

Prevalence and risk factors of teat end hyperkeratosis in cows from the Urals region of Russia

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ABSTRACT

Diseases of the dairy herd negatively affect the period of economic use of animals, the volume of milk production and its quality. In this regard, early diagnosis of diseases and their causes will reduce the incidence of dairy herds and prevent economic losses. The research of the state of udder teats in cows during lactation has been conducted in the farms of the Ural region containing Holsteinized black-motley cows with different levels of milk productivity. An analysis of the spread of teat end hyperkeratosis in different animal housing and milking technologies was carried out according to the number of lactations and the season of the year. Analysis of morbidity in farms with different levels of milk productivity showed that as animal productivity increases, more teats with signs of pathological changes are registered. Simultaneously, teat lesions are also observed when using robotic milking machines, but in smaller numbers. In autumn and winter regardless of type of milking equipment, the growth of udder teat diseases is observed. When milking by robotic system, the influence of frequency, duration of milking and other factors on the development of hyperkeratosis were analyzed. It was noted that animals with milking frequency of more than 2 times a day have a higher level of hyperkeratosis; likewise, animals with permanence in the box and prolonged milking have a higher percentage of lesions, which can be related to the narrowing of the lumen of the teat canal and excretion of a large volume of milk. Also, animals with changes in the area of the teat canal opening have changes in the qualitative composition of milk, in particular, increased protein content and a decreased lactose content, which may indicate the development of an inflammatory process in the mammary gland.

Keywords: cows; teat end hyperkeratosis; milking technology.

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INTRODUCTION

Dairy cattle breeding is an important component of animal husbandry, since it provides the population with the necessary food products, and primarily milk and dairy products. Modern intensification of dairy production is aimed at increasing the terms of economic use of animals, increasing milk production, and improving its quality. The latter depends on many factors: the conditions of keeping cows, the methods of milking them, the quality and quantity of the forage base, the breed, and the state of health.

Significant damage to the dairy cattle industry is caused by udder diseases, which is associated primarily with a decrease in animal productivity and milk quality, as well as the premature retirement of cows from the herd (Shabunin *et al.*, 2017). Pathogenic microorganisms mainly penetrate into the mammary gland through the teat canal, therefore, the condition of the udder teats and, in particular, the sphincter of the teat canal is an important indicator of animal health.

The most common teat disease of highly productive cows is teat end hyperkeratosis represented by skin thickening of varying degrees in the area of the teat canal sphincter (Bobić *et al.*, 2018; Guarín *et al.*, 2017; Sterrett *et al.*, 2013). According to some foreign authors, hyperkeratosis of udder teats is not a direct cause of mastitis, but acts as a predisposing factor (Barkova *et al.*, 2019; Bhutto *et al.*, 2010; Derakhshani *et al.* 2020;

Jamali *et al.*, 2018). Therefore, it is important to control the incidence of cows with teat end hyperkeratosis in the herd.

The health of udder and teat end skin can be significantly affected by the technology of machine milking, as well as compliance with technical regulations (Matveev *et al.*, 2018; Hamann, 1987; Sterrett *et al.*, 2013). In connection with the introduction of modern robotic milking systems into production, which perform automatic donning, individual extraction of milking cups according to milking rates, and consequently, minimize the influence of the human factor, it is relevant to assess the impact of different milking equipment on udder teat condition, and consider various factors contributing to the occurrence of lesions in the teat canal opening area (Besier *et al.*, 2017; Hovinen and Pyörälä, 2011).

In this regard, early diagnosis, and, consequently, timely treatment and preventive measures can reduce the incidence of dairy herds and avoid economic losses.

Therefore, the purpose of our study was to study the spread of nipple hyperkeratosis in cows, the factors of its occurrence and progression, as well as the impact of the presence of nipple hyperkeratosis on milk yield and milk quality.

MATERIALS AND METHODS

Studies on the distribution of udder teat end hyperkeratosis were conducted in 2009-2018 on the basis of 17 agricultural

