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

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Epidemiological examinations of *Campylobacter* transmission routes in broiler flocks on selected poultry farms in Switzerland

Abbreviated running title: *Campylobacter* sources in broiler flocks

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Abstract

On 15 poultry farms, broiler flocks, other farm animals, and the environment were examined for *Campylobacter*. Flocks were examined weekly for six or three rearing periods. Of the 5’154 collected samples, 311 (6%) from 14 farms were *Campylobacter* positive. Positive samples originated from broiler flocks, the broiler houses, cattle, pigs, bantams, a horse, a laying hen flock, and a mouse. Amongst them, 288 tested positive for *C. jejuni* and 92 for *C. coli*. The analysis of 917 isolates by flagellin gene typing and pulsed-field gel electrophoresis, and of 15 assorted strains by amplified fragment length polymorphism allowed the following conclusions: (i) on eight farms (A, D, H, K, L, M, O, P) identical genotypes were isolated from broilers and other farm animals emphasizing their importance as reservoirs and risk factors for flock colonization and retrieving the role of personnel moving between areas as potential vectors; (ii) on four farms (C, D, I, L), indications of persistent contamination of the broiler house were evident and thereby the importance of efficient cleaning and disinfection was underlined; (iii) the previously described sources for broiler flock colonization could be excluded for certain genotypes from eight farms suggesting the existence of more potential vectors or niches; (iv) especially on farms with extensive outdoor flocks, multiple genotypes were found within a rearing period; and (v) some genotypes were identical across farms. The significance of such strains remains to be elucidated.

Keywords: Campylobacter, broiler, environment, transmission, genotyping
1. Introduction

*Campylobacter*, mainly *C. jejuni* and *C. coli*, are recognized worldwide as major cause of acute bacterial food-borne gastroenteritis (World Health Organization: [http://www.who.int/mediacentre/factsheets](http://www.who.int/mediacentre/factsheets)). In the European Union (EU), a total of 195'419 confirmed human cases have been reported in the year 2005 (EFSA, 2006). The incidence of human campylobacteriosis in the EU (51.6/100’000 inhabitants) 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, O’Brien & Madsen, 2007).

*Campylobacter* colonize the intestinal tracts of a large number of mammals and birds. Broiler chickens are often carriers of *C. jejuni*. Chicken guts, particularly ceca, can be colonized at very high levels and usually the entire flock is colonized once an infection becomes established (Newell & Fearnley, 2003). In the year 2005, 0.2% to 86.3% of the poultry flocks in the EU got colonized, and 23.0% of the Swiss poultry flocks tested positive (EFSA, 2006). This may lead to contamination of carcasses during the slaughter process (Ono & Yamato, 1999; Jørgensen et al., 2002; Stern & Robach, 2003; Allen et al., 2007). Consumption and handling of poultry products has been identified as important risk factor for human disease (Friedman et al., 2004; Siemer, Nielsen & On, 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, Nielson & On, 2001; Cox, Stern, Hiett, Berrang, 2002; Callicott et al., 2006). Horizontal transmission is generally believed to be the common way for flock colonization (Sahin, Morishita & Zhang, 2002; Newell & Fearnley, 2003; Bull et al., 2006). To reveal transmission routes to the broilers, more knowledge on the diversity and stability of Campylobacter in the environment and the distribution of different clones is necessary (Johnsen, Kruse & Hofshagen, 2006).

In a previous study, the dynamics of Campylobacter spread within broiler flocks were investigated (Ring, Zychowska & Stephan, 2005). The aim of the present study was to investigate the prevalence and genetic diversity of Campylobacter in broilers and the environment during several rearing periods. To establish genetic relationships and to reveal potential transmission routes, strains were characterized by flaA restriction fragment length polymorphism (RFLP), pulsed-field gel electrophoresis (PFGE), and amplified fragment length polymorphism (AFLP) analysis.

