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Multiple-locus variable-number tandem-repeat analysis (MLVA) typing of Salmonella enterica serovar Typhimurium DT41

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During the past 12 years, 17 outbreaks of *Salmonella enterica* serovar Typhimurium DT41 have been registered in Danish broiler parent flocks. Molecular typing has so far not allowed safe conclusions as to the source of infection and possible transmission between flocks just as possible transmission to humans have remained uncertain. MLVA analysis demonstrated high resolution in typing of *Salmonella* Typhimurium (Torpdahl et al. 2007. Emerg Infect Dis. 13, 388-95) and allows comparison between laboratories. For the same reason the aims of the present investigation were to characterize outbreak isolates by MLVA and compare results obtained with those from a broad reference collection of *S.* Typhimurium DT41 including human isolates. The comparison of outbreak isolates and reference collection will enable us to investigate if outbreaks can be traced back to a common source and to what extent isolates from poultry are related with those obtained from affected humans.

A total of 22 isolates from Danish poultry and 38 unrelated isolates of veterinary origin from Germany and England were included for comparison. In addition 30 human isolates were included that all originated from Danish patients affected during 1998-2008. Phage typing was performed according to the phage typing scheme developed by Callow and extended by Anderson. MLVA analysis was carried out as reported previously (Torpdahl et al. 2007). Data collection and preprocessing were performed with GENESCAN. Fragment sizes for all loci were analyzed in BioNumerics 5.1 and MLVA repeats were calculated and clusters visualized by an UPGMA dendrogram.

The reproducibility of MLVA was 100% for the isolates investigated. The dendrogram constructed showed two large clusters. One cluster consisted of 19 of the 30 human isolates and three isolates from pig, pig meat and a reptile. The other cluster included the remaining animal isolates, including all the Danish outbreak isolates. The differences between these two clusters were two alleles which are often very conserved. Four farm outbreaks were only separated by a single allele; however, all strains isolated from the same outbreak were identical by MLVA indicating that outbreaks were due to different sub-populations of DT41. Transmission from rearing farms to PS was not demonstrated supporting the negative results of the national surveillance programme. Thirteen out of 17 outbreaks were registered during autumn and winter months indicating that feed might represent a risk factor for acquiring S. Typhimurium DT41 as previously reported (Angen et al. 1996. Prev. Vet. Med. 26, 223-237). When comparison is made to isolates included from Germany and England, DT41 seems to be ubiquitous in distribution and the wild fauna might represent a risk factor for poultry. This, in combination with the high frequency of outbreaks during autumn and winter, indicates that animals might be attracted to feeding near poultry houses and this behavior could be a risk factor. The sources of the outbreaks remained unidentified and additional investigations are needed for clarification.

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