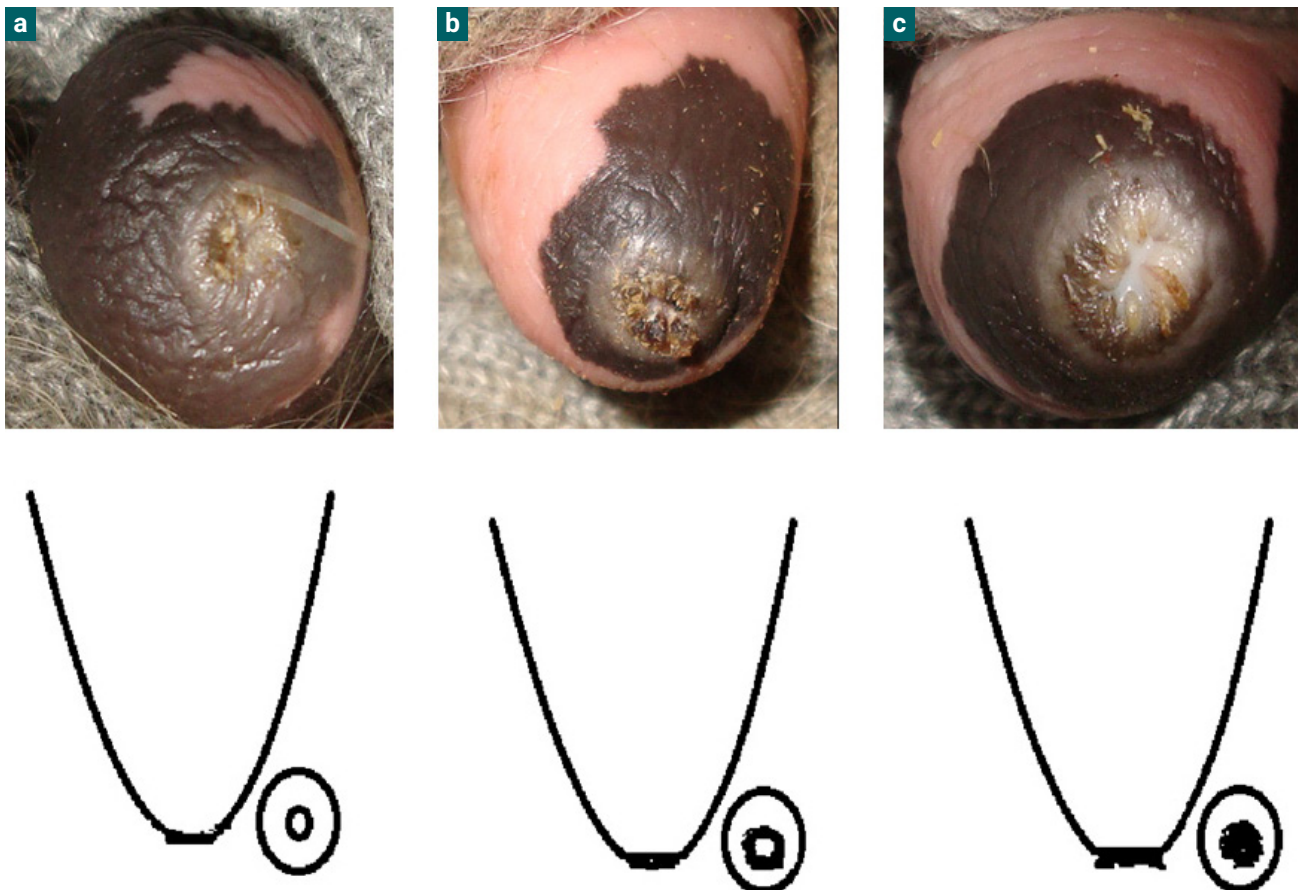


Figure 1. Diagnostic scale of udder teat lesions: a - physiological tissue reaction, b - hyperkeratosis, c - hyperkeratosis complicated by radial cracking.

enterprises of the Ural region, containing Holsteinized black and white cattle of different blood ratios, with productivity from 4 to 10 thousand kg of milk per forage cow. In total the state of udder teats of 3567 cows during lactation was investigated.

Assessment of mammary gland condition was performed using a diagnostic scale, which is a panel of 3 photographs and 3 schematic images (figure 1).

To determine indices of milk yield, milking rate, time of milk yield and other indices on robotic milking units we used data obtained from the program of Lely T4C milking robot. The research was conducted on the basis of two farms: farm 1 - with cow productivity of more than 10 thousand kg of milk per forage cow; farm 2 - with animal productivity of about 7.5 thousand kg of milk per lactation.

To detect changes in raw milk quality parameters in the presence of udder teat lesions, a chemical analytical study of 785 milk samples was conducted using a combined system of Bentley Instruments (USA), including a Bentley 2000 infrared analyzer.

The presence of hidden blood was determined by examining was performed by examining a residual collection sample of milk obtained from all quarters of the udder immediately after removal of the milking cups. For this purpose, 40-50 ml of milk were collected in a plastic container. The study was performed within 3 hours after sampling. Therefore, the milk was cooled for transportation and then heated to a temperature of 20-22°C, just before centrifugation. The presence of occult blood was determined by centrifugation. After thoroughly mixing the sample, 5-7 ml of milk was poured into a test tube and centrifuged for 10 minutes at 3000 rpm. The result was assessed by the presence of a visible sediment at the bottom of the tube in the form of a red ring (figure 2).



Figure 2. Presence of occult blood after centrifugation of a milk sample.

The data were statistically processed using Microsoft Excel 2010 and STATISTICA 10 with the calculation of arithmetic mean, standard deviation, and mean error of the indices by the parametric Student's t-criterion, for independent groups by the nonparametric Mann-Whitney criterion.

