Acta vet. scand. 1997, *38*, 283-293.

Experimental Vaccination of Pigs with an *Actinobacillus pleuropneumoniae* Serotype 5b Capsular Polysaccharide-Tetanus Toxoid Conjugate

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Andresen, L. O., M. J. Jacobsen and J. P. Nielsen: Experimental vaccination of pigs with an *Actinobacillus pleuropneumoniae* serotype 5b capsular polysaccharide-tetanus toxoid conjugate. Acta vet. scand. 1997, 38, 283-293. – The protective efficacy of an *Actinobacillus pleuropneumoniae* serotype 5b capsular polysaccharide-tetanus toxoid conjugate (Ap5bCP-TT) against homologous challenge of pigs was investigated. Four pigs were non-vaccinated controls (group A), 4 pigs were injected with adjuvant without antigen (group B) and 8 pigs were vaccinated with Ap5bCP-TT and adjuvant (group C). Pigs vaccinated with Ap5bCP-TT developed antibody responses to the capsular polysaccharide from *A. pleuropneumoniae* serotype 5b (Ap5bCP). After challenge, all pigs in groups A and B had severe clinical signs of disease and were euthanized. In group C, 3 out of 8 pigs showed severe symptoms and were euthanized. Five pigs in group C survived throughout the study. The post challenge observation period was 72 h. All pigs were subject to necropsy and results from gross pathological findings and microbiological examination are described. Pigs vaccinated with Ap5bCP-TT had statistically significant reduced values of the mass ratio of affected to unaffected lung tissue compared to pigs in groups A and B ($p = 0.01$ and $p = 0.007$, respectively). The results showed that Ap5bCP-TT-vaccination had considerable protective efficacy against lethality and pulmonary lesions caused by experimental infection with *A. pleuropneumoniae* serotype 5b.

capsular polysaccharide-protein; vaccine.

Introduction

Actinobacillus pleuropneumoniae causes pleuropneumonia in pigs (*Nicolet* 1992). This disease is of importance for the pig industry all over the world (*Nielsen* 1982, *Sebunya & Saunders* 1983). The clinical and pathological signs of the disease are well described (*Nielsen* 1982, *Shope* 1964) and reviewed in the literature (*Sebunya & Saunders* 1983, *Nicolet* 1992). Today 12 serotypes of *A. pleuropneumoniae* biotype 1 are known and serotype 5 is divided into subtypes 5a and 5b (*Nielsen* 1990). Different serotypes are dominating in different countries (*Sebunya & Saunders* 1983). In Denmark serotypes 2, 5, 6 and 7 are the most common. The different serotypes of *A. pleuropneumoniae* have different virulence in pigs (*Nielsen* 1982, *Desrosiers et al.* 1984). The serotype is determined by the structure and composition of the capsular polysaccharide of the bacteria (*Perry et al.* 1990).

Infection with *A. pleuropneumoniae* is treated with antibiotics and controlled by vaccination and by employing suitable management methods (*Nicolet* 1992). Most commercially avail-

able vaccines are bacterins containing killed and washed whole cells. Such vaccines have limited and often unsatisfactory effect. They may protect against clinical pleuropneumonia and death but do not in all cases prevent lung lesions when homologous challenge is used (*Nielsen* 1976, *Higgins et al.* 1985). Bacterins containing whole cell antigens from one serotype do not protect against other serotypes of *A. pleuropneumoniae.* Bacterins effective against more than one serotype must contain whole cell antigen from all the serotypes in question (*Nielsen* 1984). The lipopolysaccharide content of the whole cell antigen limits the quantity of antigen from each serotype that can be included in each dose and, therefore may affect the efficacy of the bacterin.

Several reports on the use of capsular polysaccharide-protein conjugates as immunogens have been published (*Robbins & Schneerson* 1990, *Peeters et al.* 1991, *Schneerson et al.* 1992, *Byrd & Kadis* 1992, *Fattom et al.* 1993). The conjugation of polysaccharide to immunogenic protein enhances the immune response to the less immunogenic polysaccharide (*Schneerson et al.* 1986).

