

CAMPYLOBACTER JEJUNI IN SLAUGHTERED CHICKENS FROM THE VIEWPOINT OF FOOD HYGIENE

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Abstract

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A total of 440 slaughtered chickens coming from 27 farms were examined for presence of *Campylobacter jejuni* between 1st Feb. 1990 and 31st Jan. 1991. The relevant specimens were taken from the outer and inner carcass surfaces, ileum contents, liver parenchyma and bile from groups of 10 birds each week. Isolation attempts yielded 366 *C. jejuni* strains. Of the 38 and 47 (10.3 % and 12.8 %) were isolated from the outer and inner carcass surfaces, respectively, 121 (35 %) from ileum contents, 92 (25 %) from liver parenchyma and 68 (18.6 %) from bile.

The proportions of contaminated chicken carcasses showed two peaks, reaching 62.5 % and 62 % in May and October, respectively. An almost parallel trend was recorded for the isolations from ileum contents and liver parenchyma where the highest number of strains (45 % and 30 % respectively) coincided with the May peak, whereas the highest proportions of isolations from bile (30 %) coincided rather with the second peak. The proportions of *C. jejuni* isolated from the outer and inner carcass surfaces were relatively low, averaging 9.4 % and 11.4 % respectively, and did not show much fluctuation. The proportions of *C. jejuni* isolated from bile averaged 13.8 %.

In 82 (22.4 %) *C. jejuni* strains solitary colonies were observed also after control aerobic incubation at 42 °C, but attempts at their further passage under these conditions yielded negative results.

C. jejuni carriers and shedders as well as the incidence of asymptomatic *C. jejuni* infection among chickens do not urge either the farmer or the veterinarian to take any measures, but the consumption of *C. jejuni*-infected chicken meat constitutes a major health hazard for man.

Campylobacter jejuni, slaughtered chickens, food hygiene

Campylobacter jejuni was first described as *Vibrio jejuni* (Jones et al. 1931) and is also known under the names of *Vibrio hepaticus* (Mathey and Rissberger 1964) or *Campylobacter fetus* subsp. *jejuni* (Smibert 1974). It is a causative agent of abortion in sheep, diarrhoea in calves and lambs, campylobacter hepatitis in chickens and febrile enteritis in man.

A point of particular epidemiological importance is the fact that *C. jejuni* can also be isolated from the intestinal tract of clinically healthy cattle, sheep, goats, pigs, rabbits, domestic fowls, turkeys, ducks, pigeons, dogs, cats, monkeys, sparrows, blackbirds and starlings (Smibert 1984). From the intestinal tract it can make its way to the liver and, upon evisceration, also to the outer and inner surfaces of farm animal carcasses. In the domestic fowl this possibility has been demonstrated:

- (1) on the outer surface after scalding and plucking (Baker et al. 1987) in 20 % of the carcasses;
- (2) on the inner surface after evisceration (Pěgřimková 1986, Marinescu et al. 1987; Míčková 1987) in 28.7 % to 92 % of the carcasses;
- (3) in the liver (Khalafalla 1990) in 40 % of the carcasses.

Examination of the bile has yielded negative results (Oosterom et al. 1983).

Further fate of these bacteria depend on the mode of carcass processing and on culinary processing of chicken meat.

Campylobacteriosis of this aetiology in man was demonstrated in Europe (Belgium, England, Holland, Sweden), Africa (Zaire, South Africa) and Australia in the seventies. In our country it has been described by Tesařová and Kubecová (1982), Kahlich et al. (1983) and Kováčová (1986), but in all these cases direct isolation of *C. jejuni* from the incriminated food is lacking. According to Oosterom (1985) the main cause of the disease is the consumption of chicken meat or chicken meat products.

Clinical signs of the disease are observed after a 3- to 5-day incubation period. They include pyrexia, headache, back-pain and muscle ache, soon followed by pain in the abdominal region and diarrhoea. The stools are thin to liquid and occasionally bile or blood-tinged. The diarrhoea generally subsides after 2 to 3 days and the disease as a whole lasts 1 to 2 weeks. Non-medicated patients shed the bacteria for as many as 5 weeks (Butzler and Skirrow 1979).

The present study was designed to obtain information on *C. jejuni* occurrence in flocks of domestic fowls and on *C. jejuni* contamination of chicken meat and giblets in our country.

