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The Microbiocenosis Analysis Of Suppurative-Necrotic Ulcers In The Area Of Hooves In Cows By PCR Method (Real – Time).

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ABSTRACT

The microbial landscape has been studied in this work isolated from ulcer lesions in the area of hooves in cows when using the PCR method in real time. To identify DNA "A reagents kit for DNA identification from biological samples" was used (Litekh, Moscow). The lysis of bacterial cells, DNA sorption on silica gel and DNA washing with an alcohol and saline buffer was the cornerstone of this method. A kit SEPTOSKRIN for PCR diagnostics was used for studies. 3 groups of cattle were formed up to 10 heads with the diagnosis "suppurative - necrotic ulcer in the field of hooves". Treatment was performed according to the phases of a lesion process: in a hydration phase – biologically active sorbents developed by LLC M. K. Aseptika, in a dehydration phase – ointment application "5% - dioxydine ointment". With the conducted research we clearly established that in purulent and necrotic ulcers in distal areas of extremities in cattle the following microorganisms are frequently isolated Enterococcus faecalis and E. Faecium, Staphylococcus aureus, Proteus spp., Enterobacter spp., Klebsiella spp., Escherichia coli. Other microorganisms were not so frequent. In general the used treatment regimens of orthopedic pathologies contribute to creating favorable conditions for the fastest arresting of inflammatory processes and acceleration of angenesis.

Keywords: cows, microorganisms, ulcer, PCR diagnostics, necrobacillosis.

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INTRODUCTION

Diseases of a non-contagious etiology of different organs and systems for the last decades have also remained at a high level. Proceeding from the data of veterinary statistics in animal husbandry non-contagious diseases constitute 80 …. 85% of which 40 … 50% are surgical pathologies. In many industrial type farms the diseases of distal section of extremities occupy a considerable place in the list that is a burning issue for the veterinary service [3, 5, 6, 7, 10, 12].

In literature there is a significant amount of scientific works devoted to etiology, pathogenesis, methods of prevention and treatment of purulent - necrotic lesions of tissues in a distal part of extremities in animals. However the data which have been accumulated earlier do not meet current requirements of production due to emergence of new ideas about the origin and course of diseases of extremities in the context of large industrial complexes in view of a sufficient diet, a high dairy productivity. Therefore the development and introduction of a more effective combined orthopedic therapy should be viewed as an urgent and timely direction [1, 2, 4, 9, 11, 13, 14, 15, 16, 17].

The purpose of this work is the comparative analysis of specific structure of microorganisms identified in cows ill with purulent - necrotic ulcers in the field of hooves by the Real - Time PCR method.

RESEARCH TECHNIQUE

Experimental studies were conducted at LLC, Agricultural complex “Krasnaya Zvezda” (Red Star) of the Ulyanovsk district in the Ulyanovsk region. From the examined animals of the black and motley breed aged from 4 up to 10 years, with a live weight of 500 … 550 kg, 30 heads of cattle were selected with diseases of distal section of extremities, with the diagnosis of purulent - necrotic ulcer. Three experimental groups with ten animals in each were created, of them two experimental and one control. Maintenance conditions, conditions of feeding and care were similar.

In control group, in a hydration phase, oxytetracycline was applied locally in the form of a powder, in a phase of dehydration 3% tetracycline ointment was used.

Animals of the first experimental group, in a hydration phase, were treated locally with diotevin, a powder (with an anti-septic agent dioxidine and proteolytic enzyme terrylitine), in a phase of dehydration 5% dioxydine ointment was applied. In the second experimental group, in a hydration phase, diovine, the powder was applied locally on a lesion(with an anti-septic agent -dioxydine), in a phase of dehydration 5% dioxydine ointment was used. Medicines -Diovine (Aseptisorb D) and Diotevine (ASEPTISORB DT) belong to biologically active draining sorbents, produced by LLC M. K. Aseptika (Moscow). For DNA identification "A reagents kit for DNA identification from biological samples" was used (Litekh, Moscow), the lysis of bacterial cells, sorption of DNA on silica gel and DNA washing with an alcohol-saline buffer is an underlying principle in this method. For studies SEPTOSKRIN, a kit for PCR-diagnostics was used (Enterobacter spp., Klebsiella spp., Enterococcus faecalis and E. faecium, Escherichia coli, Proteus spp., Pseudomonas aeruginosa, Serratia spp., Staphylococcus aureus, Streptococcus spp.) and NUKLEAPOL – RV a kit for PCR-diagnostics (Fusobacterium nucleatum).

To carry out PCR a detecting DT-96 thermocycler was used ("DNK-Technology", Moscow). Polymerase chain reaction was carried out in microtest tubes with a capacity of 0,2 ml, ready-made reaction mixtures of SEPTOSKRIN and NUKLEAPOL – RV were used to which 10 мк of DNA matrix were added. Test tubes with a ready PCR mixture stirred up in the mixer, all droplets from the walls were pelleted during 5-10 sec.

