Dóra Bekő[1], Péter Póti[1], László Bárdos[1], Ágnes Sramek[1], Ferenc Pajor[1]

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Udder health investigations in a Hungarian Fleckvieh small-scale herd, related to food safety

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1. SUMMARY

Data about the presence of pathogenic bacteria in the udders of Hungarian Fleckvieh cows and about their milk quality parameters is fairly lacking. The aim of this research was to evaluate the prevalence of mastitis pathogens in Hungarian Fleckvieh milk. This study was carried out in a small-scale (n=20) dairy farm in Pest county, Hungary. The cows were milked twice a day in a milking parlor with three stalls. Milk samples were taken from cows at similar stages of lactation and of similar age (n=14) three times during the lactation (beginning, midpoint and end of lactation) from udder quarters (n=168) at the beginning of milking for analyzing pathogenic udder bacteria, and from fully milked udders (n=42) for analyzing milk composition and somatic cell count. The 42 milk samples were divided into four groups by the type (minor or major) and prevalence of the pathogenic bacteria by udder quarter:

1 – all four udder quarters negative;
2 – minor pathogenic bacterial species in one udder quarter;
3 – minor pathogenic bacterial species in two, three or four udder quarters;
4 – major pathogenic bacterial species detected regardless of the case count.

It was determined that the mean somatic cell count was 123 thousand cells/ml, moreover pathogenic udder bacterial species have been found in 31% (n=52) of the milk samples. In the investigation the mastitis pathogen most frequently encountered was coagulase-negative *Staphylococcus (CNS)*, which was present in two thirds of the positive samples (n=33). Of the major udder pathogens, *Streptococcus uberis* (n=13) and *Staphylococcus aureus* (n=2) were detected in the 168 samples. When major pathogenic bacterial species were detected even in one udder quarter, it significantly affected the mean somatic cell count of the milk of Fleckvieh cows. The elimination of pathogenic and spoilage bacteria is important not only from a food safety point of view, but it is also of paramount importance for the production of high quality milk products. Based on our results, milk with a low somatic cell count and of favorable quality can be produced when compliance with appropriate hygienic practice is ensured under small-scale farm management conditions.

2. Introduction

To date, mastitis in dairy cows is a major problem in dairy farms, as it is one of the most common and costly diseases, resulting in significant losses due to reduced income from the sale of milk, the cost of culling and treatment (e.g. courses of antibiotics), or the burden of milk dumping because of it **[4, 11, 16]**.

Based on domestic studies, in Hungary the average udder health loss per cow is approximately USD 105.9 (HUF 28,000-29,000 – Today this sum is approximative 34,000 HUF. The Editor.). On a farm with 1,000 cows, this could be as much as HUF 30 million per year **[15]**.

[1] Szent István University, Faculty of Agricultural and Environmental Sciences

The development of mastitis is usually caused by some kind of pathogenic udder bacterium that multiplies and damages the tissues when entering the udder quarters. If, at the onset of the disease, there is no change in the udder quarter and there is no increase in the somatic cell count of the milk produced by it, then the inflammation is in the subclinical stage. When we are talking about a *clinical* mastitis, an increase in the temperature of the udder quarter can be observed, it becomes red, swollen, hard and sensitive and, in addition, the milk extracted from the udder quarter contains flakes or lumps, and the consistency of the milk becomes water-like.

Bacteria that cause mastitis are divided into two groups, one of which is the so-called major udder pathogens, while the other group is the so-called minor udder pathogenic bacteria **[2]**. Major udder pathogens include *Staphylococcus aureus*, *Streptococcus uberis*, *Streptococcus dysgalactiae* and Enterobacteria (e.g., *E. coli*). These dangerous pathogens are the most common causes of clinical mastitis **[1, 3, 12, 14]**. The most common udder pathogens are coagulase-negative *Staphylococcus* (CNS, in short) and *Corynebacterium spp.* **[19]**.

Subclinical mastitis is most often caused by these minor pathogens **[9]**. Based on all this, it is clear that providing the hygiene background of the livestock and its control are important issues from the point of view of the safety of milk and the dairy products made from it. It is well known that the processability of poor quality milk (presence of udder pathogenic bacteria, increased somatic cell count) is greatly reduced **[21]**. The destruction of various harmful microorganisms can be achieved by the pasteurization of milk. In addition to food safety considerations, various heat treatments are also used to achieve safer production **[10]**.

The Fleckvieh is a breed of cattle originating from cross-breading of Central European stocks with Simmental cattle, and it can be integrated into small farm conditions. The weight of the Fleckvieh cow is 600-700 kg, and that of the bull is 900-1,300 kg **[22]**. Annual milk production of the breed is 5,000-5,500 kg, with a fat content of 3.9 to 4.1% **[5]**. According to Hungarian authors **[22]**, in terms of the amount of milk produced, Fleckvieh is classified as a medium-performing breed among all world breeds. In addition to the above-mentioned traits, the Fleckvieh has excellent genetic characteristics, and with the professionalism and commitment of the breeders, all the conditions are in place for the breed to be the winner of the challenges of the future **[6]**. The incidence and frequency of mastitis vary and show significant differences in small, medium and large farms, and there are also differences between the breeds. In high yielding breeds, the incidence is as high as 40%, while in small family farms (e.g., in the case of the Fleckvieh) mastitis occurs less frequently (10-20%) **[13]**. Previous studies, using the majority Holstein Friesian cows, have shown the importance of evaluating udder health from a bacterial point of view, whereas very little data are available on the prevalence of udder pathogenic bacteria in the Fleckvieh breed and on the effect of the bacteria on the somatic cell count.

