

## Comparison of Protection Efficacy of Toxoid and Whole-Cell Vaccines Against Porcine Pleuropneumonia Caused by Endotracheal Infection with *Actinobacillus pleuropneumoniae*

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### Abstract

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Porcine pleuropneumonia caused by *Actinobacillus pleuropneumoniae* poses a continuous risk of contagious disease in large production units of pigs. This study was focused on comparison of protectivity of whole-cell inactivated vaccine containing *Actinobacillus pleuropneumoniae* serotype 9 and a toxoid vaccine containing Apx I, Apx II, Apx III toxins and outer membrane protein (OMP). Twenty-one piglets from a commercial pleuropneumonia-free herd were used in the experiment. Piglets were randomly divided to three groups, seven animals in each. Animals of the first group were vaccinated with the toxoid vaccine and those of the second group were treated with the whole-cell inactivated vaccine. The third group was an untreated control. Piglets aged 6 weeks were vaccinated for the first time and revaccinated at the age of 10 weeks. Two weeks following revaccination i.e. at the age of 12 weeks, piglets were inoculated endotracheally with a field strain *A. pleuropneumoniae* serotype 9, to yield an inoculum of  $3.2 \times 10^7$  CFU/pig. Experimental infection resulted in the development of clinical pleuropneumonia in all animals. The survival rates were 100%, 71.4% and 14.3% in the first, second and third group, respectively. The clinical course of infection differed among the groups. In animals vaccinated with the toxoid vaccine clinical state turned to normal from day 3 following infection, in those vaccinated with whole-cell vaccine it was from day 5, and in untreated animals the symptoms persisted over the whole experimental period. The experiment was terminated 7 days following infection by sacrificing the piglets that survived the infection, and the degree of pulmonary lesions was determined at necropsy. Pulmonary scores were 6.3, 17.8 and 29.0 in animals vaccinated with the toxoid vaccine, whole-cell vaccine and non-vaccinated ones, respectively. Microbiological examinations confirmed the presence of *A. pleuropneumoniae* following the infection in tonsils.

*Lung lesions, tonsils, pig, porcine pleuropneumonia, vaccine protectivity*

In the recent 20 years porcine pleuropneumonia has been worldwide estimated as a disease causing great economic losses within swine herds. The etiological agent of the infection *Actinobacillus pleuropneumoniae* causes highly contagious disease of swine, which is characterized by acute or chronic fibrinohaemorrhagic necrotizing pleuropneumonia (Sebunya and Saunders 1983). Significant differences in virulence were found among 12 *A. pleuropneumoniae* serotypes. Different stage of virulence in certain serotypes is predominantly connected with secretion of Apx toxins (Nielsen 1984). The serotypes 1, 5, 9 and 11, which produce strongly haemolytic ApxI toxin and partly haemolytic ApxII toxin, induce severe infections, characterized by high mortality and development of large lesions in lungs. One of the roles of ApxI and ApxII toxins is breaking the immune system of the host (Fre y 1995). As Apx toxins are immunogenic, they form the essential part of efficient vaccines (Inzana 1991). Vaccination with a whole-cell bacterin, capsular extract, lipopolysaccharide and outer membrane protein reduces mortality and

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morbidity but fails at elimination of subclinical tonsillar carriers, and does not confer cross protection against heterologous serotypes (Higgins et al. 1985; Rapp and Ross 1988). The aim of this study was to compare protectivity of certain vaccine types, especially their ability to prevent deaths, moderate clinical course of the disease and reduce the extent of lung lesions. Our study also comprised observations of endotracheal infection and consequent colonization of tonsils.

## Materials and Methods

### Experimental model

A total of 21 piglets, crossbreeds of the Improved White breed and Landrace aged 6 weeks from a commercial pleuropneumonia-free herd were used in the experiment. The piglets were serologically negative for antibodies to *A. pleuropneumoniae* or *Mycoplasma hyopneumoniae*. The animals were housed in boxes with straw bedding, seven animals in each box. They were fed a dry complete feed mixture, and water was provided from drinkers *ad libitum*.

