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Research Article

Production of an Oily Adjuvant Vaccine against the Swine Erysipelothrix

Veterinary Medicine

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Abstract

Infection with Erysipelothrix rhusiopathiae in swine has a significant economic impact on pig production systems worldwide. Since a character zoonotic disease, it poses a problem for public health and veterinary services. the clinical and pathological features of the disease have been well-described. Inactivated and attenuated vaccines are available to prevent development of clinical signs of swine erysipelas. In Albania, repeatedly has had outbreaks in swine erysipelas. For too long in our country has been produced a liquid alive attenuated vaccine with strains VR2. This vaccine has a short validity and cannot be used in any epizootic situation. For these reasons we proposed to produce a oily inactivated vaccine, against erysipelas in pigs with a long validity and can be used in any situation epizootic. This constitutes also main purpose of this paper.

Introduction

Swine erysipelas is an important bacterial disease of pigs caused by infection with Erysipelothrix rhusiopathiae. Since a character zoonotic disease, it poses a problem for public health and veterinary services. the clinical and pathological features of the disease have been well-described (Wood and Henderson, 2006). Swine erysipelas found in literature in different languages such as Schweinerotlauf, Vlekziekte, Rouget du porc, Mal Rossino, erysipelas del cerdo, etc. Causative agent of swine erysipelas is *Erysipelothrix rhusiopathiae*. In 1876 *Erysipelothrix muriseptica* was isolated from the blood of a mouse with septicemia by Koch. In 1966, the name was changed to *E. rhusiopathiae.*

The genus *Erysipelothrix* is now subdivided into two major species, *E. rhusiopathiae* (Migula 1900; Skerman et al. 1980) and *Erysipelothrix tonsillarum* (Takahashi et al. 1987). In addition, there are other strains that constitute one or more additional species currently known as *Erysipelothrix* sp.-1 (Takahashi et al. 1992, 2008), *Erysipelothrix* sp.-2 (Takahashi et al. 1992, 2008), *Erysipelothrix inopinata* (Verbarg et al. 2004), and *Erysipelothrix* sp.-3 (Takahashi et al. 2008). *Erysipelothrix* spp. strains can be differentiated by precipitation reactions using hyperimmune rabbit antiserum into at least 28 serotypes (Kucsera 1973; Wood and Harrington 1978). Field cases of swine erysipelas throughout the world are predominantly caused by *E. rhusiopathiae* serotypes 1a, 1b, or 2, while less common serotypes of *E. rhusiopathiae* typically have lower virulence for swine. The organism is presented in the form of rods, straight, angled, in the form of the letter "V" or "X" or spiral with 0.2-0.4 x 0.8-2.5µ.

Erysipelothrix spp. is a gram positive microorganism *Erysipelothrix* are nonmotile, nonsporulating, non-acid-fast, slender gram-positive rods (Brooke and Riley 1999). All the members of the genus are facultative anaerobes and grow between 5°C and 44°C, with optimal growth occurring between 30°C and 37°C (Brooke and Riley 1999; Carter 1990; Sneath et al. 1951).

The causative organism of swine erysipelas, *Erysipelothrix rhusiopathiae*, was first isolated from a pig in 1882 by Louis Pasteur. In 1885, *E. rhusiopathiae* was isolated from pigs in the United States (Smith 1885).

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 (Jeffrey J. Zimmerman. 2012) Most bacterins are serotype 2 (Eamens et al. 2006; Wood 1979) and most attenuated live vaccines contain serotype 1a isolates (Opriessnig et al. 2004).

For the prevention of this disease in pigs in our country used a live attenuated vaccine. Its practical application has some difficulties. This vaccine is applied only in swine herds free from disease. In herds where infection has erupted, the application of this vaccine aggravate the situation. The assessment of effectiveness of its carried out only on animals homologue, consequently has a high financial cost. In order to improve the parameters of this vaccine we undertook the study with the above title.

Materials and Methods

The study of biological properties of strains of Erysipelothrix rhusiopathiae avaiable was conducted by test Spearman – Karber. The study of biological properties of the strains was conducted on white mouse weighing 18-20 grams as follows:

From 24-hour bacterial cultures of each strain of E. rhusiopathiae became the dilutions up to 10^{-6} dilution (acting according to the classic model of dilution 9 + 1). With each dilution was injected into the subcutaneous route 7 groups of rats, the 5 heads of each group.

Calculation of the Lethal Dose

To calculate the dose Letale 50% was used statistical methods Reed –Muench. At first it calculates the distance proportional (DP) between the dilution that gives a percentage over 50% and that gives a percentage of infected under 50%.

Production of Emulsions of Vaccines

By bottles containing vaccine, strains selected by Dose Letale 50%, were carried out planting in the solid terrains agar, liquid terrain and Taroc terrain. Then, the bacterial cultures were placed in incubator for incubation for 24 hours at temperature 37^{o}C. After incubation, the cultures were pulled from the incubator and were controled macroscopically and microscopically for purity. After control for purity with each of the selected strains were planted by 10 plates Roux. Roux plates were placed in incubation at 37^{o}C for 24 hours. After incubation plates were pulled from the thermostat and were controlled for purity (macroscopically). Plates were washed with water physiological at concentration 0.5% formol. For each strain were collected separately base emulsions and were placed in thermostat at 37^{o}C to population bacterial killing. To verify the killing of this bacterial population, 0.1 ml of each solution was planted on solid agar terrain and the terrain Taroc in the interval 3, 5, 6 and 7 days. Bacterial emulsions were considered killed when cultural terrains mentioned above remained sterile. Emulsions were controlled for their microbial concentration by means of Mac Ferland optical standard (dilution based 1/10). For determining the microbial concentration through optical standard Mac Ferland were conducted for each emulsion dilutions 1/10, 1/100, 1/150, 1/200, 1/250, 1/300, which was leveled the score at with optical standard. Vaccine emulsions were corrected so that 1 ml emulsion have a microbial concentration of 3 billion.