The samples were transferred into a thermocycler and reaction was carried out. In parallel with the studied samples a negative control sample was made (with deionized water). The accounting of results was carried out separately on each of the channels, according to the instruction to the device. Results interpreted on the basis of existence (or absence) fluorescence curve crossings with a threshold line set at an appropriate level (0,05) (treshhold) of the threshold cycle value "Ct". The sample was considered negative if a Ct value on the Fam channel was absent.

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Bacteriological studies were conducted prior to treatment and at the end of treatment. Sampling was made by means of a special rod with an endpiece from hygroscopic material by which a smear was made from the surface of a pathological nidus and then placed in sterile test tubes.

Figure 1 – Ulcer lesions in the area of hooves in cows

Purulent - necrotic depositions served as a biological research material for the diagnostics of necrobacillosis. They were scraped with a Folkman spoon from affected tissues to healthy tissue layers. Bacteriological studies were carried out according to "Methodical recommendations on laboratory diagnostics of necrobacillosis" [8].

RESULTS AND THEIR DISCUSSION

While studying the species structure of microorganisms isolated from purulent - necrotic foci in the field of hooves it was established (table 1) that at the beginning of treatment in control group Staphylococcus aureus was found in 3 samples (30%), Enterococcus faecalis and E. faecium in 5 samples (50%), Escherichia coli in 5 samples (90%), Proteus spp. in 4 samples (40%). Streptococcus spp., Pseudomonas aeruginosa, Serratia spp., Enterobacter spp., Klebsiella spp. were not found. In 3 animals 1 species of microorganisms was identified and in 7 animals – 2 species of microorganisms.

In the first experimental group before the beginning of the experimental treatment regimen Staphylococcus aureus was found in 4 samples (40%), Enterococcus faecalis and E. faecium in 1 sample (10%), Serratia spp. in 1 sample (10%), Proteus spp. in 2 samples (20%), Enterobacter spp., Klebsiella spp. in 4 samples (40%), Escherichia coli in 4 samples (40%). Pseudomonas aeruginosa and Streptococcus spp. were not found. In 2 animals 1 species of microorganisms was identified, in 3 animals – 2 species of microorganisms and in 2 animals – 3 species of microorganisms. In 3 animals microorganisms were not identified.

In the second experimental group before the treatment Staphylococcus aureus was found in 2 samples (20%), Enterococcus faecalis and E. faecium in 2 samples (20%), Proteus spp. in 3 samples (30%), Enterobacter spp., Klebsiella spp. in 3 samples (30%), Escherichia coli in 1 sample (10%). Streptococcus spp., Pseudomonas aeruginosa and Serratia spp. were not found. In 7 animals 1 species of microorganisms was identified, in 2 animals – 2 species of microorganisms. Microorganisms were not identified in one animal.

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Table 1 – Study of species structure of microorganisms in cows with orthopedic disorders before treatment with

Identification Manure samples Key	Before treatment						End of treatment					
	1 – experim group		2 – experim group		Control group		1 – experim group		2 – experim group		Control group	
	Cmp, Fam	Result	Cm, Fam	Result	Cmp, Fam	Result	Cmp, Fam	Result	Cmp, Fam	Result	Cmp, Fam	Result
1_ Strep		-		-		-		-		-		-
1_ Staph		-		-	19,0	+		-		-		-
1_ Enteroc		-		-	29,1	+		-		-	24,0	+
1_ Pseu aer		-		-		-		-		-		-
1_ Serr		-		-		-		-		-		-
1_ Prot		-	15,2	+		-		-		-		-
1_ Ent, Kleb		-		-		-		-		-		-
1_ E,coli		-		-		-		-		-		-
2_ Strep		-		-		-		-		-		-
2_ Staph		-		-		-		-		-		-
2_ Enteroc		-		-		-		-		-		-
2_ Pseu aer		-		-		-		-		-		-
2_ Serr		-		-		-		-		-		-
2_ Prot		-		-	23,0	+		-		-		-
2_ Ent, Kleb	29,1	+		-		-		-		-		-
2_ E,coli		-	32,8	+	25,0	+		-		-		-
3_ Strep		-		-		-		-		-		-
3_ Staph		-		-		-		-		-		-
3_ Enteroc		-		-		-		-		-		-
3_ Pseu aer		-		-		-		-		-		-
3_Serr		-		-		-		-		-		-
3_ Prot	25,3	+		-	30,4	+		-		-		-
3_ Ent, Kleb	26,5	+		-		-		-		-		-
3_ E,coli	28,5	+		-	13,1	+		-		-	21,3	+
4_ Strep		-		-		-		-		-		-
4_ Staph		-	33,2	+	30,4	+		-		-		-
4_ Enteroc		-		-		-		-	17,3	+		-
4_ Pseu aer		-		-		-		-		-		-
4_ Serr		-		-		-		-		-		-
4_ Prot		-		-		-		-		-		-
4_ Ent, Kleb		-	15,0	+		-		-		-		-
4_ E,coli		-		-		-		-		-		-
5_ Strep		-		-		-		-		-		-
5_ Staph	21,5	+		-	20,6	+		-		-		-
5_ Enteroc		-		-	20,8	+		-		-		-
5_ Pseu aer		-		-		-		-		-		-
5_ Serr	26,0	+		-		-		-		-		-
5_ Prot		-		-		-		-		-		-
5_ Ent, Kleb		-	29,7	+		-		-		-		-
5_ E,coli	25,5	+		-		-		-		-		-
6_ Strep		-		-		-		-		-		-
6_ Staph		-		-		-		-		-		-
6_ Enteroc		-	29,9	+		-		-		-		-
6_ Pseu aer		-		-		-		-		-		-
6_ Serr		-		-		-		-		-		-
6_ Ent, Kleb		-		-		-		-		-		-
6_ E,coli		-		-	22,4	+		-		-		-
6_ Prot		-	33,2	+		-		-		-		-
7_ Strep		-		-		-		-		-		-
7_ Staph	23,1	+		-		-	22,5	+		-		-
7_ Enteroc		-	15,3	+		-		-		-		-
7_ Pseu aer		-		-		-		-		-		-

