Uterine lavage microbiological composition of dairy cows suffering from subclinical endometritis

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Abstract. The article presents the data on the dairy cows’ uterine lavage microbial composition characteristics suffering from subclinical endometritis. Subclinical endometritis is accompanied by the normal microbiota composition changes in the cow uterus and the disease-causing and opportunistic microorganisms’ growth. To perform uterine contents lavage, polystyrene disposable pipettes connected to a 50 ml disposable syringe using a plastic adapter were used. Subclinical endometritis was diagnosed in 22 cows (17.19%). Sixty-one dairy cows out of 128 (47.66%) were healthy. Microorganisms of the Enterobacteriaceae family: E. coli, P. vulgaris, S. enteritidis, pathogenic streptococci: S. agalactiae, S. pyogenes and staphylococci: S. aureus, S. epidermidis were selected. The total microbial contamination of the lavage samples was 64.72±8.80 CFU/ml. The most common microorganisms were microorganisms of the Enterobacteriaceae family: E. coli, S. enteritidis and P. vulgaris with 2069 CFU /ml. Pathogenic streptococci amounted to 1989 CFU /ml and staphylococci to 1,579 CFU /ml.

1 Introduction

Endometritis is one form of manifestation of metritis in cows. Endometritis in cattle worldwide is the most common pathology of reproductive organs in cows [1-3]. According to Parmar K H endometritis is the uterine mucosal inflammation without systemic features related to chronic postpartum uterus contamination by pathogenic bacteria [1]. This disease leads to losses due to problems with the dairy farming profitability, with reproductive losses and long-term fertility restoration [2-5].

Clinical and subclinical endometritis are differentiated. Subclinical endometritis is more difficult to diagnose due to the lack of obvious clinical features (fever, depression and vulval discharge). Subclinical endometritis is accompanied by the normal microbiota composition change (lactobacilli) in the cow’s uterus and the disease-causing and opportunistic microorganisms’ growth in it [6-7]. These pathogens stimulate the inflammation development in the uterus [8-10]. Additional factors of uterine invasion by microflora are mechanical injury during obstetrics, placental retention, contaminated with

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microorganisms’ obstetric instruments, hands and clothes. According to numerous data of foreign and national researchers the reported incidence of endometritis during lactation ranges from 7.8 to 61.6% [1-10].

The purpose of this research was to investigate the uterine microbiota of dairy cows suffering from subclinical endometritis.

2 Materials and research methods

This research was carried out at the service territory of the Novoaksaysky, Tsimlyansky and Shabalinovsky veterinary plots of the Oktyabrsky municipal district, Volgograd region of the Russian Federation. The studies were carried out on 128 dairy cows of Holstein-Friesian breed in the Volgograd region of the Russian Federation in 2020-2023. The animal owner’s written consent was obtained in advance for all studies. A vaginal mirror for cows was used for visual diagnosis of endometritis. Cows with clinical features of endometritis (mucopurulent and purulent discharge) and those treated with anti-infective drugs were eliminated from the experiment. Cows were diagnosed with subclinical endometritis by microscopy of endometrial cytological smears according to the proportion of polymorphonuclear neutrophils ≥18% on days 21-28 after calving using the methodology first described by Kasimanickam R et al (2004) [11].

To perform uterine contents lavage, polystyrene disposable pipettes "LITE" (LLC Venera-Vet®) 445 mm long were used. They were connected to a 50 ml disposable syringe by means of a plastic adapter. Lavage was performed by introducing 30 ml of warm (38-40°C) 0.9% physiological NaCl solution into the uterine cavity using the above-described device. After rectal massage of the uterus (10-15 sec), the fluid was aspirated into a syringe. Samples were transferred into sterile 50 ml screw-capped tubes (Deltalab®) and delivered to the laboratory within 3 hours. All lavage samples were numbered anonymously in sequential order.

Standard microbiological tests were conducted in the laboratory to select and identify microbial cultures using liquid and solid nutrient media: Pepted Meat Broth, Pepted Meat Agar, Egg Yolk Agar, Selenite Enrichment Broth, Serum Agar, SDS-broth, Loeffler medium, Kitt-Tarozzi medium & Muller medium. Differentiation of bacterial genera to species was performed with diagnostic plates "DS-DIF-STAIFI-16", "DS-DIF-ENTERO-24", "DS-DIF-SALMONELLA" (Ltd NPO "Diagnostic systems"®) and STREPTOtest 16 ("MicroBio" LLC®).

The total microbial contamination was determined as the sum of all isolated microorganisms of each uterine lavage sample of cow’s, expressed in colony-forming units/ml (CFU/ml).

Statistical significance was discussed using the STATISTICA 10 software (StatSoft®) applying Tukey's post hoc test for samples of different sizes.

3 Research results

The postpartum diseases distribution based on the results of diagnostics performed on 128 dairy cows is shown in Figure 1.
Fig. 1. Distribution diagram of postpartum diseases in dairy cows (n = 128): CEND - clinical endometritis, SEND - subclinical endometritis, RP - retained placenta, PC - premature calving, T - twinning, H - healthy cows.

So clinical endometritis was diagnosed in 26 cows (20.31%), subclinical endometritis was diagnosed in 22 cows (17.19%), retention of the placenta with its manual separation was determined in 12 cows (9.38%), premature calving was registered in 2 cows (1.56%) up to 280 days of pregnancy. The twins were born in 5 cases (3.91%). Sixty-one milking cows out of 128 (47.66%) were healthy.

Figure 2 shows the monitoring data of microflora from uterine contents lavage of dairy cows.

Fig. 2. Microflora monitoring from uterine contents lavage of dairy cows.

