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RARE CAUSATIVE AGENTS OF MAMMARY GLAND INFECTION: *CANDIDA LAMBICA* -CASE REPORT-

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Abstract

A brief case report on bovine subclinical mastitis caused by yeast species *Candida lambica* is presented in this article. Basic cultural, microscopic and biochemical traits of this rare agent implicated in bovine mammary infection are described. Identification of isolates was performed using an *Integral System Yeasts Plus* test, a commercial kit for identification of yeasts of importance in medicine. The available literature offers only sporadic reports on *C. lambica* infection in both humans and animals.

Key words: bovine mastitis, *Candida lambica, Integral System Yeast Plus* test

RETKI UZROČNICI INFEKCIJE MLEČNE ŽLEZDE KRAVA: *CANDIDA LAMBICA* -PRIKAZ SLUČAJA-

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Kratak sadržaj:

U radu je dat kratak prikaz slučaja subkliničkog mastitisa krave izazvanog kvasnicom *Candida lambica.* Prikazane su osnovne kulturelne, mikroskopske i biohemijske karakteristike ovog retkog uzročnika infekcije

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mlečne žlezde krava. Identifikacija izolata izvedena je primenom *Integral System Yeasts Plus* testa, komercijalnog sistema za identifikaciju medicinski značajnih kvasnica. U dostupnoj stručnoj literaturi, postoje samo sporadični izveštaji o infekcijama koje kod ljudi i životinja izaziva *C. lambica.*

Ključne reči: bovine mastitis, *Candida lambica, Integral System Yeast Plus* test

INTRODUCTION

Wide application of antibiotics and corticosteroids in both human and veterinary medicine throughout the period since the 1950s resulted in an increased incidence of yeast infections. The fungi, particularly yeasts, are considered opportunistic pathogens for mammary gland. Previous administration of antibiotics, treatment with contaminated antibiotic preparations, as well as syringes are prerequisites for the occurrence of the infection (Krukowski and Saba, 2003; Zaragoza *et al*., 2011). Prevalence of mycotic mastitis is usually low, 1-12% off all mastitis cases (Krukowski and Saba, 2003). However, if the infection remains unidentified and the factors implicated in its development are not eliminated on time, yeast mastitis may reach even epizootic proportions (Krukowski and Saba, 2003; Costa *et al*., 2012). During the past several years, increased incidence of subclinical and clinical mastitis caused by yeasts from the genus *Candida* has been reported (Zaragoza *et al*., 2011; Dworecka-Kaszak *et al*., 2012). Microbiological examination of milk samples is inevitable in the diagnostics of mycotic mastitis, and identification at species level is performed according to morphologic features (formation of chlamydoconidium, pseudohyphae and germinal tube development), growth in the presence of 0.1% cyclohexamide, acidic pH tolerance and carbohydrates assimilation and/or fermentation.

Mastitis in dairy cows is associated with a variety of fungal species. Though the yeasts of the genus *Candida* are most commonly isolated species (Zaragoza *et al*., 2011; Milanov *et al*., 2014) there are only sporadic reports on the isolation of *C. lambica* (Spanamberg *et al*., 2008; Zaragoza *et al*., 2011). In human medicine, only several cases of *Candida lambica* infections have been reported so far: bloodstream infections (Pfaller *et al*., 2004; Vervaeke *et al*., 2008), infection in patients with hematologic malignancies (Kruger *et al*., 1998), arthritis, probably acquired from a contaminated wound, in a patient with chronic alcoholism (Trowbridge *et al*., 1999).