The capsule of *A. pleuropneumoniae* serotype 5 has been shown to be a virulence factor by acting inhibitory to the bactericidal activity of serum and to be antiphagocytic to polymorphonuclear leucocytes (*Inzana et al.* 1988). In the same study passive immunization of pigs with serum monospecific to capsule was not fully protective against homologous intratracheal challenge. Recent studies have shown that live non-capsulated mutants of *A. pleuropneumoniae* serotype 1 and 5 did not cause disease in pigs challenged intranasally or intratracheally with 10-20 times the LD_{50} of the parent strain (*Inzana et al.* 1993).

As the capsular polysaccharide of *A. pleuropneumoniae* may be considered a virulence factor, we found it appropriate to investigate if vaccination with a capsular polysaccharide-tetanus toxoid conjugate from *A. pleuropneumoniae* serotype 5b could induce protective immunity in pigs experimentally infected with live aerosolized *A. pleuropneumoniae* serotype 5b.

Materials and methods

Bacterial strain and growth media

A. pleuropneumoniae serotype 5b reference strain L20 was used for preparation of capsular polysaccharide and challenge of pigs. *A. pleuropneumoniae* was grown on meat broth agar plates supplemented with 5% bovine blood (*Jacobsen & Nielsen* 1995) with a non-haemolytic *Staphylococcus aureus* as NAD nurse-strain or on modified PPLO-agar plates (*Nicolet* 1971) as solid medium. Liquid Trypticase-yeast extract medium for propagation of *A. pleuropneumoniae* consisted of 30 g/l Trypticase® soy broth (BBL 11768, Becton Dickinson, Cockeysville, MD, USA) supplemented with 10 g/l yeast extract (Oxoid L21, Unipath Ltd., Basingstoke, UK) and 0.01% (w/v) NAD (Sigma Chemical Co., St. Louis, MO, USA), pH was adjusted to 7.2. Broth cultures were grown at 37 °C with shaking at 130 rpm in a Queue Orbital Shaker Model 4710 (Queue Systems, Parkersburg, WV, USA). The strain was stored at –80 °C as a 6 h culture in Trypticase-yeast extract medium supplemented with 10% glycerol. Liquid medium for fermentation contained per litre 18.0 g casamino acids (Difco, Detroit, MI, USA), 14.0 g yeast extract, 11.0 g glucose, 2.50 g NaCl, 0.50 g KH_2PO_4, 50 mg $MgCl_2$, 0.10 g $CaCl_2$, 4 mg $FeSO_4$, 3 mg $CuSO_4$, 40 mg L-cysteine/HCl and 0.25 g NAD. The pH was adjusted to 7.2.

Fermentation conditions

A 7-litre MBR Labor Bioreactor (MBR Bio Reactor AG, Switzerland) containing 4 litre fermentation medium was inoculated with a 500

ml *A. pleuropneumoniae*-culture grown in Trypticase-yeast extract medium for 3 h in a shaking flask. The temperature was maintained at 37 °C and pH was maintained at 7.2 by automatic addition of 3.0 M sodium hydroxide. Foam was controlled by addition of silicon emulsion. Aeration was initially set at 20% pO_2 and gradually raised to 50% pO_2. Aeration was automatically regulated by sparging sterile atmospheric air. Agitation was initially set at 300 rpm and gradually raised to 500 rpm. After 4 to 6 h of cultivation the optical density (OD) at 600 nm was approximately 6.0 and the culture was cooled to 25 °C. Cells were harvested by centrifugation at 17,000×g for 30 min at 4 °C. The supernatant was discharged and the cell pellet was collected and used for preparation of capsular polysaccharide.

