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Actinobacillus Pleuropneumoniae Serotype 5, Subtypes a and b: Cross Protection Experiments

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Nielsen, R: Actinobacillus pleuropneumoniae serotype 5, subtypes a and b: Cross protection experiments. Acta vet. scand. 1988, 29, 67-75. - Vaccination of pigs with a killed culture of A. pleuropneumoniae serotype 5, strain K17 (subtype a) afforded a high degree of protection against challenge with strains L20 and T928 (subtype b). The reverse experiment showed that strain L20 gave good protection against challenge with strain K17 whereas strain T928 did not afford an acceptable protection against challenge with this strain.
The considerable cross immunity shown to exist between strains K17 and L20 indicates a high degree of homogeneity of the antigenic determinants of the two strains involved in induction of protective immunity and suggest that antibodies to capsular subtype specific determinants may not play a significant role in the specific defence against A. pleuropneumoniae strains belonging to serotype 5. The finding that a vaccine prepared from strain T928 did not afford an acceptable protection against challenge with strain K17 indicates a variable expression among serotype 5 strains of the antigenic determinants which induce protective immunity against A. pleuropneumoniae infection.

Vaccine; cross immunity.

Introduction

The cellular components of Actinobacillus pleuropneumoniae which play a role in protective immunity have not yet been fully defined. Serotype specificity of the organism is related to capsule associated antigens of polysaccharide (PS) and lipopolysaccharide (LPS) nature (*Gunnarsson* 1979, *Nielsen & O'Connor* 1984, *Fenwick et al.* 1986). Both PS and LPS seem to be able to raise antibodies in pigs infected with A. pleuropneumoniae (*Rapp et al.* 1986, *Mulks et al.* 1986). Experimental studies of cross immunity in pigs have revealed that the protection obtained after parenteral immunization is related to serotype-specific antibodies (*Nielsen* 1985). Also, evidence have been obtained to indicate that both antibodies to type-specific capsular PS and LPS are important in the specific defence against A. pleuropneumoniae (*Bendixen et al.* 1981, *Nielsen* 1985).
Strains assigned to A. pleuropneumoniae, serotype 5 can be subdivided into two subtypes a and b, based upon a capsular antigenic determinant of PS nature which is specific for each subtype. In addition, the strains share capsular antigenic determinants of both PS and LPS nature (*Nielsen* 1986).
The present study was undertaken to compare the immunizing capacity of the two subtypes in cross protection experiments.

Materials and methods

Antigens

The strains of A. pleuropneumoniae used in vaccines and for challenge were K17 (subty-

Table 1. Actinobacillus pleuropneumoniae serotype 5 strains used in experiments and as antigen in the modified complement fixation test.

Subtype	Strain designation	Isolated from	Source
a*	K17	Arthritis, lamb	L. Frazier, Davis USA
b*	L20	Pneumonia, pig	L. Frazier, Davis USA
b	T928	Pleuropneumonia, pig	F. S. Hsu, Taiwan

* The strains were received from Dr. A. Gunnarsson, Uppsala.

pe a), L20 (subtype b) and T928 (subtype b). The origin of the strains is given in Table 1. The bacteria used for inoculation of pigs were grown at 37°C for 24 h on modified PPLO agar plates (*Nicolet* 1971). The growth was harvested in 0.9% saline. The density of the suspension was adjusted to 10^{10} organisms per ml by comparison with an opacity standard.

Antigens for the complement fixation (CF) test were prepared as described earlier (*Nielsen* 1982).

Animals

A total of 37 nine-week-old pigs were used (29 vaccinates and 8 controls). The pigs were derived from the laboratory herd which is being maintained as an SPF unit. The pigs were divided into 6 groups (c.f. Tables 2 to 7).

Vaccines

Vaccines were made from 6-h whole cells of A. pleuropneumoniae with Freund's incomplete adjuvant (3:1) as described earlier (*Nielsen* 1976, 1982).

Vaccination procedure

The experimental pigs were vaccinated on arrival and given a booster injection 3 weeks later. Injections were given subcutaneously in the neck. Doses were 2 × 2 ml. Non-vaccinated controls were kept in contact with the vaccinates.

Challenge

Three weeks after the last vaccine injection all pigs were inoculated intranasally with 10^{10} viable organisms, as described earlier (*Nielsen* 1979).

Post mortem examination

Three weeks after challenge (p.ch.) the pigs were sacrificed and subjected to a full post mortem examination as described earlier (*Nielsen* 1979).

Serology

Blood samples were taken from the anterior vena cava before and after vaccination, before challenge and at sacrifice. Sera were tested by the CF test (*Nielsen* 1974, 1982).