2. Materials and methods

2.1. Farms

Between March and December 2006, chicken flocks and the environment of 15 poultry farms (Table 1) were examined for Campylobacter. All farms were part of an integrated system (parent animal farms, hatcheries, broiler units, defined feedstuff, slaughterhouse). As usual in Switzerland, each farm maintained other branches of animal farming and only contained one broiler house with one flock at a time. A hygiene barrier was located in the anteroom and required change of clothes and boots, putting on a headdress, and washing and sanitizing hands. On the farms A to I, M, and N, each broiler stable had a winter garden built
adjacent to it. This is a paved, fenced, roofed, and sealed up outdoor area. The broiler houses of the farms K, L, and P were connected to an additional outdoor enclosure measuring 1 m² per chick (extensive outdoor flocks). At the age of 21 days, chickens were allowed to use the outdoor areas during daytime. All houses were equipped with automatic feeding and drinking systems. As litter, wood shavings, straw chaff, and straw pellets were used. According to the broiler houses and rearing types, flock sizes varied between 3’000 and 14’800 birds. Rearing periods lasted between 35 and 58 days.

2.2. Sample collection

Sampling comprised three to six rearing periods (Table 1) and each flock was sampled weekly from the third or fifth week of age (extensive outdoor flocks) until slaughter. In the broiler stable, each sampling included three fresh fecal samples (cecal droppings), 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. From other farm animals, fecal samples were collected regularly from cattle (farms A to O), pigs (farms A, C, D, I, P), sheep (farms B, C, G), horses (farms B, K, P), rabbits (farm B), laying hens (farm L), bantams (farm G), and sporadically from dogs (farms B, C, G, I, O). Moreover, mice (feces), earthworms, arthropods, and the supply air to the broiler houses were examined. In the idle time, litter and surfaces in the stable were sampled. Feces, dust, and surface samples were collected with swabs (Transwab, MW 170, Medical Wire & Equipment, Wiltshire, UK). Flies were caught by sticky strips placed in the anteroom, winter garden, broiler house, or cattle stable. Other arthropods and earthworms were examined if found in the outdoor areas. Air samples were obtained by the air sampler MAS 100 (MBV AG, Microbiology & Bioanalytic, Stäfa, CH).
2.3. Isolation of Campylobacter

Swabs were put in 10 ml Campylobacter Selective Broth (CSB, Difco 0495-17-3, Becton Dickinson, Sparks, USA) with Campylobacter Enrichment Supplement (Oxoid SR84, Oxoid Ltd., Hampshire, UK) and Skirrow Campylobacter Selective Supplement (Oxoid SR69, Oxoid Ltd.). The 40 ml of water samples were mixed with 40 ml CSB with supplements. Feed, litter, earthworms, and arthropods were mixed up in a weight ratio 1:10 with CSB with supplements and 10 ml of this suspension were processed. All enrichments were incubated at 42°C during 48 h under microaerophilic conditions (CampyPack Plus, Becton Dickinson). Subsequently, samples were plated on Campylobacter Selective Agar (CSA, Difco 0964-17-5, Becton Dickinson) with lyzated horse blood (Oxoid SR48, Oxoid Ltd.) and Butzler Campylobacter Selective Supplement (Oxoid SR85, Oxoid Ltd.) and incubated as described. In the air sampler, CSA with supplements and sheep blood agar (Difco Laboratories, Becton Dickinson; 5% sheep blood, Oxoid Ltd.) were used. The later were sampled with moistened cotton swabs, which were enriched as described. From every sample with presumptive colonies, three colonies were subcultured on CSA with supplements. These isolates were verified by PCR as \textit{C. jejuni (hipO)} or \textit{C. coli} (gene encoding aspartokinase) (On & Jordan, 2003).

2.4. Genotyping

\textit{Fla-RFLP} was performed using primers A1 and A2 (Nachamkin, Ung & Patton, 1996). The amplicon, a 1.7 kb \textit{flaA} fragment, was then digested with \textit{DdeI}. Macrorestriction profiling using \textit{SmaI} and subsequent PFGE were performed as outlined by Ribot, Fitzgerald, Kubota, Swaminathan and Barrett (2001). Fifteen assorted isolates were further analyzed by AFLP (Duim et al., 2000). \textit{HindI} and \textit{HhaI} were applied for DNA digestion and staining was performed using a DNA Silver Staining Kit (Amersham Biosciences, Freiburg, D). Visually
identical patterns on the same gel were grouped. One isolate of each group was used to compare with groups from other gels. Thereby, isolates were divided into final groups. Moreover, patterns analyzed by BioNumerics 3.0 (Applied Maths, Kortrijk, B) and the unweighted paired group method (arithmetic means) was used for dendrogram construction.

