



THE IMPACT OF MASTITIS ON REPRODUCTIVE PARAMETERS OF DAIRY COWS

Zigo, F.¹, Adamová, A.¹, Vasil', M.¹, Elečko, J.¹
Ondrašovičová, S.², Zigová, M.³, Kudelková, L.⁴

¹Department of Animal Husbandry

²Department of Anatomy, Histology and Physiology

University of Veterinary Medicine and Pharmacy, Komenského 73, 041 81 Košice

³Department of Pharmacology, Faculty of Medicine, Pavol Jozef Šafárik University, 040 11 Košice
Slovakia

⁴Department of Animal Husbandry and Animal Hygiene

University of Veterinary and Pharmaceutical Sciences Brno, Palackého tř. 1946/1, 612 42 Brno
Czechia

frantisek.zigo@uvlf.sk

ABSTRACT

The aim of this study was to evaluate the occurrence of mastitis and its impact on the reproductive parameters in a herd of 180 dairy cows. Based on the herd records 127 cows of Slovak spotted cattle and their crosses with red Holstein were selected for study between 1–2 months after calving. The examination of the health status of the mammary glands consisted of: the clinical examination of the udder, the California mastitis test (CMT) supplemented by the collection of mixed milk samples, and the laboratory examination of bacterial pathogens causing the mastitis. In addition to the mammary investigation, reproduction indicators such as the length of the insemination interval, the service period, the intercalving period and the insemination index were also analyzed. The results of this study indicated: a high

incidence of mastitis (41.6 %), especially latent (21.2 %), subclinical (15.7 %) and clinical (4.7 %) forms were most common in the herd. The most frequently isolated bacteria from the infected milk samples were: coagulase negative staphylococci (54.1 %), *S. aureus* (16.9 %), *Streptococcus* spp. (15.0 %), *A. viridans* (7.5 %) and *Ent. faecalis* (6.4 %). According to the available literature, the optimum values of the intercalving period were 365–400 days, the insemination interval 55–80 days, the insemination index 1.2–2 and the service period 60–110 days. In comparison, our results showed increased, unsatisfactory reproductive values in the group of dairy cows with clinical mastitis. While in healthy cows as well as in groups of cows with latent and subclinical mastitis, all of the reproductive indicators were within the optimal levels.

Key words: dairy cows; intercalving period; mastitis; pathogens; service period

INTRODUCTION

Cattle rearing is a very important part of livestock production. Its role in agriculture involves mainly the production of milk and meat which are essential components of human nutrition. From the point of view of the economy, cattle breeds with high milk yield [20] are of greatest interest in the Slovak Republic, but due to their high milk production, the reproductive indicators essential for repeated pregnancy are often worsened [3].

Securing the reproductive cycle requires a lot of attention and skill from the breeder. It is important that the farmer be able to control the reproductive cycle of cows (Fig. 1), manage the detection of oestrus, maintain an identification system with records of cows mated and calved. It is also necessary to use high quality semen and the correct time of insemination and pay attention to the formation of groups of dairy cows according to the appropriate age category. In addition, close observation of animal hygiene requirements on the farm is required in order to ensure the good health of these productive animals [17].

The economic value of dairy cows is determined mainly by their milk yield and longevity, because milk is the main source of income on Slovak dairy farms [20]. The economic losses are mainly due to the prolongation of the service period and intercalving period the nonstandard length of which reduces milk yield during the subsequent lactation. Losses that arise due to poor fertility are affected by more

factors such as the milk production, the purchase price of milk, the price of calves, the course of the lactation curve, individual characteristics of the cows and more [15].

In addition to reproduction management, one of the important roles of dairy farmers is to control the incidence of diseases, especially mastitis. Often due to the lack of symptoms, the identification of subclinical mastitis is a frustrating problem for dairy farmers and veterinarians to overcome in order to ensure not only the animal health but also the hygienic quality of the produced milk [6, 19].

Based on the intensity and severity of the clinical signs, mastitis is usually divided into subclinical and clinical disease. In clinical mastitis (CM), the signs range from mild to severe and can be systemic, local, or milk related, whereas in subclinical mastitis (SM) no signs are observed. The most prominent signs of CM are swelling, heat, hardness, redness or pain of the udder. The milk of a cow with CM has a watery appearance, and flakes, clots or pus is often present. During SM the udder and milk appear normal, but the infection is still present [16]. Subclinical mastitis is more common than CM. It is estimated that in the herd of dairy cows there are approximately 15-40 undetected cases of SM for each case of CM. The increase in somatic cell counts (SCC) are associated with reduced milk production to the tune of 60 to 140 litres per cow per year in animals with SM [1].

