

**Brief Communication**

**SEROLOGICAL CHARACTERIZATION OF HAEMOPHILUS  
PLEUROPNEUMONIAE (ACTINOBACILLUS PLEUROPNEU-  
MONIAE) STRAINS AND PROPOSAL OF A NEW SEROTYPE:  
SEROTYPE 10**

Until now 9 serotypes of *H. pleuropneumoniae* have been identified (Nicolet 1971, Gunnarsson *et al.* 1977, Nielsen 1982, Rosendal & Boyd 1982, Nielsen & O'Connor 1984, Nielsen 1985). Recently a hitherto unrecognized serotype was isolated from 2 Danish outbreaks of pleuropneumoniae in pigs. The present study describes their serological properties and compares them with those of 7 strains isolated from outbreaks of pleuropneumonia in the United Kingdom (Table 1).

Table 1. Origin of strains examined in serological tests.

| Strain designation | Isolated from        | Country | Source     |
|--------------------|----------------------|---------|------------|
| D13039             | Pleuropneumonia, pig | Denmark | R. Nielsen |
| D11815             | "                    | Denmark | R. Nielsen |
| B22009             | "                    | U.K.    | A. Jones*  |
| B271               | "                    | U.K.    | A. Jones   |
| B391               | "                    | U.K.    | A. Jones   |
| B491               | "                    | U.K.    | A. Jones   |
| B860               | "                    | U.K.    | A. Jones   |
| B861               | "                    | U.K.    | A. Jones   |
| B1254              | "                    | U.K.    | A. Jones   |

\* The strains were collected by Dr. H. J. Riising, Nordisk Droge & Kemikalie, Copenhagen.

The cultural and biochemical characteristics of the strains were consistent with earlier descriptions of *H. 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 9 were: Shope 4071, S1536, S1421, M62, K17, Femø, WF83, 405, CVJ 13261.

Sheep red cells sensitized with capsular extracts (non-heat-treated or heat-treated) of the 9 strains were agglutinated to high titers (1:640 to 1:10,240) by antiserum D13039 and B22009 (Table 2). Agglutination was not observed with antisera for serotypes 1 through 9.

Table 2. Cross agglutination tests (IHA) involving 2 Danish and 7 British strains.

| Antigen    | Antiserum* |        |
|------------|------------|--------|
|            | D13039     | B22009 |
| B22009, Ce | 10.240     | 10.240 |
| Ce 100°    | 2560       | 2560   |
| D13039, Ce | 10.240     | 2560   |
| Ce 100°    | 5120       | 2560   |
| D11815, Ce | 5120       | 2560   |
| Ce 100°    | 1280       | 1280   |
| B271, Ce   | 5120       | 5120   |
| Ce 100°    | 1280       | 1280   |
| B391, Ce   | 2560       | 5120   |
| Ce 100°    | 1280       | 1280   |
| B491, Ce   | 5120       | 2560   |
| Ce 100°    | 1280       | 640    |
| B860, Ce   | 5120       | 2560   |
| Ce 100°    | 1280       | 640    |
| B861, Ce   | 5120       | 2560   |
| Ce 100°    | 1280       | 640    |
| B1254, Ce  | 5120       | 2560   |
| Ce 100°    | 1280       | 1280   |

Ce = Capsular extracts.

Ce, 100° = heat-treated capsular extracts.

Titers are given as reciprocals of the highest serum dilution giving positive reaction.

\* Rabbit immune sera were produced against whole-cell antigens.

Cross absorptions involving strains D13039 and B22009 and their respective antisera resulted in complete removal of agglutinating activity towards the 2 strains and towards strains D11815, B271, B391, B491, B860, B861 and B1254 (Table 3).

Table 3. IHA titers obtained with various antigen preparations of strains D13039 and B22009 against rabbit antisera produced against whole-cell antigens (6-h cultures). Sera were tested before and after homologous and heterologous absorption.

| Antigen    | Antiserum             |               |        |                      |               |        |
|------------|-----------------------|---------------|--------|----------------------|---------------|--------|
|            | D113039<br>unabsorbed | absorbed with |        | B22009<br>unabsorbed | absorbed with |        |
|            |                       | D13039        | B22009 |                      | D13039        | B22009 |
| D13039, Ce | 10.240                | —             | —      | 2560                 | —             | —      |
| Ce, 100°   | 5120                  | —             | —      | 2560                 | —             | —      |
| B22009, Ce | 10.240                | —             | —      | 10.240               | —             | —      |
| Ce, 100°   | 2560                  | —             | —      | 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 (heat-treated and non-heat-treated) of strains D13039 and B22009 were used as antigens against their homologous antisera 2 serotype-specific precipitation lines were seen: one broad and fuzzy line situated near the antigen well and another more dense peripheral line (Fig. 1). In addition 2 precipitation lines showing reaction of identity between all serotypes were seen with non-heat-treated antigen. An example is given in Fig. 2. In comparative analyses of the 9 strains against antisera D13039 and B22009 the strains proved identical. Fig. 2 gives an example of this. Cross absorptions involving the 9 strains resulted in removal of all precipitins from antisera D13039 and B22009.

