

## Assessment of udder health in lame cows

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### Abstract

The objective of this study was to assess the udder health in lame dairy cows. The study was performed on 35 dairy cows which were admitted to the Clinic of Ruminants. The most frequent claw diseases were white line abscess (28.6%) and toe necrosis (28.6%), followed by digital dermatitis (17.1%), toe ulcers (14.3%), and sole ulcers (11.4%). The prevalence of mastitis in lame cows was 74.3% (26); clinical mastitis was detected in 5 (19.2%) and subclinical mastitis in 21 (80.8%) dairy cows. Distribution of mastitis was similar in terms of front (52.54%) and rear quarters (47.46%). For statistical analyses of mastitis grade in lame dairy cows, a six-point Mastitis Score was created based on physical examination of the mammary gland and results of California Mastitis Test. The Mastitis Score tended to be higher in cows with corium inflammation than in those with digital dermatitis (10.0 and 6.5, respectively). Microbiological culture of 24 positive milk samples was performed and intra-mammary pathogens were isolated in 18 (75%) samples. The most prevalent bacteria were coagulase negative staphylococci (50%), followed by *Enterococcus* spp. (16.7%), *Proteus* spp. (11.1%), and *Aerococcus viridians* (11.1%). Of the total bacteria, contagious pathogens were determined in two milk samples (11.1%). In conclusion, high prevalence of mastitis was detected in lame cows suffering from claw diseases without a significant effect of the type of claw diseases on the mastitis grade. Moreover, the dominating isolation of environmental pathogens may be a result of longer lying period of animals affected with lameness.

*Mastitis, lameness, claw disease, dairy cattle*

Bovine mastitis is a large-scale infectious disease with significant impact on the economy of milk production and causes significant direct and indirect economic losses in dairy industry world-wide (Halasa et al. 2007; Awale et al. 2012). Mastitis-related financial losses are associated with reduction in milk yield, increased treatment costs, higher discarded milk, increase in culling and replacement rates, impaired reproductive performance and financial penalties for exceeding legal milk quality limits (Hortet and Seegers 1998; Kocak and Ekiz 2006; Gunay and Gunay 2008). The incidence of mastitis in dairy herds results from a complex interaction between the infectious agents, poor management practices, genetic and environmental factors, stressing the defence of the udder (Nakov et al. 2014). It is well known that many factors may change the susceptibility to mastitis, negatively influencing the cow's local and systemic barriers and defence, or increasing the exposure of the udder to micro-organisms (Burvenich et al. 2003; Nyman et al. 2007). The most common potential risk factors for mastitis on dairy farms are classified as quarter, cow and environmental risk factors (Nayan et al. 2014). A great number of quarter risk factors for mastitis have been described, including previous bacterial infection, immunity of the udder (Sordillo et al. 1997), injury to the udder and teat, pendulous udder, defect of the teat sphincter (trauma previous to infection, quarters with hyperkeratosis, udder quarters position, udder shape, teat shape, size, and position udder (Breen et al. 2009, Zadoks et al. 2001). Cow-level risk factors reported for mastitis include the age (Steenefeld et al. 2008), breed (Elbers et al. 1998), genetic factors, parity, stage of lactation, lactation number (Zadoks et al. 2001), milk yield, leaking milk (O'Reilly et al. 2006), checking

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foremilk before attaching the clusters, immune status, physical conditions, hygiene of cows/cow cleanliness (Ward et al. 2002), periparturient diseases and lameness (Peeler et al. 1994; Sato et al. 2008). In the current literature, many environmental risk factors for mastitis are identified by researchers. The environmental risk factors include nutrition (Perkins et al. 2001), feeding, housing system, type of bedding (Peeler et al. 2000), herd size, pasture condition, season, climate, poor management practices, sanitary conditions and milking methods/milking hygiene (Nyman et al. 2007; Valde et al. 2007). It has been hypothesized that lameness with a longer lying time increases the risk of bacterial contamination of the teat canals and that cows that lie down immediately after milking would be at most risk of acquiring intra-mammary pathogens. Therefore, lameness may be viewed as one of the most risk factors responsible for distribution of mastitis in herds with increased prevalence of lameness. The relationship between lameness was studied by several authors, who confirmed the effect of poor hoof health and decreased milk yield, affected milk composition and prevalence of mastitis (Arvidson 2000; Olechnowicz and Jankowski 2012; Singh et al. 2018)

