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Occurrence and genotypes of *Campylobacter* in broiler flocks, other farm animals, and the environment during several rearing periods on selected poultry farms

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ABSTRACT

On 15 Swiss poultry farms, broiler flocks, other farm animals, and the environment were examined during consecutive rearing periods to investigate the occurrence and genetic diversity of *Campylobacter*. Of the 5154 collected samples, 311 (6%) from 14 farms were *Campylobacter* positive by culture. Amongst the positive samples, 228 tested positive for *Campylobacter jejuni* and 92 for *Campylobacter coli*. Positive samples originated from broilers, the broiler houses, cattle, pigs, bantams, laying hens, a horse, and a mouse. Feed, litter, flies, and the supply air to the broiler house tested negative. By flagellin gene typing (*fla*-RFLP) and pulsed-field gel electrophoresis (PFGE), 917 *Campylobacter* isolates were genotyped. Additionally, amplified fragment length polymorphism (AFLP) analysis was performed on 15 assorted strains. On eight farms, matching genotypes were isolated from broiler flocks and other farm animals: Certain genotypes from cattle (farms H, K, L, and M), pigs (farms D and P), or laying hens (farm L) were subsequently found in the broiler flocks, whereas other genotypes initially present in the broiler flocks turned up in cattle (farms A, D, and O). These results emphasize the importance of other farm animals on poultry farms for broiler flock colonization. Indications of persistent contamination of the broiler house were evident on four farms (C, D, I, and L) where matching genotypes were detected in consecutive broiler flocks, but not concurrently in other samples. By *fla*-RFLP, PFGE, and confirmed by AFLP, some genotypes proofed to be identical across different farms.

1. Introduction

Campylobacter, mainly *C. jejuni* and *C. coli*, are worldwide recognized as major cause of acute bacterial food-borne gastroenteritis (World Health Organization: www.who.int/mediacentre/factsheets). In the European Union (EU), a total of 175,561 confirmed human cases have been reported in the year 2006 (EFSA, 2007). The incidence of human campylobacteriosis in the EU has increased over the past years and recently exceeded that of *Salmonella* in many countries. Although campylobacteriosis is usually a self-limiting diarrheal disease, severe complications such as septicemia, reactive arthritis, and Guillain–Barré syndrome sometimes occur (Humphrey et al., 2007).

Campylobacter colonize the intestinal tracts of a large number of mammals and birds. Broilers are often carriers of *C. jejuni*. Chicken guts, particularly ceca, can be colonized at high levels and usually the entire flock is colonized once an infection becomes established (Newell and Fearnley, 2003). In the year 2006, 0.0% to 83.2% of the poultry flocks in the EU got colonized, and 25.9% of the Swiss poultry flocks tested positive (EFSA, 2007). This may lead to contamination of carcasses during the slaughter process (Ono and Yamato, 1999; Jørgensen et al., 2002; Stern and Robach, 2003; Allen et al., 2007). Consumption and handling of poultry meat has been identified as important risk factor for human disease (Friedman et al., 2004; Siemer et al., 2005; Humphrey et al., 2007). Major efforts are therefore attempted to reduce the number of colonized flocks being delivered for slaughter.

The epidemiology of *Campylobacter* in broiler production is still incompletely understood. There is a degree of dispute over which are the most important sources for flock colonization (Humphrey et al., 2007). Vertical transmission, carryover from previous flocks, and horizontal transmission via contaminated water, domestic and wild animals, personnel working in the broiler house, and the external environment have been implicated. The importance of vertical transmission from parent flocks to their offspring remains unclear (Petersen et al., 2001; Cox et al., 2002; Callicott et al., 2006). Horizontal transmission is generally believed to be the common way for flock colonization (Sahin et al., 2002; Newell and Fearnley, 2003; Bull et al., 2006). However, more knowledge on the diversity and stability of *Campylobacter* in the environment and the distribution of different clones are necessary (Johnsen et al., 2006).