Preparation of crude capsular polysaccharide
A crude preparation of capsular polysaccharide from *A. pleuropneumoniae* serotype 5b (Ap5bCP) was prepared from cells grown under fermentation conditions as described above by phenol extraction as described earlier (*Nielsen et al.* 1996). After phenol extraction and treatment with Proteinase K the majority of the lipopolysaccharide was removed from the suspension by centrifugation at 4 °C and 25,000 rpm in a Beckman L8-70M ultracentrifuge (Beckman, Palo Alto, CA, USA) using a SW25 rotor. The supernatant was concentrated by ultrafiltration (10,000 Da cut off) in an Amicon ultrafiltration cell (Amicon Corp., Daverns, MA, USA) at 4 °C. The carbohydrate content of the concentrated supernatant containing the crude capsular polysaccharide was determined. Ap5bCP was used for preparation of conjugate and as antigen in an indirect ELISA after derivatization with 2-phenylethylamine.

Conjugation of Ap5bCP to tetanus toxoid
Conjugation of Ap5bCP to tetanus toxoid (TT) was achieved by periodate oxidation and reductive amination with cyanoborohydride by the technique described by *Gray* (1978). Twenty five ml of crude Ap5bCP (5.5 mg/ml) in distilled water was added 1.05 ml freshly prepared 0.1 mM $NaIO_4$ in 10 mM NaH_2PO_4, pH 7.0 and was left at room temperature for 60 min. Thereafter 20 µl ethylene glycol per ml was added to reduce excess of periodate. The preparation was dialysed extensively against 0.2 M KH_2PO_4, pH 7.0 over night. TT (Statens Serum Institut, Copenhagen, Denmark) (0.15 mg/ml) and $NaBH_3CN$ (Merck, Darmstadt, Germany) (8.0 mg/ml) was added and the mixture was incubated for 10 days at 37 °C with gentle end-over-end tumbling in a tightly sealed container. After 10 days the mixture was dialysed extensively against 0.2 M KH_2PO_4, pH 7.0. The dialysed reaction mixture containing the conjugate was passed through two serially connected Sepharose CL-6B (Pharmacia, Uppsala, Sweden) gel filtration columns (2.5 by 35 cm) at 4 °C. The conjugate was eluted with 0.2 M KH_2PO_4, pH 7.0. Fractions of 10 ml were collected and those containing protein were identified by measuring OD at 280 nm. Void volume fractions containing both protein and carbohydrate were pooled. Conjugate was concentrated by addition of six volumes of 96% ethanol and precipitation at –20 °C for 18 h. The conjugate was collected by centrifugation at 17,000×g for 60 min and resuspended in 0.2 M NaCl.

Quantification of carbohydrate
Carbohydrates were quantified by the phenol-sulphuric acid colorimetric method described by *Dubois et al.* (1956) using glucose as a standard.

Derivatization of Ap5bCP with 2-phenylethylamine
In order to facilitate the adsorption of Ap5bCP to the polystyrene surface of ELISA plates

Ap5bCP was derivatized with 2-phenylethylamine using a method described by *Sørensen et al.* (1988). Briefly, Ap5bCP was activated by reaction with cyanogenbromide. Twenty ml containing 2 mg/ml crude Ap5bCP dissolved in sterile water was adjusted to pH 11.0 with 1.0 M NaOH. The polysaccharide was activated by addition of cyanogenbromide (Merck) dissolved in 1-methyl-2-pyrrolidone (Merck) (0.10 g/ml) to a final concentration of 0.1%. The pH was maintained at 10.9 with 0.1 M NaOH for 6 min using a ABU 80 autoburette and a TTT 60 titrator (Radiometer, Copenhagen, Denmark) at room temperature with constant stirring. After activation pH was adjusted to 7.0 and 25 µl 2-phenylethylamine (Merck) was added and pH was maintained at 8.5 for 24 h by automatic titration with 0.1 M HCl. The phenylethylamine derivatized Ap5bCP (Ph-Ap5bCP) was precipitated with six volumes of ethanol and was left at –20 °C over night. The precipitate was recovered by centrifugation at 17,000×g for 60 min and resuspended in sterile distilled water. Ph-Ap5bCP was stored at –20 °C for subsequent use in indirect ELISA.