RESULTS AND DISCUSSION

The analysis of the condition of the teat end in 3567 lactating cows from 17 farms of the Ural region with different milk production levels, holding and milking technologies revealed that 11,9-56,6% of all the examined quarters have significant changes in the external opening of the teat canal. Hyperkeratosis complicated by radial fissures and teat canal gaping was recorded in 9.3% of the examined cows, and changes in the form of its uncomplicated form were recorded in 18.7%. During studies, it was noted that animals in high-yielding herds were more susceptible to the disease of hyperkeratosis of the udder teat end.

The epidermal thickening, which is visualized and palpated as a relief ring of varying severity, can be attributed to the physiological response of tissues to the vacuum system, as there is no involvement of the teat canal tissues in this process.

Changes in the form of hyperkeratosis and its complicated radial fissures are accompanied by deeper changes in the teat end. There is a circular thickening of tissues in the area of the teat canal opening, which, accompanied by the presence of strong roughness, can protrude above the skin surface and be determined when examining the udder from the side. At this stage of the disease the mucous membrane of the teat canal is involved in the process associated with impaired regeneration of horny cells, which can lead to its partial obstruction and appearance of tightness in cows (figure 3).

Histological examination of the teat canal revealed thinning of the epidermis, partial destruction and desquamation with perifocal round-cell infiltration (figure 4). The infiltrate is represented by lymphocytes, plasma cells, and macrophages. Leukocytes nuclear segmentations are detected in small numbers. An epidermal cyst is detected in the projection of the lesion, the lumen of which contains fragments of detached epithelium. In the dermis, granulation tissue infiltrated by lymphocytes and plasma cells is detected. Thus, due to trauma, granulation is formed in the area of the nipple canal, and signs of productive inflammation develop.

Histological examination of the skin at the nipple apex showed focal hyperkeratosis with significant thickening of the stratum corneum (figure 5).

To determine the impact of milk productivity on the condition of udder teats a study of 1682 cows in 9 farms of the Ural region with different levels of milk productivity, maintenance and milking technology was carried out. Depending on the average annual milk productivity the enterprises were subdivided into the following groups: Group 1 - 1 farm with cow productivity up to 5 thousand kg of milk per lactation (ADM-8); Group 2 - 3 farms with animal productivity of 5-7 thousand kg of milk per forage cow (ADM-8, DeLaval, milking room, Group 3 - 3 farms with cow productivity of 7-9 thousand kg (ADM-8, DeLaval, monorail, Lely Astronaut, robot); Group 4 - two farms with productivity of cows of 9 thousand kg of milk per lactation and more (Lely Astronaut, robot, DeLaval linear unit).

Analysis of the obtained data showed that with the productivity of cows of 4-5 thousand kg and milking by linear milking



Figure 3. Changes in the area of the teat canal opening: a - physiological condition of the teat canal; b - destruction of the teat canal in the complicated form of hyperkeratosis.

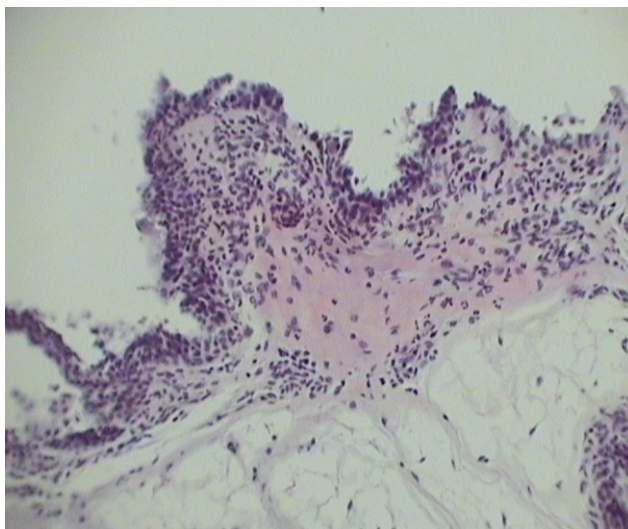


Figure 4. State of the teat canal in the uncomplicated form of hyperkeratosis. Hematoxylin and eosin staining, magnification x200.

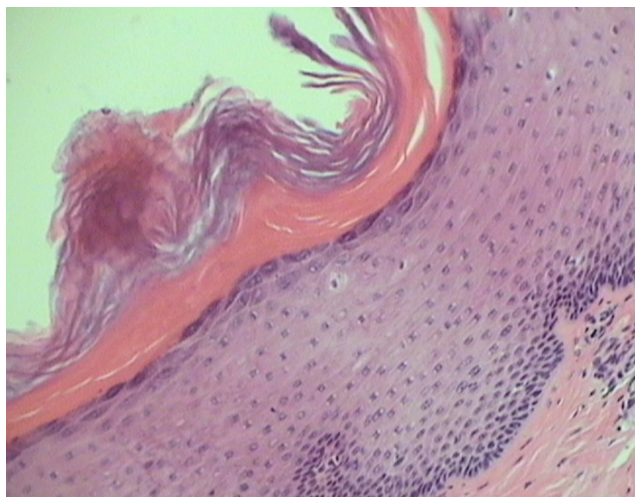


Figure 5. Epidermis in the area of the teat canal opening in the uncomplicated form of hyperkeratosis. Hematoxylin and eosin staining, magnification x200.

unit the physiological condition of teat end of udder was observed in 65.8-67.1% of the teats examined. Hyperkeratosis was registered on 24.5-27.6% of teat ends, and its complicated form - on 6.6-8.4%.