3. Results

3.1. Prevalence and distribution of Campylobacter

Of the 5'154 samples, 311 originating from 14 farms were Campylobacter positive. The proportion of positive samples ranged from 0.0% to 23.4% (Table 1). Positive broiler flocks were detected on 11 farms, including all farms with extensive outdoor flocks (Figure 1). Beside the broiler flock, Campylobacter were detected on nine farms in the broiler house (dust from the flocks, water from the nipples, clean floor and boots in the anteroom). In the environment, positive samples were detected in fecal samples from bantams, cattle, laying hens, pigs, a horse, and a mouse. Amongst the positive samples, 228 tested positive for C. jejuni and 92 for C. coli. Highest proportions of C. jejuni or C. coli positive samples were seen in those compartments in which fecal samples had been collected (Figure 2).

3.2. Fla-RFLP and PFGE

Of the 311 positive samples, 917 isolates were genotyped. Where the same genotype was observed among the subcultures, only one isolate was used for cluster-analysis. Fla-RFLP was successfully performed on 804 isolates, generated 46 different profiles, and 18, 17, and 11 profiles belonged to C. jejuni, C. coli, and both species, respectively. PFGE was successfully performed on 807 isolates, generated 47 different profiles, and 25, 14, and 8 profiles belonged to C. jejuni, C. coli, and both species, respectively.

3.2.1. Matching genotypes from broilers and other farm animals
Fla-RFLP and PFGE profiles initially present in the cattle (C. jejuni) appeared in the broilers and on the boots on farm H (Table 2), in the broilers, dust from the flock, and water from the nipples on farm K, and in the broilers on farm M. Similarly, bovine PFGE profiles (C. jejuni) from farm L were detected in the broilers during two rearing periods. Furthermore, profiles initially present in the pigs (C. coli) appeared in the broilers on farm D (Table 3), and in the broilers and on the boots on farm P. Another fla profile (C. coli) from farm P was detected in pigs during the first and on the boots during the second rearing period. Finally, certain profiles persisting in the laying hens (farm L) were consecutively found in the broilers and the anteroom. Interestingly, the genotype from a horse (farm P) was simultaneously present in the broilers and the anteroom.

On the other hand, fla-RFLP and PFGE profiles (C. jejuni) from broilers and boots on farms A and D, and broilers, water and the anteroom on farm O were subsequently detected in the cattle. Similarly, a certain fla-RFLP profile (C. jejuni) from the broilers and the anteroom on farm L was also detected in the laying hens.

3.2.2. Matching genotypes from consecutive broiler flocks

Identical genotypes were observed in the broilers (i) on farm D (Table 3), (ii) between the second and third rearing period on farms C (fla-RFLP; C. coli) and L (fla-RFLP; C. jejuni), and (iii) between the third and fourth rearing period on farm I (PFGE; C. jejuni).

3.2.3. Genotypes from broilers and the broiler house

On farms C, D, H, I, L, N, O, and P, broiler flocks harbored certain isolates with genotypes not present in samples from the environment. Even though genotypes from the broiler house were generally also found in the flocks, additional profiles were obtained during certain rearing periods (i) from the boots on farms H and P, (ii) from the clean floor in the anteroom on farms I and P, and (iii) from the water on farm I. Interestingly, the mentioned boot profiles from farms H and P were also detected in cattle and pigs, respectively.
3.2.4. Different genotypes within one positive sample

Twenty-three (7.4%) of the *Campylobacter* positive samples showed multiple profiles during a certain rearing period and 13 of them originated from farms P and L. Multiple genotypes were found in broilers from farms D, L, N, and P, in pigs from farms A and D, in cattle from farm M, in laying hens from farm L, in a mouse from farm A, in the water from farm I, and on the clean floor and the boots from farm P.

