

# Influence of cow reproductive tract microbiota on formation of calf upper respiratory tract microbiota

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**Abstract.** Diagnosis of latent endometritis and predicting the effect of the inflammatory process on the course of pregnancy and its outcome is a necessary measure when conducting clinical examination of animals. Lack of diagnostic criteria, including microbiological one, cause difficulties for veterinarians in assessing latent endometritis. Often, animals suffering from latent inflammation of the reproductive tract are infertile and cannot be treated. And from cows with dysbiosis of the genital tract, sick young animals are born, lagging behind in growth and development. The aim of the work was to establish the relationship between the genital tract microbiome, the number and species composition of commensals and opportunistic microorganisms on the ability to carry pregnancy. Washes from the oral and nasal cavities were taken from the young born to study the microbiome: lactic acid microorganisms in combination with opportunistic pathogens. As a result of the research, it was found that animals with a low number of lactic acid microorganisms and an increased titer of opportunistic microorganisms were infertile, which led to their culling. In young animals, they are present in the respiratory tract in all samples, together with coccal microorganisms. The study of lactic acid microorganisms is advisable in the diagnosis of inflammatory processes of the reproductive tract and upper respiratory tract.

## 1 Introduction

Inflammations of cattle pelvic organs cause economic damage to agricultural enterprises due to early culling of cows, shortage or receiving sick non-viable young animals. Microorganisms are transmitted from the parent stock to the offspring one in two ways: intrauterinely and/or exogenously through objects of the external environment. Commonly, absence of pronounced clinical manifestations, lead to the fact that latent inflammation of the pelvic organs is not provided with proper and timely treatment, and consequently to intrauterine infection of calves. Sick livestock releases pathogens into the environment with

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physiological and pathological secretions of the upper respiratory tract, gastrointestinal tract, urinary tract, thereby contributing to echogenic infection of healthy animals.

Timely diagnosis of latent endometritis can help to avoid culling of animals. Considering that this disease has a microbial etiology, it is necessary to take into account the species and biochemical properties of the microbial community when carrying out diagnostic measures. At the same time, the state of the indigenous microflora, its quantity and species composition is of importance as well.

The aim of the study is to establish the relationship between the composition and properties of the microflora of the cows' genital tract and the microflora biotypes' colonization of the calves obtained from these cows.

## 2 Materials and methods

A study of swabs from the cervical canal of cows of different ages was carried out in the amount of 20 samples before artificial insemination. Sampling is done with a sterile cotton swab from the cervical canal.

From 10 calves, at the age of 1-2 months, were washed with a sterile swab from the mucous membranes of the pharynx and nose. A visual examination of the animals revealed that the animals are clinically healthy, the appetite is preserved, the integrity of the mucous membranes of the pharynx and nose is not disturbed, and the color of the mucous membranes is pale pink.

After sampling in a laboratory, successive 10-fold dilutions from 10<sup>1</sup> to 10<sup>10</sup> are prepared. Dilutions of 0.1 ml are inoculated into sectors of the following plate media: meat-peptone agar with the addition of 5% erythrocytes of ram, Endo, Ploskirev, yolk-milk-salt agar, Enterokokkagar, Saburo and Zeissler agar with incubation of the latter in an anaerostat. At the same time, inoculations are carried out on liquid, poured in a high column into test tubes, media: Blikfeldt and Bifidum medium, sugar broth. Crops are grown at 37 ° C for 24-72 hours (on Saburo the temperature is 24 ° C - 5 days).

## 3 Literature review

Functional and inflammatory diseases of the genital organs of cows, such as cervicitis and endometritis, cause significant economic damage to farms. Taking into account the fact that every year more than 30% of cows are asymptomatic with endometritis, the inflammatory process in these animals turns into a chronic stage, leading to infertility. There is a hypothesis that the development of inflammatory diseases is caused by changes in the ratio of representatives of normal microorganisms and opportunistic pathogens, which together constitute the microbiome of the genital tract [2, 3].