By agglutination and immunodiffusion tests *Nicolet* (1971) and *Gunnarsson et al.* (1978) showed that serotypes 1 through 5 of *H. pleuropneumoniae* possessed type-specific antigenic determinants which were of capsular origin. In immune diffusion tests *Gunnarsson* (1979) found that at least 2 type-specific precipitation lines were regularly identified.

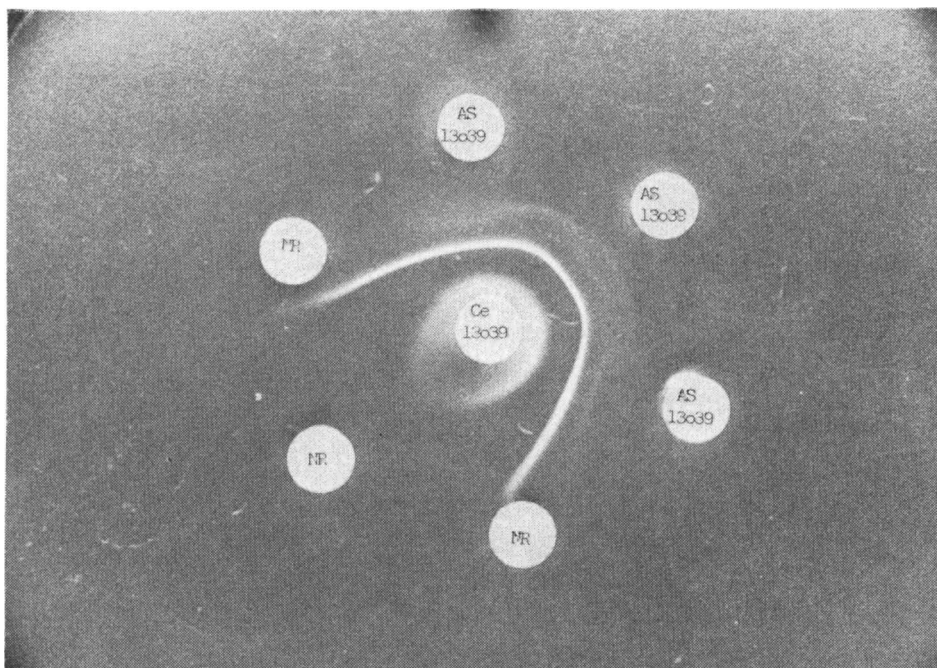


Figure 1. Capsular extract (Ce) of strain D13039 in the center well. In the peripheral wells are antiserum D13039 (AS 13039) and serum from a normal rabbit (NR).