The objective of this study was to assess the udder health of lame dairy cows admitted to the Clinic of Ruminants of the University of Veterinary Medicine and Pharmacy in Kosice.

## Materials and Methods

### Animals

The study was conducted on all Holstein-Friesian dairy cows ( $n = 35$ ) with locomotor disorders, hospitalized at the Clinic of Ruminants of the University of Veterinary Medicine and Pharmacy in Kosice, Slovak Republic, between 2011 and 2014. The cows were referred from four dairy farms in Eastern Slovakia.

### Orthopaedic examination

Each animal was examined in the trimming crush for causes of the lameness. Based on the clinical findings, a diagnosis of the limb health disorder was made using the updated orthopaedic terminology for cattle (Egger-Danner et al. 2015). Subsequently, the lesions were treated and all the data were recorded for statistical analysis.

### Examination of mammary gland

Clinical examination of the udder was performed by visual inspection and palpation, using standard physical methods of examination. The initial milk stripped from each teat was discarded and individual milk samples from lactating cows were physically examined (Rosenberger et al. 1979). Clinical mastitis was diagnosed by the presence of observable signs of inflammation in the affected quarter such as swelling, heat, pain or redness and by presence of macroscopically altered milk in one or more quarters.

To detect subclinical mastitis, California mastitis test (CMT) was performed on each milk sample free of visible abnormalities. The CMT results were interpreted according to the CMT scoring system: negative, trace, 1, 2, and 3 (Jackson and Cockcroft 2002).

### Bacteriological examinations

Microbiological culture was performed with 24 milk samples which were positive either by clinical or by CMT examination. Briefly, milk samples (10  $\mu$ l) were incubated at 37 °C using Columbia blood agar base with 5% of defibrinated blood, Staphylococcal medium N° 110, Baird-Parker agar, Edwards Medium, MacConkey Agar (Oxoid, (OXOID Ltd., Basingstoke, Hants, UK) for 18–24 h. The following assays were used to determine bacterial species: pigment and coagulase production, catalase activity, haemolysis, Gram staining, and other virulence factors. Commercial kits STAPHYtest 24; STREPTOtest 24, resp. ENTEROtest 24 (Erba Lachema, Brno, ČR) were used to identify the bacterial strains. All kits were evaluated by the programme TNW ProAuto 7.0® (Erba Lachema,

Brno, Czech Republic) according to the manufacturer's instructions. Coagulase-negative staphylococci (CNS) were identified by typical colony morphology and negative coagulase reaction.

Table 1. Mastitis Score (MS).

Score	Description	California Mastitis Test
1	Healthy milk	Negative
2	Doubtful mastitis of one quarter	Trace: 200,000 – 400,000
3	Weak Subclinical mastitis	1: 400,000 – 1,200,000
4	Distinct Subclinical mastitis	2: 1,200,000 – 5,000,000
5	Strong Subclinical mastitis	3: over 5,000,000
6	Clinical mastitis	

### Statistical analysis

For statistical analyses of mastitis grade in lame dairy cows a six-point Mastitis Score (MS) was created based on physical examination of the mammary gland and CMT (Table 1).

