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A one-year study of campylobacter carriage by individual Danish broiler chickens as the basis for selection of *Campylobacter* spp. strains for a chicken infection model

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SUMMARY

From February 1999 to February 2000, 1250 individual broiler chickens representing 125 broiler flocks originating from 62 broiler farms in Denmark were screened for campylobacter carriage. Every month, 10 flocks were tested for campylobacter carriage. The swabs were tested individually and as a pooled sample representing the flocks. *Campylobacter* spp. carriage was detected from 512 (40·9%) broiler chickens originating from 63 (50·4%) positive flocks. Campylobacter carriage by both individual chickens and flocks showed seasonal variation. *Campylobacter jejuni* was the dominant species (95·5%). Campylobacter isolates were typed using Penner heat-stable serotyping and *flaA*-typing methods. Data of campylobacter carriage by individual chickens and data generated by the use of different typing methods contributed to a better understanding of the dynamics of campylobacter infection within the broiler flocks. *C. jejuni* Penner heat-stable serotype HS2, *flaA*-type 1 was the most common type found in Danish broiler chickens.

INTRODUCTION

Campylobacteriosis is one of the most common bacterial intestinal disorders of humans in many industrial countries.

In Denmark, the number of campylobacterosis cases in humans increased by 5% from 4164 cases in 1999 to 4386 in 2000 and campylobacteriosis recently became more prevalent than salmonellosis with 82 cases per 100 000 inhabitants. Since 1992, registered cases of human campylobacterosis in Denmark have increased fourfold, and the same trend has been observed in many industrialized countries [1].

A number of sources such as pork, pork products, raw ground beef, unpasteurized milk, surface water as well as contact with dairy cattle and puppies may contribute to campylobacter infection in man [2–9]. However, undercooked poultry or poultry products are considered to be a major source playing an important role in human campylobacteriosis [10–14].

Campylobacter carriage in broiler flocks has been reported from many countries. A low prevalence of campylobacter carriage in broiler flocks has been reported from Scandinavian countries with 10% positive flocks from a survey in Sweden [15] or 18% positive flocks from a study in Norway [16]. Surveys from other European countries show a much higher prevalence. In the Netherlands 82% positive flocks were found in one study [17], and 57% in another [18], whereas 76% were reported in one survey in the United Kingdom [19] and 36% in another [20]. In Denmark, a national surveillance programme for *Campylobacter* spp. in broilers, hens and ducks has been in operation since the beginning of 1998. At slaughter, ten birds per flock are examined through the collection of cloacal swabs. The swabs

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are examined in pools of ten. The prevalence of campylobacter infected broiler flocks in Denmark has recently been reported as 37·7% in the year 2000 [1], the dominant species (86% of isolates) being identified as *C. jejuni* [21].

In most of these studies, the samples were tested in pools. Testing of pooled samples has been shown to be a useful tool for large-scale epidemiological studies [17, 22, 23] as well as for mass screening programmes [21]. However, testing pooled samples usually implies that only one or a few colonies from the positive samples will be picked and identified. This contributes to an overall picture of campylobacter infections in poultry, but lacks detailed epidemiological data of campylobacter infection in relation to individual chickens within the flocks as well as of the flocks on a farm. The inherent danger in this type of investigations may lie in the fact that culture of composite samples may select the strains that are most fit for laboratory culture conditions, but maybe not the strains that are the most abundant colonizers of the gut of broiler chickens.

Little is known about the relative distribution of different types carried by individual broiler chickens within a flock on the basis of e.g. serotyping, or on the basis of molecular typing methods such as flagellin gene typing (*Fla*-typing), macrorestriction profiling pulsed-field gel electrophoresis (MRP–PFGE), amplified fragment length polymorphism (AFLP) and others.