The piglets were randomly divided into three groups of seven animals each. Animals of the first group were vaccinated with a toxoid vaccine, whole-cell inactivated vaccine was administered to animals of the second group, and the third group were non-vaccinated control animals. Fourteen days following revaccination at the age of 12 weeks the piglets were infected with a field strain *A. pleuropneumoniae* serotype 9 (KL 2-2000) which was isolated from lungs of a pig who succumbed to acute pleuropneumonia. The experiment was terminated 7 days following the infection. Animals that survived the infection were sacrificed by intravenous administration of 5 mL T61 (Intervet, the Netherlands).

### Immunization

The first vaccination of piglets was carried out at the age of 6 weeks and revaccination 4 weeks later with 2 mL of the vaccine injected intramuscularly in the neck. A commercial vaccine PORCILIS APP<sup>R</sup> (Intervet, the Netherlands) was used as a toxoid vaccine, and the reference strain *A. pleuropneumoniae* serotype 9 (CAPM 3888) was used for preparation of the whole-cell vaccine.

### Preparation of whole-cell vaccine

Vaccination strain *A. pleuropneumoniae* serotype 9 (CAPM 3888) was cultured in a medium containing Soya peptone 20.0 g (Oxoid, England), Trypton 10.0 g (Oxoid, England), distilled water ad 1 000 mL, and supplemented with 10 µg·mL<sup>-1</sup> NAD and 1% (w/v) of glucose. Culture was performed in the shaking machine at 37 °C for 8 h. After finishing, samples were taken for microbiological and bacteriological examinations. Then the culture was inactivated with formaldehyde to a final concentration of 0.12% and centrifuged at 3 000 rpm for 60 min. After centrifugation the supernatant was decanted and the sediment was resuspended in PBS to obtain the concentration of *A. pleuropneumoniae* 5.10<sup>9</sup>. Concentration was determined photometrically, optical density OD<sub>550</sub> = 1. A thorough stirring resulted in homogenization with an adjuvant. In our study Emulsigen (MVP laboratories INC, USA) at final concentration of 12% was used as adjuvant. After homogenization pH was adjusted to 7.2 using 10M NaOH solution and sterility was examined.

### Experimental infection

The infection dose consisting of 1 mL culture of *A. pleuropneumoniae* serotype 9 (KL 2-2000) at a concentration of 3.2 × 10<sup>7</sup> CFU was diluted with PBS to a final volume of 10 mL. The strain of *A. pleuropneumoniae* was cultured at 37 °C for 18 h in BHI supplemented with 10 µg/mL NAD.

Prior to infection, sedation of the animals was performed intramuscularly using STRESNIL<sup>R</sup> (Janssen, Belgium) at a dose of 1 mL/10 kg. Piglets were then anesthetized intravenously with THIOPENTAL ICN (ICN, Czech Republic) in the form of 5% solution at a dose of 5 mg/kg. The infection dose was applied by an endotracheal catheter 3.3 mm in diameter, under a visual control.

### Clinical observations

Evaluation of clinical state was made using a modified method of Hensele et al. (2000). Clinical signs including body temperature were recorded in each animal during 7 days following infection. The state of the animals was assessed daily based on increased respiratory rate, dyspnoea, anorexia and lethargy using the scale 0–3 : 0 = normal state, 1 = moderately impaired, 2 = markedly impaired, 3 = severe course of infection.

### Assessment of lung lesions

Lung lesions were assessed based on the method of Hannan et al. (1982). The extent of lung lesions observed at post mortem examination was drawn as accurately as possible onto a lung diagram. Each lobe was arbitrarily allotted a maximum possible lesion of 5. The pneumonic area of each lobe was then assessed and expressed as a fraction of 5 to give the pneumonic score per lobe. The maximum total score possible for each complete lung was 35. The extent of lung lesions was not assessed in piglets that succumbed following infection during the experiment.

### Microbiological examinations

Samples from pathologically changed parts of lung, tracheobronchial lymph node and tonsil were taken for microbiological examination for the presence of *A. pleuropneumoniae*. The samples were cultured on blood agar with a strip of *Staphylococcus aureus*, under microaerophilic conditions with 10% CO<sub>2</sub> for 18 h at 37 °C.

## Results

No clinical signs were observed in pigs prior to infection. Neither local nor general post-vaccination reactions were observed over vaccination, and no complications occurred at anesthesia, the pigs aroused from narcosis within 1 h.

