

been increasing despite a reduction in annual submissions. *S. Typhimurium* U308a has been isolated with increased frequency since 1999. The incidence of antimicrobial resistance for all *Salmonella* isolates from pigs showed increasing resistance trends to tetracycline and sulphamethoxazole/trimethoprim.

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References:

- Shipp, C.R. B. Rowe (1980). A mechanised microtechnique for *Salmonella* serotyping. *J. Clin Pathology* **33**: 595-602.
- Smerdon, W.J., Adak, G.K., O'Brien, S.J., Gillespie, I.A., Reacher, M. (2001): General outbreaks of infectious intestinal disease linked with red meat, England and Wales, 1992-1999. *Commun Dis Public Health* **4**: 259-267
- Sojka W.J., Slavin, G., Brand, T.F., Davies, G. (1972) A survey of drug resistance in *Salmonella* isolated from animals in England and Wales. *British Vet Journal* **128**: 189-198.
- Way C, Beedell, Y.E., McLaren, I.M. (1991) A survey of antimicrobial resistance in salmonellae isolated from animals in England and Wales during 1984-1987. *British Vet Journal* **147**: 356-369.

O 22 Investigations of potential transfer of *Campylobacter coli* between hogs and turkeys.

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Summary: Hogs are often grown in close proximity to turkey farms in North Carolina, and the potential exists for transfer of pathogens, including *Campylobacter*, from one host animal to another. The aim of this study was to obtain evidence for possible transfer of *Campylobacter coli* from hogs to turkeys, or vice versa. Strains from four paired hog and turkey farms were isolated and characterized in terms of their antibiotic resistance profiles, and by molecular subtyping utilizing PCR-RFLP of *flaA*. Certain strains were found to be shared between hogs and turkeys, suggesting possible transfer. In spite of identical molecular subtypes, such strains commonly differed in antibiotic resistance profiles. The results are consistent with the hypothesis that strains of *C. coli* may transfer between hogs and turkeys, or that certain strain subtypes may independently colonize these animals through unidentified reservoirs.

Keywords: Strain subtypes, antibiotic resistance, PCR-RFLP, reservoir, prevalence

Introduction: *Campylobacter* spp., especially *Campylobacter jejuni* and *Campylobacter coli*, are recognized as leading bacterial causes of acute human gastroenteritis (Campylobacteriosis). *Campylobacter* is a zoonotic pathogen, which colonizes meat animals (poultry, hogs, cattle and others) and becomes transmitted to humans primarily through meat contaminated during slaughter and processing (Friedman et al., 2000).

Although various meat animals are known to be commonly colonized by campylobacters, a degree of host adaptation appears to exist. Poultry are most frequently colonized by *C. jejuni*, followed by *C. coli*, whereas cattle and swine are colonized almost exclusively with *C. jejuni* and *C. coli*, respectively (Aarestrup et al., 1997; Saenz et al., 2000; van Looveren et al., 2001). However, circumstantial evidence exists for possible transfers among hosts, and common strain types between *Campylobacter* from broilers and other animals (cattle, swine) have been reported (Aeschbacher and Piffaretti, 1989; Meinersman et al., 1997; On et al., 1998).

Our laboratory has been investigating the prevalence and strain types of *Campylobacter* from turkey flocks since 2001. We have found that turkey flocks in eastern North Carolina are frequently colonized by *C. coli*. The high frequency of *C. coli* in turkeys is of interest, as this bacterium is normally associated with swine (Aarestrup et al., 1997; Saenz et al., 2000). In other surveys of turkey colonization by *Campylobacter*, *C. coli* was found only in 9% of the isolates in one study (van Looveren et al., 2001), and not at all in another (Wallace et al., 1998).

Eastern N. Carolina is a major production region in the United States for both hogs and turkeys, and turkey husbandry often operates side-to-side with hog production. Although different service people and veterinarians typically serve turkeys and hogs, the potential exists for contact between the turkey and hog operations through the farmer, farm staff, and vehicular traffic (the farmer's truck etc).

The objective of this study was to investigate the potential transfer of *C. coli* from hogs to turkeys in hog-turkey production systems that operate in close proximity to each other. Four paired hog-turkey farms were examined in terms of the strain subtype and antibiotic resistance profile of *C. coli* that colonized the animals. Our results suggest the possibility of transfer of the organism from hogs to turkeys, with subsequent acquisition of different antibiotic resistance determinants.