Furthermore, the avian intestinal tract is considered the natural environment of *Campylobacter* spp. The chicken therefore represents an appropriate model to obtain more knowledge of the factors involved in the colonization and survival of campylobacter in the intestinal environment. Several studies have shown that a very small dose of campylobacter is sufficient to colonize day-old chickens [24–27]. A great variation in the ability of different campylobacter strains to persist in the intestinal tract of chickens has been observed [24, 28]. When placing infected chickens in the same enclosure to facilitate interchange of strains it was observed that one strain was dominant and able to displace other strains [28]. Stern et al. 1988 [24] showed that by passages several times through the chicken gastrointestinal tract, a non-colonizing *C. jejuni* was turned into a strong colonizer. However, in almost all of these studies, the campylobacter strains used were human clinical isolates [24, 29], type strains, or mutants derived from certain strains [25, 30, 31].

Attempting to set up a chicken infection model that can be applied to study the colonization, the infection of campylobacter, and to develop a challenge model for tests of new vaccine developments. In the present paper, we have first studied the prevalence of campylobacter carriage by individual chickens within broiler flocks during a period of 1 year. Data of campylobacter carriage by individual chickens in the flocks will contribute to a better understanding of campylobacter infection dynamics within individual chickens in broiler flocks, the seasonal variation of campylobacter infection, the distribution of different species etc. The diversity of campylobacter isolates carried by individual chickens within the flocks was studied in more detail using different typing methods. On the basis of the typing data, the most common type of *Campylobacter* spp. in Danish broiler chickens was determined. This is a starting point for the selection of strains for a future chicken infection model, based on the most common and therefore the most strongly colonizing strain(s) present in Danish poultry.

MATERIALS AND METHODS

Poultry samples

A total of 1250 individual chickens representing 125 broiler flocks originating from 62 broiler farms were screened for campylobacter carriage during February 1999 through February 2000. The location of broiler farms was 6 farms in Bornholm, 2 in Sealand, 4 in Funen and 50 in Jutland (Fig. 1). Nine abattoirs were included in the study. Every 4 weeks, 10 cloacal swabs from each of 10 broiler flocks were randomly collected from the samples taken from broilers at the abattoir immediately prior slaughter. The swabs were transferred to the laboratory in screw-cap centrifuge tubes with 15 ml transport medium containing Brain Heart Infusion (BHI) broth (Difco) 37 g l^{-1}, sterile defibrinated calf blood 5% (v/v) and agar (Oxoid) 0·5%, pH 7·4. The samples included in the present study were a part of 6557 Danish broiler flocks examined in the Danish national surveillance for thermophilic campylobacter in animal production in 1999.

Microbiological culture of campylobacter

On arrival in the laboratory, the swabs were immediately subjected to laboratory processing. The swabs were transferred to 0·3 ml of sterile water and left at

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Fig. 1. Location of broiler farms included in this study.

room temperature for 10–20 min to release the bacteria from the swabs. The suspension of faeces and bacteria was used directly for culture of campylobacter. The 10 swabs were tested both individually and as one pooled sample.

Primary isolation of campylobacter was carried out on Charcoal Cefoperazone Deoxycholate Agar (CCDA (Oxoid CM 739) with cefoperazone selective supplement (SR 155E)). The medium was prepared according to the manufacturer's instructions.

Subcultures were grown on CCDA as previously described [32].

Isolation and identification of thermophilic *Campylobacter* spp.

Thermophilic *Campylobacter* spp. was isolated according to the recommendations given by the Nordic Committee on Food Analyses [33]. Briefly, a loop full (10 μl) of the faeces and bacteria suspension was spread on the surface of CCDA. The plates were incubated under microaerobic conditions (6% O_2, 6% CO_2, 4% H_2 and 84% N_2) at 42 °C for 48 h. Colonies suspect of campylobacter were examined for cell morphology using a phase-contrast microscope (Olympus B201) and purified further on CCDA and BA. The plates were incubated under the conditions mentioned above. A single colony was picked and streaked on two wet BA plates to prepare for identification of the isolate and for further analysis. All the isolates were characterized by their catalase reaction, ability to hydrolyse hippurate and indoxylacetate and by their susceptibility to nalidixic acid and cephalothin as described by [34–37].

DNA techniques

Bacterial chromosomal DNA was extracted from 24 h BA plate cultures using the QIAAmp kit (QIAGEN, Germany). The DNA was eluted in 200 μl of preheated (65 °C) sterile water. DNA concentrations were measured on a spectrophotometer (Ultrospec 2000, Pharmacia Biotech, Cambridge, UK) and stored at −20 °C. The DNA was used for flagellin (*flaA*) gene PCR (see below).