Materials and Methods

A total of 440 chickens coming from 27 farms were examined for the presence of *C. jejuni* between 1st Feb. 1990 and 31 Jan. 1991. The tissue specimens were taken from the (1) outer surface of plucked chicken carcasses, (2) inner surface of eviscerated carcasses, (3) ileum contents, (4) liver parenchyma and (5) bile in a poultry slaughter-house from groups of 10 birds each week. Five farms were represented on 2 occasions, two farms on 3 occasions and one farm on 9 occasions. The origin of each group of 10 birds was recorded.

Swabs taken from wet outer and inner carcass surfaces, ileum contents and liver parenchyma as well as 2 to 3 drops of bile obtained with a syringe were each placed into test tubes containing circa 7 ml transport medium (thioglycolate medium IMUNA) completed with 1 % liquid supplement "C" enriched with antibiotics (cephalotin, vankomycin) and trimethoprim. After 24-h incubation at 42 °C inoculations were made into blood agar prepared from IMUNA base No. 4, 7 % defibrinated horse blood and 1 % supplement "C". Incubation was carried out under microaerophilic conditions at 42 °C for 48 h in a modified apparatus according to Hussels as cited by Hallman (1955).

From suspect colonies (generally showing a tendency to creeping growth) pure cultures were started on the blood agar as described above except that supplement "C" was replaced with its 1 % non-antibiotic version "A". The two supplements were obtained from the Department of Medical Microbiology, Medical Faculty Hospital, Motol, Prague 5.

The nutrient base used for testing for nitrate reduction, indole production, hydrogen sulphide production, glucose oxidation and growth in 1 % glycine contained 2.5 g tryptose peptone, 0.75 g beef extract, 1.25 g NaCl, 0.25 g Na HPO₄ and 250 ml distilled water. After addition of the test substrates, dispensing, sterilization and inoculation we carried out incubation under microaerophilic conditions at 42 °C.

The isolated strains were identified according to the latest edition of Bergey's Manual (Krieg and Holt 1984) and in the light of the published data on newly accepted campylobacters (Benjamin et al. 1983; Gebhart et al. 1985; McClung et al. 1983; Neil et al. 1985; Roop et al. 1985; Steele and Owen 1988; Tanner et al. 1981 and Totten et al. 1985). See Table 1.

Results

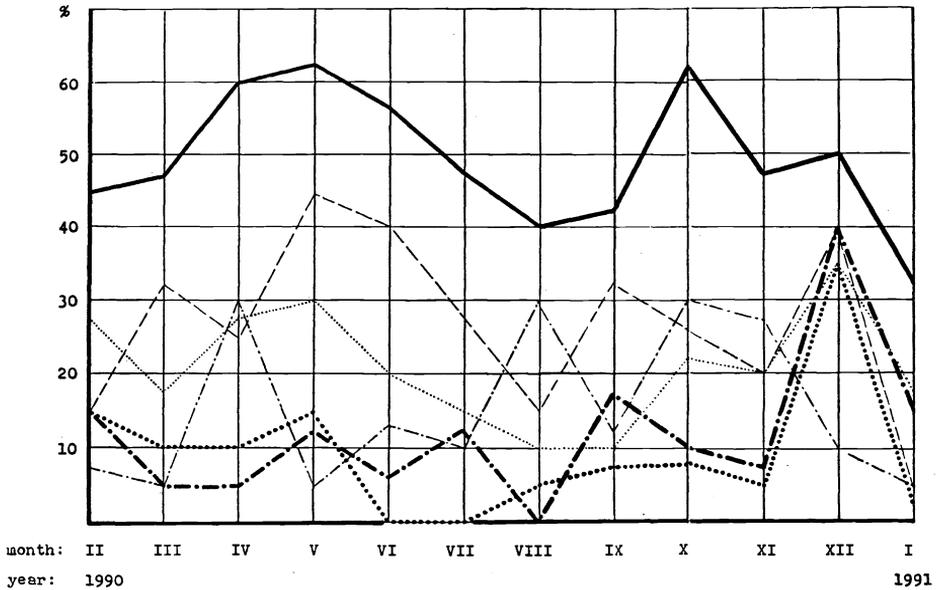
A total of 366 strains of *C. jejuni* were isolated from 440 chicken carcasses during 12 months. Of these 38 and 47 were isolated from the outer and inner carcass surfaces, respectively, 121 from the ileum contents, 92 from the liver parenchyma and 68 from bile.

The positive results varied from week to week and from farm to farm, ranging from 0 to 100 % (with an average of 50 %) without any clear trend. Nevertheless, it is of interest to note that out of 44 batches of 10 chickens 3 (6.8 %) batches were without *C. jejuni* findings but only one farm proved to be uninfected.