In various literary sources, information on the number and species composition of representatives of the resident microflora differs. But the authors state that the normal microflora of the genital organs is diverse and is represented by aerobes, facultative and strict anaerobes. The basis of the normal microflora of the genital tract is microorganisms of the genus *Lactobacillus* spp. It is generally accepted that normally lactobacilli are sown in 80–100% of cases in an amount of more than 106 CFU/ml. Research Gordeeva I.V. (2005) show that normal microbiocenosis of the genital tract of clinically healthy cows contains: bifidobacteria (73.5%), coagulase-negative *S. xylosus* (14.6%), lactobacilli (4.2%), anaerobic microorganisms *Bacteroides* spp. (2.9%) and fungi of the genus *Candida* (2.1%) [1, 5, 15, 16, 18, 19].

Against the background of imbalance between the resident and opportunistic microflora of the genital tract, cervicitis and endometritis develop. With inflammation of the genital

tract, a sharp decrease in the number of lactobacilli and bifidobacteria is observed, to their complete absence, and opportunistic microorganisms act in dominant positions with the formation of polymicrobial associations. Most often, when the microocenosis is disturbed, bacteria of the genera *Staphylococcus* spp. and *Candida* spp. develop quickly. In clinically sick animals, several species were found in the reproductive tract: *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Proteus vulgaris* [5, 8, 12, 13, 14, 16].

L.N. Kosolovich (2013), examining cows with acute catarrhal-purulent endometritis, found that the main causative agents of the disease are: *S. aureus* (81%); *S. pyogenes* (12%), *E. coli* and associations of these microorganisms (5%), which is consistent with the data of many authors. For example, I. Gordeeva (2005) determined that acute endometritis occurs in the presence of polymicrobial associations consisting of *S. pyogenes* (40.2%), *S. aureus* (18.3%) and *P. vulgaris* (0.01%), with chronic form - *P. aeruginosa* (11.0%) and *S. aureus* (2.3%), with subclinical - *P. aeruginosa* (2.5%) and *S. aureus* (18.2%). Microorganisms of the species *S. epidermidis*, *S. xylosus*, fungi of the genus *Candida*, as well as anaerobic *Bacteroides* spp. and *Fusobacterium* spp. were found regardless of the form of the course of the disease. With the course of the inflammatory process, an increase in the number of opportunistic microflora and the proportion of gram-negative bacteria in its composition was observed [4, 7, 11, 17, 19, 22].

The research by Lyzikova Yu.A. (2005) confirms the hypothesis about the influence of dysbiotic states on the manifestation of inflammatory processes: in chronic endometritis, gram-positive cocal microorganisms prevail in the endometrial tissues, while in the body where chronic endometritis is not diagnosed; lactobacilli are the prevailing group of microorganisms [8, 21].

In this case, it is advisable to resort to laboratory diagnostics of the microbiome of the cervical canal and endometrium, which consists in studying the change in the ratio of the number of microbiological indicators of normal representatives of the microbiota to opportunistic microorganisms [6, 9, 24, 25].

It should be borne in mind that contamination with opportunistic strains of microorganisms occurs much easier with dysbiotic changes in the microbial community of biotopes. An increase in the number of transient microbiota and a decrease in the number of obligate microbiota indicate an equalization of the importance of members of the microbial community. Thus, inflammatory diseases of the reproductive tract are characterized by changes in the qualitative and quantitative characteristics of the microbial community. In this case, the study of the microbiome of biotopes shows an increase in the total number of bacteria along with an increase in the frequency of occurrence of various microbial associations [10, 26].

Fundamental importance for the development of infectious pathology, in addition to reducing the amount of obligate microflora, has the presence of pathogenic properties in transient microorganisms. Degtyareva S.S. (2008) showed that cultures with hemolytic activity (35.5% of cultures) gave a positive plasma coagulation reaction (18.3% of cultures), and 51% of cultures were pathogenic for laboratory animals [6].

When studying diseases of the reproductive organs of cows, it is necessary to take into account the likelihood of intrauterine infection of calves, with the subsequent long-term persistence of the pathogen and the development of diseases of the respiratory and gastrointestinal tracts. Perinatal transmission of pathogens of infectious diseases in farm animals is confirmed by many studies [23].

