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Research article

Carry-over of thermophilic *Campylobacter* spp. between sequential and adjacent poultry flocks

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ABSTRACT

Nineteen flocks of four poultry species were monitored at a veterinary field station to investigate the distribution and spread of *Campylobacter* genotypes between sequential and adjacent flocks. Caecal and liver samples were obtained at frequent intervals from birds of all flocks and examined for *Campylobacter*. Amplified fragment length polymorphism (AFLP) analysis was performed to genotype *Campylobacter* isolates. Of the 1643 caecal and liver samples investigated, 452 (27.5%) caecal samples and 11 (0.7%) liver samples contained *Campylobacter*. Of the caecal isolates 76.3% were identified as *Campylobacter jejuni* and 23.7% were identified as *Campylobacter coli*. Poultry flocks were largely colonized by more than one AFLP type and an intense exchange of *Campylobacter* genotypes between different poultry flocks occurred. These findings indicate that multiple genotypes can constitute the *Campylobacter* population within single poultry flocks, hinting to different sources of exposure and/or genetic drifts within the *Campylobacter* population. Nevertheless, in most flocks single *Campylobacter* genotypes predominated. Some strains superseded others resulting in colonization by successive *Campylobacter* genotypes during the observation period. In conclusion, the data demonstrate that the large genetic diversity of *Campylobacter* must be considered in epidemiological evaluations and microbial risk assessments of *Campylobacter* in poultry.

1. Introduction

Poultry and poultry meat is regarded as one of the main sources of thermophilic *Campylobacter* (*C.*) spp. infection in humans (Humphrey et al., 2007). Even though many studies focussed on the epidemiology of *Campylobacter* spp. in poultry processing, the epidemiology of *Campylobacter* spp. in poultry flocks is still poorly understood (Messens et al., 2009). That lack of understanding limits attempts to control *Campylobacter* colonization in poultry. It is assumed that horizontal transmission via domestic animals, wild animals, contaminated water, rodents, insects and other environmental sources is largely responsible for flock colonization (Hald et al., 2004; Guerin et al., 2007). Vertical transmission of the bacteria is rather unlikely to occur, even though some experimental evidence supports that hypothesis (Petersen et al., 2001; Callicott et al., 2006). Soon after introduction into the flock, the bacterium spreads rapidly (Evans and Sayers, 2000). Several studies indicate that flocks may be simultaneously colonized with different *Campylobacter* clones at any time (Ridley et al., 2008a; Messens et al., 2009). Often one or two clones dominate other strains, possibly due to a better colonization potential. Nonetheless, restricted sampling strategies and strain characterization limit the identification of sources and the characterization of strain diversities (Newell et al., 2001; Nadeau et al., 2002; Bull et al., 2006).

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Accurate und highly discriminatory typing methods for *Campylobacter* spp. are needed to detect sources of colonization and to study the population structure. In recent years, a number of reliable and highly discriminatory genotyping methods have been developed and applied to *Campylobacter* spp. PCR-RFLP analysis of the flagellin locus (*fla*A-typing), automated ribotyping, pulsed-field gel electrophoresis (PFGE), amplified fragment length polymorphism (AFLP), multilocus sequence typing (MLST) and sequencing of the *fla*A short variable region (SVR) are the typing methods most often applied (Wassenaar and Newell, 2000).

The objective of this study was to detect the *Campylobacter* genotype distribution in poultry flocks by applying AFLP analysis and to describe a potential carry-over of *Campylobacter* strains among sequential and adjacent poultry flocks.