Materials and methods: The turkey and hog farm pairs were typically within 100 m of each other, and each pair consisted of a growout turkey farm and a finishing hog farm. Fresh fecal samples were collected in sterile tubes, and transported to the laboratory on ice. Ca. 0.1 g from the interior of the sample was directly plated on a modified CCDA plate (Oxoid) and incubated at 42 AC microaerobically for 48 h. Purifications were on sheep blood agar plates (Remel). Antibiotic resistance determinations employed the disk diffusion test.

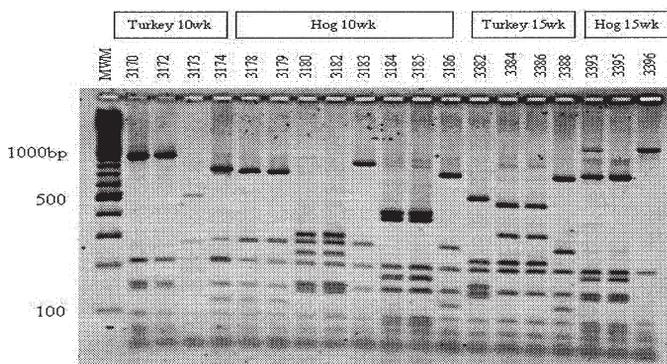
Genomic DNA was extracted with the Dneasy kit (Qiagen) and polymerase chain reaction (PCR) with primers for *hip* (Chan et al., 2000) and for *ceuE* (Gonzalez et al., 1997) was used to identify *C. jejuni* and *C. coli*, respectively. Molecular subtyping of the bacteria utilized PCR-restriction fragment length polymorphism (PCR-RFLP) of *flaA*, involving amplification of *flaA* and subsequent digestion with restriction endonuclease *DdeI* (Nachamkin et al., 1993). The restriction fragments were separated by agarose gel electrophoresis (1 % agarose in Tris Borate EDTA buffer) and photographed.

Results: All four pairs of hog-turkey production systems were found to be colonized by *Campylobacter* at high prevalence when sampled. Upon direct plating, *Campylobacter* was isolated from 50-85 % of the hog fecal samples, and from 55-95 % of the turkey samples. All *Campylobacter* strains from hogs were *C. coli*. Prevalence of *C. coli* among the turkey isolates was 68%, on the average (range among farms, 35 – 100 %), with the remainder being *C. jejuni*.

Resistance to tetracycline (30 mg/L) was uniformly high in both turkey and hog derived isolates of *C. coli* (93 and 96 %, respectively), and resistance to ampicillin (100 mg/L) was 75 % for *C. coli* from turkeys and hogs. However, significant differences were found in the incidence of ciprofloxacin and nalidixic acid resistance. *C. coli* strains from three of the hog farms were much less frequently resistant to these antibiotics (4, 31, and 18 %) than *C. coli* from the corresponding turkey farms (60, 67, and 60 %, respectively). Resistance was rare in the remaining farm pair.

Several different strain *flaA* strain subtypes of *C. coli* were isolated from the hogs. In each farm, several samples (20-60 %) were found to harbor bacteria of the same *flaA* type. Unique strain subtypes, encountered only once among the samples from each farm, and not shared among farms, constituted 11, 26, 32, and 35% of the strain subtypes. Although each of the four farms had its characteristic prevalent strain subtypes, one subtype, designated cc5, was encountered among all four farms (Table 1). However, strains of the same subtype frequently had different antibiotic resistance phenotypes, and generally the antibiotic resistance profiles of such strains differed from farm to farm.

Fig. 1. Strain subtypes of *C. coli* from a hog-turkey production system.



To evaluate the genetic similarity between strains from turkeys and from hogs, we employed *flaA* typing. Fig. 1 shows some of the strain subtypes of *C. coli* isolates from hogs of one farm, and of the corresponding turkey isolates. It can be seen that certain strain types were identical between hogs and turkeys (e.g. strains 3174 (turkey), 3178 (hog), 3179 (hog), 3186 (hog) and 3388 (turkey)). Interestingly, the antibiotic resistance profiles of the strains differed. The hog-derived strains were resistant to tetracycline, ampicillin, streptomycin and erythromycin, whereas the turkey-derived strains were additionally resistant to kanamycin, ciprofloxacin and nalidixic acid. It is worthy of note that the hog samples at the first time point (at which time the turkeys were 10 wk old) all had the same antibiotic resistance markers (resistance to tetracycline, streptomycin, ampicillin and erythromycin), even though they included 4 distinct strain subtypes, one of which (cc5, detected in 3 of the 8 strains) was also detected in the turkey samples.