7_ Serr		-		-		-		-		-		-
7_ Prot		-		-		-		-		-		-
7_ Ent, Kleb		-		-		-		-		-		-
7_ E,coli	27,9	+		-	22,9	+		-		-	21,0	+
8_ Strep		-		-		-		-		-		-
8_ Staph		-		-		-		-		-		-
8_ Enteroc		-		-	23,2	+		-		-		-
8_ Pseu aer		-		-		-		-		-		-
8_ Serr		-		-		-		-		-		-
8_ Prot	22,6	+	36,5	+		-		-		-		-
8_ Ent, Kleb	27,3	+		-		-		-		-		-
8_ E,coli		-		-	30,9	+		-		-		-
9_ Strep		-		-		-		-		-		-
9_ Staph	30,2	+		-		-		-		-		-
9_ Enteroc		-		-	19,0	+		-		-	19,0	+
9_ Pseu aer		-		-		-		-		-		-
9_Serr		-		-		-		-		-		-
9_ Prot		-		-	30,2	+		-		-	24,5	+
9_ Ent, Kleb		-	33,8	+		-		-		-		-
9_ E,coli		-		-		-		-		-		-
10_ Strep		-		-		-		-		-		-
10_ Staph		-	33,7	+		-		-		-		-
10_ Enteroc	21,2	+		-	23,1	+		-		-		-
10_ Pseu aer		-		-		-		-		-		-
10_Serr		-		-		-		-		-		-
10_ Prot		-			30,5	+	13,1	+		-	23,6	+
10_ Ent, Kleb	24,8	+		-		-		-		-		-
10_ E,coli		-		-		-		-		-		-
K+_ Strep	25,5	-	31,9	+	22,3	+	11,8	+	15,8	+	22,6	+
K+_ Staph	27,5	-	31,7	+	26,2	+	13,2	+	13,4	+	27,1	+
K+_ Enteroc	28,3	-	29,0	+	24,5	+	6,3	+	11,0	+	24,7	+
K+_ Pseu aer	27,5	-	28,8	+	29,1	+	27,0	+	7,9	+	29,8	+
K+_Serr	26,9	+	28,7	+	29,9	+	6,3	+	6,1	+	30,1	+
K+_ Prot	26,0	+	28,6	+	30,7	+	10,4	+	7,4	+	19,5	+
K+_ Ent, Kleb	13,0	+	32,1	+	31,4	+	10,5	+	12,1	+	23,7	+
K+_ E,coli	24,8	+	32,4	+	25,2	+	22,8	+	5,7	+	23,0	+
K-		-		-		-		-		-		-

As a result of the treatment conducted in the control group Enterococcus faecalis and E. faecium were found in 2 samples (20%), Proteus spp. in 1 sample (10%), Enterobacter spp., Klebsiella spp. in 1 sample (20%), Escherichia coli in 2 sample (20%). In 4 animals 1 species of microorganisms, in 2 animals 2 species of microorganisms were identified.

In the first experimental group after conducted therapy of purulent and ulcer lesions of soft tissues in the area of hooves Proteus spp was found in 1 sample (10%) and Staphylococcus aureus in 1 sample (10%). In the second experimental group at the end of the experimental treatment Enterococcus faecalis and E. faecium were found in 1 sample. In 3 animals of experimental groups 1 species of microorganisms was identified.

Examination of samples for existence of Fusobacterium nucleatum showed lack of pathogenes of necrobacillosis in experimental animals both before and after treatment.

Thus, results of our researches showed that in 26 samples or 86,7% taken from purulent - necrotic sites of soft tissues of hooves in all experimental sick cows prior to treatment, there were the following associations of microorganisms: Enterobacter spp., Klebsiella spp., Enterococcus faecalis and E. faecium, Escherichia coli, Proteus spp., Serratia spp., Staphylococcus aureus. After the performed treatment a decline of number of samples with microorganisms – 9 or 30% becomes perceptible. Pseudomonas aeruginosa and Streptococcus spp. in the studied samples were not found. Necrobacillosis pathogen during all experimental period was not

found. The obtained data confirm a beneficial effect of biologically active sorbents used in complex treatment schemes on healing of purulent - necrotic ulcers in cows.

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