Study on pathogenity and immunogenous capacity of *Fusobacterium necrophorum*

Studiu privind patogenitatea și capacitatea imunogenă a lui *Fusobacterium necrophorum*

Andrei Nanu, Constantin Chiurciu, Costin Stoica
Romvac Company S.A.

nanu.andrei.vet@gmail.com

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Abstract

The present study is aimed for veterinarians, researchers and sheep, goats and cattle breeders of and presents an effective prophylactic method in the fight with necrobacilar pododermatitis. The pathogenicity and the determination of the minimum lethal dose in mice, facilitated the demonstration of the immunogenic capacity and the protective effect of an inactivated suspension of *Fusobacterium necrophorum.* Vaccination of sheep and goats against necrobacillosis induced an increase of antibodies with at least 2log$_2$, evidenced by the slow seroagglutination reaction in tubes. The decrease until the disappearance of the cases of necrobacillary pododermatitis in sheep flocks certifies the immunizing efficacy of the vaccine suspension.

Rezumat

Prezentul studiu este adresat medicilor veterinari, cercetătorilor cât și crescătorilor de ovine, caprine și bovine, și are ca scop prezentarea unei metode profilactice eficiente în lupta cu pododermatitele necrobacilare. Patogenitatea și stabilirea dozei minime letale la șoareci, au facilitat demonstrarea capacității imunogene și efectului protector a unei suspensii inactivate de *Fusobacterium necrophorum*. Vaccinarea ovinelor și caprinelor contra necrobacilozei a indus o creștere a anticorpilor cu cel puțin 2log$_2$, evidențiată prin reacția de seroaglutinare lentă în tuburi. Scăderea până la dispariție a cazurilor de pododermatită necrobacilară în efectivele de ovine certifică eficacitatea imunizantă a suspensiei vaccinale.

Introducere

The importance and aggressiveness of anaerobic and non-sporogenic micro-organisms in various animal diseases has been known since the beginning of the last century, but nonetheless, the interest in these pathogens has increased sharply in recent years.

The main reason is the increasing incidence of clinical cases produced by these microorganisms, this generating a negative economic impact, especially in the case of non-acrobatic pododermatitis.

Fusobacterium necrophorum is one of the infectious agents on which the efforts of specialists who were looking for data on the pathogenic mechanism in necrobacillosis have been much sought.

This microorganism is a companion of the digestive tract to animals but is also found in the female genital tract.

Consequently, the reservoir or source of this infectious agent will be continuous and could not be permanently sterilized or removed. Due to its strictly anaerobic nature, pretentious status and marked sensitivity, isolation, identification and study of this

bacillus is much more difficult compared to other Gram-negative bacteria.

Fusobacterium necrophorum does not show a form of resistance, therefore once outside its development environment, the oxygen-rich outer atmosphere destroys it within minutes.

However, certain factors favoring the external environment, such as the increased humidity of the litter, the paddocks, the pastures and the doves, favor the creation of a favorable environment for the survival of the pathogen.

At the same time, other favorable factors such as animal deficiencies, untreated lesions, excessive horn and in general their poor hygiene lead to the appearance of non-acrobatic pathologies, among which the pododermatitis of sheep, goats and cattle is frequently listed.

In view of the aspects described above and the countless requests from animal breeders as well as practicing veterinarians, this study welcomes them with positive solutions and results.

The study followed particularities related to the pathogenicity of the microorganism *Fusobacterium necrophorum* and in particular its immunogenic capacity.

The pathogenicity study was performed on laboratory mice and the aspects related to the clinical expression of the disease, the anatomopathological changes, the re-isolation and identification of the inoculated microorganism from the samples taken, the determination of the minimum lethal dose (DML) and the absolute lethal dose (DL_{100}) were followed.

The study of the immunogenic capacity of the bacterium was performed both on laboratory animals (mice and rabbits) and on the target species - sheep and goats.

The immunizing value in mice was tested by controlling infection and in rabbits and target species - by measuring the level of antibodies before and after vaccination

1. Materials and Methods

Animals

In the experiment, mice from NMRI and CD1 strains were used, males weighing 20-30 grams, type of nonconsanguineous growth and diet free of specific pathogens (source - SPF Animalia, Baneasa Station, Cantacuzino Institute). The mice were housed 5, in plastic cages, with feeding and watering at the discretion.

Tulpina bacteriană

Fusobacterium necrophorum "ATCC 25286" TL2 / 2017, stored in liquid nitrogen bath at -196 °C. For testing, a 24-hour bacterial culture with a concentration of 1x105 Colony-forming Units (CFU) / ml and 1x107 CFU / ml was prepared.

Bacterial culture and inoculation

Inoculum cultures were prepared by two successive replications at 48 and 24 hour intervals on special culture media for the development of anaerobic bacteria.

After reaching maturity, the total germ count of the cultures was performed, and from the decimal dilutions performed the mice were inoculated subcutaneously, with 0.5 ml/sample.

Pathogenicity testing and determination of the minimum lethal dose were performed in 3 experiments, with different bacterial cultures prepared separately.

The number of mice inoculated with different bacterial concentrations is shown below, in Table 1.

Table 1

Number of mice inoculated with different bacterial concentrations

Bacterial Concentration / 0,5 ml	$5x10^6$ UFC	$5x10^5$ UFC	$5x10^4$ UFC	$5x10^3$ UFC	$5x10^2$ UFC
No. of inoculated mice	10	15	15	10	5

As controls, 3 groups of 5 mice were used, housed separately but under the same conditions as above.

One of them was not inoculated and the other two were inoculated with suspension of Salmonella gallinarum strain 9R, with 0.5 ml / head (equivalent to 2x107 CFU).