For example, S.A. Aksenenko. (2006) found that calves with gastrointestinal tract pathology are born from mothers with endometritis 2 times more often than from healthy cows [1].

In addition, the composition of the nasopharyngeal microbiota in dairy calves is directly proportional to the mother's vaginal microbiota. The resistance of animals to respiratory

diseases depends on the composition and species diversity of microorganisms. In the presence of microorganisms of the genus *Lactobacillus* in the respiratory tract, animals have no signs of respiratory diseases, while the presence of bacterial pathogens such as *Mycoplasma bovis*, *Mannheimia haemolytica* or *Pasteurella multocida* causes upper respiratory tract disease (URT) in cattle. The respiratory tract of cattle is represented by a complex bacterial ecosystem known as the bacterial microbiome. The microbiome develops and forms under the influence of many factors: the mother's vaginal microbiota, environment, age, feeding, treatment used and stressful events [17, 23, 26].

The respiratory tract of cattle is colonized by various microorganisms immediately after birth. Airway colonization in dairy calves begins immediately after birth and develops rapidly during the first weeks of life. The number of bacteria in the nasopharynx increases from birth to 14 days of age, and then slightly decreases until the 35th day or remains unchanged until 42 days.

The microbiome of the nasopharynx of calves at the age of 3 and 14 days is characterized by the presence of proteobacteria up to 70% of the specific weight of the total number of microorganisms. After the 14th day, the species diversity increases, starting to include such divisions of microorganisms as Tenericutes, Firmicutes, Actinobacteria and Bacteroidetes. The most numerous genera of bacteria in the nasopharynx of dairy calves are *Mannheimia*, *Moraxella*, *Mycoplasma*, *Psychrobacter* and *Pseudomonas*. The relative abundance of these genera changes over time: the abundance of *Moraxella* decreases between the 14th and 35th day, and the relative abundance of *Mannheimia* and *Mycoplasma* simultaneously increases significantly. Thus, the nasopharyngeal microbiota of cattle changes significantly between weaning and the first weeks of feeding. This evolution may explain why cattle are more susceptible to respiratory tract disease during the first 40-60 days of feeding, as unstable microbiota is less resistant to colonization by pathogens [14].

When studying diseases of the upper respiratory tract and lungs of cattle, most scientists pay attention only to pathogenic biological agents, without studying the role of beneficial microorganisms in the immune response during the development of an infectious process. As a result, there is not enough information in the information field about the structure of the microbiota of the oropharynx and nasopharynx and its role in maintaining health.

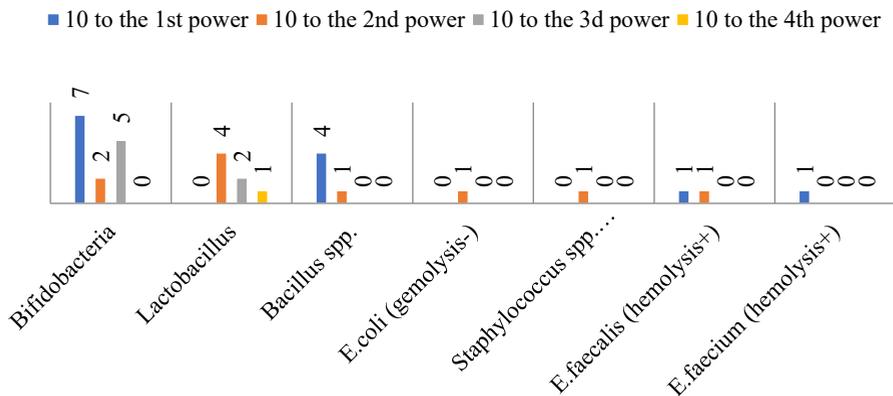
Various studies show that pneumonia develops in cattle, when the number of commensal bacteria in the URT organs decreases. Normally, the types Proteobacteria and Firmicutes predominate, with smaller proportions of Actinobacter, Bacteroidetes and Tenericutes. Of the dominant genera, the most frequently identified are *Mycoplasma*, *Moraxella*, *Pasteurella*, *Psychrobacter*, and *Mannheimia* [15, 23].

