Porcine pleuropneumonia: the first evaluation of field efficacy of a subunit vaccine in Croatia

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ABSTRACT: A vaccine for porcine pleuropneumonia caused by *Actinobacillus pleuropneumoniae* was studied in Croatia on a farm infected by agent serotypes 2 and 9. Vaccination with a commercial subunit vaccine was initiated in the second half of 1998 due to the immense economic damage caused on the farm by this disease. All prefattening and fattening pigs kept on the farm during the first three months of 1999 were allocated in two groups: vaccinated and control. In the control and vaccinated group, 226 and 35 animals (5.78% and 0.96% of the average number of prefattening and fattening pigs in control and vaccinated group), respectively, died from pleuropneumonia. The vaccine efficacy was 83.5%. Examination of the randomly selected lungs on the slaughter line revealed significant reduction in the lesions specific for the chronic form of pleuropneumonia in the vaccinated group (vaccine efficacy 78.6%). The tested vaccine significantly decreased the death rate and pulmonary lesions due to *A. pleuropneumoniae*.

Keywords: porcine pleuropneumonia; immunoprophylaxis; vaccine efficacy

Porcine pleuropneumonia caused by *Actinobacillus pleuropneumoniae* is an important, severe, contagious disease of pigs that entails great economic losses in industrialized swine breeding worldwide. Two biovars, i.e. β-nicotinamide adenine dinucleotide (β-NAD) dependent biovar 1 and β-NAD independent biovar 2, and 15 serotypes (Blackall *et al.*, 2002) based on serotyping of capsular polysaccharides and lipopolysaccharides (LPS) have been identified, while serotype 5 is subdivided into serotypes 5a and 5b (Nicolet, 1992). There are significant virulence differences between the various serotypes of *A. pleuropneumoniae.* Serotypes 1, 5, 9 and 11 are highly virulent, causing severe outbreaks of the disease with high mortality and major pulmonary lesions. Other serotypes are generally less virulent and have low mortality, although they produce similar pulmonary lesions to those induced by more virulent serotypes (Nicolet, 1992). The disease occurs in a peracute, acute, subacute, and chronic form (Sebunya and Saunders, 1983).

Although the virulence of *A. pleuropneumoniae* is multifactorial, molecular epidemiology data show it to be strongly related to the production and secretion of a number of exotoxins, ApxI, ApxII and ApxIII, which belong to the family of RTX toxins (Beck *et al.*, 1994; Frey, 1995a,b). The presence of individual genes on the three toxin operons is constant in a given serotype and reflects the production of as well as the potential to secrete the ApxI, ApxII and ApxIII toxins (Beck *et al.*, 1994).

The first epidemics of porcine pleuropneumonia on large swine breeding farms in Croatia were reported in 1983, and they caused moderate outbreaks with a low mortality (Bilić *et al.*, 1983). At that time, the majority of isolated *A. pleuropneumoniae* strains were identified as serotype 2 (Bilić *et al.*, 1983). Some strains isolated from the farms in eastern Croatia belonged to serotype 7 (Habrun *et al.*, 1998).

In 1997, 80 gilts were imported from West Europe to a farm in northeast Croatia. Before this animal import, *A. pleuropneumoniae* serotype 2 was endemically present at the farm, however, no major losses due to pleuropneumonia were recorded (Habrun *et al.*, 1998). Several months after the import of gilts, considerable losses due to the fast onset, peracute form and high mortality of the disease began to occur on the farm, especially among prefattening and fattening pigs. Serotyping

showed most of the strains isolated from dead pigs from the farm to belong to serotype 9, which belongs to *A. pleuropneumoniae* group characterized with highest virulence, while a minor proportion of isolates belonged to serotype 2, which was continuously present at the farm as an endemic (Habrun *et al.*, 1998). The isolated serotype 9 strains possessed the genes encoding for the synthesis, activation and secretion of ApxI and ApxII toxins, which is a characteristic of the most virulent *A. pleuropneumoniae* serotypes (Habrun *et al.*, 1998).

Until 1998, immunoprophylaxis for porcine pleuropneumonia was not performed on any of the farms in Croatia, however, great losses caused by pleuropneumonia on the above mentioned farm stimulated us to introduce the measures of pleuropneumonia prophylaxis using a commercial subunit vaccine (Porcilis APP, Intervet, Boxmeer, The Netherlands).

This vaccine is considered to provide a good protection from *A. pleuropneumoniae* infection with all serotypes and biovars (Van den Bosch *et al.*, 1992; Kobisch and Van den Bosch, 1992), but in experimental conditions the vaccine induced partial protection against severe challenge (Van Overbeke *et al.*, 2001).

The aim of this study was to assess the vaccine efficacy in reducing the morbidity and mortality due to porcine pleuropneumonia on the farm affected with *A. pleuropneumoniae* serotypes 2 and 9.

MATERIAL AND METHODS

Vaccine

The vaccine (Porcilis APP, Intervet, Boxmeer, The Netherlands) contains ApxI, ApxII and ApxIII toxoids, which are the major virulence factors in *A. pleuropneumoniae*. It also contains 42kDA outer mambrane proteins, common to all serotypes and biovars of *A. pleuropneumoniae*. Alfa-tocoferol-acetate is used as adjuvant.

Pigs

The study was performed on a farm in northeast Croatia, with some 1 500 sows and a respective number of boars for artificial insemination. The farm is a closed farrowing-finishing unit. All technological phases are based on the all-in-all-out principle. During the study period, the farm had 6 800–8 370 prefattening and fattening pigs, and was free from O.I.E. A and B list diseases. All pigs were vaccinated against classical swine fever, pseudorabies, erysipelas and mycoplasmal pneumonia.