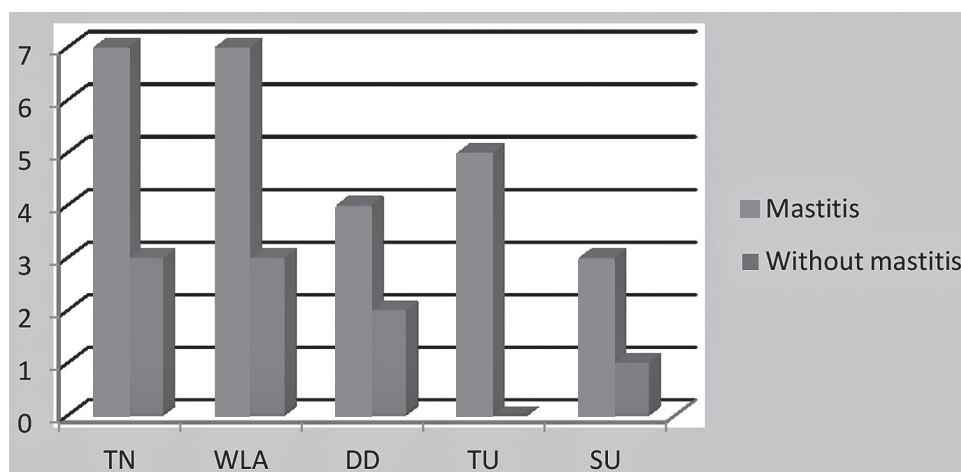
The final value of MS is a sum of points given to each udder quarter. Thus, the minimal MS is 4 and maximal 24 points. The animals were divided according the orthopaedic diagnosis into two groups: corium inflammation (toe ulcer, sole ulcer, toe necrosis, white line abscess) and digital dermatitis. The significance of differences in MS between the groups was tested by Student's *t*-test. The level of significance was set at  $P < 0.05$ .

## Results

Lameness in studied dairy cows was caused by toe necrosis (10 cows, 28.6%), white line abscess (10 cows, 28.6%), digital dermatitis (6 cows, 17.1%), toe ulcer (5 cows, 14.3%), and sole ulcer (4 cows, 11.4%; Table 2; Fig. 1). The corium inflammation and digital dermatitis groups consisted of 29 (83%) and six animals (17%), respectively. Claw diseases affected rear limbs in 31 cows (86%) and only in four cows (14%) the front limbs were affected. Mastitis was found in 26 dairy cows (74.3%); the subclinical and clinical form of mammary gland inflammation was found in 21 and 5 animals, respectively (Table 2). Distribution of mastitis was similar between front (52.5%) and rear quarters (47.5%). There were no differences ( $P = 0.17$ ) between the mastitis rate in cows with corium inflammation and digital dermatitis (69% and 67%, respectively). The statistical analysis of MS did not reveal any significant difference between cows with different types of claw inflammation (Table 3).

Table 2. Prevalence of mastitis in lame dairy cows hospitalized at the Clinic of Ruminants of the University of Veterinary Medicine and Pharmacy.

	Number of dairy cows	Mastitis	Clinical mastitis	Subclinical mastitis
Toe necrosis	10	7 (70%)	2	5
White line abscess	10	7 (70%)	3	4
Digital dermatitis	6	4 (66.7%)	0	4
Toe ulcer	5	5 (100%)	0	5
Sole ulcer	4	3 (75%)	0	3
Total	35	26 (74.3%)	5	21



TN - toe necrosis, WLA - white line abscess, DD - digital dermatitis, TU - toe ulcer, SU - sole ulcer

Fig. 1. Distribution of mastitis in lame cows affected by certain claw diseases.

Table 3. Mastitis score in lame dairy cows.

	Number of dairy cows	Mastitis score (x ± SD)
Corium inflammation	29	10.0 ± 5.83
Digital dermatitis	6	6.50 ± 2.59

x – mean; SD – standard deviation

18 (75%) samples. There was no growth in 6 milk samples (25%). The most prevalent bacteria were coagulase-negative staphylococci (50%), followed by *Enterococcus* spp. (16.7%), *Proteus* spp. (11.1%), and *Aerococcus viridians* (11.1%). Of the total bacteria contagious pathogens were determined in two milk samples (11.1%, Table 4).