3.2.5. Matching genotypes across farms

Fla-typing revealed eight profiles and PFGE nine profiles that proofed to be identical and widely distributed across different farms and compartments.

3.3. AFLP

AFLP analysis of 15 isolates generated patterns from 23 to 25 bands and divided *C. jejuni* and *C. coli* into two clusters with an average similarity of 27% (Figure 3). Groups of isolates sharing more than 90% similarity were considered as genetically related and grouped to form a phenon (Olive & Bean, 1999). Among *C. jejuni*, four phena were identified and similarity was between 95.2% and 99.2% within these phena.

4. Discussion

During the investigated rearing periods, *Campylobacter* were detected in 6% of the samples, especially in fecal samples. The majority of isolates were identified as *C. jejuni*. Following fecal shedding, *Campylobacter* could be found in the dust from the flocks and the water from the nipples became contaminated. The role of water and the water system as source of chicken colonization remains controversial (Pearson et al., 1993; Herman, Heyndrickx, Grijspeerdt, Vandekerchove, Rollier & De Zutter, 2003; Zimmer, Barnhart, Idris & Lee, 2003). Boots became contaminated the same way and led to the contamination on the
clean site of the hygiene barrier. Comparable to other studies, the organisms were not detected
in feed or in fresh litter (Jacobs-Reitsma, van de Giessen, Bolder & Mulder, 1995; Ring et al.,
2005; Bull et al., 2006). Although the role of airborne transmission in the spread of
*Campylobacter* was recently raised (Bull et al., 2006), all samples from the supply air to the
broiler house tested negative. As indicated by several studies, insects and other wild animals
might play an important role as vectors for (Jacobs-Reitsma et al., 1995; Berndtson,
Emanuelson, Engvall & Danielsson-Tham, 1996; Newell & Fearnley, 2003; Hald et al., 2004;
Waldenström, On, Ottvall, Hasselquist & Olsen, 2007). In the present study, *Campylobacter*
were not detected in any of the examined arthropods, especially flies. Low detection rates in
flies were also reported in two recent studies (Johnsen et al., 2006; Hansson, Vågsholm,
Svensson & Olsson Engvall, 2007). However, due to the desiccation sensitivity of
*Campylobacter*, collecting of flies by sticky strips must be reconsidered.

Several methods have been compared for *Campylobacter* subtyping but the genetic
instability makes it difficult to establish universally applicable methods. Applying *fla*-RFLP
and PFGE, which both were successfully used for studies in the broiler production (Ring et
al., 2005; Bull et al., 2006; Hansson et al., 2007), resulted in a comparable discriminatory
power but the distribution of the RFLP and PFGE profiles was slightly different. The
untypability of about 12% of the isolates might result in PFGE from the production of DNAse
and in RFLP from a *fla* type that could not be recognized by the primers used (Thomas, Long,
Good, Panaccio & Widders, 1997). AFLP on the other hand is almost insensitive to genome
rearrangements and ensures reliable results over a long time. Consequently, subtyping
methods should be selected according to the epidemiological problem addressed.

Identification of the sources of flock colonization would enable measures to be targeted
towards the areas posing the greatest risk. On eight of the 15 farms, identical genotypes were
isolated from broiler flocks and other farm animals. Thereby, certain genotypes present in
cattle on farms H, K, L and M, in pigs on farms D and P, and in laying hens on farm L were subsequently found in the broiler flocks. These results emphasized the importance of farm animals as reservoirs and risk factors for broiler flock colonization. Otherwise, genotypes from the flocks appeared in cattle on three farms and in the laying hens. Because the results illustrate only punctual time events, the direction of spread could not be verified in either case. Despite the strict hygiene prescriptions of the poultry integrations, personnel working in the broiler houses represent the probable vector for *Campylobacter* transmission from the environment to the chickens or vice versa (Gibbens, Pascoe, Evans, Davies, & Sayers, 2001; Hiett, Stern, Fedorka-Cray, Cox, Musgrove & Ladely, 2002; Sahin et al., 2002; Newell & Fearnley, 2003).