Similar findings were obtained with the other farms. Although certain strains derived from hogs had identical strain types to those derived from the corresponding turkey farms, the antibiotic resistance profiles of the strains were usually different. Exceptions were occasionally noted. For instance, the hog-derived strain of subtype cc5 from farm 3 had a phenotype identical to several cc5 strains from turkeys, and was resistant to all antibiotics in the panel, including fluoroquinolones.

Discussion. The observed relatively high prevalence of *C. coli* in the turkeys is consistent with the hypothesis of transfer of this organism from hogs to turkeys. Since the antibiotic treatment regimens are distinctly different between hogs and turkeys, there would be selection for resistance to antibiotics used during turkey husbandry. In such a scenario, the strains would differ in antibiotic resistance but would be otherwise closely related genetically.

Although our data do provide evidence for strain subtypes that may be shared between hogs and turkeys in these production systems, further work is needed to confirm the putative transfer and determine its direction. *C. coli* typically colonizes hogs, which could therefore serve as a *C. coli* reservoir for other animals, such as turkeys. Turkeys have been described to be primarily colonized by *C. jejuni* (Wallace et al., 1998; van Looveren et al., 2001), and transfer from hogs, directly or indirectly, may account for the observed relatively high frequency of *C. coli* among the turkey samples in this study. Such transfer would be facilitated when turkeys and hogs are grown in close proximity, as was the case with the paired hog and turkey farms surveyed here. However, we have detected relatively high frequency of *C. coli* in turkeys from a number of different farms in eastern North Carolina, a hog-dense region (S. Kathariou and D. K. Carver, unpublished). One may speculate that, once turkeys become colonized by *C. coli*, the bacteria could transfer back to hogs. Results from such transfers may be evidenced by the isolation of strains with antibiotic resistance profiles typical of those of turkey-derived strains, such as the cc5 hog-derived strain from farm 3.

A key question that is generated by our findings concerns the origin of the shared strains. Further work is needed to determine the natural reservoir of strains with subtype cc5, which were found in both hog and turkey-derived strains in this study. Subtype cc5 has been repeatedly detected among *C. coli* isolates from turkeys from this geographical region, and, in addition, among *C. coli* recovered from flies in turkey and hog farms (B. C. Lee, L. Zurek and S. Kathariou, unpublished). Further work is needed to clearly determine whether these strains actually transfer from hogs to turkeys (and possibly vice versa) or whether hogs and turkeys receive them independently from other sources, that are currently not identified.

The results from this study identify some of the complexities of the ecology of *Campylobacter* in hog production, and are being followed by additional investigations in our laboratory, which are expected to elucidate some of the issues raised above.

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References:

