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LOCATION OF THERMOPHILIC CAMPYLOBACTER SPP IN VARIOUS PARTS OF CHICKEN INTESTINES

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Tavuk barsaklarının çeşitli bölümlerinde termofilik Campylobacter türlerinin yerleşimi

Özet: *Tavuk barsaklarının duodenum, jejunum, ileum ve caecum bölümlerinde termofilik Campylobacter türlerinin dağılımı incelendi. Selektif izolasyon yöntemi ile incelenen* 80 *tavuğun* % 86.3*'ünün bir veya daha fazla barsak bölümünde Campylobacter'lerin varlığı saptandı. Duodenum, jejunum, ileum ve caecum'dan Campylobacter izolasyon oranları sırasıyla* % 52.5, % 63.8, % 78.8 *ve* % 81.3 *olarak belirlendi. Seksen tavukda bulunan toplam* 69 *Campylobacter suşunun* % 50.7*'si C. jejuni,* % 49.3*'ü C. coli olarak identifiye edildi. Caecum ve ileum'un, duodenum ve jejunum'a göre daha fazla sayıda Campylobacter hücresi içerdiği bulundu. Buna karşın, kalın barsaktan yapılan ekimlerde, diğer bölümlere kıyasla daha fazla sayıda ve yoğunlukta kontaminant mikroorganizma ürediği gözlendi.*

Summary: *The distribution of thermophilic Campylobacter spp. in duodenum, jejunum, ileum and caecum of chicken intestines was investigated. Of* 80 *chickens examined by selective isolation technique,* 69 (86.3 %) *were found to be harboured Campylobacter sp in one or more parts of their intestines. Isolation rates of Campylobacter sp from duodenum, jejunum, ileum and caecum were* 52.5, 63.8, 78.8 *and* 81.3 *per cent, respectively. Of* 69 *strains of Campylobacter sp. isolated from* 80 *chickens,* 35 (50.7 %) *were identified as C. jejuni and* 34 (49.3 %) *as C. oli .It was found that caecum and ileum carried campylobacters in large numbers relative to duodenum and jejunum. Lower part of intestine contained more contaminant microorganisms which grew on selective medium.*

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Introduction

The importance of animal origins in the epidemiology of Campylobacter infections is based on the fact that many mammalian and avian species carry campylobacters as part of their intestinal microflora (2, 7). *Campylobacter jejuni* and *C. coli*, known as thermophilic campylobacters, are the common inhabitants of the gut of healthy poultry and wild birds (5, 21). These microorganisms often seem to be harboured in the large intestine of broiler chickens (12, 15, 18). Recent studies suggest that contamination may be nearly universal (6, 10, 17). The organism spreads through the flock from one or more sources. Potential sources for entry of campylobacters into a flock include infection of newborn chicks from older birds, contaminated feed, water or litter (8, 9, 17). Infection is usually without obvious signs of illness and long term carriage is frequently present (8, 12). During the process of slaughtering, *C. jejuni* and *C. coli* spreads from the intestinal content to the carcasses (4, 16, 20).

Campylobacter colonization in the intestinal tract of young chicks takes place about two weeks after hatching (9, 17). Experimental studies have showed that resistance of young specific pathogen free chicks to *C. jejuni* has been substantially increased by early exposure to native gut microflora (19).

Disease conditions caused by *Campylobacter sp.* are not clear in poultry. A vibrio-like organism, now considered to be *C. jejuni*, has been isolated from chickens that showed degenerative liver changes and depressed egg production (11). This condition, termed avian vibrionic hepatitis, has been frequently encountered during the 1950's and 1960's in USA and Europe (1, 3), but is now diagnosed less frequently. Recently, it has been demonstrated that low level mortality and diarrhoea follow infection of 3 days old chicks with *C. jejuni* (13).

The present study was undertaken to compare the location of thermophilic *Campylobacter spp.* in various parts of chicken intestine.

Materials and Methods

Sampling and isolation: Whole intestines of 80 broiler chickens from 8 different flocks were collected during slaughtering(Ten broiler chickens had been randomly selected from each flock). Specimens taken from the following parts of each intestine were used as inocu-

lum: duodenum, jejunum, ileum and caecum. Outer surface of selected part of intestine was disinfected and a longitudinal incision was made. Then a steril swab introduced into lumen to collect intestinal content.

Samples were cultured directly for *Campylobacter sp.* on selective medium comprising Blood Agar Base no. 2 (Oxoid) containing 5–7 % defibrinated sheep blood and Preston Selective Supplement (Oxoid). The inoculated media were incubated at 42 °C for 2 days under reduced oxygen and increased carbon dioxide tension (5 % 0_2, 10 % CO_2 and 85 % H_2).

Identification and evaluation: Typical swarming colonies of thermophilic *Campylobacter sp.* were examined for cell morphology and motility by phase-contrast microscopy. Where possible, only one single colony was taken from the selective medium for subculture onto sheep blood agar. Suspect colonies were further confirmed as *C. jejuni* or *C. coli* by the following tests: oxidase and catalase production, nitrate and sodium selenite reduction, H_2S production, resistance to cephalothin (30 mcg), sensitivity to nalidixic acid (30 mcg), tolerance for 1 % glycin but failure to grow at 25 °C. *C. jejuni* was differentiated from *C. coli* by its ability to hydrolyse hippurate. Reference strains of *C. jejuni* and *C. coli* were used as controls in each test.