From the frequency of *C. jejuni* findings in the individual weeks and organs it appears that the mean contamination of the farms amounted to 50 %, while

Table 1
Differentiation of campylobacters

Growth at 42 °C	Catalase test	Growth at 30.5 °C	H ₂ S production	Growth in 1 % glycine	Sensitivity to nalidixic acid	Sensitivity to cephalotin	Species or subspecies:	Literary data
+	+	+	+	+	+	-	<i>C. Coli</i>	5, 27
+	+	+	+	+	-	+	<i>C. hyointestinalis</i>	9
+	+	+	+	+	-	-	<i>C. laridis</i>	2
+	+	-	+	+	+	-	<i>C. jejuni</i>	11, 20
+	-	.	+	-	d	+	<i>C. mucosalis</i>	16, 28
+	-	+	+	+	-	+	<i>C. sputorum</i> subsp. <i>sputorum</i>	36
-	+	.	-	+	+	+	<i>C. cinaedi</i>	35
-	+	.	+	+	+	+	<i>C. concisus</i>	33
-	+	.	-	+	+	+	<i>C. cryaerophila</i>	23
-	+	.	-	+	+	+	<i>C. fennelliae</i>	35
-	+	.	d	+	-	+	<i>C. fetus</i> subsp. <i>fetus</i>	8, 33
-	+	.	+	+	+	+	<i>C. fetus</i> subsp. <i>venerealis</i>	8
-	.	d	-	+	+	+	<i>C. jejuni</i> subsp. <i>doylei</i>	32
-	.	.	+	+	+	+	<i>C. nitrofigilis</i>	18
-	.	.	+	+	d	+	<i>C. sputorum</i> subsp. <i>bubulus</i>	17, 29



Explanations:

- = outer surface = liver
 -.-.-.- = inner surface -.-.-.- = bile
 - - - - = ileal content ————— = total number of contaminated chickens

Fig. 1: Frequency of *C. jejuni* in slaughtered chickens during the year

the mean contamination demonstrated from the outer carcass surface, inner carcass surface, ileum contents, liver parenchyma and bile was 9.4 %, 11.4 %, 27.7 %, 21 % and 13.8 %, respectively. The results obtained during the whole year are presented graphically in Fig. 1. It can be seen that the curve representing total percentages of contaminated chickens had two peaks, one in May and the other in October, during which months the proportions of *C. jejuni* — contaminated chickens reached 62.5 % and 62 %, respectively. The first peak coincided with the highest percentages of *C. jejuni* contamination demonstrated in the ileum and liver (45 % and 30 %) and the second peak coincided more or less with the highest proportion of *C. jejuni* contamination demonstrated in bile (30 %). The findings of *C. jejuni* from the outer and inner carcass surfaces were relatively low, ranging from 0 to 17.5 %, and showed little fluctuation during the year except in December when the number of chickens was only half that examined in previous months and the contamination was generally demonstrated in the same birds and organs.

In 82 (22.4 %) *C. jejuni* strains solitary colonies were observed also after control aerobic incubation at 42 °C, but attempts at their further passage under these conditions yielded negative results. They were recorded occasionally throughout the year: 9 times (11 %) from the outer surface, 10 times (12.2 %) from the inner surface, 30 times (36.5 %) from the ileum contents, 16 times (19.5 %) from the liver parenchyma and 17 times (20.7 %) from bile.

Discussion

The information on *C. jejuni* published to date is extensive and a certain gap in the relevant literature regarding the investigations carried out in chickens became apparent. Essentially the same can be said about the characteristics used for the differentiation of campylobacters (see Table 1) where vacancies appear mainly in the column "Growth at 30.5 °C".

The cultivation on the nutrient media used in our study and the evaluation of the results presented no difficulties thanks, among other things, to parallel inoculations of *C. jejuni* CCM 6207 type strain. The findings of solitary colonies growing also under aerobic conditions were due to the fact that we included this control incubation of the isolated strains. Their frequency with respect to the individual organs was more or less equal, amounting to 22.4 ± 5 %. It is of interest to note that similar observations were made in the strains of *Campylobacter cryaerophila* the subcultures of which were reported to grow under both aerobic and anaerobic conditions (Neil et al. 1985).

The seasonality in the occurrence of *C. jejuni* strains in our study became apparent particularly in the total percentages of contaminated chicken carcasses peaking in May and October. A similar observation was reported by Doyle (1984) in his study based on the investigation of faeces from laying hens.