At an average milk yield of 5-7 thousand kg of milk and milking with a linear milking machine the level of teat end hyperkeratosis is 23.5-24.6%, while in the milking parlor it is 12.8% (1.9 times less).

In farms with cows productivity of 7-9 thousand kg of milk when milking by linear milking unit the level of teat end affection is 22.7-40.2%, when milking by robotic system it is 22%.

In the group of farms with animal productivity of more than 9 thousand kg of milk per lactation at stall system of keeping and milking into the milk duct by ADM-8 the level of hyperkeratosis is 38.5%, at robotic system of milking and milk productivity by body more than 10 thousand kg of milk the number of

teats with lesions as complicated and uncomplicated hyperkeratosis is at the level of 23.7%.

Thus, with an increase in milk productivity and the use of linear milking equipment, there is a gradual increase in the number of teat end lesions in the herd. However, a sufficiently high level of hyperkeratosis was detected with productivity of more than 10 thousand kg of milk per lactation and with the use of a robotic milking system - 10.9 and 12.8% respectively.

An analysis of the incidence of teat hyperkeratosis depending on daily milk yield showed that in 23.4% of cows with a productivity of up to 18 l / day, hyperkeratosis was found in one quarter of the udder; in 25.4% of cows with a productivity of 18-29 l/day, hyperkeratosis was found in two quarters of the udder; in 23.5% of cows with a productivity of more than 29 l/day, hyperkeratosis was found in three or more quarters of the udder.

Analysis of the distribution of hyperkeratosis in the udder quarters depending on daily milk yield showed no pronounced dependence, which we primarily attribute to the fact that the lactation stage of an animal was not recorded.

To determine the effect of the number of lactations on the udder teat condition in cows, we studied 1069 cows with different levels of milk production and milking technology. The results showed that as the number of lactations increased in cows, there was a slight increase in udder teat lesions (figure 6).

Thus, during 6 lactations, lesions of teat end hyperkeratosis and its complicated form were recorded in 36.7% of all cows examined. However, in animals with more than 9 lactations, hyperkeratosis of udder teat ends is practically not observed (7.7%) and the complicated form of the disease is

not detected. The least spread of udder teat diseases is recorded in cows of 1 lactation, in which changes in the form of hyperkeratosis are registered in 10.1% and its complicated form in 2% of cows.

A detailed study of the distribution of hyperkeratosis by udder quarters revealed the same trends (figure 7). The smallest number of quarters with pathological teat changes was found in cows of 1 lactation - 16.3% and cows 9 lactations and older - 7.7%, and no form of hyperkeratosis complicated by radial fissures was found in the older age group.

The highest number of quarters with teat end hyperkeratosis and its complicated form are registered in cows of 6 lactations and amount to 28.1%, as well as in cows of 2 lactations - 22.8% of quarters.



Figure 6. Influence of the number of lactations on the distribution of teat ends hyperkeratosis in cows.

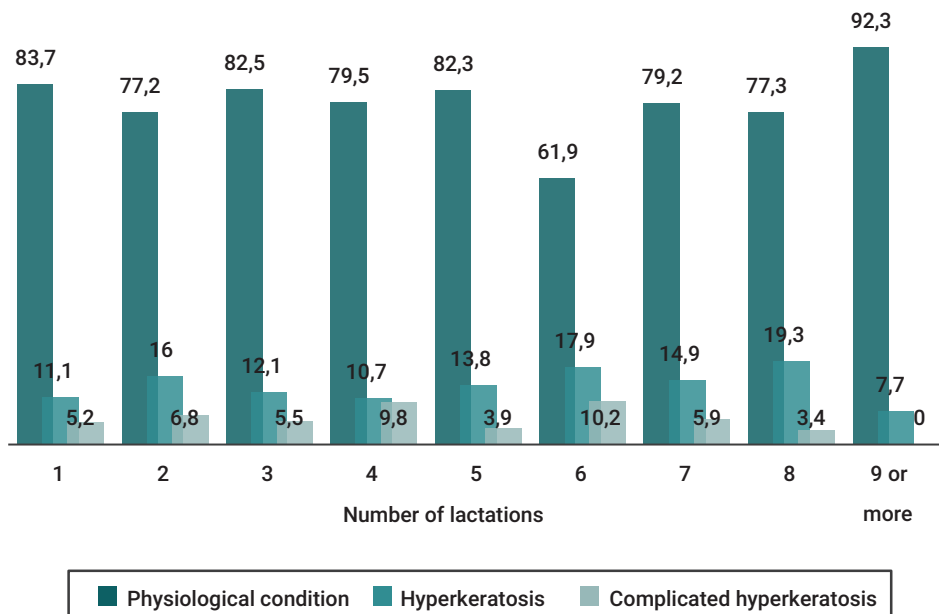


Figure 7. Distribution of teat end hyperkeratosis by udder quarters in cows depending on the number of lactations.

