Study on the pathogenicity and immunogenic capacity of the microorganism *Fusobacterium necrophorum*

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

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

nanu.andrei.vet@gmail.com

Key-words: Fusobacterium necrophorum, necrobacillary pododermatitis, ovine, vaccine, immunization.
Cuvinte cheie: *Fusobacterium necrophorum*, pododermatite necrobacilare, ovine, vaccin, imunizare.

Abstract

This study is aiming veterinarians, researchers and breeders of sheep, goats and cattle, and aims to present an effective prophylactic method in the fight against necrobacillary pododermatitis. The pathogenicity and the determination of the minimum lethal dose in mice facilitated the demonstration of the immunogenic capacity and protective effect of an inactivated suspension of Fusobacterium necrophorum. Vaccination of sheep and goats against necrobacillosis induced an increase in antibodies by at least 2log2, evidenced by the slow seroagglutination reaction in the tubes. The reduction to extinction of cases of necrobacillary pododermatitis in sheep herds 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.

Introduction

The importance and aggressiveness of anaerobic and non-sporogenic microorganisms in various animal diseases has been known since the beginning of the last century, but nevertheless, interest in these pathogens has increased rapidly in recent years. The main reason is the increasing incidence of clinical cases caused by these microorganisms, which has a negative economic impact, especially in the case of necrobacillary pododermatitis.

Fusobacterium necrophorum is one of the infectious agents on which the efforts of specialists looking for data about the pathogenic mechanism in necrobacillosis have relied heavily. This microorganism is a commensal of the digestive tract in 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 sterilized or permanently removed. Due to its strictly anaerobic nature, pretentious status and marked sensitivity, the isolation, identification and study of this bacillus is much more difficult compared to other Gram-negative bacteria.

Fusobacterium necrophorum does not show any form of resistance, therefore once outside its development environment, the oxygen-rich outer atmosphere destroys it in a few minutes. However, certain favorable factors related to the external environment, such as the high humidity of bedding, paddocks, pastures and savannahs favor the creation of an environment conducive to the survival of the pathogen.

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

Considering the aspects described above and the countless requests from animal breeders but also from practicing veterinarians, this study meets them with positive solutions and results.

The study looked at particularities related to the pathogenicity of Fusobacterium necrophorum and especially its immunogenic capacity.

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

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 the infection and in rabbits and the target species - by measuring the level of antibodies before and after vaccination.

Materials and Methods

Mice

In the experiment, mice from NMRI and CD1 strains were used, males weighing between 20-30 grams, nonconsanguineous growth type and free pathogen-specific diet. (from *Animaleria SPF, Băneasa Station, Cantacuzino Institute*). The mice were accommodated 5 in plastic cages, with fodder and watering at discretion.

Bacterial strain

Fusobacterium necrophorum "ATCC 25286" TL_2/2017, stored in a liquid nitrogen bath at -196 ℃. For testing, a 24-hour bacterial culture was prepared with a concentration between 1x105 Colony Forming Units (CFU) / ml and 1x107 CFU / ml.

Bacterial culture and inoculation

The inoculum cultures were prepared by two successive transplantings at intervals of 48 and 24 hours 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 / head. 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 in Table 1

Table 1.
Number of mice inoculated with different bacterial concentrations

Bacterial concentration / 0,5 mL	$5x10^6$ CFU	$5x10^5$ CFU	$5x10^4$ CFU	$5x10^3$ CFU	$5x10^2$ CFU
Number of inoculatd mice	10	15	15	10	5

As Control, 3 lots of 5 mice were used, accommodated separately but in identical conditions to the above. One of them was not inoculated and the other two were inoculated with a suspension of Salmonella gallinarum strain 9 R, with 0.5 ml / head each (equivalent to 2x107 CFU).

Vaccine and imunisation

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. 3 batches of 5 mice were inoculated subcutaneously with 0.5 ml of vaccine, twice at 14-day intervals.

Another 3 batches of 5 mice were used as unvaccinated controls. After 2 weeks from the booster vaccination, the control infection was performed, all six batches being inoculated with live bacterial suspension of Fusobacterium necrophorum, in different concentrations, these being shown in table 2.

Table 2.

Inoculation with live bacterial suspension of Fusobacterium necrophorum, in different concentrations

Group / Category	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6
	vaccinated	M / unvaccinated	vaccinated	M / unvaccinated	vaccinated	M / unvaccinated
Inoculated concentration	$5x10^6$ UFC		$5x10^5$ UFC		$5x10^4$ UFC	

The testing of the immunizing 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 given after 4 weeks in sheep and goats and in rabbits - after 14 days from the first inoculation.

Blood samples collected before the first vaccination and two weeks after the booster vaccination were tested for antibody levels.

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

The sheep and goats tested were part of 5 herds totaling 1879 animals, being also included in the vaccination program. Those units were included in the testing program because the incidence of necrobacillary pododermatitis was high and frequent.

