ACTA SCIENTIARUM POLONORUM *Acta Sci. Pol., Zootechnica 7 (1) 2008, 3–10*

ETIOLOGICAL AGENTS OF MASTITIS IN DAIRY COWS ON A FARM IN THE WEST POMERANIAN REGION

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Abstract. Four hundred milk samples were collected from cows. In 78 samples (19.50%) an increase of somatic cells was detected employing the California Mastitis Test (CMT). Therefore, those milk samples were plated (0.1 mL) onto bacteriological and mycological media. Cultures were incubated at 37°C and 20°C for 24h to 72h. Biochemical features of bacteria and fungi were determined in API tests and/or on liquid O-F Medium. The presence of coagulase in *Staphylococcus* spp. was investigated by tube test using rabbit plasma. For identification of *C. albicans* the germ tubes test was conducted. Based on microbiological investigation and increase of somatic cells, mastitis was confirmed in all cows, whose milk was selected for further research. The presence of bacteria was confirmed in 64.10% of samples, bacteria and yeast in 34.62% and yeast alone in 1.28%. Sixteen bacterial species were isolated: *Staphylococcus* spp. (5 species), *Enterobacteriaceae* (5 species), *Streptococcus* spp. (4 species), *Bacillus cereus* and *Pseudomonas aeruginosa*. Among yeasts, 15 species were identified: *Candida* spp. (9 species), *Blastoschizomyces capitatum*, *Dipodascus ingens*, *Geotrichum candidum*, *Trichosporon asahii*, *Saccharomyces cerevisiae*, *Torulopsis conglobata*. The most frequent isolated yeasts were *Candida* spp., mainly *C. guilliermondii* (6 strains), *C. kefyr* (4 strains) and *C. butyri* (4 strains). The most frequent isolated bacteria were *Staphylococcus* spp. (37 strains), *Streptococcus* spp. (28 strains) and *Enterobacteriaceae* (23 strains).

Key words: bacterium, dairy cow, mastitis, milk, yeast

INTRODUCTION

Bacteria, yeasts, moulds [Capurro et al. 1999], viruses and *Prototheca* [Malinowski et al. 2002] are considered to be etiological agents of mastitis. *Candida* spp. is the most often isolated yeast from cows' milk [Lagneau et al. 1996]. In mastitic milk, apart from *Candida* spp., *Rhodotorula* spp., *Trichosporon* spp. [Krukowski et al. 2001], *Saccharomyces* spp. *Cryptococcus* spp. [Keller et al. 2000] are also isolated. Yeasts are supposed to be the main fungal agents of mastitis and they are very often isolated with pathogenic bacteria. Yeasts develop well in high humidity and in a wide range of temperatures. In natural circum-

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stances, their growth rate depends on hydrogen ion concentration and on the amount of organic compounds. They prefer an acidic environment (pH 3.5 – 6.5) to a neutral, or slightly alkaline one [Corbo et al. 2001]. Although all pathogenic yeasts come from the environment, the alimentary tract is their major habitat. The diversity of *Candida* species is connected with its common presence in nature. Fungal transmission appears directly from animal to animal and indirectly from the environment – through contaminated objects [Cannon et al. 1995]. *C. albicans* was the only one which was thought to be pathogenic for a very long time. Therefore, 16 out of 196 known species are considered pathogens. The amount of pathogenic species is still on the increase. The differentiation of yeasts and bacteria isolated from mastitic milk was the aim of this study.

MATERIAL AND METHODS

The examination was conducted at one dairy farm located in the West Pomeranian Region, Poland. Milk samples were taken from 400 cows. In further investigations, 78 samples, which were positive in the California Mastitis Test (CMT), were considered. 0.1 mL of each were plated onto: Sabouraud Dextrose Agar, Corn Meal Agar, Edwards Medium, Mannitol Salt Agar, MacConkey Agar, Schiemann CIN Medium, Wilson-Blair Medium and Nutrient Agar with 5% of sheep blood, respectively. Cultures on Sabouraud Dextrose Agar, as well on Corn Meal Agar, were incubated at 37°C and at 20°C for 72 h, while all the others were incubated at 37°C for 24 h. Cultures on Wilson-Blair Agar were incubated for 48 h in anaerobic conditions. Biochemical features of bacteria and yeasts were determined in API tests (BioMérieux, France). The presence of coagulase in *Staphylococcus* spp. strains was investigated in a tube test with rabbit plasma (Biomed Kraków, Poland). The fungal ability of carbon assimilation was verified in API 20C AUX, whereas fermentation of glucose, maltose, saccharose, lactose and galactose was analyzed on liquid O-F Medium [Ewing 1987]. Furthermore, *C. albicans* ability of a germ tubes production was conducted according to the Campbell et al. [1996] method.