Since the robotic system provides quadruple milking, we evaluated the time of milk retention and recoil depending on the lesion directly on the udder quarters. The results showed that as the severity of udder teat lesions increased, the time of milk retention increased by 1.1-1.5 times compared to teats with no changes in the teat canal opening area.

There are also more severe changes of udder teats with increasing duration of milking. On average, the duration of milking of quarters with a complicated form of hyperkeratosis is longer by 30-80 seconds relative to quarters without pathological changes, which may be associated with the difficulty of milk evacuation through the teat canal.

The study of the distribution of hyperkeratosis between the anterior and posterior quarters of the udder in robotic milking and in linear milking in one farm showed that in robotic milking the lesions of udder teats on anterior and posterior teats are at the same level of 22-25%. At milking by linear semiautomatic system, the lesions of front and back teats of udder are 39-42.2%, thus, the complicated form of hyperkeratosis on front teats is registered 1.1 times more often than on back ones. The same trend is observed when milking cows with linear milking unit ADM-8 (1.2 times).

The influence of the duration of being in the box during milking on the condition of the udder teat end in cows was noted. On farm 1 with the productivity of cows over 10,000 kg of milk, the number of teats with physiological condition was 85%, with pathological changes - 15% when the duration of stay in the box was up to 5:55 minutes (25% of animals). When cubicles lasted from 5:56 to 8:24 minutes (50% of cows), the level of teat end hyperkeratosis was 19.9%. With a duration of more than 8:25 minutes (25% of animals), the level of uncomplicated and complicated hyperkeratosis increased significantly to 41.3% of the teats.

On farm 2 with the productivity of about 7.5 thousand kg of milk per forage cow, the number of teats with pathological changes amounted to 8.3% when the duration of stay in the box was up to 4:39 minutes (25% of cows). When staying from 4:40

to 6:45 minutes (50% of cows), 24.8% of teats with changes in the form of hyperkeratosis and its complicated form were registered. When cows were in the box for more than 6:46 minutes (25% of animals), the level of hyperkeratosis increased to 29.6%.

Analysis of teat end condition depending on average and maximum milking rates showed that in physiologically normal teats, average and maximum milking rates were on average 1.2 times higher compared to those with pronounced pathological changes.

A study of robotic milking frequency showed that the average number of milkings per cow was 2.4 per day, with 26.3% of cows having an actual number of milkings less than 2, 54.3% of cows - in the interval of 2 to 3, 19.4% of cows - more than 3 milkings per day (figure 8).

In this connection, we studied the effect of the frequency of milking on the condition of the udder teat in cows. Animals were divided into groups with the number of milkings per day less than 2 times, from 2 to 2.9 times and more than 3 times. The results showed that cows in both farms showed an increase in the number of teats with lesions of varying degrees of hyperkeratosis with increasing frequency of milking (figure 9). Thus, with the frequency of milking 2-2.9 times a day, the number of teats with lesions in the form of hyperkeratosis increases by 1.4 times and remains virtually unchanged with further increase in the frequency of milking.

At the same time in the groups with less than 1.9 milkings per day the average milk yield per milking was 11.3 and 8.1 kg, 2 to 2.9 times - 12.4 and 9.6 kg, more than 3 times - 9.9 and 9.7 kg of milk respectively. The days of lactation averaged 245, 174 and 127 days respectively.

The estimation of teat state depending on the lactation duration showed that the level of teat affection is practically unchanged and makes 20.1-23.7%, in the period from 241 to 300 days of lactation this indicator increases in 1.2-1.6 times and is at the level of 27.4-31.4%. When lactation lasts for more than 301 days, the number of teats with pathological changes in the teat canal decreases to 17-21.7%.