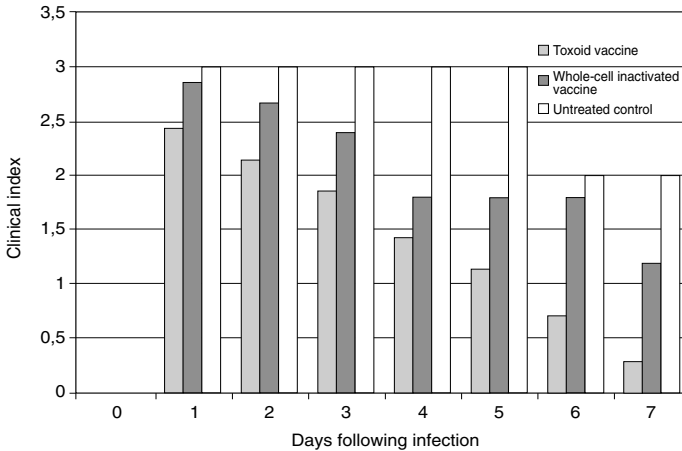


Fig. 1. Clinical observations

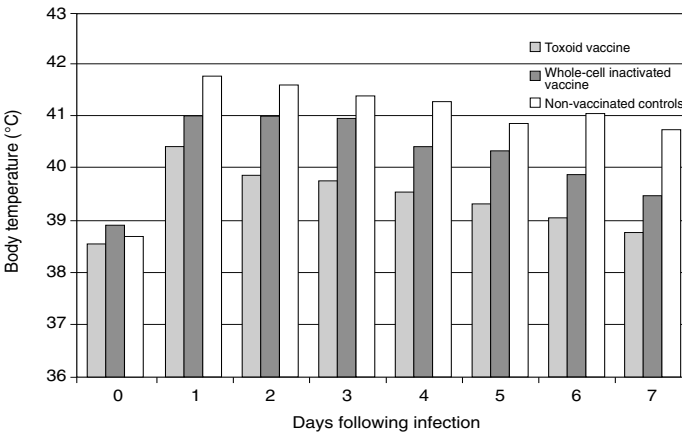


Fig. 2. Average body temperatures following infection

Four animals died in the untreated group one day following infection, and one animal in the group vaccinated with whole-cell vaccine. In all other animals clinical signs of infectious respiratory disease developed, including a sudden increase of body temperature, apathy, dispense, increased respiratory rate and loss of appetite. In animals vaccinated with toxoid vaccine, the clinical course of infection was milder compared to both remaining groups (Figs 1 and 2).

Two pigs in control group died two days following infection. In other animals signs of severe respiratory infection were observed.

Three days following infection one pig of the group vaccinated with whole-cell vaccine died. In both non-vaccinated animals and those vaccinated with whole-cell vaccine pronounced clinical signs persisted; the animals were apathic, suffering from dyspnoea and laying most of time. In animals vaccinated with a toxoid vaccine a markedly improved state was observed. Intake of feed became normal and apathy was less marked.

Five days following infection, health stabilization was observed also in animals vaccinated with whole-cell vaccine. Clinical signs persisting till the end of experiment were recorded in non-vaccinated control. At the end of the experiment, the state of vaccinated pigs was stabilized, feed intake was normal and dyspnoea occurred only at irritation and movement. Death rates and survivals of pigs in individual groups after the infection are shown in Fig. 3.

A characteristic fibrino-haemorrhagic pleuropneumonia was found at necropsy of all succumbed animals (six from non-vaccinated control group and two from group vaccinated with whole-cell vaccine). In animals that died 1 and 2 days following infection, a bloody