2. Materials and methods

2.1. Farm management and flock characteristics

The birds included in this study consisted of four different avian species and were kept in the veterinary field station of the University of Veterinary Medicine Hannover. All birds were supplied by commercial hatcheries and placed into four conventional poultry houses at the first day of life. The four houses were located within a distance of approximately 26 m between each other. All flocks were subsequently reared under commercial conditions. Altogether, five pekin duck flocks (P1–P5), four muscovy duck flocks (M1–M4), two turkey flocks (T1–T2) and eight broiler flocks (B1–B8) were investigated. The samples were collected for a period of 1 year (November 2004 to November 2005). Information on flock size, room size and slaughter age of the individual flocks is given in Table 1. Each house had its own set of clothing for farm workers, a physical barrier between the entrance area and the inside clean area and disinfectant boot dips in each house. Farm workers were not assigned to a specific house. Service period length varied from 7 to 36 days. Within that period cleaning and disinfection measures were carried out and insecticides and larvicides were applied inside the houses to control flies. Rodent controls were placed outside the walls of all houses. Close to these premises, laying hens, pigs and cattle were reared (distance to poultry houses under investigation: 100–172 m).

2.2. Sampling methods

Each poultry house was checked at least twice a day. All dead ducks and turkeys were collected and cooled until a necropsy was performed. Twice a week five to ten broilers were chosen for necropsy. Weak or moribund individuals were sacrificed and examined. Altogether, 1643 birds were tested (240 pekin ducks, 256 muscovy ducks, 401 turkeys and 746 broilers). The abdominal cavity was aseptically opened for bacteriological sampling. To reduce the possibility of cross-contamination livers were aseptically removed before removal of the caeca. The sterilized instruments were changed between each step. Individual organ samples (i.e. livers and caeca) were placed into separate sterile plastic bags and transported immediately to the laboratory.

2.3. Laboratory testing

Caeca were opened and swabs with caecal material were streaked directly onto modified *Campylobacter* charcoal differential agar (mCCDA, Oxoid, Wesel, Germany). The livers were cut aseptically and a small piece of approx. 5 mm × 5 mm was taken from the internal surface and streaked directly onto mCCDA. Media were incubated for 48 h at 37 °C microaerobically, using CampyGen Kit (Oxoid) to generate an O_2-deficient, CO_2-enriched atmosphere in anaerobic jars (Oxoid).

Up to three *Campylobacter*-presumptive colonies of each sample were subcultured on mCCDA and subsequently on Columbia agar supplemented with 7% sheep blood (Oxoid) and incubated for 48 h at 37 °C under microaerobic conditions. Colonies were identified as *Campylobacter* spp. by typical morphology and motion, Gram-stain, catalase and oxidase reaction. For DNA extraction, up to three single colonies were harvested and resuspended in 500 μl distilled water. Extraction of DNA was performed by boiling the suspension for 10 min. DNA samples were stored at −70 °C. *Campylobacter jejuni* was identified by the PCR described by Marshall et al. (1999). For species verification of strains, which were not identified as *C. jejuni*, a multiplex PCR was carried out according to Wang et al. (2002).

2.4. Genotyping

AFLP analysis was carried out on *Campylobacter* spp. strains as described previously (Duim et al., 1999). Amplified DNA fragments were electrophoresed horizontally on a 12.5% SDS-gel (ExcelGel DNA Analysis Kit, GE Healthcare, Freiburg, Germany) on a Multiphor II apparatus (GE Healthcare). Following electrophoresis, gels were silver stained (PlusOne DNA Silver Staining Kit, GE Healthcare), visualized and photographed on the ChemiImager 4400 (Cell Biosciences, Santa Clara, CA, USA).

Data were collected with the AlphaEase software v5.5 (Cell Biosciences) and fragment analysis was conducted

Table 1
Details of sampled poultry flocks.

Poultry species	No. of flocks	Flock size (no. of animals)	Room size	Beddings	Slaughter age (d)
Pekin ducks	5 (P1–P5)	3400	425 m^2[a]	Straw	43–50
Muscovy ducks	4 (M1–M4)	1840–3550	425 m^2	Plastic grits with manure pit below	60–72
Turkeys	2 (T1–T2)	3100–3300	945 m^2	Wood shavings and straw	144–147
Broilers	8 (B1–B8)	18,500–18,800	945 m^2	Wood shavings	29–37

[a] Additional winter garden (173 m^2).