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SEROLOGY OF HAEMOPHILUS (ACTINOBACILLUS) PLEUROPNEUMONIAE SEROTYPE 5 STRAINS: ESTABLISHMENT OF SUBTYPES A AND B

By

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NIELSEN, R.: *Serology of Haemophilus (Actinobacillus) pleuropneumoniae serotype 5 strains: Establishment of subtypes a and b.* Acta vet. scand. 1986, 27, 49—58. — The serological characteristics of 14 strains assigned to serotype 5 were examined by the slide agglutination test, the indirect haemagglutination (IHA) test and by gel diffusion.

All the strains possessed identical capsular antigenic determinants of polysaccharide (PS) nature and lipopolysaccharide (LPS) nature. However, based upon a capsular antigenic determinant of PS nature the strains could be divided in two subtypes. It is therefore proposed to refer to the H. pleuropneumoniae strains of serotype 5 to two subtypes: subtype a with strain K17 as the subtype strain and subtype b with strain L20 as the subtype strain.

indirect haemagglutination test; gel diffusion; agglutination.

Serotype 5 of Haemophilus pleuropneumoniae was identified by *Gunnarsson et al.* (1977) by whole cell agglutination tests in rabbit antisera. Five strains from the United States were assigned to this serotype. One of the cultures which was isolated from a steer and another which was isolated from a lamb were found to be identical in their agglutination reactions with the porcine isolates of serotype 5.

In the present study the serological properties of the 5 strains were examined by means of the slide agglutination test, the indirect haemagglutination test and gel diffusion. The results were compared with those obtained with 9 strains received from Taiwan, Canada and Singapore, respectively.

MATERIAL AND METHODS

Antigens

The strains examined are listed in Table 1. The antigens for immunization of rabbits, for slide agglutination, for the IHA test and for gel diffusion were prepared as described earlier (*Nielsen & O'Connor* 1984). Heat treatment was made in a water bath at 100°C for 2 h.

Table 1. Origin of strains compared in serological tests.

Strain designation	Isolated from	Country	Source
K17 *	Arthritis, lamb	USA	H. Olander, Davis
K45 *	Brain abscess, steer	USA	L. Frazier, Davis
K98 *	Serositis, pig	USA	L. Frazier, Davis
L20 *	Pneumonia, pig	USA	L. Frazier, Davis
J45 *	Septicaemia, pig	USA	J. E. Moulton, Davis
T928	Pleuropneumonia, pig	Taiwan	F. S. Hsu, Taiwan
C1	Pleuropneumonia, pig	Canada	B. Schiefer, Canada
S12, 13, 14, 15, 16, 18, 20	Pleuropneumonia, pig	Singapore	R. Singh, Singapore

* = received from Dr. A. Gunnarsson, Uppsala.

Antisera

Rabbits were immunized as described earlier (*Nielsen & O'Connor* 1984) with strains K17, T928, L20 and K98.

IHA test

The test was made as described earlier (*Nielsen* 1974).

Gel diffusion

The technique used was described earlier (*Nielsen & O'Connor* 1984). Additionally a three basin arrangement was used. The basins were 4×6 mm and arranged with a distance of 8 mm between the two upper basins. The distance from the upper basins to the lower basin was 5 mm.

Absorption procedure

Absorption of antisera was made as described earlier (*Nielsen & O'Connor* 1984).

RESULTS

Slide agglutination

By the slide agglutination test the strains could be divided in two groups: one group comprising strains K17, J45, K45, Cl, S12, S14 and S18 which agglutinated with antiserum K17, but not with antisera L20 and T928, and another group consisting of strains L20, T928, K98, S13, S15, S16 and S20, which agglutinated antiserum L20 and T928 only (Table 2).

Table 2. Slide agglutination tests with 6-h cultures of 5 American strains (K17, K45, J45, L20, K98), 1 Canadian strain (C1), 1 strain from Taiwan (T928) and 7 strains from Singapore (S12, 13, 14, 15, 16, 18, 20).

	Antiserum		
Antigen	K17	L20	T928
K17	+++	—	—
L20	—	+++	+++
T928	—	+++	+++
K98	—	+++	+++
J45	+++	—	—
K45	+++	—	—
S12, 14, 18	+++	—	—
S13, 15, 16, 20	—	+++	+++

+++ = gross flocculae.
— = no reaction.