RESULTS

Sixteen bacterial species and 15 species of yeasts were isolated from 78 milk samples (Table 1). The presence of bacteria was confirmed in 64.10% samples, bacteria and yeasts in 34.62%, and yeast in 1.28% alone.

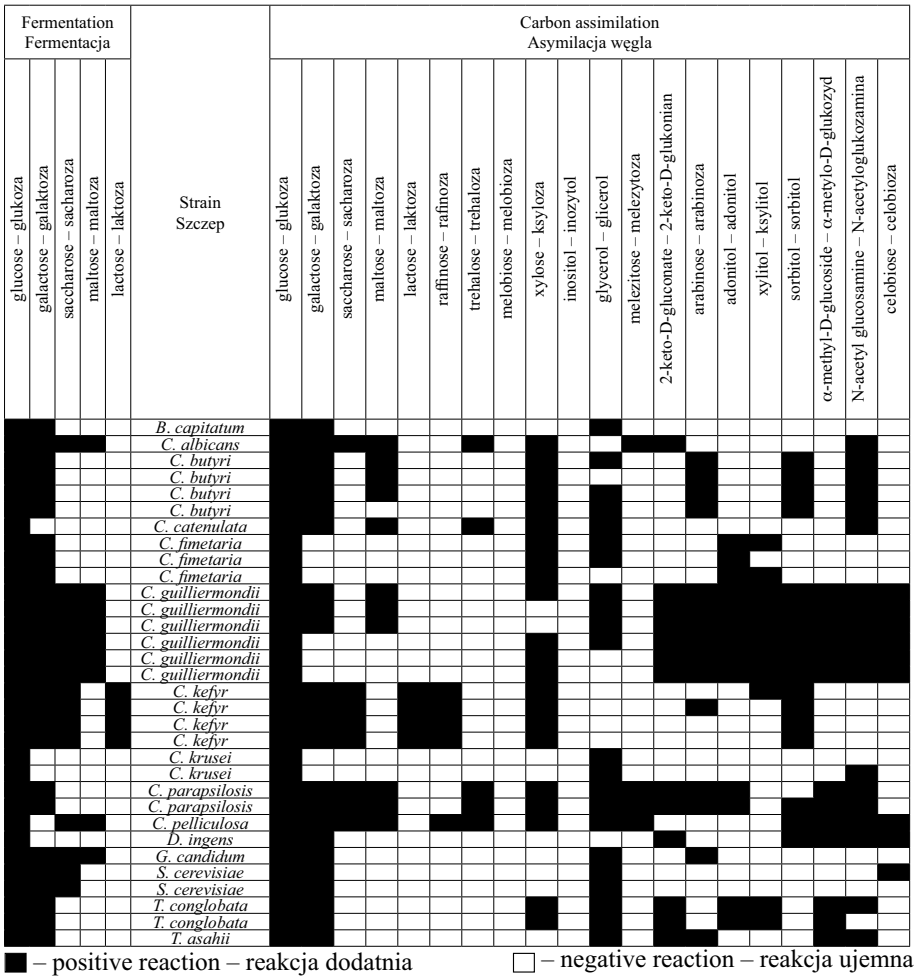
Table 1. Etiological agents of mastitis on dairy farm in the West Pomeranian region
Tabela 1. Czynniki etiologiczne zapalenia gruczołu mlekowego u krów mlecznych w regionie zachodniopomorskim

Etiological agents Czynniki etiologiczne	Number of strains Liczba szczepów	%
Blastoschizomyces capitatum	1	0.81
Candida albicans	1	0.81
Candida butyric	4	3.25
Candida catenulata	1	0.81
Candida fimentaria	3	2.44
Candida guilliermondii	6	4.88
Candida kefyr	4	3.25
Candida krusei	2	1.63
Candida parapsilosis	2	1.63
Candida pelliculosa	1	0.81
Dipodascus ingens	1	0.81
Geotrichum candidum	1	0.81
Saccharomyces cerevisiae	2	1.63
Torulopsis conglobata	2	1.63
Trichosporon asahii	1	0.81
Total Yeasts – Suma	**32**	**26.02**
Staphylococcus aureus	7	5.69
Staphylococcus chromogenes	7	5.69
Staphylococcus hyicus	10	8.13
Staphylococcus intermedius	9	7.32
Staphylococcus simulans	4	3.25
Total *Staphylococcus* spp. – Suma	**37**	**30.08**
Streptococcus agalactiae	20	16.26
Streptococcus dysgalactiae	4	3.25
Streptococcus parauberis	3	2.44
Streptococcus uberis	1	0.81
Total *Streptococcus* spp. – Suma	**28**	**22.76**
Citrobacter diversus	6	4.88
Escherichia coli	8	6.50
Proteus vulgaris	2	1.63
Serratia fonticola	6	4.88
Yersinia enterocolitica	1	0.81
Total *Enterobacteriaceae* – Suma	**23**	**18.70**
Bacillus cereus	**2**	**1.63**
Pseudomonas aeruginosa	**1**	**0.81**
Total – Suma	**123**	**100**