Postpartum diseases, especially mastitis, can have a major impact on the reproductive performance of dairy cows. Due to the signs, it is insufficient or difficult to detect the oestrus cycle in mastitis cows resulting in a prolongation of the insemination interval and service period [9]. Therefore, the aim of this work was to evaluate the prevalence of

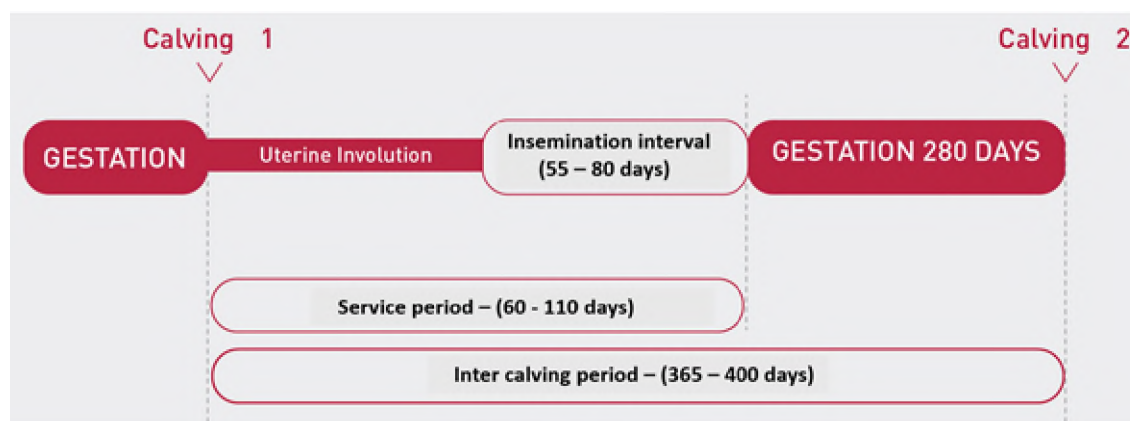


Fig. 1. Reproductive cycle of dairy cows [4]

mastitis and its impact on the reproductive parameters in dairy cows.

MATERIALS AND METHODS

Dairy cows and milking

This study was conducted on a herd of 180 cows of Slovak pied cattle breed and their crosses with Red Holstein with the average milk yield of 7,228 kg per lactation. The farm was located in the Trenčín region (west Slovakia) with two stables for dairy cows and heifers. The cows were milked twice a day in a herringbone milking parlor DeLaval 2x10 (Tumba, Sweden) (Fig. 2), with the first milking starting at 3:30 a.m. and the second at 3:30 p.m. First, the wet udder hygiene was performed with water to remove dirt from the udder and teats. Subsequently, the udder was thoroughly wiped with disposable wipes soaked in a disinfectant solution (Valiant - ABS CZ s. r. o.).

The first milk from each quarter was hand milked into a dark-bottomed pot, and the milk was subjected to sensory analysis. The milking and pulsation vacuum was set at 42 kPa. The pulsation ratio was 60 : 40 at a rate of 52 cycles per min and termination was automatically signalled when the milk flow dropped to 0.2 l.min⁻¹. After the milking process, the teats were disinfected by teat-dipping with IODERM 5000 (Hyperred Czech s. r. o.). The milk was stored in refrigerated milk tanks at +5 °C and removed daily around 11:30 a.m.

Animal selection

A total of 127 cows of the 180 dairy cows were selected for study based on the data provided from the milk recording control for the purpose of their investigation at intervals of 1–2 months after calving. The herd monitoring was carried out during one lactation period of each cow at the turn of 2017/2018. During this period, a complex examination of each cow was performed for mastitis with the sta-



Fig. 2. Herringbone milking parlour with CMT evaluation and milking



Fig. 3. Bacterial pathogens isolated from clinical mastitis cases
From the left: *S. aureus*, *S. warneri* and *Str. dysgalactiae*

tistical analysis of the reproductive indicators, such as the insemination interval, the insemination index, the service period and the intercalving period.

Udder examination and laboratory analyses

A thorough evaluation of the udder health in the 127 lactating cows included a veterinary history, clinical examination, sensory analysis of milk from forestripping of each udder quarter followed by a CMT (Indirect Diagnostic Test, Krause, Denmark) (Fig. 2). The milk from every quarter was mixed with the reagent and the result was read as trace, score 1–4 or negative depending on the gel formation in the milk sample according to Jackson and Cockcroft [7].

