USE OF MOLECULAR EPIDEMIOLOGY TO UNDERSTAND AN INCIDENT OF
SALMONELLA DERBY IN PIG HERDS IN SWEDEN

ASPAN, A.¹, WAHLSTÔM, H.² and HÄGGBLOM, P.¹

In the summer of 1996, two epidemiologically linked pig herds in the south-west of Sweden were found to be infected with Salmonella Derby. S. Derby is a serotype of Salmonella that has been reported to cause problems for pork producers in for example Sweden, Norway, Denmark, Ireland and the USA.

The occurrence of different Salmonella serotypes in animal production in Sweden has carefully been monitored. Since 1949 the National Veterinary Institute (SVA) has published reports concerning the findings of Salmonella in animals and animal feedingstuffs (Malmqvist et al., 1995). The prime aim of the Swedish Salmonella control program for production of animal feedingstuffs is to promote the production of food for human consumption free of Salmonella and other pathogens. Imported raw materials for animal feed production are occasionally reported to be contaminated with Salmonella. The necessity to monitor feed for Salmonella may be controversial, but several reports indicate that many serotypes are present in ingredients of animal feedingstuffs (Humphrey and Lanning, 1988; Malmqvist et al., 1995).

Feed as a source of S. Derby infection in pigs in Sweden may be unlikely, as there have been few findings of this serotype in imported feed raw materials. Still, as a sample of imported palm seed was found to be positive for S. Derby during the same time period as the 1996 outbreak of S. Derby, this was considered as a potential source of infection. The aim of this study was to try to establish, by molecular epidemiology, i.e. analysis of strains by pulsed-field gel electrophoresis, whether the recent out-break of S. Derby was feed-related.

MATERIALS AND METHODS

Bacterial strains: Ca 100 strains of S. Derby isolated in Sweden between 1980 and 1996, originating from domestic food producing animals, animal feedingstuffs, imported raw materials for feed production and food products, were analysed by pulsed-field gel electrophoresis. The strains were all serotyped at the SVA, and kept in serum broth at -20° C since isolation.

DNA preparation: DNA for PFGE (pulsed-field gel electrophoresis) analysis was prepared according to Christensen et al., (1994), with slight modifications. Bacteria was grown overnight at 37°C on LB-agar plates. Four colonies from each agar-plate culture was inoculated into 2 ml of LB-medium in 10 ml sterile Falcon tubes. The tubes were incubated at 37°C for 16 hours in an incubator shaker (100 revolutions per min, Heto shaking bath SBD-50). 1.0 ml of the bacterial suspension was centrifuged in sterile Eppendorf tubes at 6 000 rpm for 8 min. The pellets were washed once with 1.0 ml Pett IV (10mM Tris-HCl, pH 7.6, 1M NaCl) and

¹ Dept. of Feed Hygiene¹ and Dept. of Epizootiology², National Veterinary Institute, P O Box 7073, S-750 07 Uppsala, Sweden
resuspended in 250 μl EC-buffer (1M NaCl, 10mM Tris, 200 mM EDTA, 0.5% Sarcosyl, 0.2% sodium deoxycholate, pH 8.0) warmed to 37°C and then mixed with 350μl warm (56°C) agarose (1.5% in EC-buffer) and 30μl lysozym-stock solution (20mg/ml). The mixture was distributed into plug moulds and left to solidify for 15 min. The agarose plugs were transferred to 10ml Falcon tubes containing 5ml EC-buffer, and incubated on shaking water-bath (50 revolutions per min, Heto shaking bath SBD-50) for two hours at 37°C. Then the EC-buffer was decanted and new EC-buffer added (2.5 ml/tube), containing 1mg proteinase K/ml buffer solution. The plugs were incubated for 56°C for 20 hours or longer on shaking water-bath (50 revolutions per min, Heto shaking bath). After the proteinase K incubation, the plugs were washed with TE-buffer (10mM Tris, 1mM EDTA, pH 8.0) and then proteinase K was inactivated by adding 50μl from a stock solution of 20mM Pefablock into 5ml TE-buffer, leaving the plugs at 37°C for two hours. Finally the plugs were washed with 5 ml TE-buffer at 37°C (2x 30 min), and then stored in TE-buffer at 4°C until further use.

*Restriction endonuclease digestion and PFGE:* Restriction endonucleases were used as recommended by the manufacturer (Amersheim). The gel plugs were cut in two parts and each part equilibrated in 0.2 ml restriction enzyme buffer for 30 min. New restriction enzyme buffer was added (0.1 ml) containing 20 U of respective enzyme, and plugs were incubated over night with mild shaking. A thin slice (1mm) of the gel plug was loaded onto an 1.2% agarose gel (Agarose Prep, Pharmacia) and run in a Pharmacia Gene Navigator unit, at 175 V and 12°C with buffer circulation. The program for XbaI was 10s, 3h / 15s, 6h / 20s, 6h / 40s, 5 h / 60s, 4h. After the electrophoresis was completed the gels were stained with ethidium bromide for 20 min (0.1% in water) washed in water for 20 min and then photographed under UV-light. λ DNA (Pharmacia, Uppsala, Sweden) was used as a marker of molecular size.

*Interpretation of PFGE patterns:* Difference in presence, absence or apparent mobility of any band, between two genomic restriction profiles, was considered to be sufficient to regard the profiles as non-identical.

**RESULTS**

*Macrorestriction analysis:* Macrorestriction profiles of chromosomal DNA from S. Derby were generated by cleavage with XbaI and subsequent separation by pulsed-field gel electrophoresis. The strains could be divided into a number of different macrorestriction profiles.

**DISCUSSION**

In 1996, two pig herds in the south-west of Sweden, were reported to be infected with S. Derby. As a sample of imported palm seed was found to be contaminated with the same serotype during the same time period, this was suggested as a possible source of infection. It was also speculated about a connection with persistent infections of S. Derby, that had caused problems in, all in all, four pig herds in the south of Sweden, from the early 1970’s until 1991.
Pulsed-field gel electrophoresis have successfully been used when tracing food sources for human infection of several Salmonella serovars (Baquar et al., 1994; Lemacher et al., 1995), and thus PFGE was the method of choice in this study. Analysis ca 100 strains of S. Derby, both domestic strains and strains isolated from imported food samples, showed a high diversity in PFGE patterns between strains.

All strains, isolated from the two pig herds that were reported to be infected by S. Derby in 1996, had identical PFGE pattern. Strains analysed, isolated from available feed samples, showed PFGE patterns which differed considerably from the above mentioned pattern. Thus, these, as well as the suspected palm seed, could be ruled out as sources of infection. Also, a number of isolates from the previous findings of S. Derby in Swedish pig herds during the 1980’s, were analysed by the same method. These isolates shared identical, or in a few cases, very similar, PFGE patterns, indicating a common source of the infections, which persisted for many years in these herds. The strain found in the two pigs herds in 1996 differed considerable from these earlier findings, and thus no connection is suggested. Unfortunately, the source of infection for the 1996 findings could not be identified in this study. Similar, but not identical, PFGE patterns, were found in isolates of S. Derby from meat imported from Ireland and Greaves meal imported from Italy. This may indicate a clonal spread of strains of S. Derby within Europe.

REFERENCES


