Two *Actinobacillus pleuropneumoniae* Serotype 8 Reference Strains in Circulation

Actinobacillus pleuropneumoniae is an important pathogen, causing pleuropneumonia in pigs. Until now 14 different serotypes have been recognized. The antigenic variability among these has hampered the attempts to produce species-specific diagnostic tests. We recently described a species-specific PCR test for detection of *A. pleuropneumoniae* based on a gene coding for an outer membrane protein (*omlA*) (2). Comparison of the *omlA* sequence of all serotype reference strains revealed conserved termini and variable middle regions of the gene, which could divide the serotypes into distinct groups. Recently, we became aware that the *omlA* genes of the serotype reference strains have also been sequenced by Chevallier and Kobisch from Ploufragan, France (GenBank accession no. Y12810, Y12811, and Y12340 to Y12349). Comparisons of these *omlA* sequences with ours showed identical results with the exception of the *omlA* gene of serotype 8. Chevallier and Kobisch found serotype 8 (GenBank accession no. Y12811) to be in possession of an *omlA* gene homologous to the *omlA* gene of serotype 2. In our examination we found the *omlA* gene of serotype 8 to resemble that of serotypes 3, 4, 6, and 7. This was further supported by the analysis of four Danish field strains of serotype 8, which also were in possession of an *omlA* gene homologous to the gene found in serotypes 3, 4, 6, and 7. The presence of homologous genes in serotypes 8 and 2 have, however, been suggested in earlier publications based on hybridization experiments (1) and PCR-restriction fragment length polymorphism examination of the *omlA* gene (4). In order to elucidate the problem we analyzed different freeze-dried cultures of strain 405 used in our laboratory during the last 10 years, as well as strain 405 received from other researchers (M. Kobisch and J. Frey) and from the Collection of Animal Pathogenic Microorganisms (Brno, Czech Republic). All strains were serotyped as serotype 8. Sequencing and PCR typing suggested that strain 405 and a serotype 8 field isolate had been mixed in our laboratory in 1995. Hence, the *omlA*

gene of *A. pleuropneumoniae* serotype 8 reference strain 405 is homologous to the *omlA* gene found in serotype 2. These results have no influence on the earlier published species-specific PCR (2) as the primers used in the test are constructed from the conserved regions in both ends of the gene. These parts of the gene are identical in all the *A. pleuropneumoniae* serotypes, including the two variants of serotype 8.

In conclusion, two different *omlA* genes exist within *A. pleuropneumoniae* serotype 8. This might depend on the fact that different clones of serotype 8 exist in Denmark and Ireland where the strain 405 was isolated (3), or the difference may reflect changes in serotype 8 over time. It could be interesting to investigate the prevalence of these *omlA* types in field strains from other countries to reveal whether the variants of the gene reflect present *omlA* variations or whether the difference exists only between strain 405 and recent isolates.

REFERENCES

1. Gerlach, G. G., C. Anderson, S. Klashinsky, A. Rossi-Camos, A. A. Potter, and P. J. Willson. 1993. Molecular characterization of a protective outer membrane lipoprotein (*omlA*) from *Actinobacillus pleuropneumoniae* serotype 1. *Infect. Immun.* **61**:565–572.
2. Gram, T., and P. Ahrens. 1998. Improved diagnostic PCR assay for *Actinobacillus pleuropneumoniae* based on the nucleotide sequence of an outer membrane lipoprotein. *J. Clin. Microbiol.* **36**:443–448.
3. Nielsen, R., and P. J. O'Connor. 1984. Serological characteristics of 8 *Haemophilus pleuropneumoniae* strains and proposal of a new serotype: serotype 8. *Acta Vet. Scand.* **25**:96–106.
4. Osaki, M., Y. Sato, H. Tomura, H. Ito, and T. Sekizaki. 1997. Genetic diversity of the genes encoding the outer membrane lipoprotein (*omlA*) of *Actinobacillus pleuropneumoniae*. *J. Vet. Med. Sci.* **59**:213–215.

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