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ACTINOBACILLUS PLEUROPNEUMONIAE (APP) SUBUNIT VACCINE PROVIDES CROSS PROTECTION AGAINST VIRULENT APP CHALLENGE

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Introduction: *Actinobacillus pleuropneumoniae* (App) is the etiologic agent of pleuropneumonia in swine. At present, there are 12 recognized serotypes of App (1-12), with serotype 5 being subdivided into subtypes 5a and 5b (Nicolet, 1992). Licensed vaccines for pleuropneumonia in the USA are whole cell bacterins which provide little cross protection.

Secreted toxins (Apx Toxins) are important virulence factors for App pathogenesis (Devinish, 1990; Frey, 1993; Kamp, 1997). While some serotypes express more than one Apx toxin, at least one toxin is expressed by all serotypes of App (Frey, 1993; Kamp, 1997; Beck, 1994) The 12 serotypes of App express various combinations of the Apx toxins and can be grouped into four toxin groups based upon toxin expression patterns (Frey, 1993; van den Bosch, 1994); see Figure 1 In addition to the Apx toxins, the outer membrane protein is also an important component of App vaccines. All serotypes of App possess a 42 kDa outer membrane protein which has been shown to be a protective antigen (van den Bosch, 1994). The antigens expressed by the different serotypes of App are shown in Figure 1.

We have developed a subunit vaccine that is highly efficacious against pleuropneumonia in swine. The vaccine contains ApxI, ApxII and ApxIII toxoids purified from serotypes 10, 7 and 2 respectively, and purified 42 kDa outer membrane protein (OMP) prepared from serotype 1. The antigens are optimally formulated in our patented aqueous adjuvant, Diluvac Forte. The vaccine has been proven to be safe, efficacious and to provide cross protection not available with traditional whole cell bacterins.

Figure 1: Toxicity and Antigens Expressed by Reference Strains of App

Toxin Group	Serotype	(ApxI)	(Apx II)	(ApxIII)	OMP 42 kD
I	1	+	+	-	+
	5a	+	-	-	+
	5b	+	+	-	+
	9	+	+	-	+
	11	+	+	-	+
II	2		+	+	+
	3	-	+	+	+
	4	-	+	+	+
	6	-	+	+	+
	8	-	+	+	+
III	7	-	+	-	+
	12	-	+	-	+
IV	10	+	-	-	+

Materials and Methods: Five separate efficacy studies were done by obtaining three week old pigs free of App antibodies and randomly dividing them into Vaccinate, or Non-Vaccinate Groups. The vaccinated pigs were injected intramuscularly with Intervet's subunit App vaccine at 3 and 6 weeks of age. Two weeks after the second vaccination, the pigs were challenged intranasally with one of the following serotypes: 1, 2, 5, 7 or 10. Pigs that died from App during the 1-week observation period, post-challenge were given a score of 100%, and pigs that survived one-week post challenge were euthanized and their lungs were scored for App lesions.

Results: The vaccine provided significant protection from challenge for each of the 5 serotypes of App that were evaluated. See Figures 2-4.

Figure 2 Efficacy Testing in Pigs. Summary of 5 experiments

Group	Challenge	Mortality	Mean Lung Lesions
Vaccinate	App Sero 1	0/24 (0%)	3 %
Control	App Sero 1	10/20 (50%)	55 %
Vaccinate	App Sero 2	3/23 (13%)	10 %
Control	App Sero 2	4/12 (33%)	53 %
Vaccinate	App Sero 5	1/24 (4%)	23 %
Control	App Sero 5	2/12 (17%)	49 %
Vaccinate	App Sero 7	1/24 (4%)	16 %
Control	App Sero 7	4/12 (33%)	46 %
Vaccinate	App Sero 10	2/24 (8%)	18 %
Control	App Sero 10	18/19 (95%)	97 %

Mann Whitney Statistical Analysis of Lung Lesions
Serotype 1 $p = 0.000$
Serotype 2 $p = 0.025$
Serotype 5 $p = 0.011$
Serotype 7 $p = 0.036$
Serotype 10 $p = 0.000$

Figure 3: Lung Scores Post Challenge

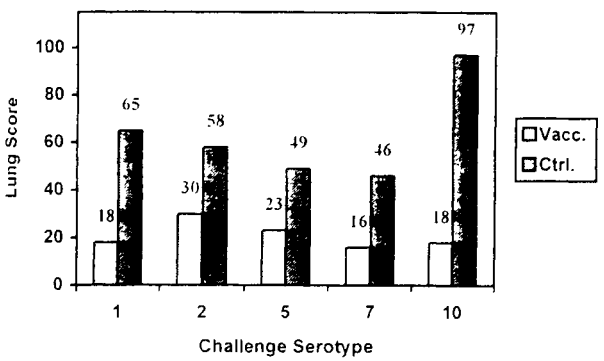
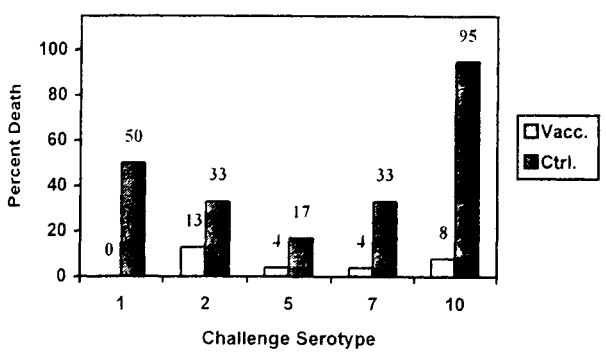


Figure 4: Death Post Challenge



Conclusions:

Our conclusion is that the vaccine is safe and highly protective against relevant North American serotypes. In addition, the vaccine is protective against challenge from at least one serotype of each of the four Toxin Groups shown in Figure 1. These efficacy studies, in conjunction with studies performed in Europe, provide evidence that the vaccine would be protective for all known serotypes of App.

References.

A. Devenish et. al. 1990. Humoral antibody response and protective immunity in swine following immunization with the 104-kilodalton hemolysin of *Actinobacillus pleuropneumoniae*. Infection and Immunity. 58:3829-3832.

B. Frey, J. et. al. 1993. *Actinobacillus pleuropneumoniae* RTX toxins: uniform designation of haemolysins, cytolysins, pleurotoxin and their genes. Journal of General Microbiology. 139:1723-1728.

C. Kamp, E. et. al. 1997. Endobronchial inoculation with Apx toxins of *Actinobacillus pleuropneumonia* leads to pleuropneumonia in pigs. Infection and Immunity. 65:4350-4354.

D. Beck, M. et. al. 1994. RTX Toxin Genotypes and Phenotypes in *Actinobacillus pleuropneumonia* Field Strains. Journal of Clinical Microbiology. 32:2749-2754.

E. Nicolet, J. 1992. *Actinobacillus pleuropneumoniae* in "Diseases of Swine", 7th Edition. pp 401-408. Iowa State University Press.

F. van den Bosch, J. F. et. al. 1994. Protection induced by a trivalent *A. pleuropneumoniae* subunit vaccine. Proceedings of the International Pig Veterinary Society. P. 194