It is becoming more and more obvious that the pathogenesis of infectious diseases of the reproductive and respiratory tract organs is influenced by several factors, including the interaction of pathogens with commensals. This interaction was the subject of this study.

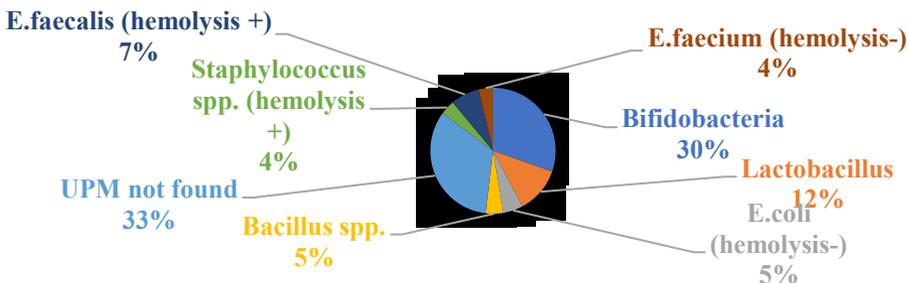
## 4 Results

When examining the microflora of the cervical canal of cows, it was found that bifidobacteria are found in an amount of  $10^1$  in 7 samples,  $10^2$  in 2 samples,  $10^3$  in 5 samples; lactobacilli in the amount of  $10^1$  were not found,  $10^2$  - in 4 samples,  $10^3$  - in 2 samples,  $10^4$  - in 1 sample; microorganisms of the family *Bacillus* spp. cultivated in a dilution of  $10^1$  in 4 samples, in a dilution of  $10^2$  in 1 sample; *E. coli* without hemolytic properties was cultivated in 1 sample at a dilution of  $10^2$ . The presence of coccal gram-positive microflora was also established: microorganisms of the family *Staphylococcus* spp. with pronounced hemolytic properties were found in 1 sample at a dilution of  $10^2$ ;

*E. faecalis* with hemolytic properties was found in 2 samples at dilutions  $10^1$  and  $10^2$ ; *E. faecium* with hemolytic properties - in 1 sample at a dilution of  $10^1$  (Fig. 1).

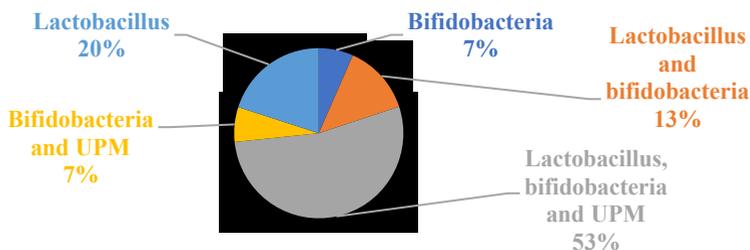


**Fig. 1.** Number of microorganisms isolated from samples of cow cervical canal.



**Fig. 2.** Specific weight of microorganisms in samples of cow cervical canal.

The specific weight of microorganisms in the samples is: bifidobacteria - 30% of the samples; lactobacilli - 12% of samples; *Bacillus* spp. - in 5% of samples; *E. coli* - in 5% of samples; microorganisms of the family *Staphylococcus* spp. - in 4% of samples; *E. faecalis* - in 7% of samples; *E. faecium* - in 4% of samples; CPB was not found in 33% of samples (Fig. 2).



**Fig. 3.** Specific weight of samples containing associations of microorganisms and microorganisms in a monoculture.

Microorganisms were cultured in associations and monocultures. Representatives of the normal flora, together with CPB, were isolated in 53% of the samples; bifidobacteria and CPB were contained in 7% of samples; monocultures of lactobacilli are isolated from 20% of samples; monoculture of bifidobacteria - in 7% of samples; only representatives of normal flora - in 13% of samples (Fig. 3).