All PCR mixtures (50 μl) contained 2·5 mmol l^{-1} $MgCl_2$, 25 mmol l^{-1} of (each) dATP, dCTP, dGTP, and dTTP, 5 μl (20 pmol l^{-1}) of each primer (DNA Technology, Aarhus, Denmark), 1 × *Taq* DNA polymerase buffer and 0·5 μl (2·5 U) of *Taq* DNA polymerase (Boehringer–Mannheim).

PCR was performed in a Peltier Thermal Cycler PTC-200 (MJ, Research Inc., MA, USA) for 25 cycles. PCR products were analysed by electrophoresis (40 min at 80 V) on 1% NuSieve GTG agarose (FMC Bio-Products, Rockland, MD, USA) in 1 × TAE

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(Tris-acetate 40 mmol l^{-1}, EDTA 2 mmol l^{-1}) buffer. Each 100 ml gel containing 2·7 μl ethidium bromide solution (10 mg ml^{-1}, Bio–Rad, Hercules, CA, USA).

Typing methods

Serotyping

Serotyping was performed according to the Penner heat-stable antigen scheme as previously described [38, 39] with the use of the full set of 66 antisera of the system (47 *C. jejuni* and 19 *C. coli* antisera). The antisera used in this study were prepared in-house (Department of Microbiology, Danish Veterinary Institute, Copenhagen, Denmark).

Fla *typing*

PCR–RFLP profiles of the *fla*A gene were performed according to the method described by [40, 41]. Briefly, 8 μl aliquot of the PCR product was digested with 12 units of the restriction enzymes *Dde*I (Gibco-BRL, Rockville, MD, USA), and *Alu*I (Boehringer–Mannheim, Germany) by using incubation buffers recommended by the manufacturer in a 20 μl reaction volume. The digest was analysed by electrophoresis (2 h at 100 V in 1 × TAE buffer) on 2% NuSieve GTG Agarose (FMC Bio-Products, Rockland, MD, USA) 0·7% Seakem Agarose (FMC Bio-Products), each 100 ml of gel containing 2·7 μl of ethidium bromide solution (10 mg ml^{-1}, Bio-Rad, Hercules, CA, USA). Computer assisted identification using 'GelCompar' (Applied Maths, Kortrijk, Belgium) was used for identification of RFLP profiles in a database based on 600 *C. jejuni* isolates and profiles were assigned to previously defined profile types [42, 43].

RESULTS

The location of 62 broiler farms included in this study is presented in Figure 1.

Abattoirs

The distribution and percentage of campylobacter positive flocks among the nine abattoirs included in the study was 25–70·6% (Table 1). As an average over the 13 months' period, 50·4% of the flocks tested were positive for *Campylobacter* spp. (Table 1).

Farms

Of the 62 broiler farms included, 36 farms (58·1%) were positive with at least one sample positive for campylobacter during the study period. Of these 36 campylobacter positive farms, 12 (33·3%) contributed with samples from only 1 flock, while 24 farms (66·6%) contributed with samples from 2 or more than 2 flocks. Of these 24 farms, 10 farms (41·6%), including 4 farms with 2 samples, 2 with 3 samples and 4 with 4 samples, were positive for campylobacter on all sampling occasions.

Table 1. *Number of broiler flocks tested, and the number and percentage of campylobacter positive flocks distributed among nine Danish abattoirs from February 1999 to February 2000*

Abattoirs	No. of flocks tested	No. of positive flocks (%)
A	12	7 (58·3)
B	34	21 (61·8)
C	8	2 (25·0)
D	3	2 (66·7)
E	11	4 (36·4)
F	17	12 (70·6)
G	2	1 (50·0)
H	20	4 (20·0)
I	18	10 (55·6)
Total	125	63 (50·4)