Afterward (10 ml) each of the mixed milk samples from each cow was collected aseptically for bacteriological cultivation in accordance with the guidelines of the National Mastitis Council [13]. The cooled samples were immediately transported to the laboratory of the University of Veterinary Medicine and Pharmacy in Kosice.

The bacteriological examinations and identifications were performed according to generally accepted principles [12]. The milk samples (10 pl) were inoculated on a Columbia Blood Agar Base (Oxoid, UK) with 5 % of defibrinated ram blood in a Petri dish and incubated for 48 h at 37 °C (Fig. 3). The dishes were examined after 24 and 48 h of incubation. The suspected colonies were inoculated and cultured on selective media and growth-confirmed colonies of *Staphylococcus* spp., *Streptococcus* spp. and *Enterobacteriaceae* spp. were further identified biochemically using the STAPHYtest 24, STREPTOtest 24, respectfully. ENTEROtest 24 (Erba-Lachema, CZ) and the software TNW Pro 7.0 (Erba-Lachema, CZ).

Mastitis forms

Based on the diagnostic methods of mastitis, the individual forms were classified as follows: Latent mastitis is characteristic only with the presence of bacterial pathogens in samples of milk without changing its consistency and somatic cell count (SCC). Subclinical mastitis is characterized by a positive CMT score, bacteriological cultivation, increased SCC, and a reduced milk yield without clinical signs. Clinical mastitis is characterized by a positive CMT score, bacteriological cultivation, higher level of SCC, a change in the consistency of the milk, and a reduced or loss of milk production with clinical signs.

Statistical analysis

The average values of the reproductive parameters of dairy cows were analysed by the one-way ANOVA. A Dunnett's Multiple Comparison was used to compare mastitis groups of dairy cows with a healthy (control) group. The level of significance was set to $P < 0.05$.

RESULTS AND DISCUSSION

Worldwide, mastitis is known as a multifactorial disease, and it is closely related to the production system and the environment. The incidence of mastitis increases when the immunological and antioxidant defense mechanisms of the mammary gland are impaired. Dairy cows are exposed to numerous genetic, physiological, and environmental factors associated with both the host and pathogens that can compromise host immunity and increase the incidence of mastitis [2, 21].

Table 1 shows the prevalence of mastitis and bacterial agents of intramammary infections (IMI) in dairy cows. The results demonstrated a high incidence of mastitis (41.6 %), especially latent (21.2 %), subclinical (15.7 %) and clinical (4.7 %) forms were most common in the study herd.

According to Ozenc et al. [14] more than 140 different microorganisms are considered to cause mastitis. Bacteria are the most common causative factor, recognized in more than 95 % of mastitis cases. Globally, the most common mastitis-causing bacteria in dairy cows and small ruminants are *S. aureus* and coagulase-negative staphylococci (CoNS), as well as streptococci and *E. coli*, which may have a similar or higher prevalence than that of staphylococci.

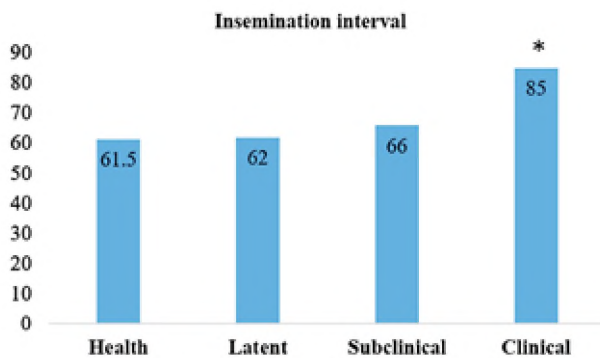
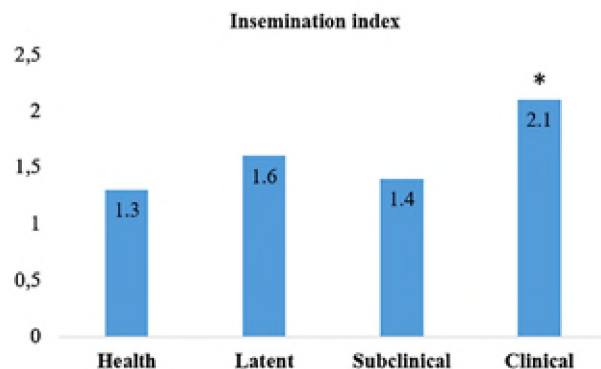
From our results, the most common bacteria pathogens were CoNS (*S. chromogenes*, *S. warneri* and *S. xylo-sus*), which were found mainly in the latent and subclinical forms. The bacteria *S. aureus*, *S. chromogenes*, *Str. dysgalactiae* and *E. coli* were the most frequently isolated from the clinical forms of mastitis (Table. 1).