In a previous study, dynamics of *Campylobacter* spread within broiler flocks were examined (Ring et al., 2005). The aim of the present study was to investigate the occurrence and genetic diversity of *Campylobacter* in broiler flocks, other farm animals, and the

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Table 1
No. (%) of *Campylobacter* positive samples during the sampled rearing periods on the different farms (n=5154)

Farm	No. (%) of *Campylobacter* positive samples during the rearing periods					
	1st rearing	2nd rearing	3rd rearing	4th rearing	5th rearing	6th rearing
A	12 (14.1%)	2[c] (4.1%)	3[c] (3.8%)	3[c] (4.5%)	ns[b]	ns
B	4[c] (6.3%)	1[c] (1.0%)	6[c] (7.4%)	1[c] (1.8%)	0 (0.0%)	3[c] (6.1%)
C	0 (0.0%)	10[d] (9.6%)	2[d] (2.2%)	4[d] (4.5%)	0 (0.0%)	12[d] (22.6%)
D	4[c] (4.0%)	1[c] (1.0%)	5 (6.2%)	8 (10.7%)	13 (16.5%)	13 (15.5%)
E	2[c] (3.0%)	1[c] (1.5%)	3[c] (6.4%)	0 (0.0%)	1[c] (1.6%)	2[c] (4.2%)
F	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
G	3[c] (4.5%)	0 (0.0%)	0 (0.0%)	5[c] (6.3%)	0 (0.0%)	1[c] (2.5%)
H	3[c] (3.4%)	2[c] (3.1%)	4[d] (5.8%)	8 (12.1%)	1[c] (1.1%)	2[c] (5.6%)
I	0 (0.0%)	0 (0.0%)	6 (13.6%)	5[d] (7.0%)	7[d] (9.9%)	0 (0.0%)
K[a]	2[c] (3.4%)	9 (9.6%)	0 (0.0%)	ns	ns	ns
L[a]	5[c] (8.1%)	14 (19.4%)	10 (11.6%)	15 (23.4%)	ns	ns
M	7[c] (13.7%)	3 (8.3%)	6[c] (11.3%)	8[c] (9.2%)	2[c] (3.7%)	1[c] (1.9%)
N	0 (0.0%)	3[d] (4.7%)	1[c] (1.5%)	1[c] (1.5%)	2[c] (5.6%)	0 (0.0%)
O	2[c] (4.4%)	0 (0.0%)	6[d] (9.7%)	8 (15.7%)	1[c] (2.6%)	0 (0.0%)
P[a]	10 (9.9%)	16[d] (20.8%)	16 (19.5%)	ns	ns	ns

[a] Extensive outdoor flocks.
[b] ns, not sampled.
[c] Positive samples only in the environment.
[d] Positive samples only in boilers and the broiler house.

environment during consecutive rearing periods. To establish genetic relationships and to reveal potential transmission routes, strains were characterized by restriction fragment length polymorphism (RFLP) of the *flaA* gene, pulsed-field gel electrophoresis (PFGE), and amplified fragment length polymorphism (AFLP) analysis.

2. Materials and methods

2.1. Farms

Broiler flocks, other farm animals, and the environment of 15 Swiss poultry farms (Table 1) were examined for *Campylobacter* (March to December 2006). All farms were part of an integrated system (parent animal farms, hatcheries, broiler units, defined feedstuff, prescribed cleaning and disinfection procedures, slaughterhouse). As customary in Switzerland, each farm maintained other branches of animal farming and only contained one broiler house with one flock at a time. The distance from the broiler house to the other farm buildings was between 50 and 150 m. The same persons were attending poultry and other farm animals. In front of the broiler house entrance was a concreted area (10×10 m). A hygiene barrier was located in the anteroom of the broiler house and required change of clothes and boots, putting on a headdress, and washing and sanitizing hands. Boots used for entering the flock were sanitized before each use. On the farms A to I, M, and N, each broiler stable had an adjacent winter garden. This is a paved, fenced, roofed, and sealed up outdoor area. The broiler house of the farms K, L, and P was connected to an additional outdoor enclosure measuring 1 m^2 per chick (extensive outdoor flocks). From the age of 21 days, chickens were allowed to use the outdoor areas during daytime. Houses were equipped with automatic feeding and drinking systems. Drinking water was not treated with disinfectives. Wood shavings, straw chaff, and straw pellets were used as litter. Flock sizes ranged from 3000 to 14,800 birds. Rearing periods until slaughter lasted 35 or 58 days (extensive outdoor flocks). After each rearing period, litter was removed, and premises and equipments were cleaned by high-pressure and sanitized with Aldekol Des® 03 (EWABO, Wietmarschen, Germany). Houses then remained empty for about two to four weeks.