Campylobacter carriage by flocks, and by individual broiler chickens

The distribution and percentage of *Campylobacter* spp. carriage by broiler flocks and by individual chickens within flocks are presented in Table 2. The mean monthly percentage of campylobacter positive flocks was 20–90% of flocks tested during the period of 13 months. At the individual broiler level 509 of 1250 individual broiler chickens tested were positive for *Campylobacter* spp. The method was designed to pick up one colony of campylobacter per chicken sample. However, if there appeared to be more than one type of colony morphology on the BA plates then all of the colony types were picked and tested further. This resulted in the additional testing of 1 isolate from each of 4 chickens; 3 of these chickens carried 2 *C. jejuni* isolates (distinct by different *flaA*-types) and 1 chicken carried 1 *C. jejuni* and 1 *C. coli*. Overall, a total of 512 (40·7%) *Campylobacter* spp. isolates were obtained (Table 2).

The number of individual broiler chickens carrying campylobacter within a flock varied from flock to flock. In the month of October 1999, for example, the

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Table 2. Campylobacter *spp. carriage by individual chickens, and at flock level*

Months and year	No. of flocks tested	No. of positive flocks (%)	No. of isolates from each positive flock (%)
1999			
Feb	5	3 (60·0)	4; 8; 12(*) (80·0)
Mar	10	2 (20·0)	9; 4 (65·0)
Apr	10	2 (20·0)	10; 10 (100·0)
May	10	3 (30·0)	10; 9; 8 (90·0)
Jun	15	7 (46·7)	10 × 5; 9; 2 (87·0)
Jul	10	6 (60·0)	10 × 2; 9 × 2; 8; 5 (85·0)
Aug	5	4 (80·0)	10 × 3; 9 (97·5)
Sep	10	8 (80·0)	10 × 3; 9; 8; 5; 3; 1 (70·0)
Oct	10	9 (90·0)	10 × 5; 9; 8; 5; 1 (81·0)
Nov	10	2 (20·0)	10 × 2 (100·0)
Dec	10	6 (60·0)	10 × 4; 5; 1 (76·6)
2000			
Jan	10	4 (40·0)	10 × 3; 3 (82·5)
Feb	10	7 (70·0)	10 × 3; 9; 3; 2; 1 (64·3)
Total	125	63 (50·4)	509 (80·8)

* Two chickens with two different isolates.

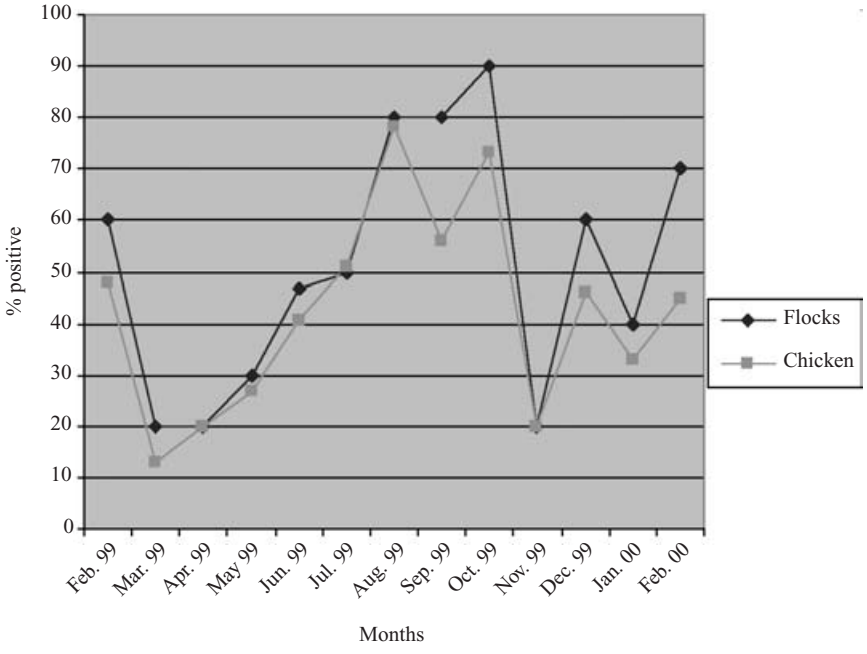

Fig. 2. Seasonal variation of campylobacter carriage by individual broiler chickens and by flocks.

numbers varied from only 1 chicken per flock in 1 of the flocks, to 5, 8, 9 or all 10 of the chickens tested for campylobacter in the others. The overall within-flock prevalence per month for campylobacter carriage in positive flocks was 80·8 (range 64·3–100·0) (Table 2).

