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Brief Communication

SEROLOGICAL CHARACTERIZATION OF ACTINOBACILLUS PLEUROPNEUMONIAE STRAINS AND PROPOSAL OF A NEW SEROTYPE: SEROTYPE 12

Until now 11 serotypes of Actinobacillus pleuropneumoniae have been described (*Nicolet* 1971, *Gunnarsson* 1980, *Nielsen* 1982, *Rosendal & Boyd* 1982, *Nielsen & O'Connor* 1984, *Nielsen* 1985, *Kamp* 1986). Recently a hitherto unrecognized serotype was isolated from 9 Danish outbreaks of pleuropneumonia in pigs. The origin of the strains is given in Table 1. From 3 herds the unrecognized serotype was found in 2 to 3 pigs submitted for necropsy at different times. The present study describes the serological properties of the 13 isolated strains.

Table 1. Origin of 13 Danish strains of Actinobacillus pleuropneumoniae examined in serological tests.

Strain designation	Isolated from	Age of pigs	Herd category
9499	Acute pleuropneumonia	4 days	Sow herd, conventional
1096	„	6 months	Sow herd, SPF*
9272	„	2 months	Sow herd, SPF
6113	„	3 months	Sow herd, conventional
2053	„	2 months	Sow herd, conventional
9680	„	2 months	Sow herd, conventional
10199	„	3 months	same as 9680
6599	„	2 months	Sow herd, SPF
16077	„	3 months	same as 6599
1831	„	2 months	Sow herd, SPF
8329	„	4 months	same as 1831
5382	„	4 months	same as 1831
11705	Septicaemia	6 weeks	Sow herd, conventional

* = specific pathogen free herd.

The cultural and biochemical characteristics of the strains were consistent with earlier descriptions of A. pleuropneumoniae (*Kilian* 1976, *Biberstein et al.* 1977, *Nielsen* 1982). The strains were examined serologically by the indirect haemagglutination test and by gel diffusion as described earlier (*Nielsen & O'Connor* 1984). Reference strains representing serotypes 1 through 11 were: Shope 4071, S1536, S1421, M62, K17, Femø, WF83, 405, CVJ13261, 56153.

Sheep red cells sensitized with capsular extracts (heated and non-heated) of the 13 strains were agglutinated to high titers (1:2560 to 1:10.240) by rabbit antiserum produced against whole-cell antigens of strains 1096 and 8329. Agglutination was not observed with antisera for serotypes 1 through 11.

Cross absorptions involving strains 1096 and 8329 and their respective antisera resulted in complete removal of agglutinating activity towards the 2 strains and towards strains 11705, 9499, 5382, 1831, 9272, 6599, 16077, 6113, 2053, 9680 and 10199 (Table 2).

Table 2. IHA titers obtained with various antigen preparations of strains 8329 and 1096 against rabbit antisera produced against whole-cell antigens (6-h cultures). Sera were tested before and after homologous and heterologous absorption.

	Antiserum					
	8329	absorbed with		1096	absorbed with	
Antigen	unabsorbed	8329	1096	unabsorbed	1096	8329
8329, Ce	10.240	—	—	5120	—	—
8329, Ce 100°	5120	—	—	2560	—	—
1096, Ce	10.240	—	—	5120	—	—
1096, Ce 100°	5120	—	—	2560	—	—

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

When capsular extracts (heated and non-heated) of strains 1096 and 8329 were used as antigens in gel diffusion tests against their homologous antisera two type-specific precipitation lines were seen: one broad and fuzzy line situated near the antigen well and another more dense peripheral line. In addition, two precipitation lines showing reaction of identity between all serotypes were seen with non-heated antigen. In comparative analysis of the 13 strains against antisera 1096 and 8329 the strains proved identical. Cross absorptions involving the 13 strains resulted in complete removal of all precipitins from antisera 1096 and 8329.

Serotypes of A. pleuropneumoniae possess type-specific antigenic determinants of capsular origin which can be demonstrated by immunodiffusion tests and by indirect haemagglutination

tests. Common species specific antigens have also been demonstrated in all serotypes (*Nicolet* 1971, *Gunnarsson* 1980, *Nielsen & O'Connor* 1984, *Nielsen* 1985).

The 13 strains of A. pleuropneumoniae examined in the present study possessed two type-specific antigenic determinants of capsular origin as well as common species-specific antigens. As the strains are antigenically homogeneous and serologically distinct from serotypes 1 through 11 the strains are proposed to be referred to a new serotype, designated serotype 12 with strain 8329 as the type strain.

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REFERENCES

Biberstein, E. L., A. Gunnarsson & B. Hurwell: Cultural and biochemical criteria for the identification of Haemophilus spp. from swine. Amer. J. vet. Res. 1977, *38*, 7—11.

Gunnarsson, A.: Haemophilus pleuropneumoniae syn parahaemolyticus. An antigenic and diagnostic study. Thesis, Uppsala 1980.

Kamp, E.: Personal communication.

Kilian, M.: A taxonomic study of the genus Haemophilus with the proposal of a new species. Thesis, Århus 1976.

Nicolet, J.: Sur l'hemophilose du porc. III. Differénciation sérologique de Haemophilus parahaemolyticus. (Haemophilus infections in pigs. III. Serological studies on Haemophilus parahaemolyticus. Zbl. Bakt. 1971, *216*, 487—495.

Nielsen, R.: Haemophilus pleuropneumoniae infection in pigs. Thesis, Copenhagen 1982.

Nielsen, R.: Serological characterization of Haemophilus pleuropneumoniae (Actinobacillus pleuropneumoniae) strains and proposal of a new serotype: serotype 9. Acta vet. scand. 1985, *26*, 501—512.

Nielsen, R.: Serological characterization of Haemophilus pleuropneumoniae (Actinobacillus pleuropneumoniae) strains and proposal of a new serotype: serotype 10. Acta vet. scand. 1985, *26*, 581—585.

Nielsen, R. & P. J. O'Connor: Serological characterization of 8 Haemophilus pleuropneumoniae strains and proposal of a new serotype: serotype 8. Acta vet. scand. 1984, *25*, 96—106.

Rosendal, S. & A. Boyd: Haemophilus pleuropneumoniae serotyping. J. clin. Microbiol. 1982, *16*, 840—843.

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