2018-15495>). Milk-derived isolates belonged exclusively to *P. zopfii* gen. 2. Among 20 isolates from animal swabs and stool, 15 isolates were *P. zopfii* gen. 2, 2 isolates were *P. zopfii* gen. 1, and 2 isolates were *P. blaschkeae*. The 4 non-*P. zopfii* gen. 2 isolates were obtained from 2 fecal samples from 2 cows with subclinical mastitis from dairy farm IV (*P. zopfii* gen. 1) and from rectum and feces of 2 control cows from dairy farm X. Of 12 environmental isolates, none were *P. blaschkeae*, 4 were *P. zopfii* gen. 1, and 8 were *P. zopfii* gen. 2.

DISCUSSION

The results from this study show that *Prototheca* spp. are the third most common cause of mastitis in dairy cows in the population we studied, after streptococci and staphylococci. Similar findings; that is, placing *Prototheca* algae among the top 3 etiological agents of bovine mastitis, have been reported in the Piedmont region of Italy; Ontario, Canada; and São Paulo State, Brazil (Costa et al., 1996; Buzzini et al., 2004; Pieper et al., 2012).

At the herd level, the prevalence of protothecal mastitis was within the range of 0 to 25%, similar to that described in the eastern United States, Brazil, Serbia and Montenegro, Japan, and Italy (Pore et al., 1987; Corbellini et al., 2001; Milanov et al., 2006; Osumi et al., 2008; Arrigioni et al., 2010; Bozzo et al., 2014). It should be noted here that our rates may be somewhat underestimated, given that in 2 herds (I and X), only a proportion of cows (48.2 and 3.7%, respectively) were examined. In the remaining herds (II–IX), an average of 83% of cows were examined (range, 66.7–95.5%).

Only a single herd had more than one *Prototheca* mastitis case confirmed (herd IV, 2 cows with SCM), thus being indicative of an outbreak. There have been a few reports of mastitis outbreaks due to *Prototheca* spp., involving 5 to 34 animals, depending on the herd size (average of 13 mastitis animals per herd; Costa et al., 1996; Abarca et al., 2001; Bueno et al., 2006; Gao et al., 2012; Ricchi et al., 2013; Wawron et al., 2013).

Protothecal bovine mastitis predominantly follows a subclinical or chronic course (Costa et al., 1996; Abarca et al., 2001; Bueno et al., 2006; Wawron et al., 2013). Based on clinical examination, SCC, and *Prototheca* spp. culture from QMS, 2 (1.2%) and 6 (3.5%) cows were classified as suffering from CM and SCM, respectively. According to previous studies, the number of cows with protothecal SCM was much higher than that of cows with CM (Abarca et al., 2001; Bueno et al., 2006). A predominance of CM cases was also documented (Gao et al., 2012; Ricchi et al., 2013).

In this study, SCC were only slightly higher in CM than in SCM (4.59 vs. 3.45×10^6 cells/mL), although other studies have reported SCC approximately 10-fold higher for CM cases (Malinowski and Klossowska, 2002; Jagielski et al., 2011). The algae were also isolated from the QMS with physiological SCC; that is, $>10^5$ cells/mL (data not shown). An elevated level of SCC ($>200 \times 10^3$ cells/mL) is frequently observed in SCM, although it is not always mandatory for the diagnosis (Tenhagen et al., 2005; Bueno et al., 2006; Bozzo et al., 2014).

Interestingly, no significant changes in blood cell counts were found between mastitis and control cows or between SCM and CM animals. This is in contrast with what was observed by Alhussien et al. (2015), where total leukocyte counts of CM cows were higher than those in SCM cows.

Half of the cows with *Prototheca* spp. (4% of all CMT-positive cows) in their milk did not yield *Prototheca* from other anatomical sites. In 8 cows (8% of all CMT-positive cows) whose milk was negative for *Prototheca* spp., the algae could be cultured from feces (4 cows), mouth, nose, rectum (3 cows each), and vagina (one cow). This clearly shows that *Prototheca* spp. are colonizers of the bovine gastrointestinal and urogenital tract without causing any harm to the host. The asymptomatic carrier state of the algae has already been documented in dairy herds (Tenhagen et al., 2005; Bueno et al., 2006).

Finding *Prototheca* spp. in different environmental sites indicates their relatively wide distribution in nature, especially in humid and wet areas containing manure and decomposing plant material (Milanov et al., 2016). In this study, the algae were found mainly