The percentage of campylobacter positive at flocks as well as at individual chickens level was higher in the late summer and autumn months (July–October), highest (78–80%) in August and was lower in the winter months (December–March), lowest (13–20%) in March (Fig. 2).

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Table 3. *Distribution and percentage of* Campylobacter *species among positive samples and flocks*

Species	No. of isolates (%)	No. of flocks (%)
Negative	738 (59·3)	62 (49·6)
Positive	512 (40·7)	63 (50·4)
Campylobacter jejuni	489 (95·5)	60 (48·0)
Campylobacter coli	13 (2·5)	4 (6·3)
Campylobacter spp.	9 (1·8)	4 (6·3)
Dead	1 (0·2)	1 (1·5)

Identification of campylobacter isolates

Identification of campylobacter isolates at species level revealed that 489 isolates (95·5%) were *C. jejuni*, 13 (2·5%) were *C. coli*, 9 isolates (1·8%) were other *Campylobacter* species which were not further identified, and 1 isolate (0·2%) died before speciation could be performed (Table 3). The 489 *C. jejuni* isolates obtained originated from 60 broiler flocks (48·0%) whereas the 13 *C. coli* isolates obtained originated from 4 different flocks (Table 3).

Distribution of Penner serotypes in broiler flocks

Two hundred *C. jejuni* and *C. coli* isolates representative of 63 positive flocks were selected for serotyping. Twenty-one different Penner heat-stable serotypes were found. Thirty-two flocks (50·8%) carried only 1 serotype, 24 flocks (38·1%) carried 2 different serotypes, 5 flocks (7·9%) carried 3 different serotypes, 1 flock (1·6%) carried 5 different serotypes and 1 flock (1·6%) carried 7 different serotypes.

Of the 32 flocks carrying only 1 serotype, 9 flocks (28·1%) carried serotype HS2; 5 flocks (15·6%) carried serotype HS4-complex; 3 flocks carried serotype HS6,7 or serotype HS27; 2 flocks carried serotype HS31, HS12, HS42 or HS21 respectively.

The different serotypes distributed on flocks and individual broiler chickens are presented in Table 4. Two serotypes (HS2, HS5) each represented more than 10% of the isolates tested, while 6 other serotypes (HS31, HS4-complex, HS12, HS59, HS27, and HS6,7) each represented more than 5% of isolates. The 47 serotype HS2 isolates were obtained from 23 broiler flocks, originating from 19 broiler farms. Two of these farms with 2 samples collected at different times over a period of 4–6 months and 1 with 3 samples collected at different times over a period of 4–8 months.

Table 4. *Distribution of Penner heat-stable serotypes in representative individual broiler chickens* (n = *200*), *and in broiler flocks* (n = *63*)

No.	Serotype	No. of chicken carried (%)	No. of positive flocks carried (%)
1	HS 2	47 (23·5)	23 (36·5)
2	HS 5	20 (10·0)	5 (7·9)
3	HS 4*	16 (8·0)	7 (11·1)
4	HS 31	15 (7·5)	8 (12·7)
5	HS 12	13 (6·5)	5 (7·9)
6	HS 6,7	13 (6·5)	7 (11·1)
7	HS 59	12 (6·0)	5 (7·9)
8	HS 27	11 (5·5)	7 (11·1)
9	HS 21	10 (5·0)	5 (7·9)
10	HS 1,44	8 (4·0)	3 (4·7)
11	HS 42	6 (3·0)	4 (6·3)
12	HS 11	6 (3·0)	4 (6·3)
13	HS 37	6 (3·0)	3 (4·7)
14	HS 29	4 (2·0)	2 (3·1)
15	HS 23,36	3 (1·5)	2 (3·1)
16	HS 18	2 (1·0)	1 (1·6)
17	HS 19	2 (1·0)	1 (1·6)
18	HS 57	2 (1·0)	1 (1·6)
19	HS 60	2 (1·0)	1 (1·6)
20	HS 22	1 (0·5)	1 (1·6)
21	HS 37,53	1 (0·5)	1 (1·6)

* HS4-complex. Reaction with one or more of the following antisera: 4, 13, 16, 43, 50, 64, 65.