A similar incidence of IMI was reported by Kivria and Nordhuzen [8] who isolated *Staphylococcus* spp. followed by *Streptococcus* spp., *E. coli* and *Klebsiella* spp. The CoNS have been increasingly isolated from dairy cows and are reported to be the leading cause of environmental mastitis. In addition to latent and subclinical forms, CoNS are often isolated from clinical and persistent mastitis [10, 18, 21].

Table 1. Bacterial agents of mastitis

Isolated bacteria	n	Latent		Subclinical		Clinical	
		n	%	n	%	n	%
<i>Staphylococcus spp.</i>							
<i>Staphylococcus aureus</i>	9	3	2.3	4	3.1	2	1.6
<i>Staphylococcus chromogenes</i>	14	11	8.6	2	1.6	1	0.8
<i>Staphylococcus warneri</i>	4			3	2.3	1	0.8
<i>Staphylococcus xylosus</i>	2	1	0.8	1	0.8	-	-
<i>Streptococcus spp.</i>							
<i>Streptococcus bovis</i>	4	2	1.6	2	1.6	-	-
<i>Streptococcus dysgalactiae</i>	4	3	2.3	-	-	1	0.8
<i>Other bacteria</i>							
<i>Aerococcus viridans</i>	7	4	3.1	3	2.3	-	-
<i>Escherichia coli</i>	4	1	0.8	2	1.6	1	0.8
<i>Enterococcus faecalis</i>	5	2	1.6	3	2.3	-	-
Total infected samples	53	27	21.2	20	15.7	6	4.7

n—number of examined mixed milk samples

Fig. 4. Insemination interval of selected dairy cows
* P < 0.05—significant differences between selected groupsFig. 5. Insemination index of selected dairy cows (in days)
* P < 0.05—significant differences between selected groups

In the studies by B l o w e y and E d m o n d s o n [2] and L a n g e et al. [10] more than 20 CoNS were isolated from mastitis milk samples; most of them were *S. chromogenes*, *S. haemolyticus*, *S. epidermidis*, *S. simulans* and *S. xylosus*.

Good fertility of dairy cows depends on the following indicators as: length of insemination interval, calving after the first insemination, insemination index, the length of service period and the intercalving period [9].

According to F a h e y et al. [5] a satisfactory insemi-

nation interval is considered to be between 60–80 days and over 80 days is considered unsatisfactory. In our study, the insemination interval was 61.5 to 85 days, with the longest interval in dairy cows with clinical mastitis and the shortest in healthy cows (Fig. 4). The insemination index indicates how many inseminations were necessary to induce pregnancy. The average insemination index ranges from 1.2 to 2.0. If it is higher than 2.0, it indicates fertility disorders, as confirmed in our study in cows with clinical mastitis (Fig. 5).

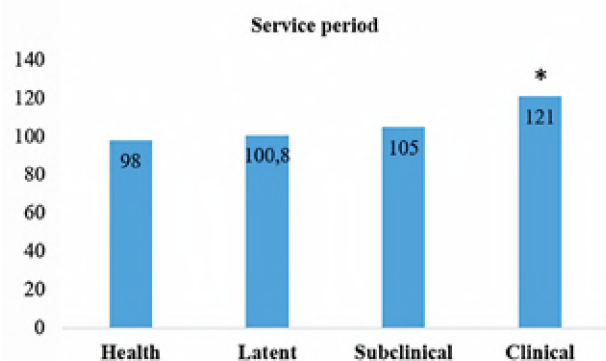


Fig. 6. Length of service period of selected dairy cows (in days)
* $P < 0.05$ – significant differences between selected groups

