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PARTICULAR MORPHOLOGICAL, MICROBIOLOGICAL, BIOCHEMICAL DATA OF ORAL AND KREVIKULAR FLUIDES IN CLINICALLY HEALTHY DOGS

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The results of laboratory tests of oral and krevicular fluids and in clinically healthy dogs (morphological composition, microbiological and biochemical parameters). On the basis of comprehensive studies of the referred to above substrates it has been satisfied: the number and species of microorganisms landform, clarified cellular composition (percentage: desquamative pavement epithelium, leukocytes - lymphocytes, neutrophils and salivary corpuscles), biochemical parameters: aspartate aminotransferase activity, alanine aminotransferase, alkaline phosphatase, lysozyme, and also the contents of crude protein, cholesterol, whole *calcium, electrolytes (sodium, potassium, chloride) and pH.*

Keywords: *a dog, oral liquid, krevikulyarna liquid, cellular and microbiological composition, biochemical parameters.*

Statement of an objective. Among the numerous preventive measures the first rank function of hygiene is the correct oral supervision. Provided to be, it is not so easy as it might seem ex facte. It is known, that oral cavity microflora and influence of individual factors of nonspecific protection impact on pathogenesis of inflammatory processes in periodontium and the course of the inflammatory reaction. At the present stage of development of veterinary dentistry there is only a small amount of basic research is devoted to the study of periodontal disease in which the authors have found the role of oral microflora in the pathogenesis of periodontal inflammation and the effect of individual factors of nonspecific protection in the course of the inflammatory response [1, 3, 6 , 16].

Analysis of major studies and publications which discuss the objects. In humane dentistry there are message about feasibility of studies in order to clarify the dynamics of cellular, biochemical and immunological parameters and microbiological - krevicular and oral fluids to enhance therapeutic effectiveness in periodontal inflammation [4, 14].

Note, however, that borrowing data in dentistry of humane medicine is currently incorrect. Therefore there is an urgent need to investigate these parameters in clinically healthy patients with periodontal disease and dogs as through in-depth study of these parameters in oral and krevicular fluids and one can make early diagnosis of disease pathogenesis and justify treatment plan.

Objective: to prove the feasibility of study of oral and krevicular fluids and in clinically healthy dogs.

Research objectives: to find a number of morphological microbiological and biochemical parameters of oral krevicular fluids in clinically healthy dogs.

Materials and methods. Studies were conducted on the base of the Department of Surgery and Obstetrics in Poltava State Agrarian Academy. For ten-days monitoring parameters Triassic and oral and krevicular fluid were selected in five clinically healthy (at the time of the study) mongrel dogs 1-1.5 years of age weighing 15-20 kg to detect specific morphological, biochemical and microbiological parameters.

Oral and krevicular fluids were sampled in the morning on an empty stomach. Oral fluid samples for microbiological, morphological, biochemical studies were obtained as follows: the liquid was taken in a sterile test tube with 2 ml syringe system "Lyuera" without needles. Surrounding tissue was dried with sterile gauze tampons before sampling. In laboratory research material was brought into culture media yolk-salt agar, Endo agar, Saburo agar and 5% blood agar [9].

Counting the number of colony forming units was performed by secretory inoculation according to Gould [12]. Species identification of microorganisms was performed by Burge determinant [7].

Morphological study of oral fluid was carried out in two ways: to calculate the total number of cells, selected oral fluid was placed in Türk solution at a ratio of 20:1, and then was stirred. The resulting solution we filled the chamber of Goryaev. The total number of cellular elements were counted in 100 large squares [4, 10].

To install the components of oral fluid, differentiation of desquamative pavement epithelium, salivary corpuscles, lymphocytes, neutrophils, a drop of collected oral fluid was fixed on a slide stained according to Romanovsky-Himza. Later the percentage was calculated by one-sample method [11].