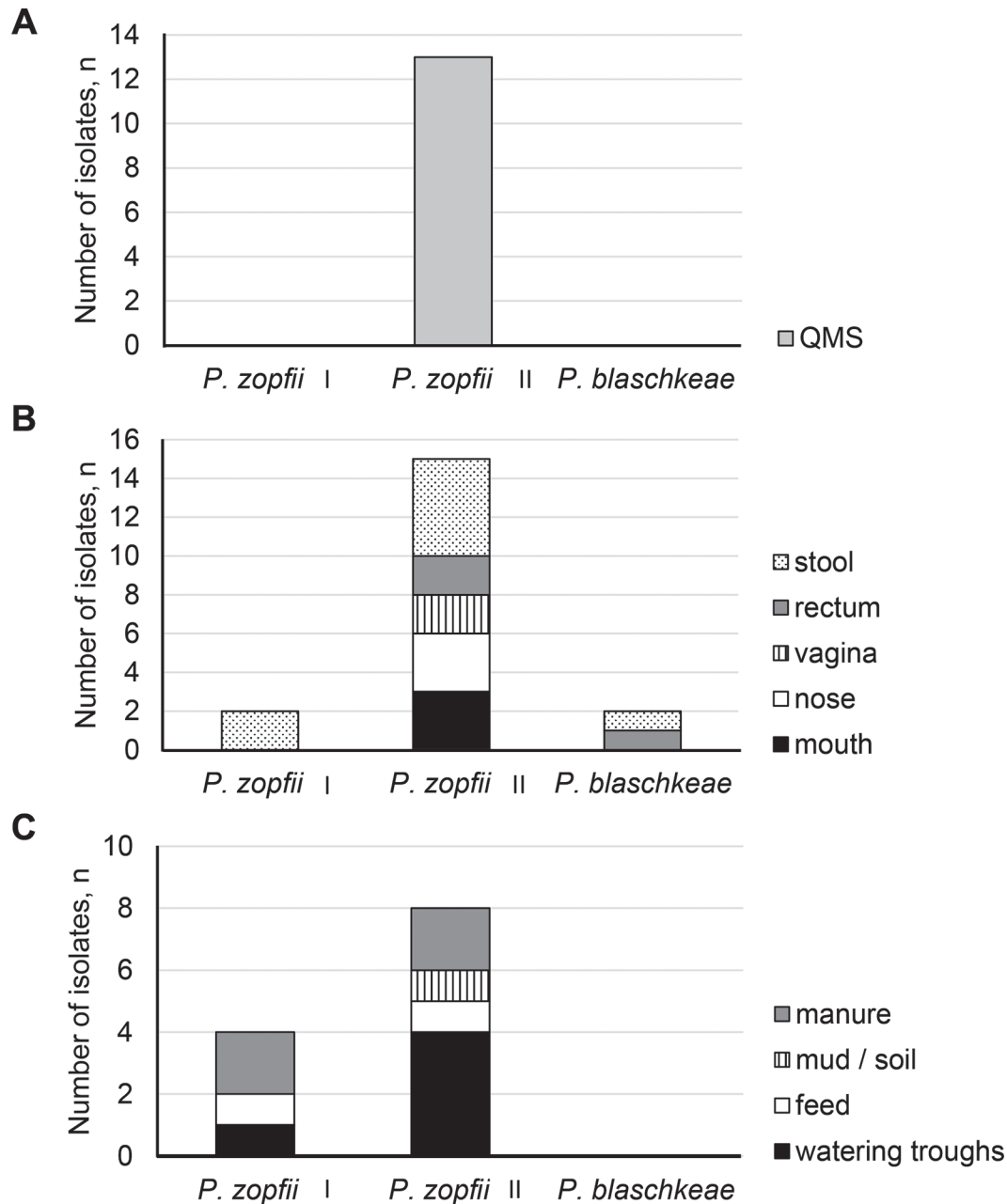


Figure 3. Species- and genotype-level identification of *Prototheca* isolates from this study in milk samples (A), body swabs (B), and environmental sources (C). QMS = quarter milk sample.

in drinking water and manure, and these sources have been their major environmental reservoir, as reported in previous studies (Pore et al., 1983; Enders and Weber, 1993; Costa et al., 1997; Scaccabarozzi et al., 2008).

At the species and genotype level, only *P. zopfii* gen. 2 was identified in milk samples, confirming its predominant role as a causative agent in protothecal mastitis in cattle (Osumi et al., 2008; Ahrholdt and Rosler, 2011; Jagielski et al., 2011; Pieper et al., 2012). *Prototheca*

zopfii gen. 2 was also recovered from most (80%) of the body site swabs, with only 2 (10%) samples yielding *P. blaschkeae*. The recovery of *Prototheca* spp. from environmental samples indicates their persistence in the dairy herd ecosystem.

The present work is a prelude to a whole-country investigation currently underway of the epidemiology of *Prototheca* bovine mastitis. Nevertheless, this study provides, for the first time in Poland, a comprehensive

and informative look at the occurrence of *Prototheca* on the regional scale.

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Update

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Corrigendum to “A survey on the incidence of *Prototheca* mastitis in dairy herds in Lublin province, Poland” (J. Dairy Sci. 102:619–628)

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In the abstract on page 619, the 13th sentence should read “All (**44**) *Prototheca* isolates recovered in this study were subjected to species- and genotype-level molecular identification.” On page 623, in the section Identification of Udder Infection, the second sentence should read “The proportion of CMT-positive cows in a sampled herd ranged from 37.5% (farm VI) to 85.7% (farm **II**), with a mean of **58.1%**.” On page 625, in the section *Prototheca* spp. Genotyping, the second sentence should read “Among **19** isolates from animal swabs and stool, 15 isolates were *P. zopfii* gen. 2, 2 isolates were *P. zopfii* gen. 1, and 2 isolates were *P. blaschkeae*. All corrections are shown in bold.

The authors regret the errors.

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