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Brief Communication

Identification of *Actinobacillus Pleuropneumoniae* Serotype 2 by Monoclonal or Polyclonal Antibodies in Latex Agglutination Tests

Porcine pleuropneumonia caused by *Actinobacillus pleuropneumoniae (A. pleuropneumoniae)* is a major problem in modern swine industry worldwide. At present 12 serotypes have been recognized (*Nielsen* 1990). Serotyping has been based upon capsule associated, heat-stabile antigens of polysaccharide (PS) and lipopolysaccharide (LPS) nature. A variety of tests have been used for serotyping including slide agglutination (*Mittal et al.* 1982), immunodiffusion (*Gunnarsson et al.* 1978, *Nielsen & O'Connor* 1984), indirect haemagglutination (*Mittal et al.* 1983a) and coagglutination (*Mittal et al.* 1983b). Recent studies report on serotyping of *A. pleuropneumoniae* strains using monoclonal antibodies in enzyme-linked immunosorbent assays (*Lida et al.* 1990, *Nakai et al.* 1990).

In the present study, a monoclonal antibody produced against whole cells of a Danish serotype 2 field strain (4226) was used in a latex agglutination test for typing of field isolates. The results were compared with those obtained with a latex agglutination test using polyclonal rabbit antisera. The polyclonal antisera were prepared against whole cells of the reference strains of *A. pleuropneumoniae* representing serotypes 1 through 12 and against 2 Danish field strains 4226 and 9008 belonging to serotype 2.

Polyclonal rabbit antisera were prepared as described previously (*Nielsen & O'Connor* 1984). The hybridoma-derived monoclonal antibody (Mab) 102-G02 used in this study was established by fusion of murine myeloma cells (P3-X63-Ag 8.653) with splenocytes of BALB/c mice immunized with whole cells of serotype 2 strain 4226. The Mab was found to recognize an epitope on the LPS O-chain of serotype 2 strain 4226 by sodiumdodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting.

The method described by *Finkelstein & Yang* (1983) for Escherichia coli was followed for sensitization of latex with polyclonal antibodies and the Mab. Briefly, 10 µl of purified Mab 102-G02 (1.2 mg/ml) or undiluted polyclonal rabbit antiserum (titer 1:256) was mixed with 190 µl glycine buffered saline solution pH 8.2. An equal volume of a latex preparation (Bacto Latex 0.81, Difco) was added and mixed thoroughly. The sensitized latex was incubated in a water bath at 37°C for 2 h and shaken at 15 min intervals. After incubation 600 µl glycine buffered saline with 0.1% bovine serum albumine (Sigma) was added. After 30 min at room temperature the mixture was ready for use. The sensitized latex particles were stable for at least 1 month.

In the latex particle agglutination test (LPAT) 1 drop (0.05 ml) of the sensitized latex was mixed on a glass slide with a small amount of bacterial mass taken from an 18 h culture on PPLO agar. The mixture was stirred

Table 1. Results obtained with *A. pleuropneumoniae* serotype 2 field isolates examined by the latex agglutination test using polyclonal rabbit antisera to strains S1536, 4226 and 9008 or Mab 102-G02 prepared against strain 4226 for sensitization of latex.

Total number of serotype 2 isolates examined:	Number of isolates positive with polyclonal antisera to strain:			Number of isolates positive to Mab 102-G02	Number of isolates negative to Mab 102-G02
	S1536	4226	9008		
786	786*	786*	786*	695*	91 (11.6% of total)

*All strains showed a very strong (+++) or strong (++) reaction

thoroughly with a wooden applicator stick to form a uniform suspension. The slide was rotated by hand for 1 to 2 min and examined against a dark background. Results were recorded as 3+ (very strong reaction), 2+ (strong reaction), 1+ (weak reaction) and 0 (no distinct reaction). Any antigen giving a 2+ reaction was considered positive. Latex particles coated with serum from a non-immunized rabbit were included as control.

Seven hundred and eighty-six *A. pleuropneumoniae* isolates from lungs with typical lesions of pleuropneumonia were examined with the LPAT. The isolates were recovered during a period of 2 years. All were identified at primary isolation as serotype 2 using polyclonal rabbit antisera in the slide agglutination test (SAT). The isolates originated from 786 different herds.

The reference serotype 2 strain (S1536) and field strains 4226 and 9008 all showed a strong reaction in the LPAT with polyclonal antisera prepared against the 3 strains. Both strain S1536 and 4226 reacted with Mab 102-G02 to 3+, whereas strain 9008 showed no reaction with the Mab-coated latex.

All 786 serotype 2 field isolates agglutinated latex coated with polyclonal antisera to serotype 2 strains S1536, 4226 and 9008. Also, the majority of the isolates reacted with the Mab-coated latex particles. However, a total of 91 (11.6%) isolates showed no reaction with the Mab (Table 1). The Mab negative isolates were recovered from both conventional and specific pathogen free (SPF) herds. No reaction was observed with polyclonal antisera prepared against the other serotypes of *A. pleuropneumoniae* or with serum from a non-immunized rabbit.

The results obtained with the LPAT using polyclonal antisera closely parallelled those of the SAT in identifying *A. pleuropneumoniae* serotype 2 strains. The failure of some serotype 2 strains to react with the Mab-coated latex seem to suggest that there exists an antigenic variation within strains belonging to this serotype. Since such strains showed a strong reaction with polyclonal antisera a possible explanation for this difference may be that they lack the epitope on LPS, responsible for binding the Mab, or that the abundance of the epitope is too sparse, leading to a negative result. In contrast, a polyclonal antiserum will detect sufficient serotype 2 specific determinants to give a positive result. Further studies are undertaken to identify the exact structure of the LPS in the Mab negative isolates.

In conclusion, the LPAT using polyclonal antisera is a reliable, rapid and economic method which is adequate in the diagnosis of serotype 2 field isolates.

Using latex coated with Mab 102-G02 it seems possible to subdivide Danish serotype 2 strains. The exact epidemiological value of

this observation is still uncertain but it might be useful in situations where acute outbreaks of pleuropneumonia are investigated for links with environmental sources.

Strain 9008 is proposed as the reference strain for the serotype 2 strains which show no reaction with Mab 102-G02. The strains are provisionally named *A. pleuropneumoniae 2x.*

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