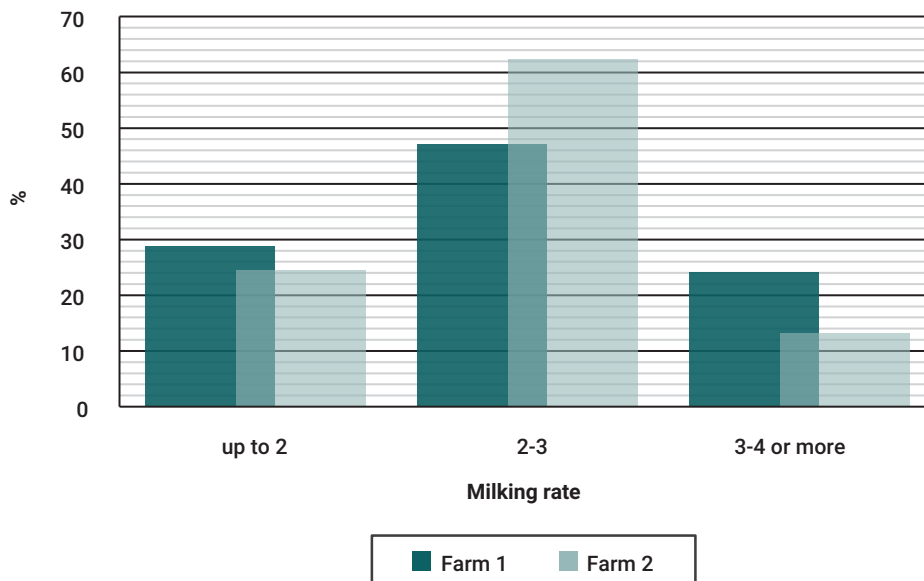


Figure 8. Distribution of cows according to milking frequency.

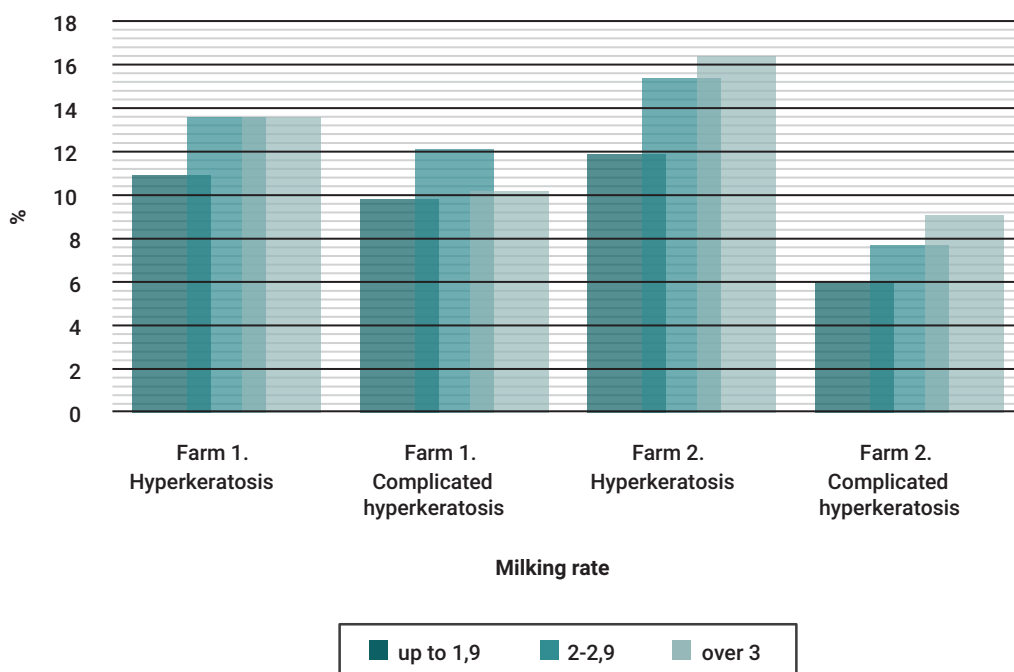


Figure 9. Effect of milking frequency on udder teat condition of cows.

The traumatic effect of milking units on mammary gland tissues was evaluated by examining 65 samples of residual milk for the presence of occult blood. The results obtained showed that when using a robotic voluntary milking system, the presence of a weakly positive reaction for hidden blood was detected in 29.4% of samples, while in cows under the tethered system and using a linear milking unit with a suspended transport system - monorail the number of positive and strongly positive samples was 2.3 times higher and amounted to 66.7% (table 1). When milking with a linear milking unit with manual removal of milking cups, there was a significant increase in the number of positive samples for hidden blood - up to 80.9% of all tested samples, which is 2.8 times higher than when milking with a robot and 1.2 times higher than when using a semi-automatic milking system.

Thus, we can conclude that the robotic milking system has a less pronounced negative impact on udder health than milking into a pipeline.

Due to the fact that periodic monitoring of udder teat condition was carried out in a number of farms, we noted the dependence of hyperkeratosis prevalence rate across the herd on the season of the year.

On a farm with tethered animals and the use of a linear milking unit for milk production, there was a 2.1-fold reduction in the level of teat lesions in the summer period (figure 10). By the middle of the autumn period, a significant increase in the number of teats with a complicated form of hyperkeratosis was recorded, reaching 23.7%, which is 4.1 times higher than in the spring and summer periods. By the end of the winter period, the number of teats with lesions in the form of hyperkeratosis remains almost unchanged, but there is a significant decrease in the number of lesions in the form of its complicated form.

