Taxonomy and Serological Identification of *Actinobacillus pleuropneumoniae*

Jacques Nicolet

The classification of bacteria has always been a subject of preoccupation for bacteriologists. Unfortunately, the aims of establishing correct taxonomy and ensuring a language of communication have not been fully realized, a fact that worries clinicians. The situation with the agent of porcine pleuropneumonia clearly illustrates this problem. The small gramnegative coccobacillary bacterium requires nicotinamide adenine dinucleotide (NAD) for its growth and, according to its biochemical and physiological properties, it was originally classified in the genus of a *Pasteurella haemolytica*-like organism, sharing the phenotypical properties of *H. pleuropneumoniae* and also causing pleuropneumonia led to some confusion concerning the etiology of this disease.

Haemophilus versus Actinobacillus

In the recent past, great interest has been shown in the taxonomy of the family *Pasteurellaceae*. These studies showed that many species, particularly of the genus *Haemophilus*, are actually more closely related either to the genus *Pasteurella* or to the genus *Actinobacillus* despite their requirement for hemin (X-factor) or NAD (V-factor). Consequently, proposals for a revaluation of the taxonomic position of certain members of the family were made and approved recently. The agent of porcine pleuropneumonia has thus been transferred to the genus *Actinobacillus* as *A. pleuropneumoniae* with its two varieties biovar 1 (NAD-dependent, syn. *Haemophilus pleuropneumoniae*) and biovar 2 (NAD-independent, syn. *Pasteurella haemolytica*-like) (2).

The agent of porcine pleuropneumonia has thus been transferred to the genus *Actinobacillus* as *A. pleuropneumoniae*

This new situation implies that, for diagnostic purposes, *A. pleuropneumoniae* must be distinguished from other haemophili occurring in pigs, e.g. *H. parasuis, Haemophilus* sp. "minor group", *Haemophilus* sp. "taxon C", and *Haemophilus* sp. "urease negative" in the case of biovar 1 (NAD-dependent) (3), and from actinobacilli commonly isolated from pigs in the case of biovar 2 (NAD-independent) (Table 1).

The Serology of Actinobacillus pleuropneumoniae

The original serological classification of *A. pleuropneumoniae* was based on capsular antigens, as far as serovars 1 to 5 are concerned (1). We now know about the existence of at least 12 serovars (ser. 11 and 12 not yet published). The exact nature of the specific antigens in serovars 6 to 12 is not yet fully understood. Some cross-reactions observed between serovars 1, 9 and 12, between serovars 4 and 7, and between serovars 3, 6 and 8 bring into question the justification of the current serological classification.

It has been recognized for many years that the different serovars can share species-specific antigens which are thought to be mainly lipopolysaccharides (LPS). On the other hand, it seems that some type-specific capsular polysaccharides (PS) are partly common between some serovars. Besides a specific capsular PS antigen, serovars 1 and 9, serovars 5a and 5b as well as serovars 3, 6 and 8 accordingly share a common capsular PS, while other cross-reactivities may be due to LPS antigens (4,5). As to the relatedness of serovars 4 and 7, there is no further information available.

Cross-reactivity between other porcine haemophili and actinobacilli has not been reported to date, except for a strain identified as *Actinobacillus suis*. The responsible cross-reactive antigens have not yet been identified; from experience we do not expect them to interfere with the recommended serotyping methods.

Recent work referring to structural studies of the capsular PS and LPS from various serovars no doubt will bring about further elucidation of the antigens involved, offering also a more comprehensive serological scheme (6,7,8) (Perry and Inzana, personal communication). Finally, the use of monoclonal antibodies directed to specific epitopes may also contribute to a more accurate serological classification. Some promising developmental work is occurring in this direction at present.

From different methods proposed for serotyping, different results were obtained either by using different antigens (whole cells, various cell extracts) or by applying different serological techniques. A critical evaluation of such methods is given in Table 1. In view of the diversity of the antigens involved and the quality of the antisera used, standardization of the serological identification of *A. pleuropneumoniae* is urgently needed. A working team has been constituted within the International Pig Veterinary Society (IPVS); by means of a collaborative study, they are trying to achieve a better understanding of the value of different serological techniques and to offer a reference structure for world-wide use.

A preliminary evaluation of the present situation led to the following recommendations: Coagglutination is currently the method of choice for routine typing of field strains. However, this technique does not allow separation of the heterogeneous serovar 8 from ser. 3 and 6, the heterogeneous serovar 9 from ser. 1, or

Institute of Veterinary Bacteriology, University of Berne, CH-3012 Berne, Switzerland

TABLE 1
Critical Evaluation of Serotyping Methods for *A. pleuropneumoniae*

Test	Antigen	Specificity	Recommended Use
Coagglutination		Satisfactory	*Routine typing*
Slide agglutination		Reasonable	Routine typing
Indirect FA	Whole cells	Moderate	Routine typing
Tube agglutination		Poor	None
CF-test		Moderate	None
Agar gel precipitation		Very good	*Final identification*
Indirect HA	Cell extracts	Satisfactory	*Final identification*
Counterimmunoelectrophoresis		Reasonable	Routine typing

the heterogeneous serovar 7 from 4. Until absorbed sera are obtained, the results are reported as group 9-1, group 8-3-6, and group 7-4 respectively. The final identification of heterogeneous serovars can only be achieved by the agar gel diffusion test and by indirect hemagglutination. Such tests not being available in every laboratory and the interpretation of the results probably being delicate, it is recommended that for atypical or nontypable strains, the service of experienced laboratories be used. In order to bring some uniformity into serotyping, reference strains and the corresponding antisera are commercially available.