Vaccine and immunization

Induction of immunity was performed with the vaccine PODOVAC, which has as active principle - antigenic mass *Fusobacterium necrophorum* "ATCC 25286", inactivated with formaldehyde and adjuvanted with aluminum hydroxide gel.

Three groups of 5 mice each were inoculated subcutaneously with 0.5 ml of vaccine, twice every 14 days.

Another 3 batches of 5 mice were used as unvaccinated controls.

After 2 weeks after booster vaccination, the control infection was performed, all six batches being inoculated with live bacterial suspension of *Fusobacterium necrophorum*, in different concentrations, they are shown in Table 2

Table 2.
Presentation of mice groups inoculated with live bacterial suspension *Fusobacterium necrophorum*, in different concentrations

No. Group	Groups: 1 no vaccine	2 control / no vaccine	3 vaccine	4 control / no vaccine	5 vaccine	6 control / no vaccine
inoculated concentration	$5x10^6$ UFC		$5x10^5$ UFC		$5x10^4$ UFC	

Testing the immunization capacity by measuring the level of serum antibodies was performed in 4 different experiments and a total of 41 sheep, 15 goats and 5 rabbits were inoculated, subcutaneously with 2 ml of vaccine suspension.

The booster vaccination was done after 4 weeks in sheep and goats and in rabbits - after 14 days after the first inoculation.

Blood samples collected before the first vaccination and two weeks after booster vaccination were tested to determine the level of antibodies.

Testing was performed by Slow Seroagglutination Reaction (SSR) in tubes with inactivated suspension of concentrated *Fusobacterium necrophorum* until turbidity corresponding to standard 1.5 McFarland (FN 1.5McF Antigen) was obtained.

The sheep and goats tested were part of 5 herds totaling 1879 animals, which were also included in the vaccination program.

These units were included in the testing program because the incidence of non-acrobatic pododermatitis was high and frequent.

2. Results

Pathogenity, DL_{100} și DML

Fusobacterium necrophorum inoculated with a concentration of over 5x103 CFU has been shown to be pathogenic to mice, presenting as clinical signs of disease - lethargy, apathy, horipilation and cyanotic inflammation at the site of inoculation.

The severity of the clinical signs was directly proportional with the increase of the inoculated concentration, ending with 100% exitus at the concentration of 5x106 CFU.

At the anatomopathological examination, edema, necrotico-inflammatory inflammation were identified and we emphasized the place of inoculation. From the liver samples collected for bacteriological examination, Fusobacterium necrophorum was isolated and identified, its cultural characteristics being superior to the mother strain.

The inoculated bacterial concentration that produced the exitus status in all animals in a lot was 5x106 CFU, representing DL100.

The minimum bacterial concentration that produced clinical signs of disease in all animals and the death of one animal in the

same batch, represents DML and is 5x104 CFU.

Both non-inoculated control mice and those inoculated with Salmonella gallinarum 9R (with a bacterial concentration 4 times higher than that of mice inoculated with DL100 of Fusobacterium necrophorum), did not show local or general reactions.

immunization

Verification of the immunity status in the batches of vaccinated mice was performed by the control infection with live culture of Fusobacterium necrophorum with bacterial concentration starting from DML to DL_{100}. Therefore, both control and vaccinated groups were inoculated according to Table 2.

Control mice inoculated with DML made clinical signs of disease at 100% and died at 20%.

In contrast, those in the vaccine group, inoculated with DML, did not show clinical signs of disease and withstood 100%.

The results of the tests performed on vaccinated and non-vaccinated mice are expressed in Table 3, as a percentage.

Table 3.

Results of tests performed on vaccinated and non-vaccinated mice, expressed as a percentage

immune status of the animals / inoculated bacterial concentration	Unevaccinated mice (non immunized)	Vaccined mice (immunized)
$5x10^6$ UFC	- 100% clinical signs of disease - 100% exitus	- 100% clinical signs of disease - 80% exitus
$5x10^5$ UFC	- 100% clinical signs of disease - 80% exitus	- 100% clinical signs of disease - 60% exitus
$5x10^4$ UFC (DML)	- 100% clinical signs of disease - 6,6% exitus	- 0% clinical signs of disease - 0% exitus
$5x10^3$ UFC	- 20% clinical signs of disease - 0% exitus	PROTECTION
$5x10^2$ UFC	- 0% clinical signs of disease - 0% exitus	

Determination of the level of serum antibodies against *Fusobacterium necrophorum* by SSR implies the conjugation of the inactivated antigen (described in the previous chapter) with the free antibodies of the animal serum.

They increased postvaccination in all rabbits by at least 3log2 compared to baseline before immunization. Vaccination of sheep and goats induced an increase in antibody level by 2log2 to 90% of the animals tested.

1. CONCLUSIONS

Expression of the pathogenic effect of Fusobacterium necrophorum is dependent on the inoculated bacterial quantity.

The minimum lethal dose in mice is 100 times lower than the absolute lethal dose.

The cultural properties of the bacterium are better expressed after re-isolating it from the samples taken, and murinization can be a solution for regaining the characteristics of this microorganism. Inactivated and prepared as a vaccine, the etiologic agent of necrobacillosis induced protective immunity in inoculated mice.

Therefore, they resisted the infection of control with the minimum lethal dose, unlike the unvaccinated ones, who made the disease in a proportion of 100%.

The increase of the detectable antibody titer in the blood of animals, certifies the immunogenic capacity of the vaccine suspension.

In the 5 sheep herds with a rich history in the incidence of non-acrobatic diseases, immunization resulted in the disappearance

and absence of specific diseases, this confirming the theoretical conclusions.

Immunization with PODOVAC vaccine is an effective prophylactic method in the fight with non-microbial pododermatitis.

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