Animals

A total of 16 (13 males and 3 females), Danish Landrace/Yorkshire pigs from a specific pathogen-free pig herd free from all *A. pleuropneumoniae* serotypes were used as experimental animals. The pigs were housed in groups and fed antibiotic free feed and offered water ad libitum in an isolation unit from one week before the first vaccination. At the first vaccination the pigs were nine weeks old and had an average body weight of 17.3 ± 2.0 (standard deviation, SD) kg.

Vaccination and blood sampling

The 16 pigs were divided into 3 groups of 4, 4 and 8, respectively. One group of 4 pigs (group A) was non-vaccinated controls. In the other group of 4 pigs (group B) each pig was injected i.m. with 2.0 ml 0.15 M NaCl with 15% (v/v) Emulsigen® (MVP Laboratories, Ralston, NE, USA). In the third group (group C) each of the 8 pigs was vaccinated i.m. with doses of Ap5bCP-TT containing 100 µg carbohydrate (Ap5bCP) in 0.15 M NaCl with 15% (v/v) Emulsigen® as adjuvant. Each vaccine dose comprised a total volume of 2.0 ml. The first vaccination was given on day zero. Pigs were revaccinated 14 days later using the same amounts and procedures. Blood samples were obtained 4 days before the first vaccination (day -4), 6 days after the first vaccination and every seventh day thereafter until day 34.

Measurement of Ap5bCP-antibodies in indirect ELISA

Antibodies to Ap5bCP were measured by indirect enzyme linked immunosorbent assay (ELISA) essentially as described by *Nielsen et al.* (1997) using Ph-Ap5bCP as antigen. Briefly, NUNC Immuno Plates F96 Polysorb nr. 4-75094 (NUNC, Roskilde, Denmark) were coated with approximately 4 µg/ml Ph-Ap5bCP in phosphate buffered saline, pH 7.0 and incubated overnight at 4 °C. Serum was diluted 1:200 in washing buffer with 2% polyvinyl pyrrolidone (Av.mol.wt. 40,000), PVP (Sigma). Samples were tested in duplicate. Secondary antibodies were peroxidase-conjugated rabbit immunoglobulins to swine immunoglobulins (Dako P0164, Dako, Glostrup, Denmark). Convalescent serum from a pig experimentally infected with *A. pleuropneumoniae* serotype 5b strain L20 was used as a positive control. The values %-OD were calculated as the mean OD-values of the samples in percent relative to the OD-value of the positive control. The convalescent serum was kindly provided by Dr. R. Nielsen, Danish Veterinary Laboratory, Copenhagen, Denmark.

Challenge with A. pleuropneumoniae
All pigs were challenged with aerosolized live *A. pleuropneumoniae* serotype 5b strain L20 by a recently described method (*Jacobsen et al.* 1995). Briefly, a suspension of 10^5 CFU/ml of *A. pleuropneumoniae* strain L20 was aerosolized and pigs were exposed to the aerosol in a challenge chamber. Pigs were challenged for 10 min on day 35 in groups of 4.

Clinical observations
Rectal temperatures of the pigs were recorded the day before vaccination, at vaccination, 4 h after vaccination and daily during the 2 days following vaccination. Rectal temperatures were recorded immediately before challenge and every 24 h post challenge. The pigs were observed clinically every 6 h during the first 2 days post challenge and signs of disease were recorded. If severe symptoms developed the pigs were euthanized to avoid unnecessary suffering.

Post-mortem examinations
Necropsy was performed immediately after euthanasia or at 72 h post challenge. Each pig was examined for macroscopic alterations of the muscular and connective tissue at the site of injection and its immediate vicinity. Lungs were examined for pathological alterations and the severity and extent of the lesions were recorded. From each pig pulmonary lesions were separated from surrounding apparently unaffected lung tissue (excluding major bronchi) and weighed separately. The mass ration of affected lung tissue (pulmonary lesions) to unaffected lung tissue of the individual pigs was calculated and used as a measure for evaluating the protection against pulmonic lesions. Samples for microbiological examination were taken from pulmonic lesions, tonsils and liver and examined as previously described (*Jacobsen et al.* 1995).