Comparing the ranking order of serotypes between flocks and individual chickens it is noted that in both cases, the serotype HS2 was recorded as the most common serotype. However, if based on the flock analysis serotype HS31 would appear to be second, while serotype HS5 would be second if based on the individual analysis.

Distribution of *fla*-types in broiler flocks

RFLP *flaA*-typing of the 489 *C. jejuni* isolates revealed 30 different *flaA*-types. At flock level, 35 flocks (55·5%) carried only 1 *flaA*-type, 16 flocks (25·4%) carried 2 different *flaA*-types, 6 (9·5%) carried 3 *flaA*-types, 1 (1·6%) carried 4 *flaA*-types, 1 (1·6%) carried 6 *flaA*-types and 1 (1·6%) flock carried 8 *flaA*-types. The distribution of the ten most commonly detected *flaA*-types of this study is presented in Table 5, while Figure 3 shows the *Dde*I RFLP *flaA*-typing profiles of these *flaA*-types. Of the 489 isolates, 136 (27·8%) isolates were identified as *flaA*-type 1, originating from 24 of the 63 positive flocks. Nine other *flaA*-types (11, 5, 8, 17, 30, 9, 2, 16 and 40) were identified from 2 to 10% of the isolates, while the remaining 20 *flaA*-types were found only in low percentages

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Table 5. *Distribution of the ten most common* flaA *types found by RFLP analysis of 489* Campylobacter jejuni *isolates*

flaA type	No. of isolates (%)	No. of flocks (%)
flaA 1	136 (27·8)	24 (38·1)
flaA 11	52 (10·6)	9 (14·8)
flaA 5	31 (6·3)	4 (6·3)
flaA 8	28 (5·7)	5 (7·9)
flaA 17	25 (5·1)	3 (4·7)
flaA 30	22 (4·4)	4 (6·3)
flaA 9	18 (3·6)	4 (6·3)
flaA 2	17 (3·5)	2 (3·1)
flaA 16	10 (2·0)	1 (1·5)
flaA 40	10 (2·0)	3 (4·7)

between 0·1 and 1·9%. The ranking order of *flaA*-types was similar when comparing flock samples and individual samples, with the *flaA*-type 1 being the most common in both categories.

Combination of both serotyping and *flaA*-typing data of the 47 serotype 2 isolates revealed that 41 isolates (87·2%) were serotype HS2, *fla*-type 1, 4 isolates (8·5%) were serotype HS2, *fla*-type 3, and 2 isolates (4·3%) were serotype HS2, *fla*-type 16.

DISCUSSION

The results of the investigations of campylobacter carriage by individual broiler chickens and by broiler flocks from February 1999 through February 2000 are summarized in Table 2. As an average over the 13 months' period, 50·4% of the tested flocks were campylobacter positive. This finding correlates well with national surveillance results for campylobacter in Danish broiler flocks which were recorded as 47·1% positive flocks in 1998, 45·0% in 1999, and 37·7% in 2000 [1]. However, the frequency of infected flocks showed considerable variation depending on the abattoir, the season, and the farm from which the samples originated. Thus, the frequency of campylobacter positive broiler flocks from different abattoirs varied between 20·0% and 70·6% (Table 1). These data may reflect various levels of education and attention to the bio-security measures on the part of the broiler producers, or differences in abattoir attention to cleaning and disinfection of vehicles, transport crates and catching equipment, as previously reported [18, 22, 23, 44].