Serotyping

Challenge strains re-isolated from lungs, tonsils and nasal cavity were subtyped using gel diffusion and indirect haemagglutination as described earlier (*Nielsen* 1986).

Evaluation of the effect of vaccination

The vaccines were considered to be protective if clinical symptoms and pneumonic lesions were absent after challenge.

Table 6. Protective effect of a vaccine prepared from a 6-hour culture of strain L20 with Freund's incomplete adjuvant (3:1). Dose of vaccine 2 × 2 ml s.c. Challenge was performed intranasally with strain T928 3 weeks after the last vaccine injection. The pigs were sacrificed 3 weeks after challenge.

Pig No.	Vaccine strain	Challenge strain	CF titers at challenge L20	CF titers at challenge T928	CF titers at sacrifice L20	CF titers at sacrifice T928	Clinical symptoms p.ch.*	Pleuropneumonia at necropsy	A. pleuropneumonia re-isolated from:
1/4	L20	T928	128	128	128	128	none	-	tonsils
2/4	L20	T928	64	64	64	64	none	-	tonsils
3/4	L20	T928	128	128	128	64	none	-	tonsils
4/4	Control	T928	0	0	32	32	severe	+++	lung, tonsils

*p.ch = after challenge
\+ = few small lesions
+++ = widespread pneumonic lesions
\- = no lesions
Titers are given as reciprocals of the highest serum dilution giving positive reaction.
0 = no reaction

Results

Group I. Five vaccinates and 1 control. Vaccine: strain K17. Challenge: strain L20 (c.f. Table 2).

Four vaccinates showed no clinical symptoms following challenge and no lesions at necropsy. One vaccinate had transient anorexia and slight respiratory distress. A small area of necrosis with an overlying fibrous pleuritis was seen at necropsy. The control had severe respiratory distress 1 day after challenge. Necropsy revealed fibrous pleuritis and widespread pneumonic lesions.

Challenge strain L20 (subtype b) was re-isolated from the lungs of 1 vaccinate and the control and from the tonsils of all but one vaccinate.

Thus, 4 out of 5 vaccinates were protected (80%).

Post vaccination titers (defined in subtext of Table II) to strain K17 were 32 to 256. Titers to strain L20 were at the same levels. None of the vaccinates had a significant rise in titer to the 2 strains after challenge. The control pig was seronegative before challenge. After challenge it became seropositive with titers of 64 to both strains.

Group II. Six vaccinates and 2 controls. Vaccine: strain L20. Challenge: strain K17 (c.f. Table 3).

Five vaccinates showed no clinical symptoms and no lesions at necropsy. One vaccinate had slight respiratory distress. A small area of necrosis with an overlying fibrous pleuritis was seen at necropsy. Both controls had severe respiratory distress 1 day after challenge. Necropsy revealed fibrous pleuritis and pneumonia with several areas of necrosis.

Challenge strain K17 (subtype a) was re-isolated from the lungs of one vaccinate and the two controls, from the tonsils of all pigs and from the nasal cavity of two vaccinates and one control.

As appears, 5 out of 6 vaccinates were protected (83%).

Post-vaccination titers to strain L20 were 32 to 256. Titers to strain K17 were at the same levels. No significant rise in titers was seen

Table 3. Protective effect of a vaccine prepared from a 6-hour culture of strain L20 with Freund's incomplete adjuvant (3:1). Dose of vaccine 2 × 2 ml s.c. Challenge was performed intranasally with strain K17 3 weeks after the last vaccine injection. The pigs were sacrificed 3 weeks after challenge.

Pig No.	Vaccine strain	Challenge strain	CF titers at challenge K17	CF titers at challenge L20	CF titers at sacrifice K17	CF titers at sacrifice L20	Clinical symptoms p.ch.*	Pleuropneumonia at necropsy	A. pleuropneumonia re-isolated from:
1/60	L20	K17	128	128	64	128	none	-	tonsils
2/60	L20	K17	64	32	32	16	none	-	tonsils
3/60	L20	K17	64	64	64	64	none	-	tonsils
4/60	L20	K17	128	128	256	256	slight	+	lung, tonsils
5/60	L20	K17	256	256	256	256	none	-	tonsils, nasal cavity
6/60	L20	K17	256	128	128	256	none	-	tonsils, nasal cavity
7/60	Control	K17	0	0	64	128	severe	+++	lung, tonsils, nasal cavity
8/60	Control	K17	0	0	16	16	severe	+++	lung, tonsils

*p.ch = after challenge
\+ = few small lesions
+++ = widespread pneumonic lesions
\- = no lesions
Titers are given as reciprocals of the highest serum dilution giving positive reaction.
0 = no reaction

Table 4. Protective effect of a vaccine prepared from a 6-hour culture of strain K17 with Freund's incomplete adjuvant (3:1). Dose of vaccine 2 × 2 ml s.c. Challenge was performed intranasally with strain T928 3 weeks after the last vaccine injection. The pigs were sacrificed 3 weeks after challenge.