Statistical methods
The %-OD obtained by testing of serum samples in the Ap5bCP-ELISA and differences in mass ratio of affected to unaffected lung tissue of the pigs were statistically analysed by Kruskal-Wallis one-way nonparametric analysis of variance. Statistical analysis of the rise in temperatures was performed by one-way analysis of variance. Calculations were performed by use of the Student Edition of Statistix Version 4.0 (Analytical Software, Tallahassee, FL, USA).

Results

Preparation of Ap5bCP-TT conjugate
Fig. 1 depicts the result of the purification of the Ap5bCP-TT conjugate by Sepharose CL-6B gel filtration. The conjugate was separated from unreacted TT and Ap5bCP and the void volume fractions 14-19 contained the Ap5bCP-TT conjugate. The carbohydrate content of the pooled fractions after precipitation and resuspension was 270 μg/ml. The conjugate was diluted in 0.15 M NaCl, mixed with adjuvant and used for vaccination of pigs.

Temperature response to vaccination
The four pigs in group B showed no significant rise in temperature after injection with Emulsigen® without antigen. The eight pigs in group C vaccinated with Ap5bCP-TT with 15% Emulsigen® as adjuvant showed a transient rise in temperature of 0.6 °C ± 0.6 °C (SD) and 1.6 °C ± 0.5 °C (SD) in average at 4 h after the first and second vaccination, respectively. The differences between group B and C in rise in temperature after the second vaccination were statistically significant ($p = 0.004$). The temperature of the pigs in group C was back to normal 24 h post vaccination.

Antibody response to vaccination
Fig. 2 shows the development of antibodies to

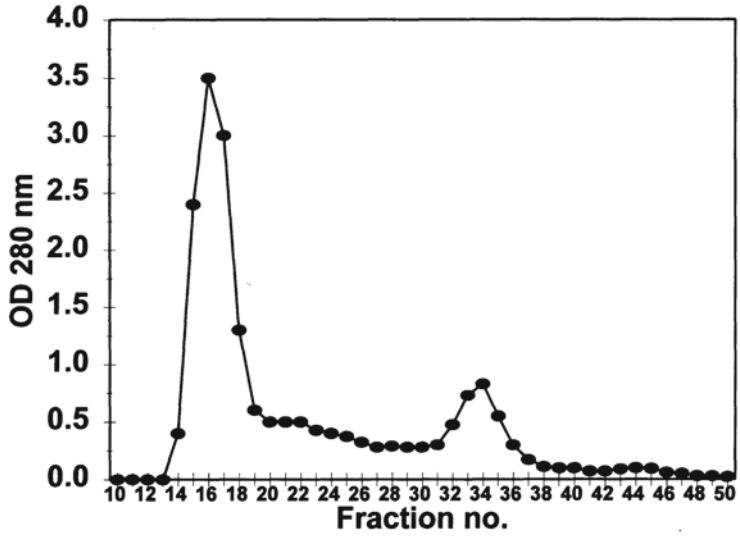

Figure 1. Results of Sepharose CL-6B gel filtration of capsular polysaccharide-tetanus toxoid conjugate from *A. pleuropneumoniae* serotype 5b. Void volume fractions 14-19 contained the conjugate.

Ap5bCP in all the pigs. The control pigs in group A had low levels of antibodies to Ap5bCP throughout the study. The 4 pigs in group B injected with Emulsigen® without antigen had a rise in %-OD measured in ELISA from 5.2% in average before vaccination to 22.5% in average (compared to convalescent serum, 100%) at day 34 after vaccination. Group C developed a varied rise in %-OD-values to Ap5bCP after the second vaccination. The response of this group seemed to peak seven days after the second vaccination and gradually declined during the following two weeks. However, the level of %-OD in group C still was significantly higher compared to the pigs both in group A and in group B at day 34 after the first vaccination ($p = 0.007$).

