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Dział Immuno- i Cytogenetyki Zwierząt, Instytut Zootechniki – Państwowy Instytut Badawczy,  
ul. Krakowska 1, 32-083 Balice, e-mail: pkrzysc@izoo.krakow.pl

PIOTR KRZYŚCIN

**Etiological factors of mastitis in Polish Merino ewes**

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Czynniki etiologiczne stanów zapalnych gruczołu mlekowego  
u maciorek rasy merynos polski

**Summary.** Etiological factors of mastitis in Polish Merino ewes were determined. The study was carried out in 2004 and 2005 in sheep farms of the NRIAP Experimental Stations in Kołuda Wielka and Pawłowice in a group of 71 and 74 ewes with an elevated somatic cell count in milk (SCC > 500,000 per cm<sup>3</sup>). During lactation, they were sampled three times for milk and a total of 610 samples were obtained. All the samples were subjected to microbiological analyses to determine pathogenic microorganisms in milk. The plate method and API tests were used for the determinations. As the somatic cell count in milk increased, the proportion of infected samples was observed to increase, and this tendency occurred on all three dates of milk collection. The following pathogenic microorganisms were detected in the milk: *Streptococcus uberis*, *Streptococcus agalactiae*, *Enterococcus* sp., *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus* sp., *Arcanobacterium pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida* sp. In the first year of the study, the most often isolated bacteria were streptococci, and *Streptococcus uberis* was detected in the greatest number of milk samples (30%). In the second year, staphylococci dominated in the infected milk, with *Staphylococcus aureus* being the most common pathogen (in ~25% of the samples).

Pathogens identified in the present study represent the microflora typical of milk, produced by mastitic udders; therefore, they can be considered as etiological factors of the subclinical and clinical forms of mastitis in Polish Merino ewes.

**Key words:** etiological factors, mastitis, sheep

INTRODUCTION

Mastitis is one of the most frequent diseases of the udder. This complex disease entity can vary in form and intensity, but it always negatively influences the productivity of affected animals. Bacterial infections account for the highest percentage of mastitis in ruminants, and the proportion of particular etiological factors is determined by the climatic zone, region, flock size and study period, among others [Tietze *et al.* 1999].

In recent years, constant quantitative and qualitative changes have been observed in cattle among mastitis-inducing microorganisms, with disappearing differences in the course of inflammations induced by particular pathogens. The possible reasons are the increased (and differentiated) resistance to antibacterial drugs and the reduced efficiency of antibiotics used to treat staphylococcus infections [Smith 2001, Malinowski *et al.* 2003]. The constant monitoring of the reasons for and the course of mastitis may enable the introduction of new prevention and therapy methods to maintain the udder in good health.

The aim of the study was to determine the etiological factors of mastitis in Polish Merino sheep.

#### MATERIAL AND METHODS

The study was carried out in sheep farms of the NRIAP Experimental Stations in Kołuda Wielka (flock of 253 ewes) and Pawłowice (flock of 262 ewes) in two successive years. A group of Polish Merino ewes (71 milked ewes in 2004 and 74 ewes in 2005 lactation) with an elevated somatic cell count in milk ( $SCC > 500,000$  per  $cm^3$ ) were investigated. During lactation, they were sampled three times for milk (at weaning and after 30 and 60 days) from both udder halves, and the samples were analysed in a laboratory. The milk was assayed for the somatic cell count using flow cytometry (Fossomatic) and for the presence of pathogenic microorganisms using the plate method (according to Instruction no. 48 of 22 September 1978 concerning the routine identification of udder pathogens, issued by the Ministry of Agriculture, Department of Veterinary Affairs).

The species of microorganisms were identified using the API tests [Malinowski and Kłossowska 2002]. Microbiological analyses of milk whose SCC did not exceed the physiological norm were also performed.

#### RESULTS AND DISCUSSION

Percentage of ewes with SCC-elevated milk in both flocks was similar; in 2004 and 2005 it occurred in 28.06% and 28.24%. During a two-year study, a total of 610 milk samples were obtained (298 in 2004 and 312 in 2005) and subjected to laboratory analysis. The incidence and percentage of pathogen-infected samples in particular ranges of elevated SCC ( $500,000 \leq SCC \leq 1,000,000$  and  $SCC > 1,000,000$ ) are given in Table 1 for the first year of the study and in Table 2 for the second year of the study.