Indications of persistent contamination of the broiler houses were observed on four farms (C, D, I, L) where identical genotypes were detected in consecutive broiler flocks, but not concurrently in other samples. Probably due to inadequate cleaning and disinfection of the stables between the flocks, *Campylobacter* have survived in certain niches. Persistent contamination of the broiler house is described as risk factor that needs to be further investigated (Petersen & Wedderkopp, 2001; Shreeve, Toszeghy, Ridley & Newell, 2002).

None of the previously described sources for broiler flock colonization could be confirmed for certain genotypes from eight flocks. This suggests the existence of other potential vectors or niches for *Campylobacter* such as wild birds, bugs, flies or even mice. On the other hand, the restriction of positive samples to the environment outside the broiler flocks on farms B, E, and G may indicate adequate hygiene prerequisites and compliance with them. Especially a more efficient and strict hygiene barrier might have averted colonization of the flocks. The importance of the hygiene barrier as critical factor to either prevent or delay flock colonization was underlined by several studies (Van de Giessen, Tilburg, Ritmeester & van der Plas, 1998; Gibbens et al., 2001; Herman et al., 2003; Hansson et al., 2007).
In flocks from stables with adjacent winter garden, merely only one genotype was detected during a certain rearing period. This might be explained (i) by a single contact during a limited time span and the spread of a strain over the flock, which then maintained its dominating position until slaughter, or (ii) by a constant colonization pressure of a certain genotype from the environment. Multiple genotypes within flocks were especially detected in extensive outdoor flocks, probably due to frequent ingress from the environment. Overall, inconsistent data exist on the heterogeneity of the *Campylobacter* within colonized broiler flocks. Similar to our results from the first rearing type, some studies revealed a low amount of genetic diversity (Berndtson et al., 1996; Shreeve, Toszeghy, Pattison & Newell, 2000; Ring et al., 2005), whereas others detected multiple genotypes within single flocks (Petersen et al., 2001; Hiett et al., 2002; Höök, Fattah, Ericsson, Vågsholm & Danielsson-Tham, 2005; Wittwer et al., 2005).

By *fla*-RFLP, PFGE, and confirmed by AFLP, some *Campylobacter* genotypes proofed to be identical across different farms. AFLP analysis showed in different studies that there exist stable strains, despite the known genome instability (Wassenaar & Newell, 2000; Manning, Duim, Wassenaar, Wagenaar, Ridley & Newell, 2001). In Switzerland, such strains are geographically widespread and show no host specificity (Wittwer et al., 2005). Currently it is not known, how these strains survive in nature or their distribution takes places. The significance of such strains remains to be elucidated.

**Acknowledgments**

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References


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Figure Captions

417  Figure 1
418  Isolation of *Campylobacter* (positive samples dark colored, n=313) during the examined
419  rearing periods (1-6) on 15 different farms
420
423  Figure 2
424  Proportional distribution of *C. jejuni* (■, n=228) and *C. coli* (▲, n=92) positive samples on the
425  farms
426
427  Figure 3
428  AFLP dendrogram of 15 selected *Campylobacter* isolates from different farms with a cut-off
429  set at 90% similarity. Isolates No. 1-8, 10-12, 14, *C. jejuni*; No. 9, 13, 15, *C. coli*. Isolates No.
430  1, 6, 10, 15 originating from cattle; No. 2, 4, 5, 8, 9, 13, 14 originating from broilers; No. 3,
431  11 originating from boots, No. 7, 12 originating from water inside the flocks
Table 1

No. (%) of *Campylobacter* positive samples during the sampled rearing periods on the different farms (n=5’154)