Clinical observations post challenge

After challenge the 4 control pigs (group A) had dyspnoea. One pig died within 24 h post challenge and two pigs were euthanized at 24 h post challenge. The fourth pig developed severe respiratory symptoms including coughing and mouth-foaming and was euthanized at 48 h post challenge.

In group B the first clinical signs of disease were apparent 24 h after challenge with severe dyspnoea in 3 out of 4 pigs and these 3 pigs were euthanized. Initially the fourth pig in group B showed minor respiratory symptoms, but later the symptoms aggravated and the pig was euthanized at 48 h post challenge.

Tree of the 8 pigs in group C showed severe symptoms 24 h post challenge and were euthanized. The other 5 pigs in this group initially showed dyspnoea, anorexia and rise in temperature of 1.7 °C ± 0.6 °C (SD) in average. The symptoms declined after 30 h post challenge and the pigs seemed clinically healthy at 54 h post challenge. The temperature of 3 of these 5 pigs was normal at 48 h post challenge and 2 of the pigs had slightly elevated temperatures even at the end of the study 72 h post challenge.

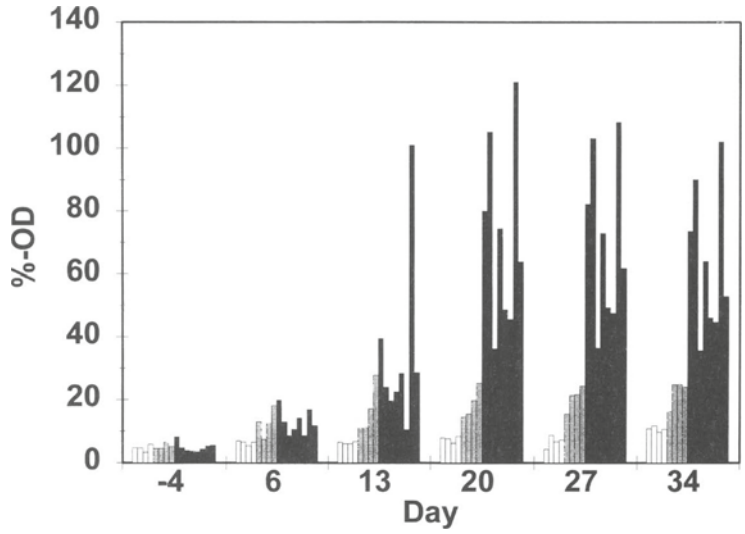

Figure 2. Development of antibodies to capsular polysaccharide of *A. pleuropneumoniae* serotype 5b (Ap5bCP) in pigs shown in % of the response of a convalescent pig in indirect ELISA (%-OD). Pigs were vaccinated on day zero and day 14. Open bars, group A: Non-vaccinated control pigs. Grey bars, group B: Pigs injected with 15% Emulsigen® in saline. Black bars, group C: Pigs vaccinated with Ap5bCP-tetanus toxoid conjugate with 15% Emulsigen® as adjuvant.

Pathology

No macroscopically detectable alterations were observed in any of the animals at the site of injection and its vicinity. Necropsy of the pigs and examination of the lungs showed that all the pigs in group A and B and the 3 pigs in group C that were euthanized 24 h post challenge had fibrinous pleuritis, pericarditis and acute multifocal necrotizing pneumonia. The 5 pigs in group C that survived challenge had fibrinous pleurisy and the number of pulmonary lesions in these pigs were 0, 1, 1, 5 and 21, respectively. The sizes of the lesions varied. The mass ratio of affected to unaffected lung tissue of the individual pigs is shown in Table 1. There were statistically significant lower values of the mass ratio of affected to unaffected lung tissue of group C vaccinated with Ap5bCP-TT compared to groups A and B ($p = 0.01$ and $p = 0.007$, respectively). *A. pleuropneumoniae* was isolated from all pigs with pulmonary lesion and from tonsils or liver from some (Table 1).