Study design

The study was performed during the first three months of 1999. At that time, *A. pleuropneumoniae* serotype 9 and to a lesser extent serotype 2 were isolated from dead pig lungs. The disease entailed considerable economic losses.

Vaccination against porcine pleuropneumonia started in second half of 1998, so that approximately a half of prefattening and fattening pigs had received vaccination by the beginning of 1999. These animals served as a vaccinated group. The animals at vaccinated group were vaccinated with 2 ml of vaccine intramuscularly in the 6th and 10th week of life, according to the manufacturer's instructions.

The rest of prefattening and fattening animals were not vaccinated during the first three months of 1999 and served as a control group. All animals that died at the farm during the period were submitted to the Croatian Veterinary Institute for pathoanatomical and bacteriological examinations to determine the exact cause of death.

Gross pathology

On the slaughter line, 100 randomly selected lungs of the control and vaccinated pigs, respectively, were examined for the lesions specific for infection with *A. pleuropneumoniae* (single hemorrhagic abscess, pleurisy lesions).

Calculation of vaccine efficacy

Vaccine efficacy was calculated by the method described by Martin *et al.* (1987). In particular, vaccine efficacy in reducing pig mortality due to the peracute and acute form of the disease, and in diminishing pulmonary lesions characteristic of the chronic form of the disease as determined at the slaughter line was assessed. The obtained results were entered in the 2 × 2 table and calculated according to the following formulas:

Relative Risk ratio (RR) = (a/(a+b))/(c/(c+d))
Vaccine efficacy (%) = (RR–1)/RR
P-value was calculated using STATA 6.0 computer package.

RESULTS AND DISCUSSION

Results are presented in Tables 1 and 2. Table 1 shows data on the vaccine efficacy in reducing mortality on the farm, while Table 2 depicts the results obtained by determination of lung lesions due to *A. pleuropneumoniae* on the slaughter line.

Discoveries on the immunogenicity of Apx toxins in infected pigs have stimulated studies trying to achieve protection from *A. pleuropneumoniae* by use of these toxins (Fedorka Cray *et al.*, 1993). Results of the studies using various combinations of exotoxins and membrane proteins (Devenish *et al.*, 1990; Bhatia *et al.*, 1991; Inzana *et al.*, 1991, 1993; Beaudet *et al.*, 1994) have shown the vaccine containing ApxI, ApxII and ApxIII toxins and external membrane protein of 42 kDa to provide most efficient protection from infection with all *A. pleuropneumoniae* serotypes and biovars (Van den Bosch *et al.*, 1992; Kobisch and Van den Bosch, 1992; Frey, 1995b).

Upon the *A. pleuropneumoniae* serotype 9 transmission to the study farm, porcine pleuropneumonia began to cause significant economic losses due to high pig mortality and morbidity. During the first three months of 1999, 226 prefattening and fattening pigs in control group (5.78% of the mean number of prefattening and fattening pigs on the control group) died from pleuropneumonia (Table 1). In vaccinated group, 35 prefattening and fattening pigs (0.96% of the mean number of prefattening and fattening pigs on the vaccinated group) died from pleuropneumonia (Table 1). The vaccine efficacy was 83.5%. Significant difference was observed between vaccinated and control group ($P < 0.001$). As pig mortality was mostly caused by peracute or acute course of porcine pleuropneumonia, we believe that vaccination considerably reduced the outbreak of the disease in its peracute and/or acute form.

The chronic form of porcine pleuropneumonia usually is clinically inapparent, but causes a decrease

Table 1. Vaccine efficacy in decreasing mortality due to *A. pleuropneumoniae* infection in prefattening and fattening pigs

	Number of pigs died from pleuropneumonia	Number of pigs not died from pleuropneumonia	Total
Control group	226	3 683	3909
Vaccinated group	35	3 629	3664
Total	261	7 312	7573

relative risk ratio = 6.052
vaccine efficacy = 83.5%

Table 2. Vaccine efficacy in decreasing lung lesions due to *A. pleuropneumoniae* as determined on slaughter line

	Number of lungs with lesions due to *A. pleuropneumoniae*	Number of lungs without lesions due to *A. pleuropneumoniae*	Total
Control group	28	72	100
Vaccinated group	6	94	100
Total	34	166	200

relative risk ratio = 4.667
vaccine efficacy = 78.6%

in gain and increase in feed conversion (Nicolet *et al.*, 1969; Nielsen *et al.*, 1976). Slaughter-line examination of the lungs of *A. pleuropneumoniae* infected pigs revealed hemorrhagic abscesses and pleurisy lesions characteristic for the chronic form of porcine pleuropneumonia in all these animals. In the control and vaccinated group, such hemorrhagic abscesses were observed in 28 (28%) and 6 (6%) pigs, respectively (Table 2). The vaccine efficacy was 83.4%. The achieved results indicated that vaccination had significantly reduced ($P < 0.001$) the proportion of pigs with the chronic form of pleuropneumonia and thus probably the number of carriers.

CONCLUSIONS

The vaccination of piglets at the age of 6 and 10 weeks with a subunit vaccine containing ApxI, ApxII, ApxIII toxins and external membrane protein of 42 kDa significantly decreased the occurrence of acute and chronic porcine pleuropneumonia at the study farm and thus considerably reduced the losses due to this disease.

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