COLONISATION OF PIGLETS BY GENOTYPES OF *CAMPYLOBACTER COLI*

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Background

In Northern Ireland (NI) *Campylobacter* spp. are the principal cause of gastro-enteritis (Anon. 1995) with *Campylobacter jejuni* responsible for 90-95% of cases and *Campylobacter coli* causing the majority of the remainder, as is the case in most developed countries. A local survey of campylobacters in deep tissues of freshly eviscerated porcine liver (Moore and Madden, 1998) showed that 50% of the *Campylobacter* spp. isolated were *C. coli* however only 6% of livers were contaminated. In contrast 71.7% of samples of retail pig liver in England and Wales carried *Campylobacter* spp. with *C. coli* dominant, being found in 42.4% of samples. Subsequent investigations of pig faeces showed high *C. coli* carriage rates, as reported elsewhere (Blaser *et al.* 1983). Pigs are a natural reservoir of *C. coli* (Weijtens *et al*. 1993) and hence pork products may represent a risk to consumers. Skirrow (1991) concluded that *C. coli* from pigs are probably an important source of clinical infection in countries where pork is eaten salted or lightly cooked.

Objectives

Pigs are known to carry a wide range of *C. coli* genotypes and one pig may carry several (Weijtens *et al*. 1997). A detailed sampling of a single litter, and their mother, over the first 10 weeks of life was undertaken to allow a better understanding of the colonization of pigs by distinct *C. coli* genotypes.

Materials and methods

All media used were obtained from Oxoid, Basingstoke, UK, and all chemicals from Sigma-Aldrich, Fancy Road, Poole, UK.

Sampling of pigs. Pigs were sampled in the farrowing unit of an experimental farm. Litters were not allowed to associate but no exceptional measure were made to avoid cross-infection of the litters. Anal sampling of pigs was based on the method previously described (Madden *et al*. 2000). Swabs were locally made and consisted of cotton gauze fixed to a flat wooden support and had a surface area of approximately 6cm^2. Swabs were moistened in sterile peptone saline diluent prior to use and each pig was swabbed once. The swab was then broken into a sterile disposable universal. All samples were stored at 4^{0}c for transport to the laboratory and analysis commenced within two hours of sampling.

Six specific piglets in a single litter were selected using the unique identifier tattooed on its ear and sampled 6 times, using anal swabs, over the first 10 weeks of life. The sow was also sampled on each visit.

Isolation of *Campylobacter* spp. Swabs were streaked onto modified charcoal cefoperazone desoxycholate agar (mCCDA) (Bolton and Robertson, 1984) and incubated (42^{0}C) in a Don Whitley Mk III anaerobic cabinet (Don Whitley Scientific Ltd, Shipley, Yorks., UK) using a gas mix of 5% O_2, 10% CO_2, 85% N_2. Plates were examined daily for up to 5d and colonies characteristic of *Campylobacter* spp. selected and purified. Five isolates per plate were picked from each of the six piglet samples and twenty from those of the sow. All colonies morphologically characteristic of campylobacters were subjected to initial phenotypic characterisation and all *Campylobacter* spp. identified to species level using standard biochemical tests (Bolton and Robertson, 1982). They were then stored using 'Cryovials' (Technical Service Consultants, Heywood, Lancs. GB) at -80^{0}C.

Genotyping. Polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP) typing of the *flaA* gene was conducted using the method of Nachamkin *et a.l* (1993) whilst random amplification of polymorphic DNA (RAPD) was performed as previously described (Madden *et al*. 1996). Pulsed field gel electrophoresis (PFGE) was by the method of Thomson-Carter *et al*. (1993) using *Sma* 1. All profiles were analysed using Gelcompar (V 4.1) software.

Results and discussion

Sampling was initiated 3d after the piglets were born and at that time the sow and 3 piglets carried *Campylobacter coli*, the remaining 2 piglets yielded no campylobacters. Subsequently *Campylobacter* spp. were isolated from all samples. All isolates from the piglets (n=165) were identified as *C. coli*. The carriage rate of 100% from 17d of age onwards is higher than that reported by Weijtens *et al.* (1997), who found 85% positive after 4 weeks and Steinhauserova *et al.* (2001) who reported 41% of healthy piglets up to 8 weeks old. However Weijtens *et al.* (1997) noted that significant differences in carriage rates were found between farms, hence this could account for the differences reported. The sow died when the piglets were 14d old and was replaced by a foster sow. The latter was sampled when the piglets were 17d and 31d old and carried *Campylobacter lanienae*, with 20 isolates and 19 isolates being found out of 20 samples taken on the respective days. One *C. coli* isolates was found on the second sampling and subsequently all isolates were of this species. Overall 120 isolates from the sows were studied.
When the piglets were 27d old some piglets from a neighbouring litter briefly gained access to the pen, and on day 31 four of the neighbouring piglets were sampled to obtain isolates for comparative purposes.
Genotyping was conducted by RAPD, PCR-RFLP and PFGE and *C. coli* isolates were characterized according to their initial source of isolation i.e. mother, neighbour or other. Based on this characterization the genotypes isolates obtained from the piglets and the sow showed the same trend hence the mean is shown below, Figures 1 and 2.