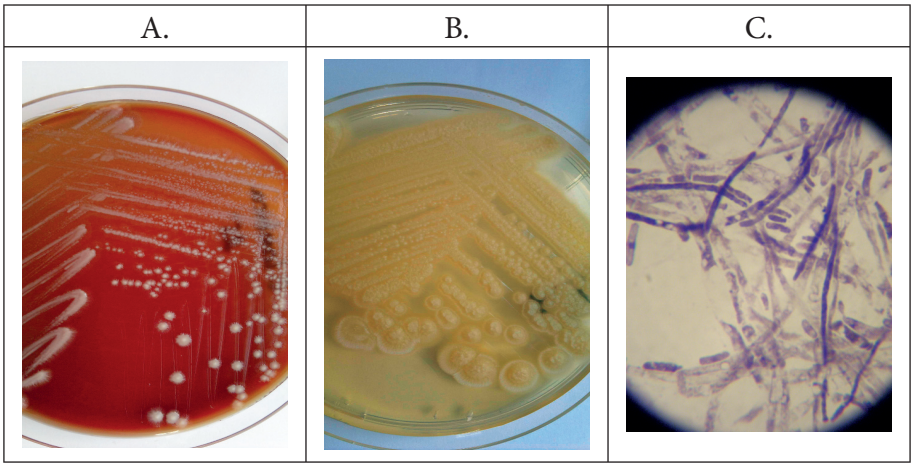
CASE REPORT

Regular monthly examination of milk sample using California Mastitis Test revealed positive finding in the single infected udder quarter in a Holstein-Friesian cow aged 3.9 years. The colour and consistency of the milk were not significantly changed, but the presence of small patches was evident in the sample. Before milk sampling for microbiological examination, the cow underwent two unsuccessful antibiotic treatments that included parenteral administration of enrofloxacin, amoxicillin and penicillin as well as intramammary administration of *cefquinome*, bacitracin, neomycin, tetracycline and prednisolone. The time period between the last antibiotic administration and collection of milk sample was more than one month. In July, when the sampling was performed, the somatic cell count per one mL of milk from infected udder was 1679000. The sampling was performed on 170th day of second lactation, and average seven-day milk yield was a 27 Litres.

Milk samples for microbiological examination were obtained separately from each of the four quarters. Disinfection of teats was performed using 70% ethanol, and the milk was collected into sterile plastic tubes, cooled and transported to the laboratory. To the purpose of bacterial isolation, aliquots of 50μL were inoculated onto Columbia blood agar base (Oxoid, Basingstoke, UK, CM0331) with 5% defibrinated ovine blood, MacConkey agar (Oxoid, CM0007) and onto Sabouraud dextrose agar (Oxoid, CM0041) and incubated during 2-3 days at 25°C and 37°C.

From the milk sample collected from the right anterior quarter, massive amounts of pure culture yeast were isolated. The growth was noticed after 24-hour incubation at all nutritive media and at both incubation temperatures (25°C and 37°C), with largest colonies observed on Sabouraud dextrose agar. Colonial appearance on blood and Sabouraud dextrose agar after 72h incubation at 25°C is presented in Figure 1 (A and B). Preparations made of 7-day old cultures grown on Sabouraud dextrose agar at 37°C. The preparations were stained according to Giemsa method and examined using light microscopy (1000x, immersion) (Figure 1, C).

Figure 1: Yeast colonies at blood agar (A) and Sabouraud dextrose agar (B) after 72h incubation at 25°C and microscopic appearance (C)



Biochemical characteristics of the isolate were examined using *Integral System Yeasts Plus* test (Liofilchem, Italy, Ref. 71822) according to manufacturer's instructions. *Integral System Yeasts Plus* test is a system for identification of most clinically important yeasts. Presumptive identification is based on assimilation reactions of sugars (glucose, maltose, saccharose, lactose, galactose, melobiose, cellobiose, inositol, xylose, raffinose, trehalose and dulcitol). Based on its assimilation features, the isolate was identified as *Candida lambica* (Table 1). Microscopic examination revealed abundant, moderately branched pseudohyphae (Figure 2, C). True hyphae *C. lambica* are not formed (http://www.doctrofungus.org/ the fungi/Candida_ lambica.php).