Determining the quality of oily protective vaccine against swine Erysipelothrix in laboratory animals (white mouse**)**

To determine the protective power of the vaccine was injected sub Cutaneous by 0.5 ml vaccine white mouse divided into 14 groups, each group was formed by 5 heads. After 21 days and 6 months after vaccination, the vaccinated mouse were infected in parallel with the other 30 mouse not been vaccinated with 0.5 ml virulent strain liquid culture RS C 43-8, and its dilution. The difference of DL 50% resulting between mice vaccinated and infected, and them to control gave the protective power of the vaccine expressed in logarithmic scale. As oily adjuvant was used Marcol 52 and Arlacel 80. It was determined the ratio of oil adjuvant (Marcol 52 and Arlacel 80) with emulsion of vaccine 9:1. It produced a vaccine with oily adjuvant at a concentration of 3 billion microbial cells per ml

Results and Discussion

With oily adjuvant vaccine were vaccinated 35 mouse in 0.5 ml dose, subcutaneous. After 21 days after vaccination, the vaccinated mouse were infected in parallel with the other 30 mouse not been vaccinated with 0.5 ml virulent strain liquid culture RS C 43-8, and its dilution. The data are presented in table n^{o} 1

Table 1: **Determination of the protective power of** oily adjuvant **vaccine against swine erysipelas in laboratory animals (white** mouse**), 21 days after vaccination**

Type of vaccine	Heads vaccinated	Dose of vaccine	Infected after 21 days post vaccination				protection	
			Infected with	Infected heads	Dead after infection	Alive after infection	Ratio N/ Total	
Vaccine with oily adjuvant	5	0.5ml	0.5ml culture	5	5	0	5/5	DL 50 % is 0.5 ml of dilution $10^{-3.68}$
	5	"	0.5 ml 10^{-1}	5	5	0	5/5	
	5	"	0.5 ml 10^{-2}	5	5	0	5/5	
	5	"	0.5 ml 10^{-3}	5	5	0	5/5	
	5	"	0.5 ml 10^{-4}	5	1	4	1/5	
	5	"	0.5 ml 10^{-5}	5	0	5	0/5	
	5	"	0.5 ml 10^{-6}	5	0	5	0/5	
Heads non vaccinated (control)	0.5 ml 10^{-1}	5	5	0	5/5	DL 50 % is 0.5 ml of dilution $10^{-4.83}$	5/5	0.5 ml 10^{-1}
	0.5 ml 10^{-2}	5	5	0	5/5		5/5	0.5 ml 10-^{2}
	0.5 ml 10^{-3}	5	5	0	5/5		5/5	0.5 ml 10^{-3}
	0.5 ml 10^{-4}	5	5	0	5/5		5/5	0.5 ml 10^{-4}
	0.5 ml 10^{-5}	5	2	3	2/5		2/5	0.5 ml 10^{-5}
	0.5 ml 10^{-6}	5	0	5	0/5		0/5	0.5 ml 10^{-6}

Difference between DL 50% of the mouse vaccinated and their unvaccinated with oily adjuvant vaccine gave protective power of the vaccine against swine erysipelas, which was 1:15 logarithmic scale, protection. This is considered nearly over 20 lethal minimal dose.

In table 2 shows the data of the control power protective of vaccine after a period of 6 months.

Table 2: Determination of the protective power of oily adjuvant **vaccine against swine erysipelas in laboratory animals (white** mouse**), 6 months after vaccination.**

Type of vaccine	Vaccinated heads	Dose of vaccine	Infected after 6 month post vaccination					
			Infected with	Infected heads	Dead after infection	Alive after infection	Ratio N/ Total	
Oily adjuvant vaccine	5	0.5 ml	0.5 ml culture	5	5	0	5/5	DL 50% is 0.5 ml of dilution $10^{-4.54}$
	5	"	0.5 ml 10^{-1}	5	5	0	5/5	
	5	"	0.5 ml 10^{-2}	5	5	0	5/5	
	5	"	0.5 ml 10^{-3}	5	5	0	5/5	
	5	"	0.5 ml 10^{-4}	5	4	1	4/5	
	5	"	0.5 ml 10^{-5}	5	1	4	1/5	
	5	"	0.5 ml 10^{-6}	5	0	5	0/5	
Heads unvaccinated (control group)			0.5 ml 10^{-1}	5	5	0	5/5	DL 50 % is 0.5 ml of dilution $10^{-4.83}$
			0.5 ml 10^{-2}	5	5	0	5/5	
			0.5 ml 10^{-3}	5	5	0	5/5	
			0.5 ml 10^{-4}	5	5	0	5/5	
			0.5 ml 10^{-5}	5	2	3	2/5	
			0.5 ml 10^{-6}	5	0	5	0/5	

Difference between DL 50% of vaccinated mice with unvaccinated gave the protective power of the oily adjuvant vaccine against swine erysipelas, which is 0:29 logarithmic scale, which is considered the protection of minimal lethal dose nearly of 2,9. We believe that the protective effect of the vaccine is good, and should be used in specific prophylaxis against this infection.

Conclusion

Was produced oily adjuvant vaccine against swine erysipelas with a one year shelf life. Protective power of the vaccine in laboratory animals 21 and 180 days after vaccination were respectively 1:15 and 0:29 DL 50%. Oily vaccine produced by us can be used in any epizootic situation.

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