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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 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%

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

TABLE 2
Complement Fixation Titers in Pigs Experimentally Inoculated with Serotypes 3, 6, 8, 1 or 9

Antigen	Antiserum (pig):				
	Serotype 3	Serotype 6	Serotype 8	Serotype 1	Serotype 9
Serotype 3	1:32	1:8	1:128	—	—
Serotype 6	1:16	1:512	1:128	—	—
Serotype 8	1:32	1:64	1:512	—	—
Serotype 1	—	—	—	1:64	1:64
Serotype 9	—	—	—	1:128	1:512

strains because the serotype pattern may change. The development in Denmark is an example of this (Table 1). Thus, for a period of more than ten years, serotypes 2 and 6 have been the only serotypes isolated, with serotype 2 dominant. However, during recent years other serotypes have emerged. This is most probably due to importation of breeding animals for the Danish cross-breeding program which was initiated in 1978. This assumption is sustained by the finding of animals seropositive to these serotypes among the imported stock.

TABLE 3
Serological Findings in Breeding Animals After Acute Herd Outbreak

Postinfection Interval	Proportion of Seropositive Animals (%)
3 weeks	44/131 ^a (33.5)
2 months	72/128 (56)
4 months	47/72 (65.4)
8 months	89/107 (83.4)

^aData are expressed as the proportion of seropositive animals/number tested

Immune Response to Various Serotypes

The immune response of the pig to infection with *A. pleuropneumoniae* has been studied in experiments and it has been shown that complement-fixing antibodies can be demonstrated approximately 10-14 days after infection, also in subclinically affected animals.

The immune response is serotype specific. In practice, this means that an outbreak caused for example by serotype 2 can only be serologically proven if sera from infected animals are tested against serotype 2 antigen.

Some serotypes share capsular antigenic determinants with other serotypes. Thus, serotype 8 shares determinants with serotypes 3 and 6, and serotype 9 with serotype 1 (10,11). This will cause cross-reactions in serological tests (Table 2). The practical implication of this is that serotypes 3, 6 and 8 will have to be considered as a group and it will not be necessary to use more than one of the types in seroepidemiological work. As for serotypes 1 and 9, both should be used as test antigens because some herds infected with serotype 9 react only with serotype 9 antigen. The reason for this is not yet fully understood, but these findings suggest the existence of serotype 9 strains which do

not have antigen in common with serotype 1.

Serotype 5 has two subtypes (14). Pigs inoculated with either of these will develop complement-fixing antibodies with titers at the same level to both subtypes. However, it has been shown with the ELISA that the best results are obtained with antigen of the subtype present in the swine population (4).

The fact that natural infection with one serotype will elicit cross immunity to all other serotypes may complicate both serodiagnostic and seroepidemiological work

Seroepidemiological Studies

Results obtained with the CFT in SPF herds have shown that the test adequately reflects the herd status.

If the animals of a herd are seronegative, the herd is free from the infection. On the other hand, the herd is fully susceptible and if the organism is introduced, an acute outbreak will follow, with high morbidity and many deaths. Two to three weeks after an acute outbreak, only a few animals show clinical signs. There will, however, be a continuous spread of the infection and after some months the majority of the animals will be seropositive, i.e. they have gone through a sub-clinical infection (Table 3).

It follows then that serological testing will give a more reliable picture of the spread of the infection within a herd than does clinical disease, since blood testing will also detect subclinically affected animals.

It is characteristic of a herd which has recently experienced an acute outbreak that a large proportion of the animals have high titers (1:160 to 1:640). Due to herd immunity, clinical disease is infrequent in chronically infected herds, and, in contrast to the situation seen after an acute outbreak, most animals have low titers (1:10 to 1:20).

The fact that natural infection with one serotype will elicit cross immunity to all other serotypes may complicate both serodiagnostic and seroepidemiological work. In chronically infected herds where a new serotype is introduced, clinical morbidity will be low and seroconversion will be seen only in animals in which the new serotype is able to invade the pulmonary tissues. It is therefore likely that a new serotype may exist in such a herd for a considerable time before it is detected, probably as a latent mucosal infection.

Danish SPF herds are monitored for serotype 2 by monthly blood testing of approximately 10 to 20 blood samples. However in 1982 a serological survey with serotype 6 antigen revealed that approximately 40% of the top breeding herds were subclinically infected with serotype 6. That the spread of this serotype has been insidious, without acute outbreaks, is difficult to explain since strains isolated from these herds possessed full virulence in experiments. Thus, when one inoculated pig was brought in contact with 50 SPF pigs, 35% of them showed severe respiratory distress, two died, and 60% had pulmonary lesions at slaughter (15).

The presence of animals seropositive to serotype 6 in closed breeding herds implies that other serotypes may lie dormant in such herds and from there spread to other herds for some time before the infection is diagnosed. This has already been observed in some herds. Thus, an acute outbreak with serotype 5 was experienced in an SPF finishing herd which received weaners from breeding herds subclinically infected with serotype 6. A serological survey of the breeding herds revealed few seropositive animals to serotype 5 among the breeding stock (0.7 to 17%).

Usually, introduction of serotype 2 into a susceptible breeding herd is followed by an acute outbreak. However, in some herds the infection has been insidious from the beginning. In one such herd, five of 23 blood samples from breeding animals were seropositive to serotype 2 with titers of 1:10 to 1:40; thirteen had titers at the same level to serotype 6.

Conclusion

In the control of breeding herds for freedom from *A. pleuropneumoniae* infection and in test-and-slaughter procedures used to eliminate the infection, the complications brought about by cross immunity between serotypes should be considered.

It should be recognized that the presence of more than one serotype in a herd may influence clinical morbidity and serology. Also, it should be recognized that cross immunity will allow for another serotype to be present in the nasal cavity or other respiratory mucosae without eliciting seroconversion, and that this may imply that the number of animals carrying this sero-

type may be higher than anticipated from the results obtained with serological testing.

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