Table 1. Assimilation ability of isolate *Candida lambica* in *Integral System Yeasts Plus* test

Identification	Glu	Mal	Sac	Lac	Gal	Mel	Cel	Ino	Xil	Raf	Tre	Dul	ID code
C.lambica	+	-	-	-	-	-	-	-	+	-	-	-	1040

After the first microbiological examination of the samples and isolation of the yeast, the sampling was repeated for finding confirmation. Namely, *C.*

lambica was found in dairy products, water, and fruits and thus may be present in the sample as a contaminant. Second sampling was performed under maximum aseptic conditions, at the very end of milking. Such procedure minimizes the probability of contamination with yeasts that are commonly present on the skin of the udder and teats (Krukowski and Saba, 2003). The repeated sampling has confirmed the previously obtained result.

C. lambica is a rare causative agent of mastitis in dairy cattle. Similar to other yeasts of the genus *Candida*, it readily grows on nutritive media commonly applied in bacteriology labs, such as blood agar, MacConkey agar and Sabouraud dextrose agar. According to cultural and biochemical traits, it is similar to the species *C. krusei,* which is more commonly isolated from the milk of cows with mycotic mastitis (Türkyılmaz and Kaynarca, 2008; Wawron *et al*., 2010; Dworecka-Kaszak *et al*., 2012). By using chromogenic agars and a commercial phenotyping gallery, *C. lambica* might be misidentified as *Candida krusei* (Vervaeke *et al*., 2008). Neither *C. lambica*, nor *C. krusei* grow on media containing cycloheximide (which differentiates them from *C.lipolytica*). Besides molecular methods that enable most reliable differentiation between these two related species, some simpler and readily available methods such as maximum growth temperature and biochemical traits determination could be successfully applied. Both species grow at incubation temperatures 25°C and 37°C; however, contrary to *C. krusei*, *Candida lambica* does not grow at 42°C. Commercial test *Integral System Yeasts Plus* used in this research enables differentiation between these two species based on their assimilation abilities (Table 2).

Table 2. Assimilation ability of *Candida lambica* and *C. krusei* in *Integral System Yeasts Plus* test

Identification	Glu	Mal	Sac	Lac	Gal	Mel	Cel	Ino	Xil	Raf	Tre	Dul	ID code
C.lambica	+	-	-	-	-	-	-	-	+	-	-	-	1040
C.krusei	+	-	-	-	-	-	-	-	-	-	-	-	1000

Some of our previous investigation of yeasts isolates from milk of cows with subclinical and clinical mastitis using the *Integral System Yeasts Plus* demonstrated that biochemical profile of the achlorophyllous alga *Prototheca zopfii* is identical to that of C. *krusei* (Milanov *et al*., 2014). Since this test is not applicable for the identification of *P. zopfii* (contrary to *P. wickerhamii*), the simi-

larity of cultural features of these organisms makes microscopic examination of the preparation unavoidable for final identification at species level.

Even though there are some therapeutic options for the management of yeast mastitis, it is rarely practiced in everyday practice. Therapeutic options in *Candida* infections include amphotericin B, fluconazole, tioconazole, miconazole and nystatin (Krukowski and Saba, 2003). Regrettably, in this case as well as in the number of other cases, yeast mastitis remains undiagnosed. It enhances spread of the infection within the herd, and commonly practiced antibiotic therapies only aggravate the situation. Milk samples are submitted for microbiological examination only after several unsuccessful treatments with diverse antibiotic classes.

CONCLUSION

The incidence of yeast mastitis in dairy cattle has been showing increasing tendency. Timely identification, control of infection spread within the herd and adequate management require microbiological examination of milk samples. Everyday practice in our veterinary clinical laboratories does not implicate identification of yeasts to the level of species. Moreover, specific test designed for application in veterinary medicine are not commercially available. *Integral Systems Yeasts Plus* test is designed for identification of yeasts of medical importance and is highly applicable for the identification of *Candida* species involved in the mastitis of dairy cows.

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