Table 4. Bacterial isolation from the milk samples (n = 24) of cows with claw diseases.

Positive		18 (75%)
Contagious pathogens		2 (11.1%)
	<i>Streptococcus agalactiae</i>	2 (11.1%)
Environmental pathogens		16 (88.9%)
	<i>Enterococcus</i> spp.	3 (16.7%) 1/3 WLA, 1/3 TU, 1/3 DD
	<i>Aerococcus viridans</i>	2 (11.1%) 2/2 SU
	<i>Proteus</i> spp.	2 (11.1%) 1/2 DD
	CNS	9 (50%)
	<i>Staphylococcus chromogenes</i>	2 1/TN, 1/DD
	<i>Staphylococcus warneri</i>	5 4/TN, 1/SU
	<i>Staphylococcus equorum</i>	2 2/WLA
Negative		6 (25%)

DD – digital dermatitis; SU – sole ulcer; TN - toe necrosis; TU - toe ulcer; WLA - white line absceses; CNS - coagulase-negative staphylococci

## Discussion

Many risk factors play a role in the development of clinical and subclinical mastitis. Lameness should be included in them. The predominant causes of severe lameness in cows are claw diseases. Dairy Herd Improvement (DHI) numbers for the University of Florida Research herd in 1995 showed a 35% incidence of clinical lameness in cows. Claw problems (sole ulcers and white line disease) accounted for 63 percent of the reported cases. Digital dermatitis and foot rot accounted for 20 percent, and 17 percent of the cases, respectively (Stokka et al. 1996). Locomotion score has been used widely to investigate the degree of lameness. Increased locomotion score is associated with an increased percentage of cows lying down; also the cows' posture in the milking parlour is associated with lameness (Olechnowicz and Jaskowski 2012). Once lameness is developed, cows spend less time standing at the feeding troughs (González et al. 2008; Gomez and Cook 2010), and they can be observed to spend more time lying down compared to healthy cows (Chapinal et al. 2010) which consequently increases the risk of mastitis. It has long been known that the onset of intra-mammary infection is dependent on contamination and hygiene not only of the environment but also of the animals' surface area. An index of environmental sanitation based on the amount of manure present on the cow skin and in its environment was defined as a predictor for the occurrence of coliform mastitis (Bartlett et al. 1992). The lowest incidence of mastitis occurred in herds with the cleanest cows and the most satisfactory beds (Ward et al. 2002). The rate of new intra-mammary infection is just related to the number of bacteria that the teat end is exposed to (Neave et al. 1966).

Microbiological culture was performed in 24 samples randomly selected from all positive milk samples (n = 40) to determine the distribution of intra-mammary pathogens in lame cows. Intra-mammary pathogens were isolated in

A study was carried out across U.S. dairy farms to detect the association between the hygiene score of the udder and legs with the occurrence of mastitis; it found a significantly higher prevalence of intra-mammary pathogens with a higher hygiene score of the udder (Schreiner and Ruegg 2003). Poorly trimmed hooves and lameness were connected with impaired lying-down and rising behaviour; this acts as a risk factor for teat tramps (Rajala-Schultz and Gröhn 1999) which in turn predicts clinical mastitis. The longest duration of lying time was observed in lame cows suffering from claw lesions affecting the sole or the junction between the sole and the wall of the claw. More time spent by lying down was associated with extreme exposition of the udder to environmental pathogens and influenced the manifestation of mastitis (Singh et al. 1993). Our results confirmed this hypothesis, whereby the most prevalent intra-mammary pathogens in positive milk were environmental bacteria (coagulase-negative staphylococci, *Enterococcus* spp., *Proteus* spp., and *Aerococcus viridians*). Poor dairy cow hygiene is consistently related to the increase of the somatic cell count (SCC) in milk. In a Canadian study, investigators discovered that SCC in milk increased with increasingly dirty stalls (De Vries et al. 2011).