- Aarestrup, F. M., Nielsen, E. M., Madsen, M., Engberg, J. (1997). Antimicrobial susceptibility patterns of thermophilic *Campylobacter* spp. From humans, pigs, cattle, and broilers in Denmark. *Antimicrob. Agents Chemother.* **41**:2244-2250.
- Aeshbacher, M., Piffaretti, J. C. (1989). Population genetics of human and animal enteric *Campylobacter* strains. *Inf. Immun.* **57**:1432-1437.
- Friedman, C. R., Neimann, J., Wegener, H. C., Tauxe, R. V. (2000). Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized nations. p. 121-138. *In*. I. Nachamkin and M. J. Blaser (ed.) *Campylobacter*, 2nd edition., American Society for Microbiology Press, Washington, D.C.
- Houng, H., O. Sethabutr, O., W. Nirdnoy, W., Katz, D.E., Pang, L.W. (2001). Development of a *ceuE*-based multiplex polymerase chain reaction (PCR) assay for direct detection and differentiation of *Campylobacter jejuni* and *Campylobacter coli* in Thailand. *Diagn. Microb. Inf. Dis.* **10**:11-19.
- Marshall, S.M., Melito, P.L., Woodward, D.L., Johnson, W.M., Rodgers, F.G., Mulvey, M.R. (1999). Rapid identification of *Campylobacter*, *Arcobacter*, and *Helicobacter* isolates by PCR-restriction fragment length polymorphism analysis of the 16S rRNA gene. *J. Clin. Microbiol.* **37**: 4158-4160.
- Meinersmann, R. J., Hesel, L. O., Fields, P. I., Hiett, K. L. (1997). Discrimination of *Campylobacter jejuni* isolates by *fla* gene sequencing. *J. Clin. Microbiol.* **35**:2810-2814.
- Nachamkin, I., Bohachick, K., Patton, C. M. (1993). Flagellin gene typing of *Campylobacter jejuni* by restriction fragment length polymorphism analysis. *J. Clin. Microbiol.* **31**:1531-1536.
- On S. L., Nielsen, E. M., Engberg, J., Madsen, M. (1998). Validity of *Sma*I-defined genotypes of *Campylobacter jejuni* examined by *Sa*II, *Kpn*I, and *Bam*HI polymorphisms: evidence of identical clones infecting humans, poultry, and cattle. *Epidemiol. Infect.* **120**:231-237.
- Saenz, Y., Zaarazaga, M., Lantero, M., Castanares, M. J., Boquero, F., Torres, C. (2000). Antibiotic resistance of *Campylobacter* strains isolated from animals, foods and humans in Spain in 1997-1998. *Antimicrob. Agents Chem.* **44**:267-271.
- Van Looveren, M., Daube, G., De Zutter, L., Dumont, J. M., Lammens, C., Wijdooghe, M., Vandamme, P., Jouret, M., Cornelis, M., Goossens, H. (2001).
- Antimicrobial susceptibilities of *Campylobacter* strains isolated from food animals Belgium. *J. Antimicrob. Chemother.* **48**:235-240.
- Wallace, J. S., Stanley, K. N., Jones, K. (1998). The colonization of turkeys by thermophilic *Campylobacter*s. *J. Appl. Microbiol.* **85**:224-230.

Table 1: Strain subtype distribution among *C. coli* from hog-turkey production systems. Strain subtypes indicated in bold were found among both hog and turkey-derived strains. Numbers in parentheses indicate the number of strains with the specific subtype.

| Farm 1 | | Farm 2 | | Farm 3 | | Farm 4 | |
|-----------------|------------|----------------|---------|----------------|------------|----------------|------------|
| Hogs | Turkeys | Hogs | Turkeys | Hogs | Turkeys | Hogs | Turkeys |
| Cc5 (7) | Cc5 (2) | Cc5 (3) | Cc5 (6) | Cc5 (1) | Cc5 (8) | Cc5 (4) | Cc5 (7) |
| GTH1 (2) | Cc4 (3) | GTH1 (4) | | GTA (15) | GTT1 (2) | Cc4 (1) | GTT3 (2) |
| GTH2 (5) | GTH3 (1) | Unique (5) | | GTB (2) | GTT2 (2) | GTA (1) | GTT4 (2) |
| GTH3 (3) | Unique (1) | | | Unique (7) | Unique (1) | GTC (2) | Cc4 (1) |
| Unique (2) | | | | | | GTH4 (3) | Unique (2) |
| | | | | | | GTH5 (2) | |
| | | | | | | GTH6 (2) | |
| | | | | | | Unique (3) | |

O 23

Comparison of two commercial ELISA kits and bacteriology for *Salmonella* monitoring in pig herds

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Summary: Samples of 'meat-juice' and serum from 170 pigs from 20 finishing farms were tested for *Salmonella* using two commercial ELISA kit tests. In parallel samples from caecal contents and pooled pen faeces from the farm were tested by culture. Both ELISA's gave significantly correlated results with each other but only ELISA B, at a 20 % calculated OD % on 'meat juice', gave a result which correlated significantly with the percentage of positive pen faeces. None of the ELISA tests correlated with caecal positives and the 10 % cut-off level was shown to be unsuitable for monitoring commercial herds.

Keywords: serology, pork, pigs, swine, culture

Introduction: Serological testing of pig herds for *Salmonella*, despite its epidemiological drawbacks, is the most widely accepted method of monitoring, largely on the grounds of convenience and cost. It is desirable that methods used for such testing should be as standardised as possible but an international ring trial has shown large differences in the performance of various tests (Heijden, 2001). The work carried out in this study was designed to evaluate two commercial ELISA kits for suitability of use for monitoring in British pig herds.

Materials and Methods: Serum and meat juice from 170 pigs originating from 20 British finishing herds were examined for anti-*Salmonella* antibodies using two commercial ELISA tests, A (Guildhay)