<table>
<thead>
<tr>
<th>Far m</th>
<th>No. (%) of <em>Campylobacter</em> positive samples during the rearing periods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st rearing</td>
</tr>
<tr>
<td>A</td>
<td>12 (14.1%)</td>
</tr>
<tr>
<td>B</td>
<td>4c (6.3%)</td>
</tr>
<tr>
<td>C</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>D</td>
<td>4c (4.0%)</td>
</tr>
<tr>
<td>E</td>
<td>2c (3.0%)</td>
</tr>
<tr>
<td>F</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>G</td>
<td>3c (4.5%)</td>
</tr>
<tr>
<td>H</td>
<td>3c (3.4%)</td>
</tr>
<tr>
<td>I</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>K</td>
<td>2c (3.4%)</td>
</tr>
<tr>
<td>L</td>
<td>5c (8.1%)</td>
</tr>
<tr>
<td>M</td>
<td>7c (13.7%)</td>
</tr>
<tr>
<td>N</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>O</td>
<td>2c (4.4%)</td>
</tr>
<tr>
<td>P</td>
<td>10 (9.9%)</td>
</tr>
</tbody>
</table>

*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
Table 2
Genotypes (No. of isolates) of *Campylobacter* positive samples from different compartments on farm H

<table>
<thead>
<tr>
<th>Rearing period</th>
<th>Broilers</th>
<th>Boots</th>
<th>Clean floor</th>
<th>Cattle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RFLP&lt;sup&gt;a&lt;/sup&gt;</td>
<td>PFGE&lt;sup&gt;b&lt;/sup&gt;</td>
<td>RFLP</td>
<td>PFGE</td>
</tr>
<tr>
<td>1</td>
<td>H-IV (9)</td>
<td>H-a (7)</td>
<td>H-IV (3)</td>
<td>H-a (3)</td>
</tr>
</tbody>
</table>

<sup>a</sup> fla-RFLP profiles: H-I to H-V;  <sup>b</sup> PFGE profiles: H-a to H-e;  <sup>c</sup> *C. coli*;
Table 3

Genotypes (No. of isolates) of *Campylobacter* positive samples from different compartments on farm D

<table>
<thead>
<tr>
<th>Rearing period</th>
<th>Broilers</th>
<th>Boots</th>
<th>Cattle</th>
<th>Pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RFLPa</td>
<td>PFGEb</td>
<td>RFLP</td>
<td>PFGE</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>D-II (5)c, D-a (5)c, D-III (2)c, D-b (4)c, D-IV (2)c</td>
<td>D-II (3)c</td>
<td>D-b (3)c</td>
<td>D-I (1)c, D-II (1)c, D-IV (1)c</td>
</tr>
<tr>
<td>4</td>
<td>D-VI (12)</td>
<td>D-c (8)</td>
<td>D-V (9)</td>
<td>D-d (4)</td>
</tr>
<tr>
<td>5</td>
<td>D-VI (9)</td>
<td>D-c (9)</td>
<td>D-VI (2)</td>
<td>D-c (2)</td>
</tr>
<tr>
<td>6</td>
<td>D-III (6)c</td>
<td>D-b (6)c</td>
<td>D-III (3)c</td>
<td>D-b (3)c</td>
</tr>
</tbody>
</table>

-superscript a* fla*-RFLP profiles: D-I to D-VII; bPFGE profiles: D-a to D-e; c*C. coli*; d*C. jejuni* and *C.
Figure 1, Zweifel et al.

<table>
<thead>
<tr>
<th>Farm</th>
<th>Broilers</th>
<th>Stable broiler house</th>
<th>Anteroom broiler house</th>
<th>Cattle</th>
<th>Pigs</th>
<th>Other positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dust</td>
<td>Water</td>
<td>Boots</td>
<td>Clean floor</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 2 3 4 5 6</td>
<td>1 2 3 4 5 6</td>
<td>1 2 3 4 5 6</td>
<td>1 2 3 4 5 6</td>
<td>1 2 3 4 5 6</td>
<td>1 2 3 4 5 6</td>
</tr>
<tr>
<td>A</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
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<sup>a</sup> ns, not sampled; <sup>b</sup> na, not available; <sup>c</sup> extensive outdoor flocks
Figure 2, Zweifel et al.
Figure 3, Zweifel et al.