Pig No.	Vaccine strain	Challenge strain	CF titers at challenge K17	CF titers at challenge T928	CF titers at sacrifice K17	CF titers at sacrifice T928	Clinical symptoms p.ch.*	Pleuropneumonia at necropsy	A. pleuropneumonia re-isolated from:
7/70	K17	T928	64	64	64	64	none	-	tonsils
8/70	K17	T928	128	64	64	64	none	-	-
9/70	K17	T928	32	32	32	32	none	-	tonsils
10/70	K17	T928	128	128	128	128	none	-	tonsils
11/70	Control	T928	0	0	64	32	severe	+++	lungs, tonsils

*p.ch = after challenge
\+ = few small lesions
+++ = widespread pneumonic lesions
\- = no lesions
Titers are given as reciprocals of the highest serum dilution giving positive reaction.
0 = no reaction

after challenge. The 2 controls were seronegative before challenge. After challenge they became seropositive with titers of 16 and 128 to strains L20 and 16 and 64 to strain K17.

Group III. Four vaccinates and 1 control. Vaccine: strain K17. Challenge: strain T928 (c.f. Table 4).

The 4 vaccinates showed no clinical symptoms and no lesions at necropsy. The control had severe respiratory distress 1 day after challenge. Necropsy revealed fibrous pleuritis and pneumonia with areas of nerosis.

Challenge strain T928 (subtype b) was re-isolated from the tonsils of all but 1 pig and from the lungs of the control.

Thus, alle vaccinates were fully protected.

Post vaccination titers to strain K17 were 32 to 128. Titers to strain T928 were at the same levels. After challenge no rise in titers to the 2 strains was seen in the vaccinates. The control pig was seronegative before challenge. After challenge it became seropositive with titers of 64 and 32 to strain K17 and T928 resp.

Group IV. Eight vaccinates and 2 controls. Vaccine: strain T928. Challenge: strain K17 (c.f. Table 5).

Three vaccinates showed no clinical symptoms and no lesions at necropsy. Three vaccinates had transient anorexia and slight respiratory distress. Few, small areas of necrosis with an overlying fibrous pleuritis were seen at necropsy. Two vaccinates and the controls had severe respiratory distress one day after challenge. Necropsy revealed fibrous pleuritis and several areas of large necroses. The severity and extent of the lesions were not markedly different in the 2 vaccinates and the control.

Challenge strain K17 (subtype a) was re-isolated from the lungs of 5 vaccinates and the 2 controls and from the tonsils of all pigs.

Table 5. Protective effect of a vaccine prepared from a 6-hour culture of strain T928 with Freund's incomplete adjuvant (3:1). Dose of vaccine 2 × 2 ml s.c. Challenge was performed intranasally with strain K17 3 weeks after the last vaccine injection. The pigs were sacrificed 3 weeks after challenge.

Pig No.	Vaccine strain	Challenge strain	CF titers at challenge T928	CF titers at challenge K17	CF titers at sacrifice T928	CF titers at sacrifice K17	Clinical symptoms p.ch.*	Pleuropneumonia at necropsy	A. pleuropneumonia re-isolated from:
1/68	T928	K17	128	128	128	256	slight	+	lung, tonsils
2/68	T928	K17	64	64	64	128	severe	+++	lung, tonsils
3/68	T928	K17	64	64	64	128	severe	+++	lung, tonsils
4/68	T928	K17	64	128	64	128	none	-	tonsils
5/68	T928	K17	64	64	64	64	none	-	tonsils
6/68	T928	K17	128	64	128	32	none	-	tonsils
7/68	T928	K17	128	64	128	64	slight	+	lung, tonsils
8/68	T928	K17	128	64	128	64	slight	+	lung, tonsils
9/68	Control	K17	0	0	16	32	severe	+++	lung, tonsils
10/68	Control	K17	0	0	64	32	severe	+++	lung, tonsils

*p.ch = after challenge
\+ = few small lesions
+++ = widespread pneumonic lesions
\- = no lesions
Titers are given as reciprocals of the highest serum dilution giving positive reaction.
0 = no reaction

As appears, only 3 out of 8 vaccinates were fully protected (37%).
Post vaccination titers to strain T928 were 64 to 128. Titers to strain K17 were at the same levels. After challenge no significant rise in titer to the 2 strains was observed in the vaccinates. The control pigs were seronegative before challenge. After challenge they became seropositive with titers of 32 to K17 and 16 and 64 to T928.