3. Materials and methods

The location of the study was a family farm in Pest county (Törtel). In the course of the investigation, Fleckvieh cows (n=20) were milked on the farm. There were two barns to accommodate the cows: one with 19 stalls, built in 2008, the other one with 9 stalls, built in 2013. The cows were milked twice a day, in a De-Laval milking parlor with 3 stalls. The cows were fed alfalfa hay, meadow hay and alfalfa haylage *ad libitum*, and could also graze from spring to late autumn. The farm has 30 hectares of pasture, 5 hectares of which is native grassland and 25 hectares is planted grassland. In addition, dairy cows also received feed supplements in a ground form (20% each of corn, wheat, sunflower, barley and triticale).

Cows (n=14) with similar lactation stages and ages, and not showing the signs of clinical mastitis during the investigation were selected for the study, from which a total of 168 samples were collected at the beginning, middle and end of the lactation period, from the udder quarters separately, with an additional 42 individual milk samples collected from fully milked udders. In the study, the correlations between the types and incidence of udder pathogenic bacteria were evaluated using the individual somatic cell count values, therefore, milk samples were not taken from each udder quarter to determine the somatic cell count. Sampling was performed by following the milking times, during the two morning and one evening milking in the course of the study period.

Prior to sampling, the teats of the selected cows were treated with a warm water cloth and then, before milking, with a disinfectant wipe to remove bacterial contaminants from the teat surfaces. After draining the first jet of milk, individual milk samples were collected from each udder quarter in 10 ml jars and from each milked udder in a 50 ml jar for each cow after milking. Bacterial species causing mastitis [including CNS (coagulase-negative *Staphylococcus*); *Corynebacterium sp.*; *Staphylococcus aureus*; *Streptococcus uberis*] were detected in the 10 ml samples by the surface spreading method in the Gödöllő laboratory of the Animal Breeding Performance Testing Kft. In the 50 ml milk samples, milk fat, milk protein, lactose, pH value and electrical conductivity were measured, and the somatic cell count was determined. The composition of milk (dry matter, milk protein, milk fat, lactose) was analyzed using a LactoScope™ (Delta Instruments Ltd., Netherlands) instrument. The pH value and electrical conductivity of milk were measured by an EC600 (Extech Instruments Ltd., USA) instrument. Somatic cell count

was determined using an MT-05 somatic cell count device. During the three sampling periods, the total plate count data of the Budapest Raw Milk Qualification Laboratory of the Hungarian Milk Research Institute Kft. were also used.

The effect of the type of udder pathogenic bacterial species (minor or major species) and their incidence by udder quarter on the somatic cell count of the individual milk samples (n=42) of the cows were evaluated. Four categories were defined according to the type and occurrence of the udder pathogenic bacterial species detected:

1. All four udder quarters are negative, i.e., no udder pathogenic bacterial species were detected,
2. A minor udder pathogenic bacterial species was detected in one udder quarter,
3. Minor udder pathogenic bacterial species were detected in two, three or four udder quarters,
4. Major udder pathogenic bacterial species were detected, regardless of the case count.

Statistical evaluation of the data was performed with the SPSS 23.0 software package (normality and homogeneity test). The normality analysis of the data was carried out using the Kolmogorov-Smirnov test. It was found that somatic cell count values did not show a normal distribution, so these data were logarithmized in order to be able to perform further statistical studies. Then parametric tests were performed during their study. ANOVA and Chi^2 tests were performed between the groups formed on the basis of udder pathogenic bacteria. Due to the difference in the number of items between the groups, the Tukey post hoc test was used.

4. Results

The nutritional value, electrical conductivity, pH, somatic cell count and the total plate count of the mixed milk are summarized in ***Table 1***.

According to several authors **[17]**, the nutritional values of milk and its microbiological condition have a decisive influence on the processability of milk and the dairy products made from it. In the case of microbiological quality, somatic cell count and plate are strict criteria, as these parameters have a large impact on the processability of raw milk. According to the criteria of Chapter I of Section IX of Annex III of Regulation (EC) No 853/2004 of the European Parliament and of the Council for raw milk, the maximum total plate count of raw cow's milk shall not exceed 100,000 cells/ml, and the somatic cell count shall not exceed 400,000 cells/ml. Our results show that, during all three measurements, the raw milk samples met the above requirements, moreover, the somatic cell count and total plate count values measured in the course of the study were favorable. This is advantageous in the processing of milk, since good quality dairy products basically cannot be produced from milk with a high total plate count, or only with a series of complicated technological steps.