It was found that out of 4 cows, without clinical signs of inflammation, from the farm of the Prokopyevsky district, 2 heads were not inseminated and as of the reporting date they are not fertile. When analyzing the protocols of laboratory tests, it was found that in these heads in the cervical canal, the amount of CPB, namely microorganisms of the *Bacillus* spp. Family, exceeds the amount of normal flora, or there is no normal flora at all. At the same time, in 2 heads, which at the reporting date are pregnant, the CPB was either absent, or the amount of normal flora exceeded the number of CPB.

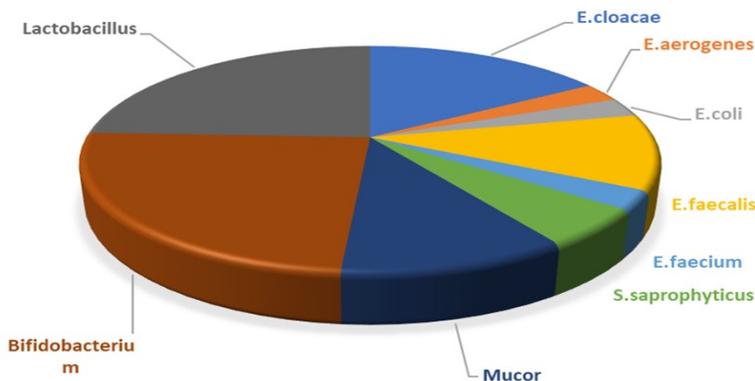
When sampling from the cervical canal of cows in the Belovsky region, 5 heads showed the presence of clinical signs of cervicitis and endometritis: hyperemia and swelling of the mucous membranes, the presence of purulent exudate with blood streaks. Analysis of these samples showed the presence of CPB: *E. coli* without hemolytic properties; *E. faecium* and *E. faecalis* with hemolytic properties (Table 1).

**Table 1.** Comparison of genital tract microbiome before artificial insemination and pregnancy

Sample number	Quantitative indicator of the normal flora representatives	Quantitative indicator of CPB representatives	Insemination performance
1	Bifidobacteria – $10^1$ ,	<i>Bacillus</i> spp. - $10^2$	No pregnancy
2	absent	<i>Bacillus</i> spp. - $10^1$	No pregnancy
3	Bifidobacteria – $10^3$	<i>Bacillus</i> spp. – $10^1$	Pregnancy
4	Bifidobacteria – $10^1$	absent	Pregnancy
5	Bifidobacteria – $10^2$	absent	Pregnancy
6	Bifidobacteria – $10^1$	absent	Pregnancy
7	Bifidobacteria – $10^1$ Lactobacillus - $10^2$	<i>Bacillus</i> spp. – $10^1$ <i>E. coli</i> (gem-) - $10^1$	Pregnancy
8	Bifidobacteria – $10^1$ Lactobacillus - $10^2$	<i>Bacillus</i> spp. – $10^1$	Pregnancy
9	Bifidobacteria – $10^3$ Lactobacillus - $10^4$	<i>E. faecium</i> (gem+) - $10^1$	Animal is culled
10	Bifidobacteria – $10^1$ Lactobacillus - $10^3$	<i>Bacillus</i> spp. – $10^1$ <i>S. cohnii</i> – $10^2$	Animal is culled
11	Bifidobacteria – $10^3$	absent	Pregnancy
12	absent	absent	Pregnancy
13	Lactobacillus – $10^1$	absent	Pregnancy
14	absent	<i>Bacillus</i> spp. – $10^1$	No pregnancy
15	Bifidobacteria – $10^2$ Lactobacillus – $10^2$	absent	Pregnancy
16	Bifidobacteria – $10^2$ Lactobacillus – $10^2$	<i>E. faecalis</i> (gem+) - $10^1$	No pregnancy
17	Bifidobacteria – $10^3$ Lactobacillus – $10^3$	absent	Pregnancy
18	Bifidobacteria – $10^1$	absent	Pregnancy
19	Lactobacillus – $10^1$	absent	Pregnancy
20	Bifidobacteria – $10^1$ Lactobacillus – $10^1$	<i>E. faecalis</i> (gem+) - $10^1$	No pregnancy