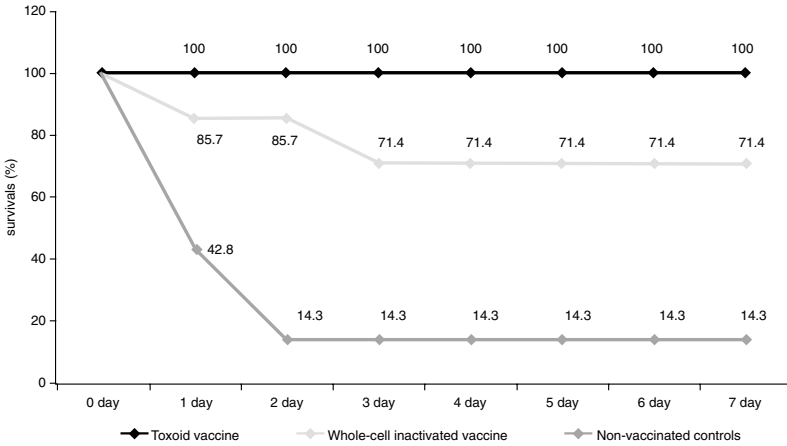


Fig. 3. Survivals following infection (%)

Table 1  
Results of microbiological examinations

Group No. 1 Immunization with toxoid vaccine				Group No. 2 Immunization with whole-cell inactivated vaccine				Group No. 3 Non-vaccinated control			
piglets	lungs	lymph node	tonsils	piglets	lungs	lymph node	tonsil	spiglets	lungs	lymph node	tonsils
9	-	+	+	6	+	+	+	20	+	+	+
10	-	-	+	1	+	-	+	21	+	+	-
16	+	+	+	12	+	+	+	25	+	+	-
17	-	+	-	15	+	+	+	26	+	+	+
18	-	-	+	29	+	+	-	27	+	+	-
21	-	-	+	8	+	+	+	30	+	+	+
23	+	+	-	3	+	+	+	31	+	+	-

+ .....isolation of *A. pleuropneumoniae*

foamy discharge from the nose and mouth were found. This sign was not observed in animals that succumbed 3 days following infection. *A. pleuropneumoniae* serotype 9 was isolated by microbiological examinations from lungs of all succumbed animals.

After sacrificing the animals, samples were taken for bacteriological examinations to detect *A. pleuropneumoniae*. Results of the examinations are shown in Table 1.

Differences were found at the assessment of lung lesions extent (Table 2). Gross lesions were found in pigs vaccinated with toxoid vaccine but a marked tendency to reparation of damaged lung tissue was apparent at the end of experiment. In the group of pigs vaccinated with whole-cell vaccine, lung tissues were more affected with necrosis, and complications caused by pleuritis occurred. One pig of the control group that survived the infection, suffered from a severe fibrino-haemorrhagic pleuropneumonia. Changes were predominantly localized to cardial and diaphragmatic lobes.

Table 2  
Results of lung lesions assessment

Toxoid vaccine		Whole-cell inactivated vaccine		Non-vaccinated controls	
piglet	Pneumonic score	piglet	Pneumonic score	piglet	Pneumonic score
9	2	6	15	11	-
10	7	1	16	21	-
16	8	12	24	25	-
17	7	15	22	26	29
18	9	29	-	27	-
22	4	8	12	30	-
23	7	3	-	31	-

## Discussion

Infection of non-vaccinated pigs with a field strain *A. pleuropneumoniae* serotype 9 resulted in peracute course of the infection and six pigs died (i.e. 85.7 %) within 48 h following infection. A severe fibrino-haemorrhagic pleuropneumonia developed in the one pig that survived the infection. Although *A. pleuropneumoniae*-induced disease in pigs is considered as one of the most severe conditions causing great losses in production units (Sebuyna and Saunders 1983), no effective therapeutic options to eliminate the bacteria exist. Different virulence levels in certain serotypes are essentially connected with production of Apx toxins (Nielsen 1984). These data on high pathogenicity and severe course of the infection induced by *A. pleuropneumoniae* producing ApxI and ApxII toxins were verified in the control non-vaccinated group of pigs.

In vaccinated groups of pigs there was a difference between the group vaccinated with whole-cell vaccine and the one treated with toxoid vaccine. The development of severe respiratory infection was observed in all animals of the former group. This is apparent from the results of clinical observations showing that the health state of pigs vaccinated with whole-cell vaccine stabilized by day 5 after infection, compared to those vaccinated with the toxoid vaccine. These pigs had moderate clinical signs and stabilized by day 3 after infection. In addition, 2 pigs succumbed in the group vaccinated with whole-cell vaccine. Hence, this type of vaccine only reduced mortality. Whole-cell vaccine reduces mortality following infection with a homologous serotype but does not prevent the development of severe clinical signs (Haesbrouck et al. 1997). Immunity acquired in this way fails at higher infection dose and cannot prevent mortality. Evaluation of lung lesions also confirms higher protectivity of a toxoid vaccine. This aspect is important especially with respect to

performance of pigs. Formation of lung lesions lowers daily weight gains and the fattening period is longer (Paisley et al. 1993). Moreover, the whole-cell vaccines do not offer sufficient protection against infections induced by other serotypes (Byrd and Kadis 1992). Therefore the incidence of certain serotypes in a herd has to be taken into account when using whole-cell vaccines.