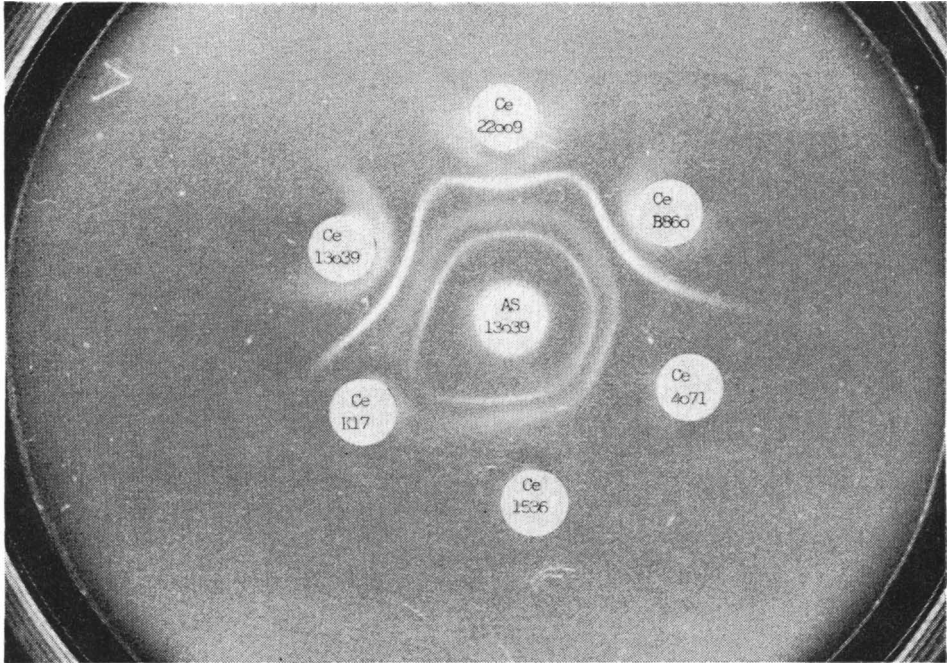


Figure 2. Capsular extracts (Ce) of strains D13039, B22009, B860, 4071 (serotype 1), 1536 (serotype 2) and K17 (serotype 5) in the peripheral wells. In the center well is antiserum (AS) for strain D13039.

The 9 strains of *H. pleuropneumoniae* examined in the present study possessed 2 type specific antigenic determinants of capsular origin as well as common species-specific antigens. The results obtained with the IHA test showed that the type-specific antigenic determinants were able to sensitize erythrocytes to the agglutinating effect of immune sera. This is consistent with a polysaccharide (PS) nature of the antigens (*Keogh et al.* 1948). With the gel diffusion test 2 type-specific precipitinogens were demonstrated. In consistence with earlier observations (*Brane-fors-Helander* 1973, *Gunnarsson* 1979, *Nielsen & O'Connor* 1984, *Nielsen* 1985) the location of the precipitates which demonstrated the presence of these precipitinogens suggested that one was of lipopolysaccharide (LPS) nature and the other of PS nature.

As the 9 strains are antigenically homogeneous and serologically distinct from other serotypes of *H. pleuropneumoniae*, it is proposed that the strains be referred to a new serotype, designated serotype 10, with strain D13039 as the type strain.

Ragnhild Nielsen

The State Veterinary Serumlaboratory, Copenhagen, Denmark.

#### 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.
- Branefors-Helander, P.*: Serological studies of *Haemophilus influenzae*. III. The endotoxic effect of various antigen preparations and the relation between this effect and demonstrable precipitogens. *Int. Arch. Allergy*, **44**, 585—600.
- Gunnarsson, A., E. L. Biberstein & B. Hurwell*: Serologic studies on porcine strains of *Haemophilus parahaemolyticus* (pleuropneumoniae). Agglutination reactions. *Amer. J. vet. Res.* 1977, **38**, 1111—1114.
- Gunnarsson, A., B. Hurwell & E. L. Biberstein*: Serologic studies of *Haemophilus parahaemolyticus* (pleuropneumoniae): Antigenic specificity and relationship between serotypes. *Amer. J. vet. Res.* 1978, **39**, 1286—1292.
- Gunnarsson, A.*: Serologic studies on porcine strains of *Haemophilus parahaemolyticus* (pleuropneumoniae): Extraction of type-specific antigens. *Amer. J. vet. Res.* 1979, **40**, 469—472.
- Keogh, E. V., E. A. North & M. F. Warburton*: Adsorption of bacterial polysaccharides to erythrocytes. *Nature* 1948, **161**, 687—688.
- Kilian, M.*: A taxonomic study of the genus *Haemophilus* with the proposal of a new species. Thesis, Århus 1976.
- Nicolet, J.*: Sur l'hémophilose du porc. III. Différenciation sérologique de *Haemophilus parahaemolyticus* (*Haemophilus* infections in pigs. III. Serological studies on *Haemophilus parahaemolyticus*). *Zbl. Bakt.* 1971, **216**, 487—495.
- Nielsen, R.*: Serological and immunological studies of pleuropneumonia of swine caused by *Haemophilus parahaemolyticus*. *Acta vet. scand.* 1974, **15**, 80—89.
- Nielsen, R.*: *Haemophilus pleuropneumoniae* infection in pigs. Thesis 1982. Commissioned by C. F. Mortensen A/S, Bülowsvej 5 C, DK-1870 Copenhagen V, Denmark.
- 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. & 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.

(Received November 6, 1985).

Reprints may be requested from: Ragnhild Nielsen, the State Veterinary Serumlaboratory, Bülowsvej 27, DK-1870 Frederiksberg C, Denmark.