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Research Article

The Study of Technology and the Production of Lyophilized Vaccine against Swine Erysipelas

Veterinary Medicine

Keywords: vaccine, lyophilised, swine, Erysipelothrix.

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Abstract

Swine erysipelas is a bacterial disease of world-wide importance. Immunization is very effective in preventing this infection. Vaccines have already been used for 50 years and are safe, efficient and easy to produce. Both inactivated and attenuated vaccines are available to prevent development of clinical signs of swine erysipelas. However, live attenuated vaccines cannot be used in any epizootic situation. For a long time in Albania it has produced an attenuated live vaccine against Erysipelothrix rhusiopathiae. For a long time in Albania it has produced an attenuated live vaccine against Erysipelothrix rhusiopathiae. This vaccine had a limited validity and occasionally gave the post vaccination incidents. To improve the parameters of this vaccine, and to extend its period of validity of we thought to produce a inactivated oily and lyophilized vaccine against Erysipelothrix rhusiopathiae. This constitutes the purpose of this study.

Introduction

Swine erysipelas is an important infectious disease of bacterial etiology, widespread in pigs of the new age. The clinical and pathological features of the disease have been well-described (Wood and Henderson, 2006). Since a character zoonotic disease, it represents a problem for public health and veterinary services.

Immunizing pigs against swine erysipelas using live or killed vaccines has been a common practice for many years and commercial products are generally considered to be efficacious (Haesebrouck et al, 2004, Neumann et al. 2008).

Prevention of swine erysipelas is best accomplished by immunization programs. Current vaccines are based on *E. rhusiopathiae* serotypes 1 or 2 and are either inactivated bacterins for intramuscular injection or attenuated (avirulent live) vaccines designed for whole herd mass treatment via drinking water. Most bacterins are serotype 2 (Eamens et al. 2006; Wood 1979) and most attenuated live vaccines contain serotype 1a isolates (Opriessnig et al. 2004). Vaccination is generally effective in preventing swine erysipelas and the duration of immunity varies between 6 and 12 months for both correctly administered bacterins and avirulent vaccines (Swan and Lindsey 1998). Vaccination may not be as effective in preventing chronic arthritis since sequestration of *E. rhusiopathiae* in the cytoplasm of chondrocytes of articular cartilage may provide protection from host immunity.

Vaccination of breeding animals reportedly reduces the incidence of periparturient vulval discharge, decreases farrowing intervals, and increases the numbers of live-born pigs in clinically affected herds (Gertenbach and Bilkei 2002).

In Albania, for immunization of pigs against *Erysipelotrirx rhusiopathiae* using a vaccine with VR2 strain of *Erysipelothrix rhusiopathiae*. This vaccine has a short-term use (effectively 45 days). Its practical application has some difficulty. Specific prophylaxis with this vaccine applied only to heads free of erysipelas infection. In herds of pigs where infection has exploded the application of this vaccine aggravate the situation. Determination the effectiveness of this vaccine performed only on animals homologue, therefore financial cost is very high. To improve the parameters of this vaccine we undertook the study with the above title.

Materials and methods

Production of vaccine emulsions

For the production of lyophilized vaccine was used *Erysipelothrix rhusiopathiae*- VR2, vaccine strain. By the vial containing the vaccine VR2 strain were carried out planting on agar media, liquid media and Taroc media. After the planting, the microbial cultures were incubated in the thermostat at 37^{o}C for 24 hours. After 24 hours, microbial cultures were controlled macroscopically and microscopically for purity. Subsequently, was carried out rinsing of microbial cultures with normal blood serum sterilized, benefiting base emulsion, which was tested for purity by means of cultures. This emulsion was used for planting of "Roux" plates. So, they were planted 20 Roux plates that were placed in thermostat for incubation. After 24 hours Roux plates were pulled from the thermostat and were controlled for microbial purity. 10 Roux plates were rinsed with protector gelatine–saccharose and 10 others with normal horse serum sterilized for 1 hour at 56^{o} C, for 3 consecutive days. After the collection of emulsions, for each protector, were carried out control of agar, and Taroc and liquid media for purity. Emulsions, resulting microbiologically pure was placed in vials of 30 ml, where each vial contained 8 ml emulsion. Vials of vaccine were entered in the freezing in temperature -40^{o}C,

Study and determination of parameters of lyophilisation of vaccine against swine erysipelas

They studied and determined parameters of lyophilized vaccine as follows:
- The temperature and the time required for freezing
- The temperature of sublimation and its duration
- The duration of conditioning of vaccine after sublimation and temperature of conditioning
- Temperature of drying and rewarming of vaccine
- The level of vacuum in which the vaccine was closed

The data are presented in table. 1

Tab. 1. Technological lyophilization processes.

Technological processes	Freezing temperatures		Time in hours	
The temperature and time required for freezing	-45^{o}C	- 55^{o}C	24	24
Temperatures and the duration of sublimation of vaccine	-40^{o}C -20^{o}C	-40^{o}C -10^{o} C	2	4
Duration of conditioning of vaccine after sublimation	- 20^{o} C	-10^{o} C	10	15
Temperature of drying and rewarming of vaccine	24^{o}C	37^{o} C	5	8
The vacuum level that needs to have the vaccine (expressed in microns)	-	-	0.5	1

Study and determination of the most appropriate protector of lyophilized vaccine

Were produced 2 series of vaccines by rinsing Roux plates with two types of protectors
- The protector gelatin-saccharose
- Normal horse serum (tyndallized at 56^{o}C for 1 hour)

In each plate were added 15-20 ml protector and were collected in balloons sterile. Vaccine emulsions were controlled for purity by means of agar cultures, and Taroc media and liquid media. Once the resulted sterile, were entered in the vial of 30 ml, where in the each vial was introduced by 8 ml emulsion of vaccine. Bottles containing emulsion of vaccine were placed in lyophilisation apparatus for drying. The formula of protector gelatin- saccharose is: Gelatin 1%, Saccharose 10%, Distilled Water until 100ml. Sterilization was carried out in the 0.5 atmosphere for 45 minutes. After completing the procedure of lyophilisation, vaccines were controlled for purity, vacuum level, the percentage of residual moisture, appearance of lyophilized mass and microbial concentration in the time immediately after drying, and in different time intervals (after lyophilisation). On the basis of the results obtained was determined which one protector appears as the most appropriate.