In 2004, the proportion of pathogen-infected samples averaged to 73.91% (milk with SCC up to  $1,000,000/cm^3$ ) and 97.45% (milk with elevated SCC). In 2005, the respective percentages were 60.87 and 87.73%. Intermediate results were obtained when analysing milk samples from Żelaźnieńska sheep; pathogenic microorganisms were found in 85.22% of the samples with SCC above 500,000 per  $1\ cm^3$  [Charon *et al.* 1996].

As the somatic cell count in milk increased, the proportion of pathogen-infected samples in both years was observed to increase, and this tendency persisted on all three dates of milk collection. These observations confirm the relationship which is known from the literature to occur in both cattle [Vishinsky and Markusfeld-Nir 1995] and sheep [Charon *et al.* 1996]. The decreasing number of milk samples analysed in the laboratory as the study went on (from 110 to 86 in 2004, and from 109 to 94 in 2005) resulted from the growing problems in sampling sufficient amounts of milk from sheep with developing mastitis.

Table 1. Incidence of pathogenic microorganisms in milk samples with elevated somatic cell count (lactation 2004)

Tabela 1. Występowanie mikroorganizmów patogennych w próbkach mleka z podwyższoną liczbą komórek somatycznych (laktacja 2004)

Milk samples Próbki mleka	Period of lactation Okres laktacji	Milk samples with SCC in thous./cm <sup>3</sup> milk Próbki mleka z liczbą komórek somatycznych, tys./cm <sup>3</sup> mleka				Total Ogółem	
		500–1.000		> 1.000			
		n	%	n	%	n	%
A	weaning	10	9.09	100	90.91	110	100.00
B	odstawienie	7	70.00	97	97.00	104	94.55
A	after 30 days	8	7.84	94	92.16	102	100.00
B	po 30 dniach	6	75.00	93	98.94	99	97.05
A	after 60 days	5	5.81	81	94.19	86	100.00
B	po 60 dniach	4	80.00	77	95.06	81	94.19
A	total	23	7.72	275	92.28	298	100.00
B	ogółem	17	73.91	268	97.45	285	95.64

A – milk samples with elevated somatic cell count in 1 cm<sup>3</sup>, B – milk samples infected with mastitis pathogens, n – number of milk samples

A – próbki mleka z podwyższoną liczbą komórek somatycznych w 1 cm<sup>3</sup>, B – próbki mleka zakażone patogenami *mastitis*, n – liczba próbek mleka

Table 2. Incidence of pathogenic microorganisms in milk samples with elevated somatic cell count (lactation 2005)

Tabela 2. Występowanie mikroorganizmów patogennych w próbkach mleka z podwyższoną liczbą komórek somatycznych (laktacja 2005)

Milk samples Próbki mleka	Period of lactation Okres laktacji	Milk samples with SCC in thous./cm <sup>3</sup> milk Próbki mleka z liczbą komórek somatycznych, tys./cm <sup>3</sup> mleka				Total Ogółem	
		500–1.000		> 1.000			
		n	%	n	%	n	%
A	weaning	33	30.28	76	69.72	109	100
B	odstawienie	19	57.58	64	84.21	83	76.15
A	after 30 days	29	26.61	80	73.39	109	100
B	po 30 dniach	18	62.07	69	86.25	87	79.82
A	after 60 days	30	31.91	64	68.09	94	100
B	po 60 dniach	19	63.33	60	93.75	79	84.04
A	total	92	29.49	220	70.51	312	100
B	ogółem	56	60.87	193	87.73	249	79.81

Symbols like in Tab. 1 – Symbole jak w tab. 1

In the milk tested, the following microorganisms were found in both years: streptococci (*Streptococcus* sp., *Str. uberis*, *Str. agalactiae*, *Enterococcus* – 35.4% samples in 2004 and 33.2% samples in 2005), staphylococci (*Staphylococcus* sp., *Micrococcus* sp.: *Staph. aureus*, *Staph. simulans*, *Staph. warneri*, *Staph. epidermidis*, *Staph. haemolyticus*, *Staph. intermedius* – 31.7 and 36.7% samples, respectively), *Arcanobacterium pyogenes*

(2.3 and 7.38% samples, respectively), colibacilli (*Escherichia coli* – 28.3 and 15.1% samples, respectively), blue pus bacteria (*Pseudomonas aeruginosa* – 4.5 and 6.71% samples, respectively), *Bacillus* sp. (18.7 and 38.9% samples, respectively), cocci (*Aerococcus*, *Lactococcus* – 68 and 73% samples, respectively) and yeast-like fungi (*Candida* sp. – 9.9 and 12.42% samples, respectively).