A marked seasonal fluctuation of campylobacter carriage was recorded for broiler flocks as well as for individual chickens within the flocks (Fig. 2). The

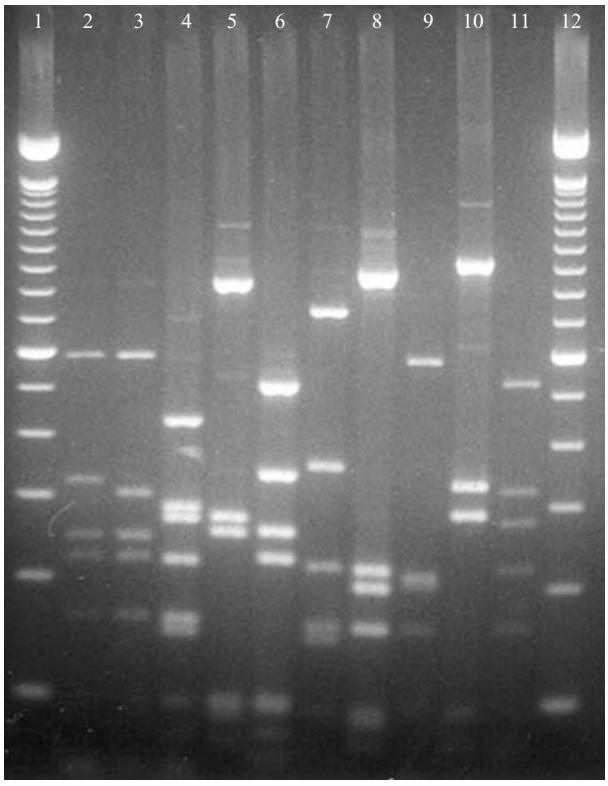

Fig. 3. Ten of the most common *fla*-RFLP/*Dde*I profiles of *Campylobacter jejuni* isolated from broiler chickens found in this study. On gel: lanes 1 and 12, 100 bp ladder molecular weight standard marker. Lane 2: *C. jejuni* DVI-SC8 *fla*-type11. Lane 3: *C. jejuni* DVI-SC245, *fla*-type 5. Lane 4: *C. jejuni* DVI-SC471, *fla*-type 8. Lane 5: *C. jejuni* DVI-SC631, *fla*-type 17. Lane 6: *C. jejuni* DVI-SC-601, *fla*-type 30. Lane 7: *C. jejuni* DVI-SC622, *fla*-type 9. Lane 8: *C. jejuni* DVI-*fla*-type 2. Lane 9: *C. jejuni* DVI-SC351, *fla*-type 16. Lane 10: *C. jejuni* DVI-SC539, *fla*-type 40 and lane 11: *C. jejuni* DVI-SC20, *fla*-type 1.

percentage of campylobacter positive flocks peaked during the summer and autumn (60–90% positive) while the lowest percentage (20%) of infected flocks was recorded during early spring (March–April). Such seasonal variation of campylobacter in broiler flocks, with a peak during the summer, has been reported [16, 17, 21, 45, 46]. In the present study smaller 'winter peaks' appear to be present in February 1999, and again in December 1999 and February 2000 (Fig. 2). Similar observations have been reported from Austria [48] and may at least in part be explained by the fact that the winters of 1999 and 2000 were unusually milk, with mean monthly temperatures being 3·5–4·0 °C above standard temperature normals [47]. A seasonal variation in the number of campylobacter isolates from individual chicken samples was also recorded (Fig. 2). The highest proportion of positive samples was 78% in August and the lowest was 13%

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in March. The within-flock prevalence of campylobacter positive flocks (Table 2) confirmed the observations of Jacobs-Reitsma et al. 1994 [17] who reported that once the infection was introduced into a broiler flock, a very high percentage of the birds would become colonized.

Species identification (Table 3) revealed that 95·5% of the campylobacter isolates obtained, were *C. jejuni* whereas only 2·5% were *C. coli*. The species distribution in this study is in line with the results from the Danish National surveillance for campylobacter (21) and the data from broiler flocks in the United Kingdom [45] reporting 94% *C. jejuni* and 6% *C. coli*. Other studies have reported higher proportions of *C. coli* from broiler flocks, e.g. 14% in Dutch broiler flocks [49] and 19% in broiler flocks from Israel [50]. These observations may reflect true regional differences. However, it should be stressed that routine species identification of *Campylobacter* spp. rests with a few biochemical reactions, and that the inclusion of additional test criteria may disclose that as many as 30% of provisionally identified *C. coli* isolates in fact belong to other species [51].