The mean proportions of contaminated chickens in our study were twice to 2.5 times lower than those reported by Baker et al. (1987) and Míčková (1987) and the mean proportion of positive findings in the liver parenchyma was half that recorded by Khalafalla (1990). The reasons of these differences are difficult to discuss because they reflect both the epizootiological situation in the flocks and some poultry slaughter-house practices such as temperature of scalding water and general sanitation standard.

For assessing the bactericidal effect of heat treatment of poultry meat products

it would have been of interest to examine the sensitivity of *C. jejuni* to temperatures used in this operation but these questions have been thoroughly considered by other investigators (Doyle and Roman 1981; Blankenship and Graven 1982; Matyáš and Tomancová 1985).

Campylobacter jejuni u jatečných kuřat z pohledu hygieny potravin

Během 12 měsíců bylo od 1. 2. 1990 do 31. 1. 1991 vyšetřeno 440 jatečných kuřat na nosičství *C. jejuni*. Každotýdenní šarže 10 kuřat pocházela z některé ze 27 drůbežích farem a příslušné vzorky byly odebrány z vnějšího a vnitřního povrchu trupu, obsahu ilea, z hloubky jater a ze žluče.

Dosažené výsledky:

1. Z jednotlivých vzorků bylo izolováno celkem 366 kmenů *C. jejuni*, a to 38 a 47 (10,3 a 12,8 %) z vnějšího a vnitřního povrchu trupu, 121 (35 %) z obsahu ilea, 92 (25 %) z jaterního parenchymu a 68 (18,6 %) ze žluče.

2. Průběžné počty kontaminovaných kuřat představovaly dvouvrcholovou křivku s maximy v měsících květnu a říjnu, kdy frekvence nálezů dosáhla 62, 5, resp. 62 %.

3. Téměř paralelní trend byl pozorován také u *C. jejuni* v obsahu ilea (max. = 45 %) a v jaterním parenchymu (max. = 30 %), zatímco v souvislosti s druhým vrcholem to bylo spíše ve žluči (max. = 30 %).

4. Záchyt *C. jejuni* na vnějším i vnitřním povrchu byl relativně řídký (s průměry 9,4 a 11,4 %) a dosti vyrovnaný. Jeho průměrný záchyt ve žluči činil 13,8 %.

5. U 82 (22,4 %) kmenů *C. jejuni* se objevily ojedinělé kolonie i v kontrolní aerobní pasáži při 42 °C, ale jejich další přeočkování za těchto podmínek vyznělo negativně.

Nosičství a vylučovatelství *C. jejuni*, stejně jako bezsymptomní průběh infekce u kuřat nenuť chovatele ani veterinárního lékaře k žádným zásahům, ale pro člověka-konzumenta znamená taková drůbež vážné zdravotní nebezpečí.

Campylobacter jejuni боенских цыплят с точки зрения гигиены пищевых продуктов

В течение 12 месяцев - с 1-го февраля 1990 г. по 31-ое января 1991 года - исследовали 440 боенских цыплят, чтобы определить носительство *C. jejuni*. Еженедельная партия 10 цыплят происходила из одной из 27 птицеферм и соответствующие образцы получали с наружной и внутренней поверхности тела, содержания подвздошной кишки, из глубинных слоев печени и желчи. Полученные результаты:

1. Из отдельных образцов изолировали в итоге 366 штаммов *C. jejuni*, а именно 38 и 47 (10,3 и 12,8 %) из наружной и внутренней поверхности тела, 121 (35 %) из содержания подвздошной кишки, 92 (25 %) - из паренхимы печени и 68 (18,6 %) из желчи.

2. Систематически проводимые подсчеты численности контаминированных цыплят представляли собой двухпиковую кривую с максимумом в мае и октябре, когда их частота достигла 62,5 или 62 %.

3. Почти параллельная тенденция наблюдалась также у *C. jejuni* в содержании подвздошной кишки (максимально 45 %) и в паренхиме

печени (максимально 30 %) между тем как в связи со вторым пиком максимум сводилось скорее к желчи (30 %).

4. Выявление *C. jejuni* на наружной и внутренней поверхности тела встречалось сравнительно редко (в среднем 9,4 и 11,4 %) и отличалось уравнищенностью. Среднее наличие в желчи достигало 13,8 %.

5. У 82 (22,4) штаммов *C. jejuni* появились отдельные колонии также в контрольном аэробном пассаже при 42 °С, однако их повторная прививка в данных условиях была негативной.

Носительство и выделение *C. jejuni* а также бессимптомный ход инфекции у цыплят не заставляет птицеводов ни ветврачей прибегать к вмешательствам, однако для потребителя такие птицы представляют серьезную угрозу здоровью.

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