Krevicular fluid samples for microbiological and morphological studies was obtained according to methods of S. Eryna, S. Dyachkova and also E. Zhulev, A. Serov. [5, 8], adapted by us by taking samples from the gingival groove approximative surfaces of teeth using sterile endodontic paper points ISO 30 (Meta Biomed, United Kindom) previously the surrounding tissue were drained with sterile gauze tampons, 10 points were administered to the bottom of a groove and left there for 5 minutes. Then, microbiology samples were transferred into a sterile test tube containing transport medium and the mixture was brought to the laboratory and for morphological studies points was transferred to a test tube containing 200 ml of physiological sodium chloride solution, was left for 30 minutes to flush its components, then the points were removed and within two hours research was conducted (to avoid destruction of the material).

Investigating morphological composition of krevicular fluid the total number and percentage of cells were determined. To determine the total quality of gingival fluid, diluted in a solution of NaCl, was placed in solution of Türk at a ratio of 10:1, then was stirred and brought to the camera of Goryaev. Cellular elements were counted throughout the area of the grid of chamber [10].

To determine the species composition of cells - desquamative pavement epithelium and white blood cells (lymphocytes, neutrophils) - a drop of gingival fluid, diluted in a solution of NaCl, was fixed on a slide stained according to Romanovsky-Himza. The number of cellular elements throughout the area of glass was counted, and then forms of leukocytes were differentiated [2, 8].

Determination of the intensity of gingival fluid discharge was performed by the method of [15].

Biochemical studies of oral fluid were performed on the following parameters: AsAT, AlAT, ALP, and also the contents of crude protein, cholesterol, whole calcium, electrolytes (sodium, potassium, chloride) and pH. semi-automatic biochemical analyzer BA 88, Mindray (DPRK) was used for this test.

Definition lysozyme activity of oral fluid was tested by a photoelectrocolorimeter (CPK-3) method in the modification of zoohygiene UNDIEV, but as a test culture was used M. Lisodeicticus (Strain 2655) [13].

The experimental material worked on by variation statistics with the definition of arithmetic (M) and standard deviations (m).

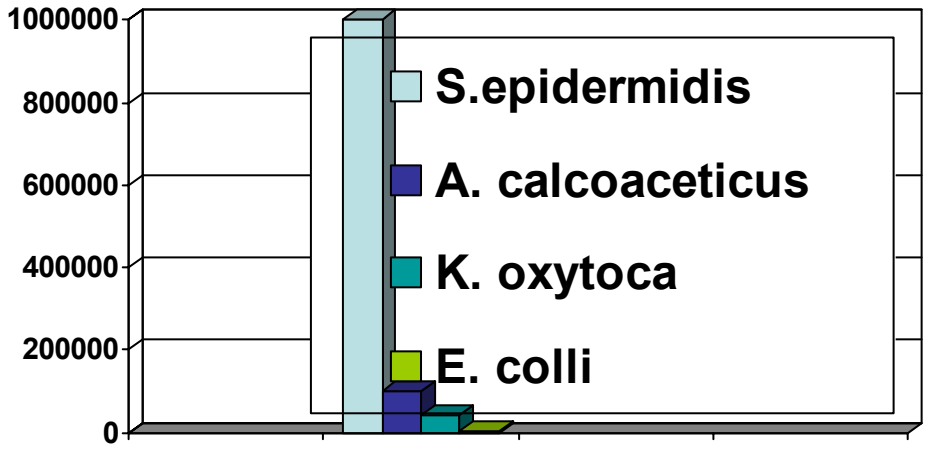
Studies: As a result of laboratory tests of selected oral fluid samples we received the growth of microorganisms on nutrient media genus Staphylococcus (S. epidermidis), Acinetobacter (A. salcoaceticus), Klebsiella (K. oxytoca), Esherichia (E. colli).

We found that the yolk-salt agar growth recorded S. epidermidis as a whitish smooth convex colonies.

In Endo identified bacteria species A. calcoaceticus, K. oxytoca, E. colli. The first type germinated as mucous pink colonies with smooth edges in diameter 2.5 mm. Species K. oxytoca germinated on nutrient medium in the form of round pink mucous colonies with smooth edges from 0.5 to 2 mm (Table 1) and species E. solli was diagnosed on the basis of the formation of red colonies with smooth rounded edges with a characteristic metallic luster and the size of daily colonies was 2 mm.