IHA test

Sheep red cells sensitized with capsular extracts (non-heat treated or heat-treated) of strains K17, J45, K45, C1, S12, S14 and S18 were agglutinated to high titers (1:640 to 1:2560) by antiserum K17 and T928. Lower levels of antibody titers were observed with antiserum L20 and K98 (1:80 to 1:320).

Strains T928, L20, K98, S13, S15, S16 and S20 agglutinated to high titers (1:640 to 1:5120) by antiserum T928, L20 and K98 whereas low titers were observed with antiserum for strain K17 (1:80 to 1:160). Examples of results obtained in cross-agglutination studies are given in Table 3.

Table 3. Cross agglutination tests (IHA) involving strains of serotype 5.

Antigen	Antiserum			
	K17	T928	L20	K98
K17 Ce	2560	2560	320	320
K17 Ce 100°	640	640	80	80
J45 Ce	2560	1280	320	320
J45 Ce 100°	1280	640	80	80
K45 Ce	2560	1280	160	160
K45 Ce 100°	1280	1280	80	80
C1 Ce	2560	2560	320	320
C1 Ce 100°	1280	640	80	80
L20 Ce	160	5120	5120	5120
L20 Ce 100°	80	1280	2560	640
T928 Ce	160	2560	5120	2560
T928 Ce 100°	80	2560	2560	640
K98 Ce	160	1280	5620	5620
K98 Ce 100°	80	640	2560	1280

Ce = capsular extract.
Ce 100° = heat-treated capsular extract.
Titers are given as reciprocals of the highest serum dilution giving positive reaction.

Cross absorptions

Homologous absorption removed all agglutinating activity from serum K17. Absorption with strain T928 or L20 removed agglutinins for these two strains and left homologous titers slightly reduced.

Cross-absorptions involving strain L20 and T928 and their respective antisera resulted in complete removal of agglutinating activity towards the two strains.

Absorption of antiserum L20 with strain K17 removed agglutinins for that strain. Homologous titers and titers for strain T928 were reduced. Absorption of antiserum T928 with strain K17 removed agglutinins for that strain. Homologous titers and titers for strain L20 were significantly reduced (Table 4).

When strains K45, J45, C1, S12, S14 and S18 were used as antigens against the absorbed sera the same results were seen as those obtained with strain K17 as antigen. With strains K98, S13, S15, S16 and S20 as antigens the results were analogous to those obtained with strain T928.

Table 4. IHA titers of antisera K17, T928 and L20 before and after absorption with strains K17, T9

Antigen	K17 unabsorbed	Absorbed with			T928 unabsorbed	Absorbed with			L20 unabsorbed	Al
		K17	T928	L20		K17	T928	L20		K17
K17, Ce	2560	—	640	640	2560	—	—	—	320	—
K17, 100°	1280	—	320	320	640	—	—	—	80	—
T928, Ce	160	—	—	—	2560	320	—	—	5120	1280
T928, 100°	80	—	—	—	2560	320	—	—	2560	640
L20, Ce	160	—	—	—	5120	640	—	—	5120	2560
L20, 100°	80	—	—	—	1280	160	—	—	2560	640

Ce = capsular extract.
100° = heat-treated capsular extract.
— = no agglutination.
Titers are given as reciprocals of the highest serum dilution giving positive reaction.

Thus, the results of the absorption experiments show that, in addition to serotype-specific capsular antigenic determinants shared by all strains of serotype 5, one group of strains represented by strains K17, J45, K45, C1, S12, S14 and S18 possesses a capsular determinant specific for these strains. Another group represented by strains L20, T928, K98, S13, S15, S16 and S20 possesses a capsular determinant different from the one possessed by the first group.