In samples from the throat of calves, the presence of the following opportunistic microorganisms (CPB) was established:

- Enterobacteriaceae: *E. cloacae* - 60%, incl. 5 cultures (83.3%) with hemolytic properties; *E.aerogenes* 10%; *E. coli* - 10% with hemolytic properties (100%);
- Enterococcusceae: *E.faecalis* - 40%, where 4 cultures (100%) with hemolytic properties; *E.faecium* - 10% with hemolytic properties (100%);
- Staphylococcusceae: *S. saprophyticus* - 20%;
- microscopic fungi: *Mucor* - 40% (Fig. 4).



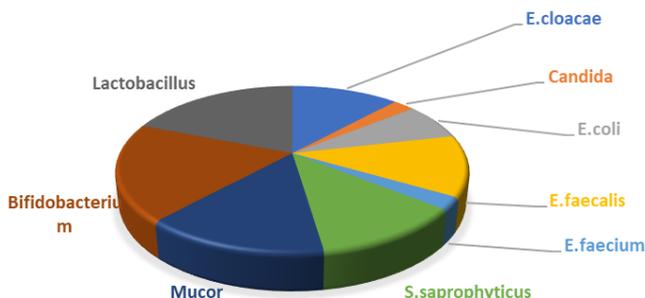
**Fig. 4.** Specific weight of microorganisms in samples from calf throat.

From the throat of some representatives of the CPB, the following quantitative characteristics were established:

- *E.cloacae* –  $10^3$  -20% of samples;  $10^6$  – 10%;
- *S.saprophyticus* –  $10^1$  -10%;
- *Mucor* –  $10^1$  – 30%,  $10^2$  – 10%,  $10^3$  – 10%.

In samples from the nasal cavity, CPB are presented in the following quantities:

- *E.cloacae* –  $10^4$  -10%;
- *E.faecalis* –  $10^1$  – 30%;
- *Mucor* –  $10^1$  – 20%,  $10^2$  – 10%,  $10^3$  – 30%;
- *S.saprophyticus* –  $10^1$  -10% (Fig.5).



**Fig. 5.** Specific weight of microorganisms in samples from calf nasal cavity

In samples from the nasal mucosa, the presence of the following CPB was adopted:

- Enterobacteriaceae: *E.cloacae* – 50%, incl. 4 cultures (80%) with hemolytic properties; *E. coli* - 30%, incl. 2 cultures with hemolytic properties (66.6%);
- Staphylococcusceae: *S.saprophyticus* – 50%;
- Enterococcusceae: *E.faecalis* – 50%, where 4 cultures (80%) with hemolytic properties; *E.faecium* - 10% with hemolytic properties (100%);

- microscopic fungi: Mucor – 60%, Candida – 10%.

Normal microflora in the pharynx is represented by lactic acid microorganisms of childbirth:

1. Bifidobacterium: in quantities of  $10^1$  – 10%;  $10^3$  – 80%.
2. Lactobacillus: in quantities of  $10^1$  – 10%,  $10^3$  – 70%,  $10^6$  – 10%.

In the nasal cavity, lactic acid microorganisms are contained in quantities:

1. Bifidobacterium: in quantities of  $10^1$  – 30%;  $10^3$  – 50%.
2. Lactobacillus: in quantities of  $10^1$  – 40%,  $10^3$  – 40%.

In most samples from the pharynx and nose (70% each), lacto- and bifidobacteria were found in a consortium with each other. In 10% of samples from the throat and 10% of samples from the nose, only representatives of the genus Bifidobacterium were found. The presence of Lactobacillus in 10% of samples was also established from the pharynx and nose.

Representatives of normal flora in 90% of samples from the pharynx and nasal cavity were in consortium with the UPM.

In 10% of samples from the pharynx, only bifidobacteria and lactobacilli with microscopic fungi of the genus Mucor were found.

In 10% of the nasal cavity, only the presence of UPM without representatives of indigenous microflora was found.