It can be concluded that the whole-cell vaccine reduced mortality in piglets at infection with a homologous serotype but did not provide protection against pneumonia and colonization of tonsils. Toxoid vaccine prevented death of pigs, minimalized the extent of lung damage but could not prevent colonization of tonsils. Limited protectivity of whole-cell vaccine is due to the absence of extracellular antigens and virulence factors in a bacterin. Therefore a vaccine containing Apx toxins and 42 kDa membrane protein is more efficient and serotype-independent (Haesebrouck et al. 1997). As neither vaccine type prevented the formation of lung lesions and colonization of tonsils, pigs which survive infection can still be carriers of the pathogen in tonsils. Lung lesions were formed in all infected animals. The course of pneumonia can be, under field conditions, affected by secondary pulmonary pathogens. Therefore in herds with *A. pleuropneumoniae*-induced acute infections with high mortality, the use of toxoid vaccine is indicated. However, neither of the vaccines can prevent formation of lung lesions (Chiang et al. 1991) and consequently invasion with secondary pathogens. Therefore it is more efficient to use whole-cell inactivated vaccines containing *A. pleuropneumoniae* serotypes and secondary pulmonary pathogens in herds with chronic course of the disease.

### **Srovnání protektivity toxoidové a celobuněčné vakcíny proti aktinobacilové pleuropneumonii prasat při intratracheální infekci**

Aktinobacilová pleuropneumonie představuje stále významné riziko ve velkochovech prasat. V této práci byla na infekčním modelu porovnána protektivita celobuněčné inaktivované vakcíny obsahující sérotyp 9 *Actinobacillus pleuropneumoniae* a toxoidové vakcíny obsahující Apx I, Apx II, Apx III toxin a vnější membránový protein (OMP). K pokusu bylo použito 21 selat z konvenčního chovu prostého aktinobacilové pleuropneumoniae. Selata byla náhodně rozdělena do třech skupin po 7 kusech. První skupina zvířat byla vakcinovaná toxoidovou vakcínou. Druhá skupina zvířat byla vakcinovaná celobuněčnou inaktivovanou vakcínou a třetí skupina byla kontrolní nevakcinovaná. Primovakcinace byla provedena ve stáří selat 6 týdnů s následná revakcinací za 4 týdny ve stáří selat 10 týdnů. Za 14 dní po revakcinaci, ve stáří selat 12 týdnů byla provedena intratracheální infekce terénním kmenem *A. pleuropneumoniae* sérotyp 9 dávkou  $3,2 \cdot 10^7$  CFU. Po infekci došlo u všech zvířat k rozvoji klinické pleuropneumonie. Ve skupině vakcinované toxoidovou vakcínou přežilo 100% zvířat, ve skupině vakcinované celobuněčnou vakcínou přežilo 71,4% a v nevakcinované skupině 14,3% zvířat. Mezi jednotlivými skupinami byl rozdílný klinický průběh. U zvířat vakcinovaných toxoidovou vakcínou docházelo k normalizaci klinického stavu od 3. dne po infekci. U zvířat vakcinovaných celobuněčnou vakcínou až od 5. dne a u nevakcinovaných zvířat přetrvávaly klinické příznaky po celou dobu pokusu. Pokus byl ukončen 7. den po infekci utracením selat která přežila infekci. U utracených selat byl stanoven rozsah plicních změn. U skupiny vakcinované toxoidovou vakcínou bylo plicní skóre 6,3, u skupiny vakcinované celobuněčnou vakcínou 17,8 a u nevakcinované skupiny 29. Mikrobiologické vyšetření potvrdilo přítomnost *A. pleuropneumoniae* po prodělaném onemocnění v tonzylách.

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