Gel diffusion

In comparative analysis with capsular extract of strain T928 against antisera K17 and T928 it was shown that the two strains shared capsular precipitinogens. These were demonstrated as a broad, fuzzy precipitation line near the antigen well and as two

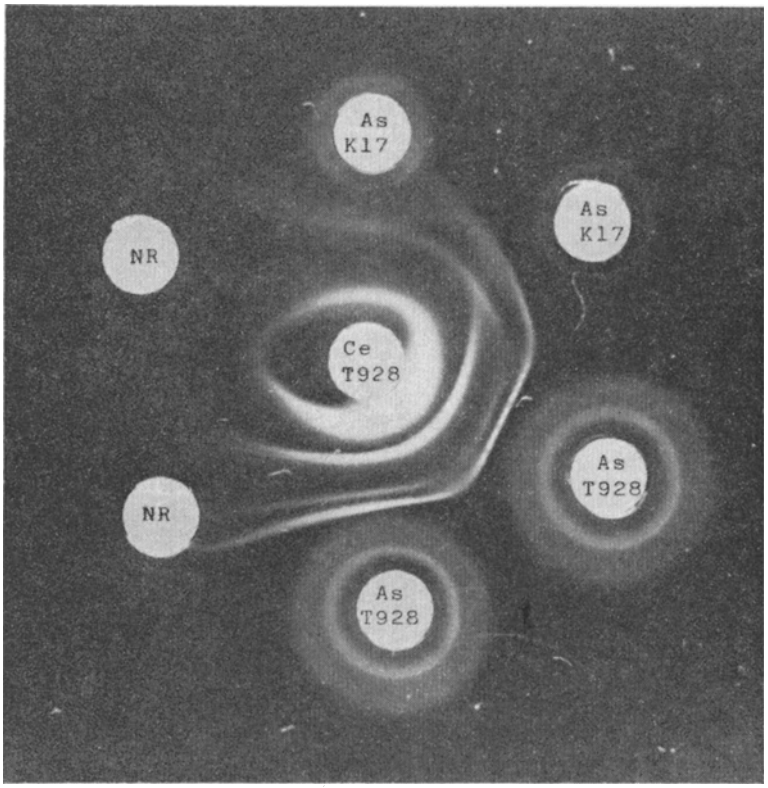

Figure 1. Capsular extract of strain T298 in the center well. In the peripheral wells are antisera (As) for strains K17, T298, L20 and from a non-immunized rabbit (NR).

peripheral, narrow lines. In addition capsular extracts of strain T928 contained a precipitinogen which was precipitated by antiserum T928 only and which was demonstrated as a broad, sharp line against homologous antiserum (Fig. 1).

With capsular extracts of strain L20 against antisera K17 and L20 the precipitation lines were analogous to those obtained wiht capsular extract of strain T928.

Comparative analyses with capsular extracts of strains T928 and L20 against antiserum T928 or antiserum L20 showed that the capsular precipitinogens of those two strains were identical (Fig. 2).

With capsular extracts of strain K17 against antisera K17 and T928 (or L20) it was shown that the capsular precipitinogens shared by the three strains formed the aforementioned pattern.

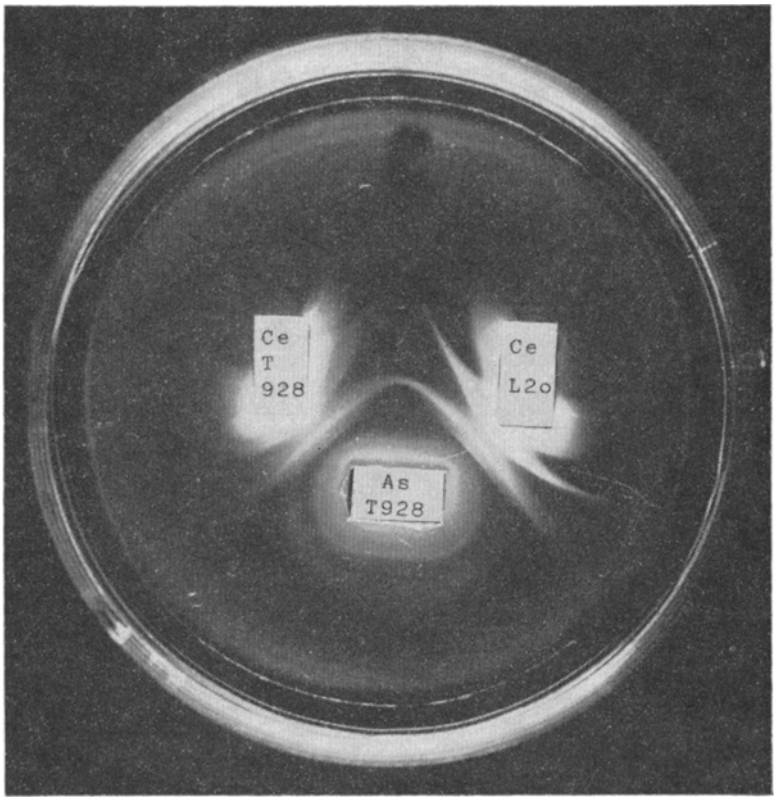

Figure 2. Capsular extracts of strains T298 and L20 in the upper basins. In the bottom basin is antiserum T928.