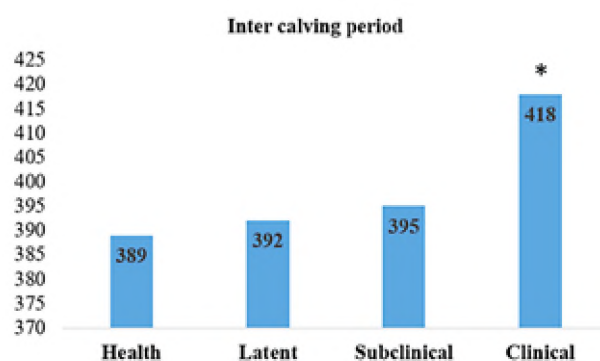


Fig. 7. Length of intercalving period of selected dairy cows (in days)
* $P < 0.05$ – significant differences between selected groups

The service period is economically one of the most important indicators. It is the period between the date of calving and the date of successful conception. The ideal service period is 85 days but may be longer in high-performance animals. The causes of service period prolongation can be found in the inadequate monitoring of oestrus, especially for non-fertilized cows, but also for physiological and health reasons. For high-yield dairy cows, a service period from 110 to 125 days can be tolerated, but only when the interval calving does not exceed 400 days [11]. The results of our study revealed that in dairy cows with clinical mastitis, the length of service period was at the cut-off value (121 days) but with a significantly increased intercalving period (418 days) which is considered unsatisfactory in both cases (Fig. 6). Conversely, the best milk yield (7,392 litres) was found in healthy dairy cows with a service period of up to 98 days. In dairy cows with clinical mastitis in addition to increased service period, we observed excessively long intercalving period which resulted in reduced milk yield (6,421 litres).

The intercalving period is the period between two calving in cows and in general, the length of this intercalving period should be between 365 and 405 days. According to a study by Ř í h a and H a n u š [15], when the intercalving period in dairy cows with milk yield above 7,000 kg of milk increases from 365 days to 405 days, 20 % of the milk production may be lost while in dairy cows with lower milk yields, the loss amounts only to about 5 %.

In our studied groups of dairy cows, the length of the intercalving period ranged from 389 to 418 days. In healthy dairy cows, the average intercalving period was 389 days

and the longest was observed in dairy cows with clinical mastitis (Fig. 7) in which it also resulted in longer insemination intervals. This indicates that the prolongation of the intercalving period also affects the length of the insemination intervals. In dairy cows with long intercalving periods, there may be less pronounced oestrus, problems with its detection, reproductive cycle disturbances, or the prolongation may be caused by other external factors.

CONCLUSIONS

All reproductive parameters were recorded as unsatisfactory only in the group of dairy cows with clinical mastitis. The clinical IMI significantly affected the health of dairy cows, not to mention the reduced milk production and increased reproductive parameters compared to the healthy cows. It should be pointed out that the effectiveness of generally established anti-mastitis methods to reduce environmental bacteria in combination with major mastitis pathogens are usually limited. Therefore, dairy farmers should still look for the most effective methods of cattle breeding using rational management of the reproductive cycle.

ACKNOWLEDGEMENT

This study was supported by the projects APVV No. SK-PL-18-0088 and VEGA No. 1-0529-19.