Thirty-two yeast strains were isolated from 28 milk samples. In 27 samples, yeasts were isolated in the presence of bacteria, but in 5 samples fungi were evidenced majority: *C. fimentaria, C. guilliermondii, C. kefyr, C. parapsilosis* and *C. krusei*. Another *C. krusei* strain was isolated in monoculture. Both the *C. krusei* strains represented the same bio-

chemical features, but differing in macroscopic analyses. The strain accompanied by *S. intermedius* and *S. hyicus* was rough, while the strain in monoculture was smooth. In each of the following 4 samples, 2 yeast strains were isolated. In each sample, *C. guilliermondii* was present*,* followed by: *C. butyri*, *C. fimentaria*, *C. kefyr*, *T. conglobata.* Among all the yeasts, *Candida* spp. was isolated most frequently (9 species in 20 milk samples). *C. guilliermondii* was the dominant one (4.88%). Fermentation facilities of strains which belonged to the same species were identical. However, they differed in ability of assimilation. Biochemical features of isolated fungal strains are described in Table 2. Yeasts accompanied by bacteria presented rough colonies, while *C. krusei* grown in monoculture, as well as other yeasts, which dominated in culture, formed smooth colonies. *C. kefyr* strain, inhibited by *P. aeruginosa,* did not produce pseudohyphae.

Table 2. f yeasts isolated from milk samples
Tabela 2. Charakterystyka biochemiczna drożdży izolowanych z prób mleka



Apart from yeasts, the following bacteria were isolated from mastitic milk samples: *Staphylococcus* spp., *Streptococcus* spp., *Enterobacteriaceae*, *Bacillus cereus* and *Pseudomonas aeruginosa*. *Staphylococcus aureus* and two *Bacillus cereus* strains were isolated in monoculture in 4 samples. Thirty-seven *Staphylococcus* strains were isolated from 25.64% milk samples. All *S. aureus, S. intermedius* and 40% *S. hyicus* strains produced coagulase. *S. intermedius* and *S. hyicus* were isolated with *C. butyri*, *C. fimentaria* and *C. guilliermondii* simultaneously, whereas *S. aureus* was isolated with *C. albicans*, and *G. candidum.* Streptococci were isolated from 23.08% milk samples. Streptococci were always accompanied by yeasts. Among them, *Streptococcus agalactiae* appeared most often. *Enterobacteriaceae* were present in 15.38% milk samples. No yeasts were found in a sample from which *Y. enterocolitica* was isolated. In the one from which *E. coli* was isolated, single colonies of *B. capitatum*, *C. catenulata*, *D. ingens*, *S. cerevisiae*, *T. conglobata* were present. *C. kefyr, C. parapsilosis* and *C. pelliculosa* were isolated in correlation to *Proteus vulrgaris* and *Pseudomonas aeruginosa*, but according to the characteristic growth of these bacteria, development of yeasts was inhibited. In samples from which *C. diversus* and *S. fonticola* were isolated, abundant bacterial growth was observed.

DISCUSSION

Staphylococcus spp. were the most often isolated bacteria. Their presence was detected in 37 (71.2%) of milk samples. Malinowski et al. [2003] isolated these bacteria from 79.2% samples of mastitic milk. In this study, strains of *S. aureus, S. intermedius i S. hyicus* produced coagulase. The most often isolated species was *S. hyicus* (19,2%). Coagulase positive strains of staphylococci are considered pathogenic. They are isolated not only from cases of clinical mastitis [Capurro et al. 1999], but also from alimentation [Majczyna and Białasiewicz 2004; Normanno et al. 2005]. Coagulase negative strains of *Staphylococcus* are isolated from clinical and subclinical cases of mastitis in dairy cows in many countries [Myllys 1995; Smith 2001; Malinowski et al. 2003]. In this research, coagulase negative staphylococci were isolated from 11 samples.

