

## Susceptibility of *Prototheca zopfii* strains isolated from cows with mastitis to chlorhexidine and iodine

Henryk KRUKOWSKI\*, Andrzej LISOWSKI, Bożena NOWAKOWICZ-DĘBEK, Łukasz WLAZŁO

Department of Animal and Environmental Hygiene, University of Life Sciences, Akademicka 13 Street, 20-950 Lublin, Poland

Received: 28.10.2011

Accepted: 06.01.2012

Published Online: 22.01.2013

Printed: 22.02.2013

**Abstract:** Bovine mastitis caused by *Prototheca* spp. can assume high significance because of economic losses and the potential risk to public health. The aim of our study was to evaluate the effect of chlorhexidine and iodine on *Prototheca zopfii* strains isolated from bovine milk. For this study, 15 *Prototheca zopfii* isolates previously obtained from cows with clinical and subclinical mastitis in Poland were used. The in vitro susceptibility tests to chlorhexidine and iodine of the strains of *Prototheca zopfii* for the determination of their minimal microbicidal concentrations (MMCs) were performed using the tube dilution method in Sabouraud dextrose broth and the evaluation of colony growth after plating in Sabouraud dextrose agar. In this study, the MMC was 0.3125%–1.25% for iodine and 0.0048%–0.0195% for chlorhexidine.

**Key words:** *Prototheca zopfii*, mastitis, MMC, chlorhexidine, iodine

Protothecosis is an infection reported in humans and in many other animal species caused by achlorophyllous algae of the genus *Prototheca*. These algae are primitive unicellular organisms considered to be chlorophyll-lacking mutants that have adopted a heterotrophic mode of nutrition. These organisms are widely disseminated in different environments of high humidity (1,2). The predominant form of protothecosis in cattle has been bovine mastitis, most commonly induced by *Prototheca zopfii*. Although bovine mastitis associated with *Prototheca* is considered a rare pathology, its prevalence is increasing. This disease is responsible for heavy economic losses due to dramatic loss in milk yield and its quality, as well as culling of the infected cows. The pathogens are isolated from a variety of environmental sources including drinking water, sewage, liquid manure, feces from both healthy animals and those infected by bovine protothecal mastitis, barn walls and floors, as well as milking machine liners and milking cups (2–4).

Chlorhexidine represents one of the most important products in dairy practice, used as a postdipping antiseptic. This antimicrobial shows a high spectrum of action. Moreover, 2% chlorhexidine solution is recommended to promote the definitive drying of the mammary gland (5).

Moreover, iodine is recognized as an efficient antiseptic. Iodine has high cell penetrating power, causing protein precipitation and oxidation of essential enzymes.

\* Correspondence: henryk.krukowski@up.lublin.pl

In dairy farms, iodine is generally indicated in pre- and postdipping for the control of bovine mastitis (6).

The aim of the present study was to evaluate the effect of chlorhexidine and iodine on *P. zopfii* strains isolated from bovine milk.

For this study, 15 *P. zopfii* isolates previously obtained from clinical and subclinical bovine mastitis were revived by subculture on Sabouraud 4% dextrose agar (bioMérieux, Poland) and incubated for 48 to 72 h at 37 °C. Identification was performed using routine culture, macro- and microscopic morphological characterization, and API 20CAUX (bioMérieux, Poland) methods (7–10). The test based on the susceptibility of each species of algae to clotrimazole according to Casal and Gutierrez (11) was also used. This test was used to distinguish between *P. zopfii* and *P. wickerhamii*.

The in vitro susceptibility tests to chlorhexidine and iodine of the strains of *Prototheca zopfii* for the determination of their minimal microbicidal concentrations (MMCs) were performed using the tube dilution method in Sabouraud dextrose broth and an evaluation of colony growth after plating in Sabouraud dextrose agar. The studies were carried out under the strict methodological protocol reported by Melville et al. (5).

Based upon morphologic features, resistance to clotrimazole, and the ability to assimilate glucose and glycerol, all of the isolates were identified as *P. zopfii*. In

**Table.** The minimal microbicidal concentration (MMC) of chlorhexidine and iodine against strains of *Prototheca zopfii*.

MMC (in %)	Iodine		Chlorhexidine	
	Number of strains	% of strains	Number of strains	% of strains
0.0048	0	0	10	66.66
0.0097	0	0	2	13.33
0.0195	0	0	3	20
0.0390	0	0	0	0
0.0781	0	0	0	0
0.1562	0	0	0	0
0.3125	4	26.66	0	0
0.6250	9	60	0	0
1.2500	2	13.33	0	0
Total	15	100	15	100

the present study, MMC for iodine was 0.3125% against 4 (26.66%) strains, 0.625% against 9 (66.6%) strains, and 1.25% against 2 (13.33%) strains. The MMC for chlorhexidine was 0.0048% against 10 (66.66%) isolates, 0.0097% against 2 (13.33%) isolates, and 0.0195% against 3 (20.00%) isolates. The results are summarized in the Table.