Group V. Three vaccinates and 1 control. Vaccine strain: L20. Challenge strain: T928 (c.f. Table 6).
The 3 vaccinates showed no clinical symptoms and no lesions at necropsy. The control had severe respiratory distress 1 day after challenge. Necropsy revealed fibrous pleuritis and widespread pneumonic lesions.
Challenge strain T928 (subtype b) was re-isolated from the tonsils of the vaccinates and from the lungs and tonsils of the control.
Thus, all vaccinates were fully protected.
Post vaccination titers to strain L20 were 64 to 128. Titers to strain T928 were at the same levels. After challenge no rise in titers were observed in the vaccinates. The control pig was seronegative before challenge. After challenge it became seropositive to strain T928 and strain L20 with titers of 32.

Group VI. Three vaccinates and 1 control. Vaccine: strain T928. Challenge: strain L20 (c.f. Table 7).
Two vaccinates showed no clinical symptoms and no lesions at necropsy. One vaccinate had slight respiratory distress. A small area of necrosis with an overlying fibrous pleuritis was seen at necropsy. The control had severe respiratory distress 1 day after challenge. Necropsy revealed fibrous pleuritis and widespread pneumonic lesions.
Challenge strain L20 (subtype b) was re-isolated from the tonsils of 2 vaccinates and the control and from lungs and nasal cavity of 1 vaccinate and the control.
Thus, 2 out of 3 vaccinates were protected (66%).
Post vaccination titers to strain T928 were 16 to 64. Titers to strain L20 were at the same level. No significant rise in titers was seen after challenge. The control pig was seronega-

Table 6. Protective effect of a vaccine prepared from a 6-hour culture of strain L20 with Freund's incomplete adjuvant (3:1). Dose of vaccine 2 × 2 ml s.c. Challenge was performed intranasally with strain T928 3 weeks after the last vaccine injection. The pigs were sacrificed 3 week after challenge.

Pig No.	Vaccine strain	Challenge strain	CF titers at challenge L20	CF titers at challenge T928	CF titers at sacrifice L20	CF titers at sacrifice T928	Clinical symptoms p.ch.*	Pleuropneumonia at necropsy	A. pleuropneumonia re-isolated from:
1/4	L20	T928	128	128	128	128	none	-	tonsils
2/4	L20	T928	64	64	64	64	none	-	tonsils
3/4	L20	T928	128	128	128	64	none	-	tonsils
4/4	Control	T928	0	0	32	32	severe	+++	lung, tonsils

*p.ch = after challenge
\+ = few small lesions
+++ = widespread pneumonic lesions
\- = no lesions
Titers are given as reciprocals of the highest serum dilution giving positive reaction.
0 = no reaction

Table 7. Protective effect of a vaccine prepared from a 6-hour culture of strain T928 with Freund's incomplete adjuvant (3:1). Dose of vaccine 2 × 2 ml s.c. Challenge was performed intranasally with strain L20 3 weeks after the last vaccine injection. The pigs were sacrificed 3 weeks after challenge.

Pig No.	Vaccine strain	Challenge strain	CF titers at challenge T928	CF titers at challenge L20	CF titers at sacrifice T928	CF titers at sacrifice L20	Clinical symptoms p.ch.*	Pleuropneumonia at necropsy	A. pleuropneumonia re-isolated from:
5/4	T928	L20	16	16	32	32	slight	+	lung, tonsils, nasal cavity
6/4	T928	L20	32	32	32	32	none	-	tonsils
7/4	T928	L20	64	64	64	64	none	-	-
8/4	Control	L20	0	0	32	32	severe	+++	lung, tonsils, nasal cavity

*p.ch = after challenge
\+ = few small lesions
+++ = widespread pneumonic lesions
\- = no lesions
Titers are given as reciprocals of the highest serum dilution giving positive reaction.
0 = no reaction

tive before challenge. After challenge it became seropositive to strain L20 and T928 with titers of 32.

Discussion

Vaccination of pigs with a killed culture of A. pleuropneumoniae strain K17 (subtype a) protected 4 out of 5 vaccinates (80%) against challenge with strain L20 (subtype b). In the reverse experiment with a vaccine prepared from strain L20 5 out of 6 vaccinates (83%) were fully protected against challenge with strain K17. The results are similar to those obtained in earlier experiments with homologous challenge (*Nielsen* 1976) and indicate a high degree of homogeneity of the antigenic determinants of strains K17 and L20 involved in induction of protective immunity. Cross protection studies with serotypes 8,3 and 6 which share capsular antigenic determinants of PS and LPS nature seem to support the concept that antibodies to both play a significant role in the protection of pigs against pleuropneumonia (*Nielsen* 1985).

