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## Effect Composition of Microbial Associations on Intensity Symptoms of Disease.

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### ABSTRACT

It is established that associations of micro-organisms *Fusobacterium necrophorum*, *Dichelobacter nodosus*, *A. pyogenes*, *S. aureus*, *C. perfringens* causes more severe damage extremities of animals by compared impact monocultures of the same organisms.

**Keywords:** hoof rot, necrobacillosis, rabbits, sheep, association of microorganisms.

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## INTRODUCTION

Hoof rot and necrobacteriosis is one of the leading places in infectious pathology the extremities of animals. Consequently, animal husbandry bear the losses from foregone production, which affects the level of development of agricultural regions [11, 12]. Often these illnesses occur simultaneously, the affected hoof is constant contact with soil and other environmental objects, which leads to their abundant colonization of bacterial flora. Besides, one characteristic of anaerobic infections is both associative effects on the body two, three or more of anaerobes and aerobes [1, 2, 3, 10]. According to some researchers, critical value for necrobacillosis and foot rot in sheep are has not so much individual pathogens *Fusobacterium necrophorum*, *Dichelobacter nodosus* as the Association of anaerobic microorganisms: *Fusobacterium*, bacteroids, clostridia, spirochetes, cocci [1, 5, 6].

*F. necrophorum* and *D. nodosus* are the main etiological factor in the occurrence of necrobacillosis and foot rot. Other members of possible Association considered as aggravating factors, contributing to the spread in the body two of the above-mentioned microorganisms [4, 7, 8, 9].

## MATERIALS AND METHODS

The work had carried out in stages.

In the first phase, conducted preparation of cultures of *F. necrophorum*, *A. pyogenes* and *S. aureus* by rabbits and was determined LD<sub>50</sub> for each culture.

The result of the conducted research it had found that LD<sub>50</sub> of cultures of *F. necrophorum*, *A. pyogenes*, *S. aureus* was respectively by 2.55, 4.0 and 5 billion microbial phone.

After the determination of LD<sub>50</sub> was experiments to confirm the negative impact of associations of microorganisms in rabbits weighing 2.0 to 2.5 kg. To this end, 24 of the rabbit were divide into 8 groups of 3 rabbits each. Animals 1, 2, 3 groups respectively were infected with cultures of *F. necrophorum*, *A. pyogenes*, *S. aureus*. All cultures were injected at a dose of 4 LD<sub>50</sub>. Group 4 animals were infected with a mixture of cultures of *F. necrophorum* and *A. pyogenes* in doses of 2 LD<sub>50</sub>. Rabbits 5 groups were infected with a mixture of cultures of *F. necrophorum* in a dose of 2 LD<sub>50</sub> и *S. aureus* at a dose of 2 LD<sub>50</sub>. Rabbits of group 6 was infected with cultures of Association of *A. pyogenes* at a dose of 2 LD<sub>50</sub> and *S. aureus* at the same dose. Animals of group 7 were infect with a mixture of cultures of *F. necrophorum* in the dose of LD<sub>50</sub>, *A. pyogenes* at a dose of LD<sub>50</sub> и *S. aureus* in a dose of LD<sub>50</sub>. Uninfected animals of the 8-th group were use as control.

The observation period for the animals was 30 days. The dead rabbits were dissect, observed pathological changes, and from internal organs and weakspots did inoculation for re-insulation original cultures.

## RESULTS AND DISCUSSION

The results of the study are had presented in table 1.

It is established that for the same amount of infectious material used for infecting the animals, at a dose of 4 LD<sub>50</sub> rabbits infected with the associations of microorganisms in various combinations, were killed significantly faster than animals infected with the monocultures, two rabbits of the second and third group during the observation period remained alive.

At autopsy of animals infected associations marked position, typical of the septic process: multiple hemorrhages in internal organs, necrosis and abscesses.

On selective media from separate bodies, manage to reinsulate and identify the source of culture microorganisms *F. necrophorum*, *A. pyogenes*, *S. aureus*.