These types of microbial growth increased by 5% on blood agar. For example, type S. epidermidis on blood agar germinated as white colonies with smooth edges that have the correct round shape, type A. calcoaceticus germinated as small whitish opaque colonies, round with smooth edges. The growth of K. oxytoca was noted germination of gray mucous rounded colonies measuring 3 mm in diameter, and E. colli - rounded, gray colonies 2 mm in diameter.

By counting the number of 1 ml liquid we obtained the following results: S. epidermidis - 10^6, A. calcoaceticus - 10^5, K. oxytoca – $5x10^4$, and E. colli – $5x10^3$ CFU (Fig. 1).



The number of colony-forming units obtained from the samples of oral fluid

1. Composition of microorganisms discharged from the samples of oral fluid during the cultivation on nutrient mediums (N=5)

SPECIES OF MICROORGANISM	MEDIUMS			
	YOLK-SALT AGAR	ENDO	SABURO	5 % BLOOD AGAR
S. EPIDERMIDIS	+	–	–	+
A. CALCOACETICUS	–	+	–	+
K. OXYTOCA	–	+	–	+
E. COLLI	–	+	–	+

In the process of samples inoculation of krevicular fluid from healthy individuals we obtained Endo growth of bacteria species E. colli - 10^4 CFU on the nutrient medium.

Analyzing the results of research (quantitative characterization and species identification of microorganisms), we can state the following: microbiological composition of oral and krevicular fluid is not homogeneous. Thus, the study identified four types of microorganisms in the oral fluid and one in the gingival fluid and it has been found that they are a group of opportunistic-pathogenic microorganisms.

Counting corpuscles of oral liquid in the chamber of Goryaeva we found that in clinically healthy animals total amount of leukocytes was 8,20 ± 1,07 G/l, the total number of desquamative epithelium cells was 13,20 ± 1,72 units 100 large squares. Accordingly, in krevicular fluid first index was 5,20 ± 0,43 G/l, and the second - 6,80 ± 0,86 units throughout the area of the grid of chamber.

In the process of counting corpuscles in stained smears obtained from oral fluid, we found that the percentage of desquamative pavement epithelium was about 44,80 ± 3,22; salivary cells 4,0 ± 0,86; lymphocytes 5,0 ± 1,50 and neutrophils 29,40 ± 5,36.

In smears made from krevicular fluid we did not count 100 cells: the average number of pavement epithelium was 12,20 ± 3,86; lymphocytes 3,60 ± 1,07; neutrophils 12,40 ± 2,14 units throughout the area of the grid in stained smears. At the stage of setting the intensity of gingival fluid discharge found that its performance in healthy animals were 4,20 ± 0,21 mg within 5 minutes.

In addition, according to the task, we conducted biochemical studies of oral fluid samples from healthy animals. The results are presented in Table 2.

2. Biochemical composition of oral fluid in clinically healthy dogs

INDEX	ANIMALS N = 5
AST, U/L	31,80±4,29
ALT, U/L	20,40±4,29
LP, U/L	55,40±13,73
LYSOZYME %	10,05±1,79
PH	7,63±0,14
CRUDE PROTEIN, G/L	0,19±0,12
CHOLESTEROL, MMOL/L	0,26±0,06
SODIUM, MMOL/L	81,78±2,23
POTASSIUM, MMOL/L	16,98±0,43
CALCIUM, MMOL/L	2,62±0,20
CHLORINE, MMOL/L	46,54±3,37

Conclusions: In the process of laboratory tests of oral and krevicular fluids and in clinically healthy dogs it has been found: quantity, species composition, cellular composition (percentage: :desquamative pavement epithelium, white blood cells - lymphocytes, neutrophils and salivary corpuscles) biochemical parameters: aspartate aminotransferase activity, alanine aminotransferase, alkaline phosphatase, lysozyme, and also the contents of crude protein, cholesterol, whole calcium, electrolytes (sodium, potassium, chloride) and pH.

In perspective, using the referred to above parameters in surgery will make possible to make prevention of oral cavity diseases in dogs, to diagnose more informatively, to start the treatment reasonable and pathogeneticaly substantiated at early stage.

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