Discussion

Byrd & Kadis (1992) have prepared polysaccharide-protein conjugates by coupling capsular polysaccharide and lipopolysaccharide from *A. pleuropneumoniae* serotype 1 to haemolysin protein (the Apx-toxins) of the same serotype. Vaccination of pigs with these conjugates and homologous challenge with *A. pleuropneumoniae* resulted in reduced mortality rate and frequency of pulmonary lesions (*Byrd et al.* 1992). The ability of the Apx-toxins (*Frey et al.* 1993) of *A. pleuropneumoniae* to induce a T-helper cell dependent immune response is not well described. Tetanus toxoid has previously been used as carrier for a number of different bacterial capsular polysaccharides to enhance the immunogenicity of these polysaccharides

Table 1. Mass ratio of affected to unaffected lung tissue, isolation of A. pleuropneumoniae serotype 5b after challenge, and time of euthanasia.

Group* and pig number	Mass ratio	Isolation of *A. pleuropneumoniae*	Time of euthanasia post challenge
Group A			
1	0.55	lung and tonsil	24 h
2	0.31	lung and tonsil	24 h
3	3.92	lung and tonsil	died within 24 h
4	0.83	lung	48 h
Group B			
1	0.44	lung and liver	24 h
2	0.42	lung	48 h
3	2.28	lung and tonsil	24 h
4	2.05	lung	24 h
Group C			
1	0.04	lung	24 h
2	0.12	lung	24 h
3	0.13	lung	24 h
4	0.07	lung	72 h
5	0.31	lung	72 h
6	0.00	no isolation	72 h
7	0.38	lung and tonsil	72 h
8	0.12	lung	72 h

* Group A: Non-vaccinated control pigs. Group B: Pigs injected with 15% Emulsigen® in saline. Group C: Pigs vaccinated with *A. pleuropneumoniae* serotype 5b capsular polysaccharide-tetanus toxoid conjugate with 15% Emulsigen® as adjuvant.

(*Robbins & Schneerson* 1990). Therefore, we chose tetanus toxoid as carrier protein for the capsular polysaccharide of *A. pleuropneumoniae* serotype 5b.

We have used a method for direct coupling of carbohydrates to protein. This technique has previously been described by *Gray* (1978). The method involves the coupling of reducing sugars to protein. The capsular polysaccharide was therefore subject to weak oxidation by exposure to periodate before coupling to tetanus toxoid by reductive amination using cyanoborohydride as reductor. The oxidation of the carbohydrate by periodate could cause destruction of some of the immunogenic determinants of the polysaccharide, but this did not seem to be of significance in this study.

The rise in %-OD from 5.2% on day –4 to 22.5% on day 34 in group B (Fig. 2) may be explained by a general rise in immunoglobulin caused by the immune stimulating effect of Emulsigen®. These immunoglobulins would then bind non-specifically in the indirect ELISA used for measuring the antibody response to Ap5bCP in this study.

Five of the 8 pigs in group C had a rise in temperature after the second vaccination in the range of 1.5 °C to 2.2 °C. The pyrogenic effect of the vaccine could be due to residual LPS and would have to be reduced before it would be acceptable for use in field trials.

Pigs vaccinated with Ap5bCP-TT were less affected with respect to development of clinical symptoms and lesions post challenge than pigs

in the other groups. Furthermore, the pigs in group C had significantly lower values of the mass ratio of affected to unaffected lung tissue. This is a strong indication of the protective effect of Ap5bCP-TT as a vaccine component.

In previous studies on various experimental vaccines against *A. pleuropneumoniae* infections in pigs different methods have been used for evaluating the protective efficacy of the vaccines against pulmonary lesions. In several of these studies rough graduations of the severity of the lung lesions of the individual animals have been used by scoring the pigs on a scale of either 0 to +++ (*Nielsen* 1976) or 0-4 (*Bosch et al.* 1990, *Kamp et al.* 1992, *Inzana et al.* 1993). In other studies (*Rosendal et al.* 1986, *Fedorka-Cray et al.* 1990, *Rossi-Campos et al.* 1992) more nuanced evaluation of the lung lesions have been applied by scoring the percentage of affected tissue of each lung giving indirectly estimated values for comparing groups of experimental animals. In the present study we have used the mass ratio of affected lung tissue to unaffected lung tissue which we consider a more objective measure for evaluating the protection against pulmonary lesions and for comparing the different groups of pigs in the experiment.