**Table 1: Infection of rabbits by associations of microorganisms**

No Groups	No Rabbits	Culture	Dose	The time of death (days)	Pathological changes	Re-insulation culture
1	1	F. necrophorum	4 LD <sub>50</sub>	5	Characteristic of sepsis	F. necrophorum
	2			7		
	3			7		
2	1	A. pyogenes	4 LD <sub>50</sub>	9	Characteristic of pyemia	A. pyogenes
	2			20		
	3			-		
3	1	S. aureus	4 LD <sub>50</sub>	19	Characteristic of sepsis	S. aureus
	2			28		
	3			-		
4	1	F. necrophorum	2 LD <sub>50</sub>	2	Characteristic of sepsis	F. necrophorum
	2	A. pyogenes	2 LD <sub>50</sub>	3		
	3			3		
5	1	F. necrophorum	2 LD <sub>50</sub>	2	Characteristic of sepsis	F. necrophorum
	2	S. aureus	2 LD <sub>50</sub>	3		
	3			4		
6	1	A. pyogenes	2 LD <sub>50</sub>	3	Characteristic of sepsis	A. pyogenes
	2	S. aureus	2 LD <sub>50</sub>	4		
	3			4		
7	1	F. necrophorum A. pyogenes S. aureus	2 LD <sub>50</sub> LD <sub>50</sub> LD <sub>50</sub>	2	Characteristic of sepsis	F. necrophorum A. pyogenes S. aureus
	2			2		
	3			3		
8	1	Control	-	-	-	-
	2			-		
	3			-		

At the second stage of the study to confirm, the influence associations of microorganisms in causing disease limb was an experiment on sheep.

In the experiment were use 18 sheep of 2 years age. All the animals were divide into 6 groups of 3 in each sheep. To infect used culture F. necrophorum, S. aureus, A. pyogenes in 20 LD<sub>50</sub> doses culture C. perfringens type A in a dose of 100 LD<sub>50</sub>. Culture D. nodosus used at a dose of 50 billion. Microbial bodies.

Infection had carried out by embrocation the infectious material in the scarified skin arch into gap of hoof followed by imposition tampon, containing infectious material, and the cover fixing bandage.

Animals from 1 group infected culture D. nodosus. Sheep 2 groups infected culture F. necrophorum. Animals of 3 and 4 groups, infected cultures A. pyogenes and S. aureus. The 5 group infected with a mixture of cultures D. nodosus, F. necrophorum, A. pyogenes, S. aureus, C. perfringens type A.

Term observation of the animals was 15 days. Three days after infection, all animals had taken off the bandage with infectious material, and conducted the first inspection of all limbs infected sheep. In the following examinations conducted on days 7, 11 and 15 days after infection. The results are had shown in Table 2.

Observed 3 grade lesions hooves.

A - Mild lesions; General slight depression, loss of appetite, sheep sometimes lift the affected limb at rest, during movement - weakly pronounced limp. On examination: redness and swelling of the skin arch and loss of skin hair gap of hoof, raising the local temperature.

C - The average degree of damage; General slight depression, lack of exercise, loss of appetite, severe lameness when moving, at rest sick animal keeps the limb elevated. On examination: in the field of skin arch gap of hoof redness, swelling, presence of erosions and ulcers covered with grayish exudate with putrid odor, increase in local temperature.

T - Heavy defeat; Clinical signs same as in the case of moderate severity (C), and the skin lesion basis hooves and the inner walls of purulent inflammation of the skin of the sole basis, exfoliation of the stratum shoe.

In the analysis of Table 2 shows that infection sheep monocultures of microorganisms are not always made animal disease or observed, mostly mild lesions. Animals infected with the associations microorganisms, sick already on the 3rd day after infection. In this case, we noted the high degree of destruction all animals, and two sheep marked subsequently heavy infestation hooves.

**Table 2: Degree disease manifestations depending the composition of microbial association**

№ group	infect culture	Number of sheep	Time observation and nature defeat of sheep limbs			
			3 days	7 days	11 days	15 days
1	D. nodosus	3	–	1/l	1/l	1/l
2	F. necrophorum	3	1/l*	2/l	2/l	2/l
3	A. pyogenes	3	–	1/l	2/l	2/l
4	S. aureus	3	–	–	1/l	1/l
5	D. nodosus, F. necrophorum	3	2/l 1/s	3/s	3/s	3/s
6	D. nodosus, F. necrophorum, A. pyogenes, S. aureus, C. perfringens type A	3	3/s	1/s 2/t	1/s 2/t	1/s 2/t

\*The numerator – the number of sick animals

The denominator – the severity of lesions:

- l – mild degree
- s – average degree
- t – severe degree

**CONCLUSION**

Experiments on rabbits and sheep, allowed concluding that the association of microorganisms inhibits significantly stronger protective systems of body in a short time, causes a severe damage to the limbs of animals, as compared to the same exposure monocultures of organisms that make up the association.

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