The number and proportion of udder pathogenic bacteria in the milk samples are summarized in ***Table 2***.

In our study, 116 negative samples were found, the average rate of which was thus nearly 70%. Furthermore, it as found that the number and proportion of positive samples increased from the initial 23% (n=13) to 39% (n=22) as lactation progressed. The average value for the period investigated was 31%. The resulting ratio is favorable compared to previous results **[8]**, where the proportion of positive samples was 27% in a study carried out by the authors with Czech Fleckvieh cows. In the same study, 42% of milk samples from a Holstein Friesian herd were positive. Some authors found similar (33.5%) values **[18]**, while others **[7]** found higher values (61-78%).

Dangerous udder pathogens such as *S. uberist* and *S. aureus* were detected only in 16 samples. Of major udder pathogenic bacteria, *Streptococcus uberis* was present in 13 samples, representing a quarter of the positive samples. *Staphylococcus aureus* was present in two samples (3.8%). Both major pathogens were present in one sample, representing 1.9% of all positive samples. The total major udder pathogens accounted for 30.8% of the positive samples and 9.5% of the 168 total samples. In previous studies, it was also the *Streptococcus uberis* and *Staphylococcus aureus* udder pathogenic bacteria that were detected by the authors **[8]**. These udder pathogenic bacteria pose a food safety risk, so it is important to use an appropriate heat treatment procedure to kill the pathogens.

According to the results of our investigations, the most common minor udder pathogenic bacterium was coagulase-negative *Staphylococcus* (CNS), similarly to previous studies **[8, 9]**. This pathogen was present in 33 samples, representing 63.5% of the positive samples and 19.6% of the total samples. In addition, other pathogens of less significance were found in 3 samples (5.7%).

In our further study, milk samples taken three times from 14 cows were divided into 4 groups based on the type of udder pathogenic bacterial species detected (minor vs. major) and their occurrence by udder quarter. Correlations were sought between the different udder pathogenic bacterium categories and the somatic cell count of the milk. The results are summarized in ***Table 3***. According to the table, in the negative samples and in the case of the presence of minor udder pathogenic bacteria, there was no difference in the somatic cell count of cow's milk or in the proportion of samples with a cell count exceeding 100,000. The lowest somatic cell counts (4.92, 4.92

and 4.90 log/ml) were measured in the milk samples classified as belonging to groups 1, 2 and 3. Minor udder pathogenic bacteria could be detected in 0, 1 or 2-4 udder quarters. The proportion of samples with a somatic cell count exceeding 100,000 was also low, ranging from 21 to 30%. If a major udder pathogenic bacterial species could be detected in a sample taken from the cows (Group 4), the somatic cell count of the milk (5.15 log/ml) and the proportion of samples with a cell count exceeding 100,000 (60%) were significantly increased.

It follows from the above that, under normal housing, feeding and hygiene conditions, no significant animal health risk is posed or economic damages caused by minor udder pathogens in the case of Fleckvieh cows, however, under unfavorable conditions, they ma make the udder more susceptible to the growth of major udder pathogenic bacteria. Good quality product cannot be prepared from milk that contains pathogenic or spoilage bacteria. Therefore, the risk to health and thus to food safety are typically reduced by processors through pasteurization. However, pasteurization must be carried out in such a way that the heat treatment applied does not significantly alter the original nature and characteristics of the milk. This requires strict supervision of the milk processing technology.

Table 4 shows the condition of the samples before the occurrence of major udder pathogens. The livestock examined by us did not qualify as an organic farm mainly due to the nature of the feed, but by way of comparison, it can be stated that in European organic farms that also have smaller herds, udder health conditions are more favorable than those in large plants, both in terms of diagnosis, treatment and its effectiveness. Although the criteria for comparison are not exactly the same, it still shows that it pays off economically as well if particular attention is paid by livestock keepers to the health of the animals **[20].**

In the milk samples in which no minor udder pathogenic bacteria were present, a smaller proportion of major pathogenic bacteria was found during the subsequent sampling. However, if minor udder pathogenic bacteria were present in the samples taken previously, then a higher proportion of major pathogens were found during the subsequent sampling. Although no significant dairy hygiene and health risk is posed by minor udder pathogens, they can increase the chances of subsequent appearance and growth of major udder pathogenic bacteria, as well as the cost of udder health treatments and the food safety risk of the raw material.

5. Conclusions

Based on our results it can be stated that the lowest somatic cell count values were measured in the case of negative samples and in the presence of minor udder pathogenic bacteria. However, when major udder pathogenic bacterial species were also detected, the somatic cell count of the milk samples increased dramatically in parallel. The presence of facultative udder pathogenic bacteria means a deterioration in udder health, as it can make the udder more susceptible and major udder pathogenic bacteria, which can also cause severe udder diseases, can more easily proliferate. Such milk lots significantly impair the financial performance of dairy farms, make it more difficult, or even impossible, to process raw milk in the food industry, and they pose a food safety risk to the consumers of the milk and dairy products.

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