2.2. Sample collection

Sampling comprised three to six rearing periods and each flock was sampled weekly from the third or fifth (extensive outdoor flocks) week of age until slaughter (Table 1). In the broiler stable, each sampling included three fresh fecal samples, three dust samples, and water from the nipples. In the anteroom, the floor on both sides of the hygiene barrier, the boots used for entering the flock, dust, drinking water, and feed were sampled. Fecal samples were also collected from cattle (farms A to O), pigs (farms A, C, D, I, and P), sheep (farms B, C, and G), horses (farms B, K, and P), rabbits (farm B), laying hens (farm L), bantams (farm G), and sporadically from dogs (farms B, C, G, I, and O). Moreover, mice (feces), arthropods, and the supply air to the broiler houses were examined. In the idle time, litter and surfaces in

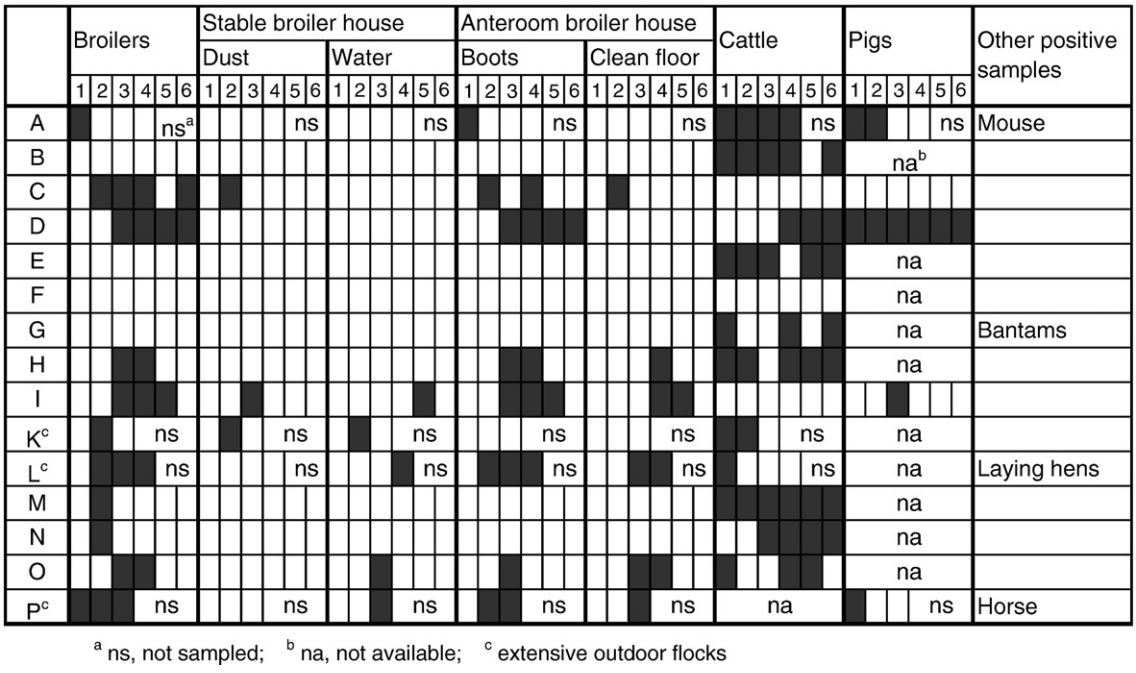

Fig. 1. Isolation of *Campylobacter* (positive samples dark colored, n=313) during the examined rearing periods (1–6) on 15 different farms.