Among the 5 species of *Prototheca* genus, *P. zopfii* is the one most responsible for bovine mastitis. The agent is widespread in the environment of dairy herds (12). *Prototheca* spp. isolated from bovine mastitis exhibit resistance to a routine treatment. The conventional antimicrobial and antifungal therapy proves to be ineffective (1,13,14).

In our study, the MMC of chlorhexidine was 0.0048%–0.0195% and was similar to that obtained in the studies by Melville et al. (5).

## References

- Lassa, H., Malinowski, E.: *Prototheca* spp. and protothecosis in animals. *Życie wet.* 2007; 82: 31–34. (Article in Polish with an abstract in English)
- Marques, S., Silva, E., Carvalheira, J., Thompson, G.: In vitro susceptibility of *Prototheca* to pH and salt concentration. *Mycopathologia*, 2010; 169: 297–302.
- Kirk, J., Mellenberger, R.: Mastitis control program for *Prototheca* mastitis in dairy cows. [http://milkquality.wisc.edu/wp-content/uploads/2011/09/mastitis-control-program\\_prototheca-mastitis.pdf](http://milkquality.wisc.edu/wp-content/uploads/2011/09/mastitis-control-program_prototheca-mastitis.pdf)
- Spalton, D.E.: Bovine mastitis caused by *Prototheca zopfii*: a case study. *Vet. Rec.*, 1985; 116: 347–349.
- Melville, P.A., Benites, N.R., Sinhorini, I.L., Costa, E.O.: Susceptibility and features of the ultrastructure of *Prototheca zopfii* following exposure to copper sulphate, silver nitrate and chlorhexidine. *Mycopathologia*, 2002; 156: 1–7.
- Salerno, T., Ribeiro, M.G., Langoni, H., Siqueira, A.K., Costa, E.O., Melville, P.A., Bueno, V.F., Yamamura, A.A., Roesler, U., da Silva, A.V.: In vitro algacide effect of sodium hypochlorite and iodine based antiseptics on *Prototheca zopfii* strains isolated from bovine milk. *Res. Vet. Sci.*, 2010, 88: 211–213.
- Costa, E.O., Ribeiro, A.R., Melville, P. A., Prada, M.S., Carciof, A.C., Watanabe, E.T.: Bovine mastitis due to algae of the genus *Prototheca*. *Mycopathologia*, 1996; 137: 85–88.
- Costa, E.O., Melville, P.A., Ribeiro, A.R., Watanabe, E.T., Parolari, M.C.F.F.: Epidemiologic study of environmental sources in a *Prototheca zopfii* outbreak of bovine mastitis. *Mycopathologia*, 1997; 137: 33–36.
- Marques, S., Silva, E., Carvalheira, J., Thompson, G.: In vitro antimicrobial susceptibility of *Prototheca wickerhamii* and *Prototheca zopfii* isolated from bovine mastitis. *J. Dairy Sci.*, 2006; 89: 4202–4204.

10. Padhye, A.A., Baker, J.G., D'Amato, D.F.: Rapid identification of *Prototheca* species by the API 20C System. J. Clin. Microbiol., 1979; 10: 579–582.
11. Casal, M.J., Gutierrez, J.: Simple new test for rapid differentiation of *Prototheca wickerhamii* from *Prototheca zopfii*. J. Clin. Microbiol., 1983; 18: 992–993.
12. Bueno, V.F.F., de Mesquita, A.J., Neves, R.B.S., de Souza, M.A., Ribeiro, A.R., Nicolau, E.S., de Oliveira, A.N.: Epidemiological and clinical aspects of the first outbreak of bovine mastitis caused by *Prototheca zopfii* in Goiás State, Brazil. Mycopathologia, 2006; 61: 141–145.
13. da Costa, E.O., Ribeiro, M.G., Ribeiro, A.R., Rocha, N.S., de Nardi, G. Jr: Diagnosis of clinical bovine mastitis by fine needle aspiration followed by staining and scanning electron microscopy in a *Prototheca zopfii* outbreak. Mycopathologia, 2004; 158: 81–85.
14. Lopes, M.M., Ribeiro, R., Carvalho, D., Freitas, G.: In vitro antimicrobial susceptibility of *Prototheca* spp. isolated from bovine mastitis in a Portugal dairy herd. J. Mycol. Med., 2008; 18: 205–209.