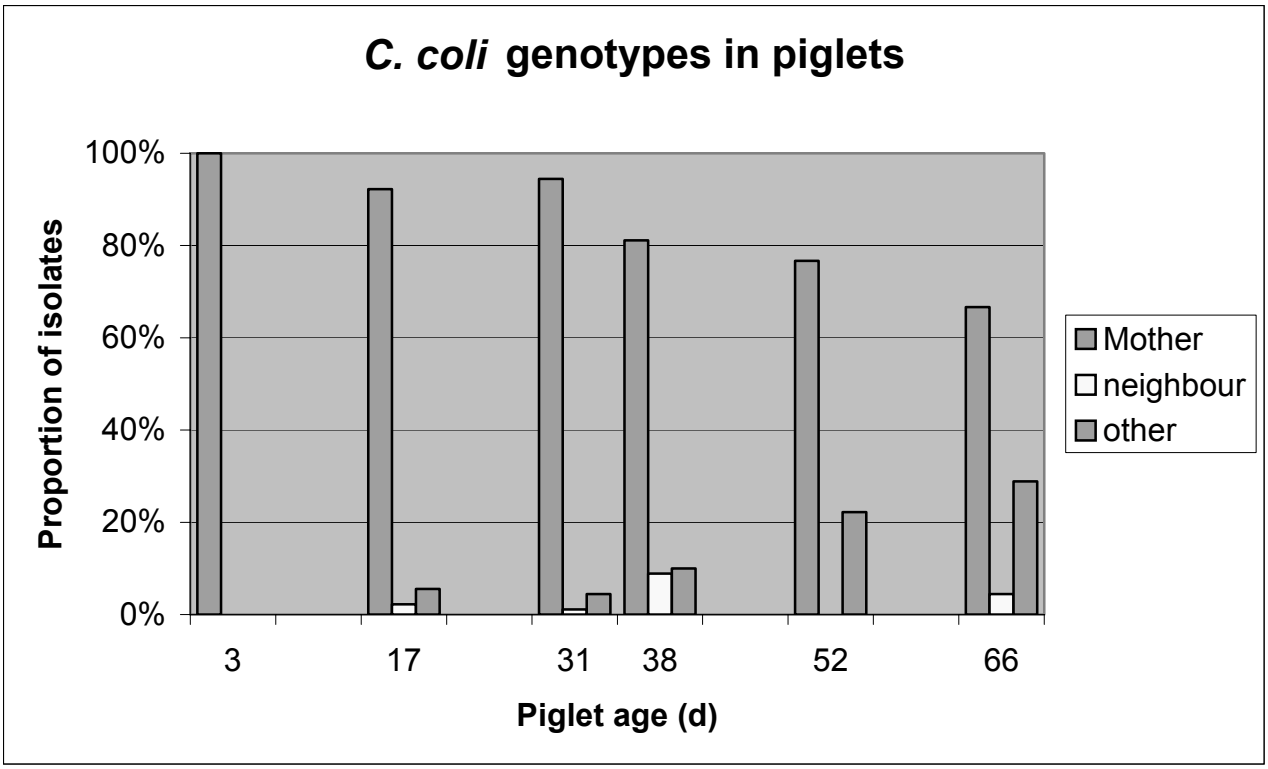

Figure 1. Distribution of *C. coli* isolates from piglets time (piglet age), based on the mean number of genotypes from three methods applied (PCR-RFLP of *flaA*, PFGE and RAPD).

The piglets show a clear trend of initially being colonized solely by *C. coli* strains from the mother but then, over the study period of 66 days, acquiring some from other sources causing replacement of the maternal genotypes. These results contrast with those of Hume et al. (2002) who reported that, in a study of 99 C. coli isolates from 3 sows and 17 piglets, there was no pattern of shared PFGE genotypes between sows and their respective piglets, nor between littermates. However in that study broth enrichment was used and enrichment conditions can affect the range of *Campylobacter* species (Madden *et al.* 2000) and genotypes (Madden et al. 2003) found. Weijtens *et al.* (1997) reported that a large diversity of campylobacter genotypes (determined by ERIC-PCR and RFLP) was found in piglets and their sows, as was the case in this study. In addition they noted that identical genotypes were found in piglets and their mothers and hence suggested that piglets became infected via their mothers, which is in agreement with the findings of this study (Fig. 1). However ,

the study reported here also shows that colonization is highly dynamic and point sampling will only record what is happening at one instant, in a very dynamic situation.