Determination of microbial concentration and validity of vaccine

From the lyophilized vaccine were made dilutions from 10^{-1} to 10^{-8}, according to the model of classic dilution 9 + 1. In Petri dishes with terrain TSA (simple agar) were made planting using 1 ml for each dilution and adding 0.5 ml of normal horse serum. Plates were held at room temperature for about 30 minutes and then were placed in thermostat for cultivation.

After a cultivation period of 48-72 hours, was the determination of microbial concentration of lyophilized vaccine for every one protector used. With the same methodology was determined the validity of vaccines in periods of 3, 6, 8 and 12 months after lyophilisation. The data obtained were processed statistically by the formula:

$$X = \frac{C1 + C2 + C3}{N1x\ 10^{-6} + N2\ x\ 10^{-7} + N3\ x\ 10^{-8}}$$

Where: X = average for 1 ml suspension colonies base
C1 = amount of colonies at 10-6 dilution
C2 = amount of colonies at 10-7 dilution
C3 = amount of colonies at 10-8 dilution
N1 = number of Petri dishes by diluting 10-6
N2 = number of Petri dishes by diluting 10-7
N3 = the number of Petri dishes by diluting 10-8

The vaccine was tested for purity, to the residual moisture, by the recognized laboratory methods.

Determination of adequate solvent of vaccine was made by injecting vaccine in laboratory mouse

Results and Discussion

Doing according to the methodology described above, the lyophilized vaccine was produced. Are defined the parameters of lyophilisation, which are presented in the table:

Tab. 1. Technological processes and defined parameters of lyophilisation

Technological processes	Temperatures of freezing		Time in hours	
The temperature and time required for freezing	-45^o C	- 55^o C	24	24
Sublimation temperature of the vaccine and its the duration (at this stage of the process the vaccine presented **as a non uniform**)	-40^o C -20^o C	40^{o}C 10^o C	2	4
Time of conditioning of vaccine after sublimation	-20^o C	-10^o C	10	15
Temperature of drying and rewarming of vaccine	24^{o}C	37^o C	5	8
The vacuum level that should have the vaccine (expressed in micron	-	-	**0.5µ**	1

The vaccine proved to be pure, safe, with residual moisture 1.2% and 0.5 micron vacuum level by all parameters specified by the relevant literature. Microbial concentration of the lyophilized vaccine was 900 million microbial cells for 1 ml, and microbial concentration of the emulsions before lyophilisation was 1.098 billion microbial cells. So there was a decrease approximately 18% of microbial cells, a level acceptable to the vaccine of this type. As for protector of the lyophilized vaccine was used protectors described of the methodology above (protector gelatin + saccharose, and normal horse tindallized serum). The data obtained are presented in the table 3.

Table 3. **Determining the concentration of microbial cell vaccine (per ml) depending on the type of protector** used**.**

Nr.	Type of protector	Microbial concentracion per ml		Vitality %
		Before lyophilisation	After lyophilisation	
1	Gelatin-saccharose	1.098.000.000	900.000.000	82
2	Normal serum of horse	1.098.000.000	724.000.000	66

From the data it resulted that protector gelatin-saccharose is more suitable for lyophilization of the vaccine, because in this case there is a decrease of microbial cells per ml approximately 16% less than when used as protector normal horse serum. Determination of shelf life of the the lyophilized vaccine was realized by determining the concentration of microbial cells for ml vaccine at different periods of time after lyophilisation. Results are presented in table 4.

Table 4. Determination of the concentration of microbial cells per ml vaccine after different periods of time

Type of vaccine	Microbial concentration after lyophilisation	Microbial concentration for 1 ml of lyophilized vaccine after different periods of time		
		3 muajsh	6 muajsh	8 muajsh
Vaccine with protector gelatin - saccharose	900.000.000	860.000.000	815.000.000	725.000.000
Vaccine with protector normal horse serum	724.000.000	640.000.000	580.000.000	420.000.000

Based on the data obtained and the concentration of microbial for ml that should be 1 ml vaccine, to provide a power protection defined (protection against 1 infectious dose or minimo lethal), the decline in concentration microbial for 1 ml of 20% to the initial concentration does not affect the protective power of the vaccine because 1 dose of vaccine to induce a certain immunity should contain from 200 million to 400 million microbial cells.

Conclusions

It was produced lyophilized vaccine against swine erysipelas with a 1 year shelf life. Protective power (immunogenicity) of this vaccine is similar to the liquid vaccine (Çarçani, 1994). To eliminate any accident vaccinated because VR2 has a certain strain virulence, used as a solvent 0,01% agar- agar. Since produced, vaccine lyophilized was tested for purity (it resulted pure) for percentage of moisture residual (was 1.2% moisture), and was determined the solvent better for it. (saline solution + 0.01% of agar that plays also an adjuvant).

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