Streptococcus agalactiae is arbitrarily pathogenic and the most virulent from all streptococci involved in mastitis, not only in cattle [Bohnsack et al. 2004]. This bacterium was detected in 20 (38.5%) of milk samples. Such a frequent isolation of *S. agalactiae* is connected with its great contagiousness [Zadoks et al. 2004].

Among *Enterobacteriaceae*, *E. coli* was the most often isolated, whereas *Y. enterocolitica* was isolated only once.

Candida strains are often responsible for mastitis [Moretti et al. 1998, Keller et al. 2000, Krukowski et al. 2001]. This phenomenon is due to frequent use of antibiotics. They are used not only in treatment, but also in the dry period as well. Antibiotic therapy leads to perturbation in udder homeostasis, inhibition of T cells and neutrophil activity, and in consequence, to stimulation of yeast growth [Sheng et al. 1987, Corti et al. 2003, Noris et al. 2007]. Half of the cows, in which yeasts contaminated milk, was treated with antimicrobial agents (penicillin, streptomycin, tetracycline, lincomycin, nystatin). Despite the

fact that yeasts are isolated even from 77% of mastitic milk [Keller et al. 2000, dos Santos and Marin 2005], 2–13% of all mastitis cases are due to yeasts [Keller et al. 2000, Corbo et al. 2001, Krukowski et al. 2001]. Typical inflammation caused by *Candida* spp. is only considered when yeasts are isolated in monoculture. Yeast infections of mammary glands were stated in 1 of the cows. *Candida krusei* was the etiological factor responsible. The employment of antibacterial agents may predispose yeasts to dominate bacterial flora. *C. fimentaria, C. guilliermondii, C. kefyr, C. krusei* and *C. parapsilosis* were predominant yeast species in mastitic milk. *C. guilliermondii* is often isolated either from the milk of healthy [Lagneau et al. 1996] or sick cows [Moretti et al. 1998]. Pathogenic yeasts like *C. albicans, C. guilliermondii, G. candidum* and *C. kefyr* also show great biochemical activity. They change pH in the udder due to carbohydrate fermentation. In consequence, they acidify the environment and inhibit development of bacterial flora.

CONCLUSION

The most often isolated bacteria were staphylococci, which is probably due to continuous increase of resistance to antibiotics used in cattle therapy.

Isolation of 32 microorganism species from 78 milk samples indicates polietiological origin of mastitis.

Isolation of many different yeast species may point their great importance in etiology of mastitis.

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CZYNNIKI ETIOLOGICZNE *MASTITIS* KRÓW MLECZNYCH UTRZYMYWANYCH W GOSPODARSTWIE NA TERENIE POMORZA ZACHODNIEGO

Streszczenie. Zebrano 400 prób mleka krowiego. W 78 (19,50%) próbach, na podstawie badania TOK, stwierdzono podwyższoną liczbę komórek somatycznych. Mleko z tych prób w ilości 0,1 mL wysiewano na podłoża bakteriologiczne i mikologiczne, po czym inkubowano od 24 do 72 godzin w temperaturze 37°C bądź 20°C. Cechy biochemiczne bakterii i grzybów określano za pomocą testów API i/lub na płynnym podłożu O-F. Aktywność koagulazy u szczepów *Staphylococcus* sprawdzano w teście probówkowym z osoczem króliczym. *C. albicans* identyfikowano na podstawie zdolności do produkcji pseudostrzępek. Obecność bakterii stwierdzono w 64,10% prób mleka, drożdży w 1,28%, a bakterii i drożdży w 34,62%. Wyizolowano 16 gatunków bak-

terii, w tym z rodzaju *Staphylococcus* – 5, rodziny *Enterobacteriaceae* – 5, rodzaju *Streptococcus* – 4, a także *Bacillus cereus* i *Pseudomonas aeruginosa.* Ponadto w mleku zdiagnozowano obecność 15 gatunków drożdży, w tym 9 gatunków z rodzaju *Candida,* a także *Blastoschizomyces capitatum*, *Dipodascus ingens*, *Geotrichum candidum*, *Trichosporon asahii*, *Saccharomyces cerevisiae*, *Torulopsis conglobata*. Najczęściej izolowanymi gatunkami z rodzaju *Candida* były: *C. guilliermondii* (6 szczepów), *C. kefyr* (4 szczepy) oraz *C. butyri* (4 szczepy). Najczęściej izolowanymi bakteriami były gronkowce (37 szczepów), paciorkowce (28 szczepów) oraz pałeczki z rodziny *Enterobacteriaceae* (23 szczepy).

Słowa kluczowe: bakteria, drożdże, mleko, krowy mleczne, zapalenie gruczołu mlekowego

Accepted for print – Zaakceptowano do druku: 10.01.2008