The IPVS reference laboratories can be contacted at the following addresses:

Serological typing:

for the USA and Canada — Dr. K.R. Mittal,
Faculty of Veterinary Medicine,
University of Montreal,
Saint-Hyacinthe,
Quebec J2S 7C6
Canada

for Europe and other countries: — Dr. R. Nielsen,
State Veterinary Serum Laboratory,
Bulowsvej 27,
1503 Copenhagen V,
Denmark

Reference strains obtainable from: — Dr. R.F. Ross,
Veterinary Medical Research Institute,
Iowa State University
Ames, Iowa 50011, USA

(Fees of approx. $50.00/strain may be charged to commercial laboratories.)

or from: — Dr. R. Nielsen
State Veterinary Serum Laboratory
Copenhagen

Commercial sera available from: — Dr. H.J. Riising
Nordisk & Kemikalie A/S,
Ragnagade 9,
2100 Copenhagen O,
Denmark

(Fees will be charged.)

Clonal Diversity

A new approach to the differentiation of bacterial strains is afforded by the analysis of outer membrane protein (OMP) profiles obtained after electrophoresis and by the detection of allelic variation in structural genes by multilocus enzyme electrophoresis.

The relationship between OMP profile patterns and the nine serovars of *A. pleuropneumoniae* studied appears to be rather consistent, since only serovars 1 and 9 and, surprisingly, serovars 2 and 6, show a similar pattern (3). Nevertheless, this study showed that the population of *H. pleuropneumoniae* is clonal, a conclusion that is supported by the detection of 32 multilocus genotypes revealed by the electrophoretic allelic profiles for 15 enzyme loci (9). The data further showed that all the serotypes occurred in association with a variety of multilocus genotypes and that, in some cases, highly divergent electrophoretic types have identical serotypes. This points out the genetic diversity of the different serovars, particularly ser. 3 and 6, although some serovars form closely related groups of clones (ser. 1 and 9, ser. 2).

The above observations confirm the complexity of the population structure of *A. pleuropneumoniae*, and they assist better understanding of the classification and, consequently, the epidemiology as well as the pathogenesis of this important swine disease.

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Seroepidemiology of *Actinobacillus pleuropneumoniae*

Ragnhild Nielsen

Introduction

To prevent and control *Actinobacillus pleuropneumoniae* infection in pigs, it is necessary to possess knowledge of the epidemiology of the infection. Serological testing has proved valuable in herd surveys not only as a diagnostic tool but also as a guide to the immune status of a herd. A prerequisite for the use of serological testing in the diagnosis of the disease and in the study of the spread of infection within a herd and from one herd to another is a serological test which holds an acceptable balance between sensitivity and specificity, i.e. there should be the lowest possible risk of detecting false-positive and false-negative animals. Also, it is important to have knowledge of the serotype pattern in the swine population to be examined and of the immune response of the pig to infection with *A. pleuropneumoniae*.

Serological Tests

Three serological tests are available today for serological studies of *A. pleuropneumoniae* infection, namely the modified complement fixation test (CFT) (1,2), the enzyme-linked immunosorbent assay (ELISA) (3,4), and the 2-mercaptoethanol tube agglutination test (2-ME-TAT) (5).

The CFT has been the accepted method for several years and is used in routine work in Switzerland, Denmark, Canada, and the USA. The test should be interpreted on a herd basis. The reason for this is that, in recently infected herds, only a few animals may have a titer, and in chronically infected herds the percentage of seropositive animals may vary considerably — from a few percent to nearly 100 percent. Although the CFT has a high degree of sensitivity and specificity, it is not widely used for routine serological work because many laboratories find it difficult to perform. In recent years, therefore, other serological tests have been developed.

Of these, the ELISA is considered to have good potential as a rapid and sensitive diagnostic test and has the additional advantage that it can be adapted to automation. Used in the field, the ELISA appeared more sensitive and as specific as the CFT (4).

The National Veterinary Laboratory, Bulowsvej 27, DK-1870 Frederiksberg C, Denmark

The 2-ME-TAT has shown promise in the control of *A. pleuropneumoniae* in finishing farms. It is of interest that the test does not detect maternal antibodies in piglets after four weeks of age. Thus, a serological response with the test in piglets older than four weeks would be an indication of recent infection. Since piglets with a recent infection are important for transmission of the infection to other herds, they should be avoided as a source to finishing farms.

No single test has a 100% specificity and sensitivity and use of a combination of tests may be preferable for the interpretation of results.

Serotypes

So far, 12 serotypes of *A. pleuropneumoniae* have been recognized (6–13).

Knowledge of the serotype pattern in a country is important for seroepidemiological work. One major reason for this is that serodiagnosis is type specific. Another reason is that natural infection with one serotype will confer a strong immunity to all serotypes.

The distribution of serotypes differs from country to country. In some countries only one serotype is present, in others several serotypes. Often, in such countries one serotype is dominant, e.g. in Canada the majority of isolated strains are serotype 1 and in Switzerland and Denmark serotype 2 is the dominant type.

It is important to follow the serotype development in a country by routine serotyping of isolated field

TABLE 1
Serotyping of 2470 Isolated Danish Strains of *Actinobacillus pleuropneumoniae*

	1970–1981	1982–1986
Serotype 2	99%	84%
Serotype 6	1%	11%
Serotype 5	—	0.8%
Serotype 1	—	0.5%
Serotype 7	—	1%
Serotype 8	—	2%
Serotype 10	—	0.1%
Serotype 12	—	0.6%