The most common association of microorganisms in samples from the pharynx (30%) and nasal cavity (20%) is represented by the following microorganisms: Mucor + E. cloacae + E. faecalis + Bifidobacterium + Lactobacillus.

**Table 2.** Microbial associations in throat samples from calves.

№	Microbial Association	Number of samples
1	E.cloacae + E.faecalis + Bifidobacterium + Lactobacillus	1
2	Mucor + E.cloacae + S.saprophyticus + Bifidobacterium + Lactobacillus	1
3	E.coli + Bifidobacterium + Lactobacillus	1
4	E.cloacae + S.saprophyticus + E.faecalis + Bifidobacterium + Lactobacillus	1
5	Mucor + E.cloacae + E.faecalis + Bifidobacterium + Lactobacillus	3
6	Mucor + Bifidobacterium + Lactobacillus	1
7	E.aerogenes + E.faecalis + E.faecium+ Lactobacillus	1
8	Bifidobacterium + Lactobacillus	1

**Table 3.** Microbial associations in nasal samples from calves.

№	Microbial Association	Number of samples
1	S.saprophyticus + E.faecalis + Bifidobacterium + Lactobacillus	1
2	Mucor + E.coli + E.faecium + Bifidobacterium	1
3	Mucor +E.coli + S.saprophyticus + E.faecalis	1
4	E.cloacae + S.saprophyticus + E.faecalis + Lactobacillus	1
5	Mucor + E.cloacae + E.faecalis + Bifidobacterium + Lactobacillus	2
6	Mucor + E.cloacae + Bifidobacterium + Lactobacillus	1
7	Candida+ Lactobacillus+ Bifidobacterium	1
8	Mucor + E.cloacae + S.saprophyticus+ Bifidobacterium + Lactobacillus	1
9	Mucor +E.coli + S.saprophyticus + Bifidobacterium + Lactobacillus	1

## 5 Discussion

1. The amount of normal microflora of the reproductive tract of cows does not exceed  $10^4$ .

2. The number of CPB does not exceed the number of  $10^2$ .

3. CPB is represented by microorganisms: *Bacillus* spp.; *Staphylococcus* spp.; *E. coli*; *E. faecium*; *E. faecalis*.

4. *E. faecium*; *E. faecalis*; *Staphylococcus* spp. possess hemolytic properties, which indicates the possibility of causing an infectious process.

5. A relationship has been established between the content of normal flora, CPB of the reproductive tract and the ability to reproduce: artificial insemination of animals with a quantitative content of CPB exceeding the amount of normal flora turned out to be ineffective.

6. The number of lacto- and bifidobacteria in the mouth and nasal cavity of calves reaches  $10^3$ .

The dominant representative of CPB is *E. cloacae*, reaching  $10^6$  in the nasal cavity and  $10^4$  in the oral cavity.

The microbial composition of the oral and nasal cavities of healthy animals is similar in qualitative and quantitative characteristics.

7. In the oral and nasal cavities, the association of bifidobacteria, lactobacilli and opportunistic microorganisms prevails.

The most common association of microorganisms in samples from the pharynx (30%) and nasal cavity (20%) is represented by the following microorganisms: *Mucor* + *E. cloacae* + *E. faecalis* + *Bifidobacterium* + *Lactobacillus*.

## 6 Conclusion

The course of the infectious process of the reproductive tract is influenced not only by the presence of the pathogen itself, but also by the presence of lactic acid microorganisms that have a protective effect in the macroorganism. An excess of the amount of CPB with a simultaneous decrease in commensal microorganisms is an unfavorable indicator for the further fertilization of animals.

In young cattle, without clinical symptoms of respiratory tract diseases, the microbiome of the upper respiratory tract consists mainly of lactic acid microorganisms: *Bifidobacterium* spp., *Lactobacillus* spp. The specific gravity of CPB, represented mainly by coccal microflora, does not exceed the specific gravity of lactic acid microorganisms.

When diagnosing diseases of the reproductive tract and upper respiratory tract using a microbiological method, it is necessary to take into account the presence of lactic acid microorganisms, along with the determination of pathogenic microorganisms.

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