The results obtained in the present study would seem to suggest that the considerable cross immunity shown to exist between strains K17 and L20 is related to the capsular antigenic determinants of PS and LPS nature shared by the 2 subtypes and indicate that antibodies to capsular subtype specific PS may not play a significant role in the specific defence against A. pleuropneumoniae strains belonging to serotype 5.

The finding that a vaccine prepared from strain K17 or strain L20 gave full protection against challenge with strain T928 (subtype b) whereas, in the reverse experiment, a vaccine prepared from strain T928 did not afford an acceptable protection against challenge with strains K17 and L20 resp. is not easily explained. In comparative gel diffusion analyses of strains L20 and T928 it was shown that the capsular antigenic determinants of the 2 strains were identical (*Nielsen* 1986) and it would therefore be justified to anticipate identical results in cross protection experiments.

Recent studies have shown that experimentally infected pigs develop antibodies to polysaccharide and outer membrane proteins (OMP) (*Rapp et al.* 1986, *Mulks et al.* 1986). Immunoblotting studies indicated antigenic differences or variable expression of these immunogens among serotype 5 strains (*Rapp et al.* 1986). Electrophoretic analyses of Haemophilus influenzae type b have revealed microheterogeneity and interstrain variation of the LPS of this organism (*Inzana* 1983). Characterization of the LPS of A. pleuropneumoniae have, so far, comprised only 2 strains (*Fenwick et al.* 1986, *Maudsley et al.* 1986). Whether variations may also be present in the LPS of A. pleuropneumoniae strains and the possible consequences of such variations for the synthesis of protective antibodies will have to await further studies.

Studies of H. influenzae type b have shown that several OMP's are immunogenic and able to induce antibodies which are protective against systemic disease in experimental animals (*Gulig et al.* 1984, *Kimura et al.* 1985). Also, it was shown that OMP's of this bacterium contained antigenic determinants capable of eliciting strain-specific protective antibodies (*Granoff et al.* 1986). So far it is not known whether antibodies directed against OMP's of A. pleuropneumoniae play a protective role against infection.

Until more it known, therefore, about the antigenic structure of A. pleuropneumoniae strains and of the protective role of the cellular components of this bacterium it cannot be ascertained whether the insufficient protection yielded by strain T928 is a question of quality or quantity of the antigenic determinants which induce protective immunity against A. pleuropneumoniae infection. From the data presented above, however, it follows that vaccine strains should be carefully chosen and the protective properties be examined before they are used as antigens in vaccines.

The control pigs inoculated intranasally with subtype a (K17) and subtype b (L20 and T928) resp. developed CF antibodies to both subtypes with titers at the same level. In practice this means that it will not be possible to obtain an exact subtype diagnosis through blood testing of infected herds. Only by examining the serological characteristics of isolated strains can a differentiation be accomplished. Subtyping of isolated strains of serotype 5 offers a useful tool for epidemiological studies.

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Sammendrag

Actinobacillus (Haemophilus) pleuropneumoniae serotype 5, subtype a and b: Undersøgelser over krydsimmunitet.

Ved vaccination af grise med en dræbt 6-timers kultur af A. pleuropneumoniae serotype 5, stamme K17 (subtype a) opnåedes en betydelig beskyttelse mod intranasal podning med stammerne L20 og T928 (subtype b).

Det omvendte eksperiment viste, at en vaccine med stamme L20 gav god beskyttelse mod intranasal podning med K17, hvorimod en vaccine med T928 ikke gav tilstrækkelig beskyttelse mod intranasal podning med denne stamme.

Disse resultater viser at de antigene determinanter der er fælles for stammerne K17 og L20 er i stand til at give en tilstrækkelig beskyttelse mod pleuropneumoni og antyder at antistoffer mod subtype specifikke antigene determinanter ikke synes at spille en væsentlig rolle for beskyttende immunitet.

Resultaterne opnået med en vaccine med stamme T928 synes at antyde, at der er kvalitative eller kvantitative forskelle i de antigene determinanter der har betydning for opnåelse af beskyttende immunitet mod stammer tilhørende serotype 5. Det er derfor vigtigt at kende potentielle vaccinestammers krydsbeskyttende egenskaber før de anvendes som antigener i vacciner.

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