REFERENCES

1. Ashfaq, K., Muhammad, G., 2008: Pathogens Associated with Bovine and Bubaline Mastitis in Peri-Urban Areas of Faisalabad, Pakistan. *Pak. J. Life Soc. Sci.*, 6, 86–88.
2. Blowey, R., Edmondson, P., 2010: *Mastitis Control in Dairy Herds*. 2nd edn., FSC, London, 259 pp.
3. Crowe, M. A., Hostens, M., Opsomer, G., 2018: Reproductive management in dairy cows - the future. *Irish Veterinary Journal*, 71, 1. DOI: 10.1186/s13620-017-0112-y.
4. Edwards, S., Rossi, M., Henney, G., 2019: *The Anatomy and Physiology of Reproduction – Cattle*. Available on: <https://www.repro360.com.au/reproductivetechologies/physiologyofreproduction>.
5. Fahey, J., Morton, J. M., Macmillan, K. L., 2003: Associations between milk protein percentage and reproductive performance in Holstein-Friesian cows in seasonal calving dairy herds. In *Proceedings of the Association for the Advancement of Animal Breeding and Genetics*, 15, 68–71.
6. Idriss, S. H., Tančin, V., Foltýs, V., Kirchnerová, K., Tančinová, D., Vršková, M., 2013: Relationship between mastitis causative pathogens and somatic cell count in milk of dairy cows (In Slovak). *Potravinárstvo* (Food Industry), 7, 207–212. DOI: 10.5219/304.
7. Jackson, P., Cockerott, P., 2002: *Clinical Examination of Farm Animals*. Oxford, UK, Blackwell Science Ltd, Wiley-Blackwell, 54–166.
8. Kivaria, F. M., Noordhuizen, J. P., 2007: A retrospective study of the aetiology and temporal distribution of bovine clinical mastitis in smallholder dairy herds in the Dar es Salaam region of Tanzania. *Vet. J.*, 173, 617–622. DOI: 10.1016/j.tvjl.2006.01.008.
9. Kvapilík, J., Pytloun, J. 2002: The economic importance of fertility, herd replacement and the production of dairy cows (In Slovak). *Náš chov* (Our Breeding), 2, 22–26.
10. Lange, C., Brito, M. A., Reis, D., Marco, A. M., Guimaraes, A., Azevedo, A. L., et al., 2015: Species-level identification of staphylococci isolated from bovine mastitis in Brazil using partial 16S rRNA sequencing. *Vet. Microbiol.*, 176, 3–4. DOI: 10.1016/j.vetmic.2015.01.024.
11. Louda, F., Vanek, D., Ježková, A., Stádník, L., Bjelka, M., Bezdiček, J., Pozdíšek, J., 2008: *The use of Biological Principles in Management of Female Reproduction in Cattle Breeding* (In Czech). Publ. Institute for cattle breeding, Rapotín, 56 pp.
12. Malinowski, E., Lassa, H., Ktosowska, A., Smulski, S., Markiewicz, H., Kaczmarowski, M., 2006: Etiological agents of dairy cows' mastitis in western part of Poland. *Pol. J. Vet. Sci.*, 9, 191–194. DOI: 17020014.
13. National Mastitis Council, 2001: *National Mastitis Council Recommended Mastitis Control Program*. <http://www.nmconline.org/docs/NMC10steps.pdf>.
14. Ozenc, E., Seker, E., Baki Acar, D., Birdane, M. K., Darbaz I., Dogan, N., 2011: The importance of staphylococci and threshold value of somatic cell count for diagnosis of subclinical mastitis in Parlak sheep at mid-lactation. *Reprod. Dom. Anim.*, 46, 970–974. DOI: 10.1111/j.1439-0531.2011.01768.x.
15. Ríha, J., Hanuš O., 2001: Important aspects of detecting the reproduction of dairy cows (In Czech), *Výzkum v chovu skotu* (Research in Cattle Breeding), 3, 12–17.
16. Sztachajńska, M., Baraiński, W., Janowski, T., Pogorzelska, J., Zduńczyk, S., 2016: Prevalence and etiological agents of subclinical mastitis at the end of lactation in nine dairy herds in North-East Poland. *Polish Journal of Veterinary Sciences*, 19, 119–124. DOI: 10.1515/pjvs-2016-0015.
17. Tamminga, S., Luteijn, P. A., Meijer, R. G. M., 1997: Changes in composition and energy content of liveweight loss in dairy cows with time after parturition. *Livest. Prod. Sci.*, 52, 31–8. DOI: 10.1016/S0301-6226(97)00115-2.
18. Thorberg, B. M., Kuhn, I., Aarestrup, F. M., Brändström, B., Jonsson, P., Danielsson-Tham, M. L., 2006: Pheno- and genotyping of *Staphylococcus epidermidis* isolated from bovine milk and human skin. *Vet. Microbiol.*, 115, 163–172. DOI: 10.1016/j.vetmic.2006.01.013.
19. Zadoks, R. N., Middleton, J. R., Mc Dougall, S., Katholm, J., Schukken, Y. H., 2011: Molecular epidemiology of mastitis pathogens of dairy cattle and comparative relevance to humans. *Journal of Mammary Gland Biology and Neoplasia*, 16, 4, 357–72. DOI: 10.1007/s10911-011-9236-y.
20. Zajac, P., Tomaska, M., Murarova, A., Capla, J., Curlej, J., 2012: Quality and safety of raw cow's milk in Slovakia in 2011 (In Slovak). *Potravinárstvo* (Food Industry), 6, 64–73. DOI: 10.5219/189.
21. Zigo, F., Elečko, J., Vasiľ, M., Farkašova, Z., Zígová, M., 2017: Occurrence of mastitis in dairy cows situated in marginal parts of Slovakia. *Folia Veterinaria*, 61, 4, 59–64. DOI: 10.1515/fv-2017-0040.

Received June 29, 2019

Accepted September 2, 2019