Thus, it was noted that the greatest amount of teat end hyperkeratosis of the udder is registered in the autumn-winter period, which is probably due to changes in the microclimate parameter of livestock buildings and, as a consequence, a decrease in the overall resistance of the cow's organism.

At the next stage of research, the qualitative parameters of milk in cows with different udder teat conditions were analyzed. In total, 785 milk samples were examined with determination of fat, protein, lactose, somatic cells.

The research revealed that in milk samples from cows with a complicated form of hyperkeratosis there was an absolute-

Type of milking system	Equipment brand	Number of samples	Positive reaction	
			Sample	%
Linear milking machine	DeLaval Monorail	6	4	66.7
	DeLaval	42	34	80.9
Robotic milking system	Lely Astronaut A4	17	5	29.4

Table 1. Presence of hidden blood in residual milk samples.

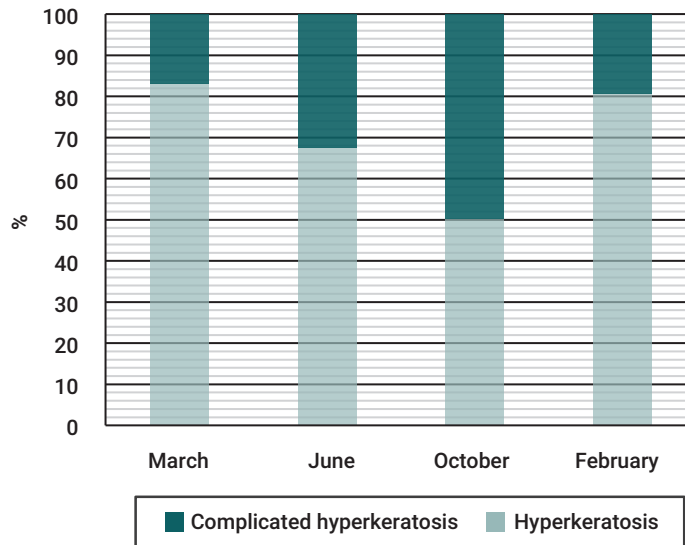


Figure 10. Prevalence of udder teat disease as a function of season when milking in a linear milking unit.

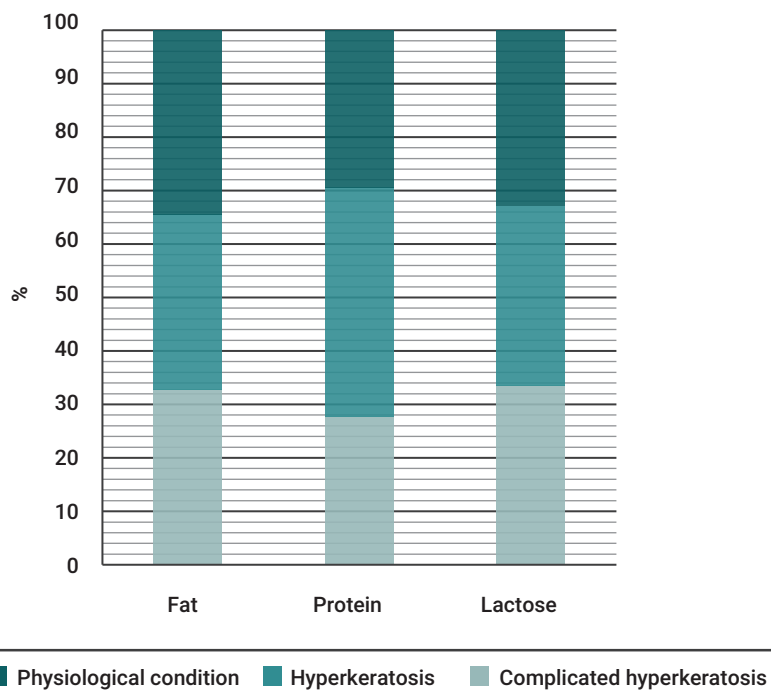


Figure 11. Results of the chemical analysis of milk.

ly reliable increase in protein content by 5.1% ($p=0.0005$), a decrease in lactose by 2.6% ($p=0.0005$) relative to cows with physiological norm. There was a high degree of reliability difference in the fat indicator - an increase of 4.5% ($p=0.1182$) relative to cows with healthy teats (figure 11).

When comparing the results obtained between the groups of cows, an absolutely reliable increase of 4.5% ($p=0.0005$) of fat level and a 3.1% (0.0081) decrease of lactose level in cows with the complicated form of teat end hyperkeratosis were re-

corded. With a high degree of reliability, there is an increase in protein level in milk of cows with the complicated form of teat end hyperkeratosis, relative to animals with its uncomplicated form by 4.6% ($p=0.2805$).