It has been hypothesized that lameness together with subsequent prolonged lying time increases the risk of bacterial contamination of the teat canals and cows that lie down immediately after milking would be at a higher risk of suffering from intra-mammary infection. Therefore, lameness may be viewed as one of the most common risk factors responsible for the distribution of mastitis in dairy herds with increased prevalence of lameness. In line with previous works (Peeler et al. 1994; Sato et al. 2008) our study noted an association between lameness and mastitis, where the prevalence of mastitis was very high in cows that suffered from claw diseases (74.3%). Similarly, a negative effect of lameness on mastitis in dairy herds was previously demonstrated in a study of ten dairy herds in the southwest of England. Clinical lameness before the first service was associated with a 1.4-fold increase in the odds of clinical mastitis (Peeler et al. 1994). According to a Danish study, claw diseases were significantly associated with the mastitis incidence rate (Sato et al. 2008). In a Swedish observational study, a close relationship was documented between poor foot health and a high incidence of clinical mastitis (Arvidson 2000). Moreover, other research reported the prevalence of the sole ulcer to be related to udder disorders (Vaarst et al. 1998). A negative association between hoof trimming and mastitis at the herd level was also found in an earlier study (Østerås and Lund 1988). Recently, a significant difference in the mastitis rate between healthy and lame cows was reported (Mudron 2016).

In contrast, in a study from Bulgaria no association between lameness and subclinical mastitis was determined, where the prevalence of mastitis in lame cows was only 5.5% (Mitev et al. 2011). No significant differences in SCC between clinically lame cows and healthy cows were determined on Polish Holstein Friesian dairy farms (Olechnowicz and Jankowski 2012). No relationship between lameness and mastitis was confirmed in a study carried out on 102 Swedish dairy herds by Hultgren et al. (2004); in accordance with this observation, statistical analysis showed only a strong relationship between teat injuries and mastitis. However, no associations were proved between the sole ulcer and clinical mastitis or high milk cell counts. This might be explained as a result of relatively mild lameness of the affected cows. Possibly, only severe lameness impairs the cow's locomotion and increases the risk of teat injuries and subsequent mastitis. The analyses indicated that teat injury was neither an intervening variable between the sole ulcer and mastitis or high cell counts, nor a confounder.

The incidence of mastitis in cattle herds is influenced by the hygiene of the udder and the environment. Similarly, the overall time spent by lying down plays an important role in the distribution of mastitis. Another study found no relationship between mastitis and lameness, and a relatively low prevalence of mastitis was observed in lame cows (Heuer

et al. 1999). In a study from the UK, a relationship among the occurrence of lameness, the locomotion score and SCC was analysed in 1,397 cows on seven dairy farms. In cows with a higher locomotion score, lower SCC was detected compared to the cows with a lower locomotion score (Archer et al. 2011).

Moreover, similar distribution of mastitis among different quarters was observed in our research. The ratio between front and rear quarters was 53:47 and no significant difference was observed in the incidence among quarters. In line with our results, no significant difference in the prevalence of mastitis between different quarters was confirmed in a study by Hashemi et al. (2011). However, several observations noted a significantly higher prevalence of intra-mammary infections (IMI) in rear quarters in cows suffering from particularly clinically manifested mastitis (Barkema et al. 1997; Berry and Meaney 2006; Maćešić et al. 2012). This variation could have resulted from udder morphology and higher milk production of rear quarters (Lancelot et al. 1997). In spite of previous research, the prevalence of mastitis could have resulted from generalized cow differences, such as individual milk yield, immune competency, mammary type characteristics, and general health state (Adkinson et al. 1993).

In conclusion, our analysis confirmed a strong association between lameness and mastitis. Lameness may be characterised as one of the risk factors contributing to the increased prevalence of mastitis in dairy herds. However, no significant differences were observed in severity of mastitis in dairy cows with different type of claw diseases.

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