E. coli were selected on average of 68.05±23.59 CFU/ml, S. enteritidis on average of 19.52±14.43 CFU/ml and P. vulgaris an average of 16.56±6.95 CFU/ml. At the same time, E. coli were selected in 20 samples out of 22. They were absent in samples No. 6 and No. 15. S. enteritidis were identified in 21 of 22 samples. They were absent in sample No. 6. P.
*P. vulgaris* were selected in 18 of 22 samples. They were absent in samples No. 10, 19, 20, and 21.

Pathogenic streptococci *S. agalactiae* were selected for an average of 52.80±28.00 CFU/ml, *S. pyogenes* were identified on average of 51.83±23.64 CFU/ml. *S. agalactiae* were selected in 20 samples out of 22. They were absent in samples №8 and №18. *S. pyogenes* were identified in 18 of 22 samples. They were absent in samples No. 11, 12, 13, and 14.

Staphylococci were selected on average of 68.05±23.59 times, *S. aureus* identified on average of 48.73±25.80 CFU/ml and *S. epidermidis* selected on average of 28.17±18.42 CFU/ml. However, *S. aureus* were identified in all 22 samples. *S. epidermidis* were selected in 18 of 22 samples. They were absent in samples No. 5, 6, 7, and 8.

The most common microorganisms were microorganisms of the *Enterobacteriaceae* family: *E. coli*, *S. enteritidis* and *P. vulgaris* with 2069 CFU/ml. Pathogenic streptococci amounted to 1989 CFU/ml and staphylococci to 1,579 CFU/ml.

The total microbial contamination of lavage samples taken from dairy cows was also different. So, the highest indicators were observed in sample №1. The total microbial contamination of lavage sample was 389.00±55.57 CFU/ml. The lowest rates of the total microbial contamination of lavage samples taken from dairy cows were observed in sample No. 6 100±25.00 CFU/ml with the average value of total microbial contamination of lavage samples averaged 64.72±8.80 CFU/ml.

Based on the statistical processing results of the data obtained in the STATISTICA 10 software (StatSoft®), Figure 3 shows a diagram of the values distribution characteristics range of the total microbial contamination of lavage samples taken from dairy cows. The diagram graphically compares the values ranges of the individual categories of the observational results, divided into groups of microorganisms identified during microbiological examination.

**Fig. 3.** Span diagram of the characteristic distribution of the total microbial contamination values of lavage samples. Post-hoc Tukey for different value selection: Non-Outlier Max – ‼, 1Q Tukey – ▽, Median – –, Average – □, 3Q Tukey – ‼, Non-Outlier Min – ‼

As can be seen from the data in the diagram, the third quartile of Tukey's criterion was 90 CFU/ml for *E. coli*, 77 CFU/ml for *S. agalactiae*, 68 CFU/ml for *S. pyogenes*, 72 CFU/ml for *S. aureus*, 47 CFU/ml for *S. epidermidis*, 26 CFU/ml for *S. enteritidis*, and 23 CFU/ml for *P. vulgaris*. The minimum (Min) Tukey criterion was 24 CFU/ml for *E. coli*, 5 CFU/ml for *S. agalactiae*, 8 CFU/ml for *S. pyogenes*, 6 CFU/ml for *S. aureus*, 3 CFU/ml for *S. epidermidis*, 1 CFU/ml for *S. enteritidis*, and 5 CFU/ml for *P. vulgaris*. The median (Median) Tukey's criterion was 70 CFU/ml for *E. coli*, 52.5 CFU/ml for *S. agalactiae*, 59
CFU/ml for S. pyogenes, 51 CFU/ml for S. aureus, 25.5 CFU/ml for S. epidermidis, 20 CFU/ml for S. enteritidis, and 18.5 CFU/ml for P. vulgaris. The maximum (Max) Tukey criterion was 99 CFU/ml for E. coli, 98 CFU/ml for S. agalactiae, 89 CFU/ml for S. pyogenes, 84 CFU/ml for S. aureus, 63 CFU/ml for S. epidermidis, 66 CFU/ml for S. enteritidis, and 25 CFU/ml for P. vulgaris.

Tukey's criterion median values were significantly different from the mean with respect to the sample in S. pyogenes by 17.31%, in S. aureus by 21.08%, and in S. epidermidis by 12.16%. In E. coli, S. agalactiae, S. pyogenes, and P. vulgaris, Tukey's criterion median values were close to the sample mean values.

4 Discussion

Microorganisms of the family Enterobacteriaceae have been reported in other studies [1-7]. Appach M. O., Wang J. And Lu W. [9] in a review article also noted that E. coli is one of the most common microorganisms causing uterine infections in cows. It is this microorganism that was identified as the most common microorganism during our study. According to the results of histologic studies, parallel sequencing data and fluorescence study in Denmark C. C. Karstrup et al. (2017) [10], the presence of microorganisms (90.7%) associated with inflammatory processes in the uterus was found inside the endometrial tissues of cows. They also concluded that the internal environment of the uterus is not sterile.

The obtained data on the isolation of Streptococcus and Staphylococcus spp. correlate with the data we obtained from earlier studies in the Volgograd region and Saratov region of the Russian Federation [8].

5 Conclusion

Endometritis in cattle is the most common pathology of the reproductive organs in cows worldwide. Subclinical endometritis was diagnosed in 17.19% of cows. The total microbial contamination of lavage samples taken from dairy cows ranged from 389.00±55.57 CFU/ml in sample No. 1 to 100±25.00 in sample №6. S. aureus were selected in all 22 samples. The total microbial contamination of lavage samples averaged 64.72±8.80 CFU/ml. The most common microorganisms were microorganisms of the Enterobacteriaceae family: E. coli, S. enteritidis и P. vulgaris.

Thus, the microbial contamination monitoring of lavage samples is a necessary diagnostic method for subclinical endometritis in dairy cows.

References