Analysis of the obtained t-criterion and the percentage ratio of the studied values allows us to assume that when cows suffer from teat end hyperkeratosis the milking reflex decreases due to unpleasant or painful sensations during milking, which affects the completeness of milking and the development of in-

flammatory udder diseases. In animals with teat end affection in the form of uncomplicated and complicated hyperkeratosis somatic cells were registered at the level of 513-736 ths./ml

In the complicated form of the disease, a reliable decrease in the lactose level by 2.6-3.1% was registered, which was accompanied by an increase in the number of somatic cells, indicating the presence of an inflammatory process or udder irritation. In addition, these differences may indicate the presence of pathological processes not related or indirectly related to udder in the animal's body.

Discussion of the results. Studies carried out in farms with different levels of milk production of cows, conditions of maintenance and milking showed that lesions in the area of the teat canal opening in the form of hyperkeratosis are widespread. At the same time, the form of hyperkeratosis complicated by radial fissures is registered in an average of 9.3% of cows, and the uncomplicated form in 18.7% of cows. These figures are higher than the average internationally accepted standards. Based on published data, a herd can be considered safe when the level of hyperkeratosis does not exceed 15%, and the presence of fissures in the apex area is registered in not more than 5% of animals (Neijenhuis, 2004). It was also noted that cows during lactation in high-yielding herds are most susceptible to this disease.

With the development of hyperkeratosis and its complicated form in the area of the nipple canal there is a significant thickening of the stratum corneum, the formation of acanthosis. There are also changes on the side of the nipple canal, corresponding to inflammation and destructive changes, which was also noted in the studies of Penry *et al.* (2017).

CONCLUSIONS

Thus, we can conclude that udder teat end diseases are widespread on farms in the Ural region. Significant changes in the skin and internal structure of the teat canal occur with pronounced hyperkeratosis of the udder teats, which may be a predisposing factor for the occurrence of mastitis.

Studies on udder health carried out on farms equipped with robotic systems and linear milking units have shown that the robotic system does have less negative impact on udder health and in particular on the development of teat end hyperkeratosis. Uncomplicated and complicated forms of hyperkeratosis averaged 23-27.5% of quarters. The reduction of permanent traumatization of udder tissues is confirmed by tests for occult blood, which showed that when using robotic milking the number of samples with apparent content of occult blood was 2 or more times lower than when milking with linear milking units.

Analysis of factors influencing the appearance of teat end hyperkeratosis revealed that with increasing productivity in cows, the risk of changes in the teat canal opening area increases significantly. Moreover, in high-yielding herds using robotic milking, the incidence of hyperkeratosis in cows is lower compared to linear units and milking parlors even when the average productivity of the herd is more than 10 thousand kg of milk per lactation. At the same time, the highest number of udder teat end pathologies was recorded when using linear milking units, which may be due to both the human factor and unstable vacuum level in the system (Mein *et al.*, 2003; Rasmussen and Hemling, 2002).

Analysis of factors influencing the appearance of teat end hyperkeratosis showed that the smallest percentage of affected animals have the first lactation with subsequent increase in the number of affected teats, which is associated with the appearance of changes in the teat canal opening and their aggravation from the beginning of the second lactation, as according to F. Neijenhuis (2004) in the presence of severe forms of hyperkeratosis, cicatricial changes are formed, in which it is impossible to restore tissues to their original state in the period of dryness (Neijenhuis, 2004).

In animals with hyperkeratosis of udder teats, a 1.5-fold increase in milk retention time was noted, indicating a decrease in the milking reflex, and an increase of 30-80 seconds in milking duration was recorded in the complicated form of hyperkeratosis relative to quarters with a physiological response to machine milking. Also, animals with hyperkeratosis had longer stays in the box during milking. With less than 5 minutes and 55 seconds in the box, hyperkeratosis was recorded on 15% of teats, and with more than 8:25 minutes, up to 41.3% of teats. Also, cows with physiological teat response had higher average and maximum milking rates.

Evaluation of the effect of milking frequency on the udder teat condition showed that with increasing frequency of milking the numbers of quarters with pathological changes in the teat canal opening increased, with the frequency of milking more than 2 times a day the number of teats with hyperkeratosis lesions in the group increased by 1.4 times and remained virtually unchanged with further increase in the frequency of milking. When the intervals between milkings are shortened, the time for tissue repair is reduced, which is a risk factor for inflammatory udder diseases (Hamann and Østerås, 1994; Rasmussen *et al.*, 2001).

Analysis of milk quality indicators in cows with different udder teat conditions showed that animals with the complicated form of hyperkeratosis showed a 5.1% increase in protein level and a decrease in lactose (2.6%), which may indicate partial destruction of epithelial cells accompanying the inflammatory process. When comparing the group of cows with hyperkeratosis with the group of cows with its complicated form, an absolutely reliable increase of fat content by 4.5% ($p=0,0005$) was registered. This also confirms the presence of an inflammatory reaction in the mammary gland tissues, as the destruction of epithelial cells facilitates the exit of fat droplets into the alveolar cavity.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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