In addition strain K17 contained a precipitinogen which was precipitated as a broad, sharp line by antiserum K17 only.

Cross absorption

Absorption of serum K17 with strain T928 or strain L20 resulted in removal of the precipitation lines shared by the three strains and left the line specific for strain K17 and its homologous serum unchanged.

Absorption of serum T928 or L20 with strain K17 resulted in removal of the precipitation lines shared by these strains. The line specific for T928 and L20 and their antisera remained unchanged.

Cross absorptions involving strain L20 and T928 and their respective antisera resulted in removal of all precipitation lines.

When capsular extracts of strains K45, J45, C1, S12, S14 and S18 were used in comparative analyses against antisera K17, T928 and L20 the same results were obtained as those seen for strain K17. With capsular extracts of strains K98, S13, S15, S16 and S20 the results were analogous to those obtained with strain T928 and L20.

Thus, by gel diffusion tests the strains were divided into the same two groups as with the slide agglutination test and the IHA test.

DISCUSSION

The results obtained in the present study have shown that strains assigned to serotype 5 can be divided into two subtypes based upon a capsular antigenic determinant specific for each subtype.

Both the capsular antigenic determinants specific for each subtype and those shared by both subtypes were adsorbable to erythrocytes and thereby rendered agglutinable by immune sera. According to the work of *Keogh et al.* (1948) this is consistent with a polysaccharide nature of the antigens.

Immunodiffusion tests with Haemophilus influenzae have shown that lipopolysaccharide (LPS) precipitates are usually located closer to the antigen well than is usual for PS precipitates (*Branefors-Helander* 1973). Although until now nothing is known about the chemical structure of H. pleuropneumoniae, studies of serotype 8 (*Nielsen & O'Connor* 1984) and serotype 9

(*Nielsen* 1985) have indicated that this interpretation is also valid for H. pleuropneumoniae.

The location of the precipitates observed in the present study suggests that the two subtypes share both LPS and PS precipitinogens and that the precipitinogens specific for each subtype are polysaccharides.

Studies of H. pleuropneumoniae, serotype 8 have shown that more than one capsular serotype may be present in H. pleuropneumoniae. Thus, in addition to serotype-specific capsular antigen, serotype 8 shares capsular antigenic determinants of LPS nature with serotype 3 and capsular antigenic determinants of PS nature with serotype 6. The capsular determinants shared by serotypes 8 and 6 were found to be related but not identical.

In contrast, all strains in the present study were found to share capsular antigenic determinants of PS nature which in cross absorption experiments proved to be identical. Also, the results of absorption experiments showed that the LPS determinants carried by the strains were identical. As the strains could be subdivided in two subtypes based upon a capsular antigenic determinant of PS nature which was specific for each subtype it seems justified to propose to refer the H. pleuropneumoniae strains of serotype 5 to two subtypes: subtype a with strain K17 as the subtype strain and subtype b with strain L20 as the subtype strain.

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SAMMENDRAG

Serologisk karakterisering af Haemophilus (Actinobacillus) pleuropneumoniae serotype 5 stammer: Oprettelse af subtyperne a og b.

Fjorten H. pleuropneumoniae stammer, identificeret som serotype 5, blev undersøgt ved hjælp af objektglasagglutination, indirekte haemagglutination og gel diffusion.

Stammerne indeholdt identiske serotype-specifikke kapselantigener af polysaccharid og lipopolysaccharid natur. På grundlag af et serotype-specifikt kapselantigen af polysaccharid natur kunne serotype 5 stammerne inddeles i to undertyper: undertype a med stamme K17 som type stamme og undertype b med stamme L20 som type stamme.

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