Data of serotyping presented in this study revealed that Penner heat-stable serotype HS2 was the most common serotype carried by Danish broiler chickens. This result was obtained regardless of whether the isolation protocol was based on 10 individual samples per flock, or whether the isolation was based on one pooled sample of 10 chickens. However, the ranking of the prevalence of different serotypes did not correspond completely. The five most common serotypes (in decreasing order) were found to be as follows: serotype HS2, HS5, HS31, HS4-complex, and HS12 if based on individual chicken isolates, while this ranking order would be serotype HS2, HS31, HS4-complex, HS27, HS6,7 if based on pooled flock samples.

The distribution of *C. jejuni* types at farm level indicated differences in campylobacter epidemiology and infection dynamics. From 50% of the positive flocks originating from 24 farms only one serotype could be isolated. Of these flocks, 9 (28·1%) flocks originating from 5 broiler farms carried serotype HS2 and 5 flocks (15·6%) originating from 3 farms carried serotype HS4-complex. These suggested that the farms may have sufficient biosecurity measures in place to avoid introductions of new types or the occupation of ecological niches and exclusion of subsequent strains but that sources on the farm may be responsible for persistent infections from one flock to the next. Such persistent infections with the same type have previously been reported [52]. However, from the other half of the positive farms 2, 3, and up to 7 or 8 different types could be identified, indicating that these farms had less efficient biosecurity measures with the resulting introduction of several new types over time.

Overall, the serotyping studies confirmed that the most common *C. jejuni* type in Danish broiler production in this study is Penner serotype HS2. Serotype HS2 is also the most commonly isolated serotype from human cases of campylobacteriosis in Denmark [1]. Interestingly the combination of serotyping and *fla*-typing data revealed that within the serotype HS2 group three combinations of subgroups: serotype HS2, *flaA*-type 1, serotype HS2, *flaA*-type 3 and serotype HS2, *flaA*-type 16 were identified. More than 80% of the serotype HS2 isolates were identified as the serotype HS2, *flaA*-type 1. The serotype HS2, *flaA*-type 1 was the most common type found in this study.

As the seasonal variation of the presence of campylobacter in Danish broiler flocks coincides closely with the seasonal distribution of human campylobacteriosis in Denmark [1], it is tempting to speculate that there is a direct link between campylobacter carriage by broiler chickens and human disease. However, it is still a question whether the majority of the serotype HS2 human isolates were also the *flaA* type 1 and furthermore, care should be taken before arriving at this conclusion. Firstly, co-variation of two variables may suggest a common as yet unknown external determinant factor, and secondly, the Penner serotype HS2 is also the most common type isolated from samples of cattle and turkey in Denmark [1]. This suggests that several reservoirs may contribute to human infections.

In conclusion, the present results obtained through a 13 months' longitudinal study of 125 Danish broiler flocks contributed a better understanding of campylobacter infection within individual chickens in broiler flocks, the distribution of different *Campylobacter* species, and of the diversity of campylobacter isolates carried by individual chickens within the flocks. The combination of Penner heat-stable serotyping and *flaA*-typing methods have established the most common campylobacter type in Danish broiler flocks as *Campylobacter jejuni*, Penner serotype HS2, *flaA*-type 1. This information was obtained regardless of whether ten individual samples, or one pooled sample, were studied from the flocks. The result

implies that isolates obtained through surveillance programmes based on pooled flock samples may be used for representative typing studies, although minor variations in the ranking order of types should be expected, in particular with regard to the more rare types. The combination of serotyping and *flaA*-typing data contributed a better foundation for selection of the campylobacter candidates for an experimental chicken infection model. Future works on the development of an experimental chicken infection model will therefore be based on one or more of the most common Penner serotype HS2, *flaA*-type 1 isolates found from this study. Once the model has been developed it will be a useful tool for comparisons of the colonization ability among different types. The developed model can be applied to study the colonization and pathogenesis of campylobacter infections, and for the testing of e.g. vaccine candidates in campylobacter immunization and challenge studies.

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