Transmission of multiresistant *Salmonella* Typhimurium DT104 between farms - investigated by the combined use of epidemiological and genotyping data

Marianne N. Skov¹, Birgitte Langvad¹, ², ³ and Dorte L. Baggesen¹

1: The Danish Veterinary Laboratory, Bülowvej 27, DK-1790 Copenhagen V, Denmark, Phone number: +45 35 30 01 00, Fax number: +45 35 30 01 20, E-mail: mns@svs.dk. 2: Institute of Veterinary Microbiology, The Royal Veterinary and Agricultural University, Copenhagen, Denmark. 3: Danish Dairy Board, Aarhus, Denmark

Abstract:
In the present investigation bacterial typing and epidemiological data was used to identify possible routes of introduction of DT104. The investigation was based on epidemiological data and isolates of 15 DT104 infected farms within a small area (15 kilometre in diameter) in Denmark, in which an almost local endemic situation has developed. In addition to the phenotypic characterisation (serotyping, phage typing, and antibiotic resistance typing) further characterisation was based on pulsed-field gel electrophoresis and plasmid profiling.

The typing results combined with the epidemiological data available indicate that a DT104 infected farm might pose a risk for having DT104 introduced at the neighbouring farms. Furthermore, the results indicated that farms owned by the same owner are at risk of having DT104 introduced if the bacteria is found at one of the farms. Finally, sharing of manure tank or agricultural machines with a DT104 infected farm might pose a risk for DT104 introduction.

Keywords:
Typing, PFGE, Plasmids, Risk factors

Introduction:
*Salmonella enterica* serovar Typhimurium (*S. Typhimurium*) phage type (definitive type) DT104 is a zoonotic bacteria and is characterised by resistance to at least five different antimicrobial agents; Ampicillin, Chloramphenicol, Streptomycin, Sulfonamides, and Tetracycline. The bacteria has been isolated world wide since its emergence in 1990 (Baggesen et al., 2000) and contaminated food of animal origin has been suggested as the main source of human infections. Thus, in order to control and reduce the level of DT104 in the animal production
knowledge of prevalence, risk factors and transmission of the infection is important.

The investigation was based on epidemiological data and isolates of DT104 collected from fifteen DT104 infected farms within a small area (15 kilometre in diameter) in Denmark, in which an almost endemic situation has developed during a 3 years period. In the present investigation bacterial typing and epidemiological data was used to identify possible routes of introduction of DT104.

Materials and Methods:
From each of the fifteen farms infected within the area one DT104 isolate were selected for further DNA based characterisation. In addition to the phenotypic characterisation further characterisation was based on pulsed-field gel electrophoresis (PFGE) and plasmid profiling.
The preparation of bacterial DNA for PFGE was performed, as prescribed by Olsen et al. (Olsen et al., 1994). Agarose plugs containing DNA were digested with the restriction enzymes BlnI and XbaI for four hours each. Electrophoresis was performed in a contour-clamped homogeneous electric field (CHEF Mapper) at 7 V/cm at 14℃ for 22½ hours. The program was as follows: first phase: beginning 7 s, end 26 s for 17½ h; second phase: beginning: 50s, end 60s for 5 h. After electrophoresis, the gels were stained and photographed. The gels were analysed and interpreted manually.
The plasmid profile was defined for all 15 isolates. The preparation of plasmids was performed by a method of Kado and Liu (1981). Electrophoresis was performed at 100 mV for 2½ h. Profiling and sizing of plasmids were performed, as prescribed by Olsen et al. (1992).

Results:
Fourteen of the included isolates shared exactly the same PFGE types when restriction enzymes BlnI and XbaI were used and analysed by PFGE. The remaining isolate (herd no. 13) showed unique PFGE types that differed from the common types with 5 and 2 bands respectively depending on restriction enzyme used.
The plasmid profile analysis divided the 15 isolates into two categories, plasmid-type-I and plasmid-type-II. The most common plasmid-type-I contained two plasmids (95 kb and 1.9 kb). The isolates from this group were collected from 13 of herds. Plasmid-type-II contained only one plasmid (95 kb) was found in two herds (herds 6 and 7).
By comparing the typing results with the common genotypes of DT104 in Denmark it is was possible to identify plasmid-type-I as a unique genotype for the case-area.
Discussion:
The identification of multiresistant S. Typhimurium DT104 in Danish food production animals is considered unacceptable by animal producer organisations and by the veterinary authorities in Denmark. Therefore, the Danish Salmonella surveillance system has been updated and adapted to identify infected animal herds (Baggesen et al., 2000). All isolates that are identified as or under suspicion of being Salmonella species are sent to the Danish Veterinary Laboratory, where they are serotyped. Identification of phage type and resistance pattern is performed for representatives of S. Typhimurium isolates.
As a consequence of this control program, S. Typhimurium DT104 was found in fifteen farms within a small area (15 kilometre in diameter) in Denmark.
In the present investigation the combined used of epidemiological and typing data has indicate that a DT104 infected farm might pose a risk for having DT104 introduced at the neighbouring farms. Furthermore, the results indicated that farms owned by the same owner are at risk of having DT104 introduced if the bacteria is found at one of the farms. Finally, sharing of manure tank or agricultural machines with a DT104 infected farm might pose a risk for DT104 introduction.

Acknowledgements:
We thank Eva Pedersen, Danish Veterinary Laboratory and Pia Mortensen, The Royal Veterinary and Agricultural University for skilful technical assistance.

References:
Olsen, J.E., Sørensen, M., Brown, D.J., Gaarslev, K. and Bisgaard, M., 1992. Plasmid profiles as an epidemiological marker in Salmonella enterica serovar Berta. Comparison of isolates obtained from humans and poultry. APMIS 100: 221-228.