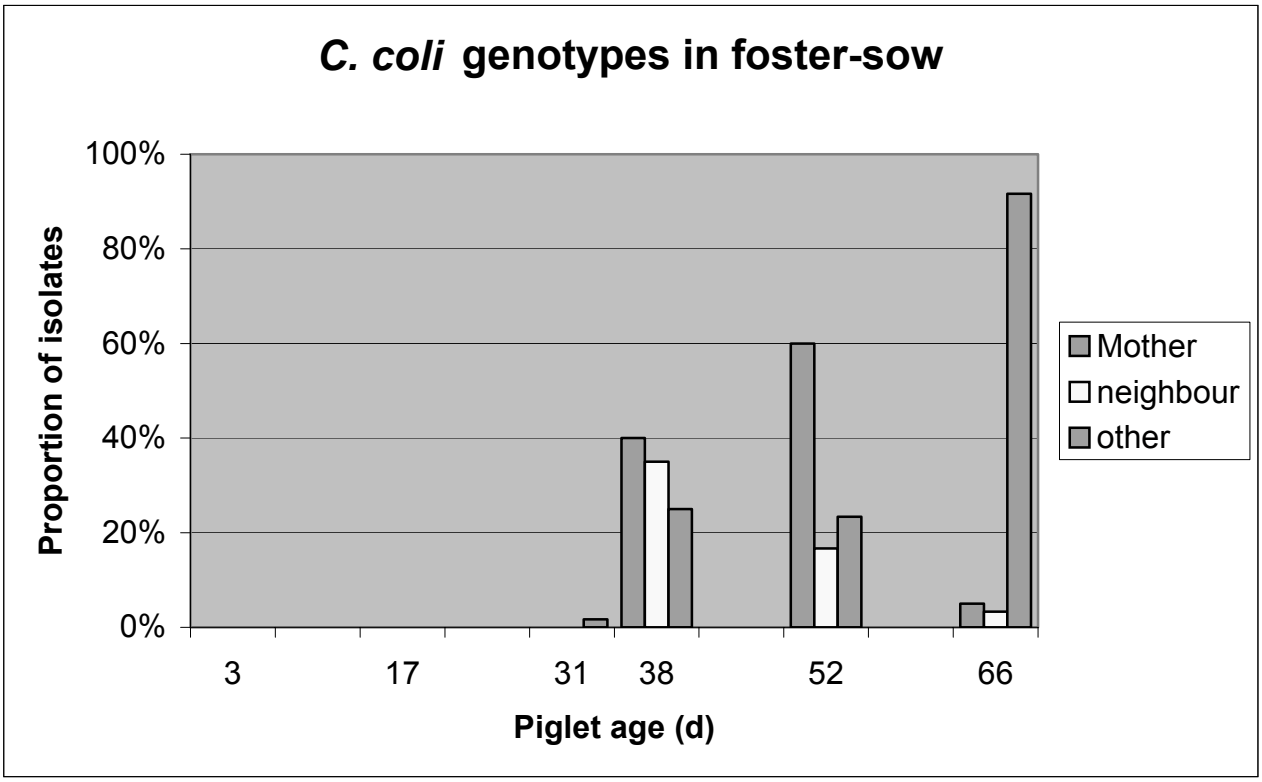

Figure 2. Distribution of *C. coli* isolates from foster-sow (introduced at age 15d of piglets) with piglet age, based on the mean number of genotypes from three methods applied (PCR-RFLP of *flaA*, PFGE and RAPD). Sampling on 17d and 31 d of age yielded 39 campylobacters, but only 1 *C. coli*.

The dynamic nature of colonization is illustrated by the results seen with the foster mother, (Fig 2). On arrival this sow was dominated by a *Campylobacter* spp. which was completely replaced (within 24d) by *C. coli* genotypes, the majority of which had been found in the original mother or the neighbouring litter. Thus whilst transmission from the sow to the litter appears to occur the process can also occur in reverse with the sow being infected from the litter, or nearby litters. Colonization by *C. coli* is highly dynamic in nature and displacement of dominant genotypes appears to occur regularly, even in adults.

Conclusions

Pigs and piglets have been reported as carrying several genotypes of *Campylobacter coli.* This study confirms that finding and shows that piglets are infected by their mother but can subsequently acquire other genotypes in a matter of days. The dominant genotypes in the piglets studied changed constantly over the 10 week sampling period, but the trend was for other genotypes to replace the maternal ones. In addition the sow can acquire new genotypes from her piglets and surroundings, and also displays a constant change in the dominant genotypes detected. Such a dynamic system means that attempts to use genotyping of *C. coli* in epidemiological studies involving pork products are unlikely to succeed. However piglets may prove a useful system in which to study genetic changes of *C. coli.*

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