Results

Patogenicity, DL_{100} and DML

Fusobacterium necrophorum inoculated in a concentration of more than 5x103 CFU proved to be pathogenic for mice, which showed 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 5 x 106 CFU.

The anatomopathological examination identified edema, necrotic-caseous inflammation and emphysema at the site of inoculation.

Fusobacterium necrophorum was isolated and identified from the liver samples collected for bacteriological examination, its cultural characteristics being superior to the mother strain. The inoculated bacterial concentration that produced the exitus status in all animals in a batch was 5x106 CFU, representing DL_{100}.

The minimum concentration of bacteria that produced clinical signs of disease in all animals and the death of a single animal in the same group is DML and is 5x104 CFU.

Both uninoculated 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.

Immunisation

Verification of the onset of immunity in vaccinated mice was performed by control infection with live culture of *Fusobacterium necrophorum* with bacterial concentration ranging from DML to DL100.

Therefore, both control and vaccinated groups were inoculated according to table 2.

Control mice inoculated with DML showed 100% clinical signs of disease and died at 20%. In contrast, those in the vaccinated group, inoculated with DML, showed no clinical signs of disease and were 100% resistant.

Table 3 summarizes the results of tests performed on vaccinated and unvaccinated mice, they are expressed as a percentage.

Determination of the level of serum antibodies against *F. necrophorum* by RSAL involves the conjugation of the inactivated antigen (described in the previous chapter) with free antibodies from animal serum.

They increased postvaccination in all rabbits by at least 3log2 from baseline before immunization.

Vaccination of sheep and goats induced an increase in the level of antibodies with 2log2 in 90% of the animals tested.

Table 3.
Test results on vaccinated and unvaccinated mice

immune Status of animals inoculated bacterial Concentration	Unvaccinated mice (unimmunised)	Vaccinated mice (immunised)
$5x10^6$ CFU	- 100% clinical sings of disease - 100% exitus	- 100% **semne clinice de boală** - 80% exitus
$5x10^5$ CFU	- 100% clinical sings of disease - 80% exitus	- 100% clinical sings of disease - 60% exitus
$5x10^4$ UFC (DML)	- 100% clinical sings of disease - 6,6% exitus	- **0%** clinical sings of disease - **0% exitus**
$5x10^3$ CFU	- 20% clinical sings of disease - 0% exitus	PROTECTION
$5x10^2$ UFC	- 0% semne clinice de boală - 0% exitus	

Conclusions

The expression of the pathogenic effect of *Fusobacterium necrophorum* is dependent on the amount of bacteria inoculated.

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 its re-isolation from the samples taken, murinization being a solution for regaining the characteristics of this microorganism. Inactivated and prepared as a vaccine, the etiological agent of necrobacillosis, induced protective immunity in inoculated mice.

Therefore, they resisted the control infection with the minimum lethal dose, unlike the unvaccinated ones, who were 100% infected.

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

In the 5 flocks of sheep with a rich history in the incidence of necrobacillary diseases, immunization led to the disappearance and absence of specific diseases, this confirming the theoretical conclusions.

Immunization with the Podovac vaccine is an effective prophylactic method in the fight against necrobacillary pododermatitis.

Bibliography

1. **Kononov AN** (2004). Eficacitatea sistemului de măsuri antiepizootice în necroza copitelor la ovine. Teză de doctorat, Stavropol - 2004.
2. **Panasyuk SD, Sidorchuk AA. Kononov AN.** (2010). Asocierea vaccinului "Nekovak" și "Novikon" în sistemul de măsuri pentru prevenirea și controlul bolilor infecțioase ale bovinelor și rumegătoarelor mici. *Veterinaria* 2010, 8:7-10.
3. **Panasyuk SD, Kononov AN.** (2015). Importanța asocierii microorganismelor în etiologia necrobacteriozei și a necrozei copitelor la ovine. Vestnik APK din Stavropol, 2015, 1, pp. 58-62.
4. **Conlon PJ, Hepper KP, George WT.** (1977). Evaluation of Experimentally Induced *Fusobacterium necrophorum* Infections in Mice. *Am Soc Microbiol Infect Immun J.*, pp. 510-517.
5. **Saginala S, Nagaraja TG, Lechtenberg KF, Chengappa MM, Kemp KE, Hine PM.** (1997). Effect of Fusobacterium necrophorum leukotoxoid vaccine on susceptibility to experimentally induced liver abscesses in cattle. *J Anim Sci.,* 75(4):1160-1166.
6. **Checkley SL, Janzen ED, Campbell JR, McKinnon JJ.** (2005). Efficacy of vaccination against *Fusobacterium necrophorum* infection for control of liver abscesses and footrot in feedlot cattle in western Canada. *Can Vet J.,* 46(11):1002–1007.