The microorganisms present in this microflora are generally considered mastitis pathogens [Charon *et al.* 1996, Malinowski *et al.* 2003, Malinowski and Kłossowska 2002, Tietze *et al.* 1999, Vishinsky and Markusfeld-Nir 1995]. Infections were detected in 285 samples in the first year of the study (over 96% samples with elevated SCC) and in 249 samples in the second year of the study (almost 80% of samples with elevated SCC).

The pathogens determined and the number of pathogen-infected samples of milk are given by year in Figure 1. In 2004, streptococci were isolated most frequently and *Streptococcus uberis* was detected in the greatest number (107) of samples. The most common staphylococci were *Staphylococcus aureus* (21% of the infected milk samples) and

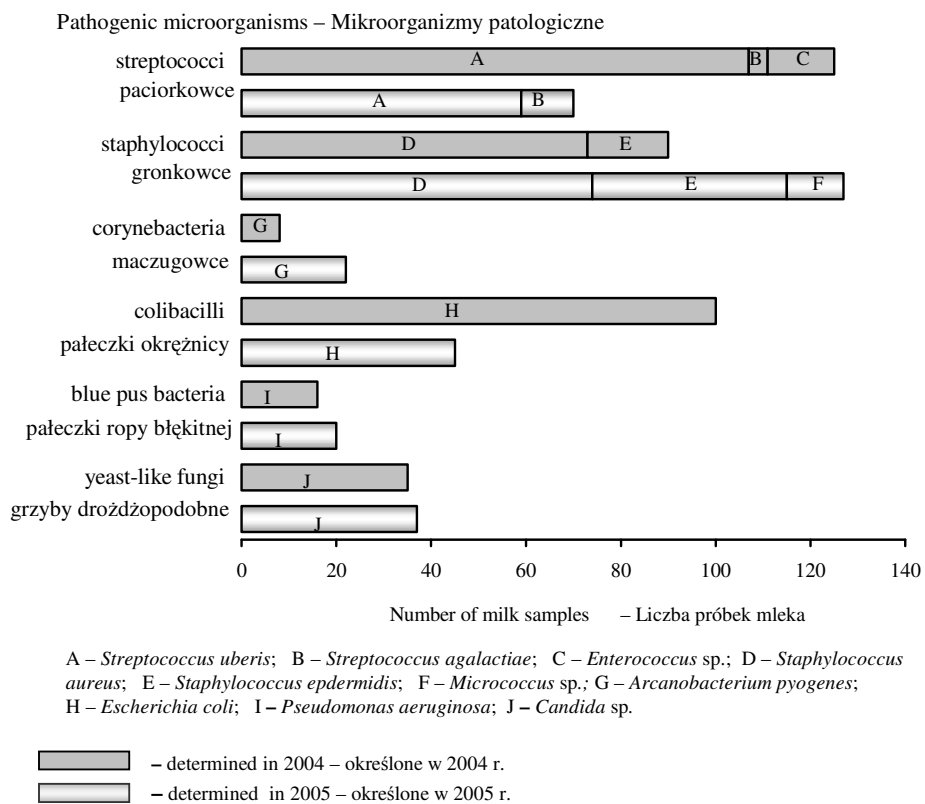


Fig. 1. Etiological factors of mastitis determined in successive years of study in milk samples with elevated somatic cell count

Rys. 1. Czynniki etiologiczne *mastitis*, oznaczone w kolejnych latach badań w próbkach mleka o podwyższonej liczbie komórek somatycznych