There have been other reports of experimental subunit vaccines using other virulence factors and components of *A. pleuropneumoniae,* e.g. the Apx-toxins and outermembrane associated proteins (*Bosch et al.* 1992, *Rossi-Campos et al.* 1992). The Apx-toxins have been shown to be important factors in the pathogenesis of pleuropneumonia (*Smits et al.* 1992). The pigs vaccinated with Ap5bCP-TT in the present study did not obtain total protection against mortality or pulmonary lesions, but improved protection may be achieved by supplementing the Ap5bCP-TT vaccine with toxoid forms of the Apx-toxins.

The results of this study showed that immunization with capsular polysaccharide from *A. pleuropneumoniae* conjugated to a carrier protein such as tetanus toxoid provided partial protection against disease in pigs subjected to homologous challenge with *A. pleuropneumoniae.* After experimental challenge 5 out of 8 pigs (62.5%) in group C not only survived but exhibited significantly reduced lung lesions compared to the challenge controls. Investigations by *Inzana et al.* (1988) have shown that the capsular polysaccharide of *A. pleuropneumoniae* serotype 5b is a virulence factor and that partial protection of pigs could be obtained by passive immunization with monospecific antiserum to capsule. In the present study it was shown that also active immunization of pigs with Ap5bCP-TT can induce partial protection. Thus, it was demonstrated that capsular antigens from *A. pleuropneumoniae* may be important vaccine components in future *A. pleuropneumoniae* subunit vaccines. The protection provided by immunity induced by Ap5bCP-TT may be due to specific antibody reactions with the capsule of the bacteria or involvement of cell-mediated host defence, e.g. opsonization. However, the actual immunoprotective mechanism remains to be elucidated.

Acknowledgements

This work was supported by the National Committee for Pig Breeding, Health and Production, Federation of Danish Pig Producers and Slaughterhouses. We wish to thank Mrs. Heidi P. Andersen and Mrs. Birgit H. Christensen for skillful technical assistance.

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Sammendrag

Eksperimentel vaccination af grise med et Actinobacillus pleuropneumoniae serotype 5b kapselpolysaccharid-tetanus toxoid konjugat.

Den beskyttende effekt af vaccination med et Actinobacillus pleuropneumoniae serotype 5b kapselpolysaccharid-tetanus toxoid konjugat (Ap5bCP-TT) over for homolog, eksperimentel infektion af grise blev undersøgt. Fire grise blev anvendt som ikke-vaccinerede kontroldyr (gruppe A), 4 grise blev injiceret med adjuvans uden antigen (gruppe B) og 8 grise blev vaccineret med Ap5bCP-TT (gruppe C). Grise, der var vaccineret med Ap5bCP-TT udviklede antistoffer overfor kapselpolysaccharid fra *A. pleuropneumoniae* serotype 5b (Ap5bCP). Efter eksperimentel infektion fik grisene i gruppe A og gruppe B alvorlig klinisk pneumoni. I gruppe C udviklede 3 ud af 8 grise alvorlige symptomer og blev aflivet. Fem grise i gruppe C overlevede infektionen. Alle grise blev obduceret, og resultaterne fra makroskopiske patologisk undersøgelser og mikrobiologisk påvisning af *A. pleuropneumoniae* er beskrevet. Grise vaccineret med Ap5bCP-TT havde statistisk signifikant reduktion af forholdet imellem vægten af patologisk forandret lungevæv og normalt lungevæv sammenlignet med ikke-vaccinerede grise og med grise injiceret med adjuvans uden antigen ($p = 0{,}01$ hhv. $p = 0{,}007$). Resultaterne viste, at vaccination med Ap5bCP-TT havde en betydelig beskyttende effekt overfor lungelæsioner og mortalitet forårsaget af infektion med *A. pleuropneumoniae.*

(Received March 4, 1997; accepted June 7, 1997).

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