*Staphylococcus epidermidis*. During the next lactation, staphylococci were dominant, with the proportion of *Staphylococcus aureus* rising to ~25% of the samples. *Streptococcus uberis* occurred in almost 20% of the samples. Colibacilli were isolated almost twice more often in the first year of the study. During the second lactation, there was an increase in the proportion of milk samples infected with *Arcanobacterium pyogenes* and, to a lesser degree, with *Pseudomonas aeruginosa* and *Candida* yeast-like fungi. The results of microbiological determinations indicate that the principal etiological factors of mastitis in the analysed populations of Polish Merino ewes were streptococci and pathogenic staphylococci. Their representatives (*Streptococcus uberis* and *Staphylococcus aureus*) dominated alternately in successive lactations among pathogenic milk microflora, which is characterized by elevated somatic cell count. A similar incidence of these two species was also observed by Charon *et al.* [1996] in the infected milk of Żelaźnieńska sheep. It is worth noting the high percentage of samples infected with *E. coli* in the first year of the study. *Corynebacteria*, blue pus bacteria and yeast-like fungi contributed to udder mastitis to a lesser degree.

The growing importance of *Staphylococcus aureus* as an etiological factor of mastitis has recently been observed in cattle [Malinowski *et al.* 2001, 2003]. The incidence of mastitis induced by *Staphylococcus aureus* on the farms of Kujawy, Pomerania and Wielkopolska almost tripled. *Staphylococcus aureus* is classified as an infectious pathogen characteristic of herds with a high somatic cell count in milk (Deinhofer, Smith). *Streptococcus uberis* (found in 30 and 20% milk samples in the present study) and *E. coli* are classified as environmental pathogens typical of populations with lower somatic cell counts in milk [Malinowski *et al.* 2001, Smith 2001].

Researchers studying the etiology of the bacterial forms of mastitis also noticed the low proportion of *Streptococcus agalactiae* and the growing role of environmental streptococci (mainly *Streptococcus uberis*) and *Staphylococcus aureus* in mastitis incidence in cattle [Deinhofer 1993, Malinowski *et al.* 2001, 2003, Smith 2001]. The results obtained in the present study indicate that similar tendencies exist in sheep populations.

#### CONCLUSIONS

The dominant etiological factors of udder mastitis in Polish Merino ewes were streptococci (34.3%), staphylococci (34.2%) and *E. coli* (21.7%).

The most common pathogens determined were *Streptococcus uberis* and *Staphylococcus aureus*.

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**Streszczenie.** Celem badań było określenie czynników etiologicznych stanów zapalnych gruczołu mlekowego u owiec rasy merynos polski. Prace prowadzono w latach 2004 i 2005 w owczarniach Zakładów Doświadczalnych IZ w Kołudzie Wielkiej i w Pawłowicach, badając, odpowiednio, grupę 71 i 74 maciorek o podwyższonej liczbie komórek somatycznych w mleku (l.k.s. > 500 tys. w 1 cm<sup>3</sup>). Trzykrotnie w okresach laktacji pobierano od nich mleko, uzyskując łącznie 610 próbek. Wszystkie próbki poddano analizom mikrobiologicznym w celu oznaczenia w mleku drobnoustrojów chorobotwórczych. Do oznaczeń zastosowano metodę płytkową oraz testy API. Wraz ze wzrostem l.k.s. w mleku obserwowano wzrost udziału zakażonych próbek, a tendencja ta była zachowana we wszystkich trzech terminach pobierania mleka. W mleku wykryto obecność następujących mikroorganizmów chorobotwórczych: *Streptococcus uberis*, *Streptococcus agalactiae*, *Enterococcus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus* sp., *Arcanobacterium pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida* sp. W pierwszym roku badań najczęściej izolowanymi bakteriami były paciorkowce, a gatunek *Streptococcus uberis* wykryto w największej liczbie próbek mleka (30%). W kolejnym roku w zakażonym mleku dominowały gronkowce, a *Staphylococcus aureus* był najliczniej reprezentowanym patogenem (w ~25% próbek).

Oznaczone w pracy patogeny należą do mikroflory typowej dla mleka, produkowanego przez wymiona ze stanami zapalnymi; mogą więc zostać uznane za czynniki etiologiczne subklinicznych i klinicznych postaci *mastitis* u maciorek rasy merynos polski.

**Słowa kluczowe:** czynniki etiologiczne, *mastitis*, owce