Observations on the growth of Campylobacter were made and arbitrarily recorded, scoring from a few colonies observed to almost confluent growth of a large number of colonies on the agar (0 = no growth, 1 = 1–5 colonies, 2 = 6–25 colonies, 3 = 26–100 colonies, 4 = < 101 colonies). All growths on selective media other than Campylobacter were considered as contamination and scored in a similar manner.

Results

Of 80 chickens examined, 69 (86.3 %) were found to be carrier of *Campylobacter sp.* in one or more parts of their intestines. Isolation rates of *Campylobacter sp.* from various parts of chicken intestines were as follows: 52.5 % (42/80) from duodenum, 63.8 % (51/80) from jejunum, 78.8 % (63/80) from ileum and 81.3 % (65/80) from caecum (Table 1).

Table 1. The distribution of *C. jejuni* and *C. coli* in different parts of chicken intestines.

	Number of positive samples			
	Duodenum[a]	Jejunum[a]	Ileum[a]	Caecum[a]
C. jejuni	21	23	33	35
C. coli	21	28	30	30
Total of strains	42 (52.5 %)	51 (63.8 %)	63 (78.8 %)	65 (81.3 %)

a = Eighty samples from each part

The distribution of *C. jejuni* and *C. coli* in different parts of intestines is also shown in Table 1. Of 69 strains of *Campylobacter sp.* isolated, 35 (50.7 %) were identified as *C. jejuni* and 34 (49.3 %) were identified as *C. coli*. In the intestine of a chicken, *C. coli* was isolated from duodenum and *C. jejuni* was isolated from jejunum, ileum and caecum.

The growth of campylobacters on selective media was scored from Grade 1 to Grade 4 according to the number of colonies. Average growth scores of Campylobacter colonies from each part of intestine were calculated by adding all grades together and dividing by the number of Campylobacter positive samples. Average scores of campylobacters from duodenum, jejunum, ileum and caecum were 1.90, 2.43, 3.11 and 3.33, respectively (Table 2).

Table 2. The growth of *Campylobacter sp.* on selective media

	Number of strains			
	Duodenum	Jejunum	Ileum	Caecum
Grade[a] 1	23	18	5	4
Grade 2	8	10	12	9
Grade 3	4	6	17	13
Grade 4	7	17	29	39
Average score	1.90	2.43	3.11	3.33

[a]Key: O = no growth, 1 = 1 - 5 colonies, 2 = 6 - 25 colonies, 3 = 26 - 100 colonies, 4 = > 100 colonies.

Colonies of contaminant microorganisms were also observed on selective media. Contamination rates of cultures inoculated with the samples from duodenum, jejunum, ileum and caecum were 28.8 %, 41.3 %, 62.5 % and 73.8 %, respectively (Table 3). Growth of contaminated microorganisms was also scored according to the num-

ber of colonies and average scores were calculated. Average scores of contamination of cultures sampled from duodenum, jejunum, ileum and caecum were 1.56, 1.54, 2.04 and 2.74, respectively (Table 3).

Table 3. The distribution and growth of contaminant microorganisms on selective media.

	Duodenum	Jejunum	Ileum	Caecum
Grade[a]1	17	22	13	5
Grade 2	2	4	24	23
Grade 3	1	7	11	13
Grade 4	3	0	2	18
Total	23	33	50	59
(%)	(28.8)	(41.3)	(62.5)	(73.8)
Average score	1.56	1.54	2.04	2.74

[a]Key: O = no growth, 1 = 1 – 5 colonies, 2 = 6-25 colonies, 3 = 26–100 colonies, 4 = > 101 colonies.

Discussion and Conclusion

In recent years, numerous workers have described the presence of *C. jejuni* and *C. coli* in poultry (6, 7, 21), most attention having been paid to processed broilers in which a high incidence has been demonstrated (4, 16). In the most of these works, cloacal swabs, caecal content and faeces have been used as the sampling sites of intestinal tract. Studies in which distribution of *Campylobacter sp.* in various parts of intestine has been compared are rather scarce.

In the present study, sample sizes were small relative to the sizes of flocks being monitored, but judging from our previous experiences using similar sample size, they were sufficient to indicate the trends of infection in flocks. *Campylobacter sp.* were usually either present or absent in all or most of the birds in any flock. This high incidence has been expressed by several other workers as a result of almost universal distribution of thermophilic *Campylobacter sp.* in poultry (12, 17).

This study has showed the variability of Campylobacter colonization among various parts of chicken intestine. Lower part of intestine seems to be predominant site of Campylobacter colonization. Frequency of campylobacters in caecum and ileum was very close to each other. But, *Campylobacter sp.* was less frequently isolated from small intestine. Soerjadi et al (18) and Oosterom et al (10) have also reported gradual distribution of *C. jejuni* in chicken intestine. High inciden-

ce of *Campylobacter sp.* in caecum reported by many workers (9, 20) also correlates with our results. Average scores of Campylobacter colonies from caecum and ileum were higher than those of upper parts of intestine. It means that the incidence of *Campylobacter sp.* is related to the number of campylobacter cells in each part of intestine. This suggestion has been confirmed by the findings of Soerjadi et al (18). It was of interest to determine the isolation of *C. coli* from duodenum and the isolation of *C. jejuni* from other parts of intestine in a chicken. It can be suggested that different strains of *Campylobacter sp.* can colonize the various parts of intestine of a chicken. And if it is practical to identify each colony grown on selective media, this phenomenon may be detected more frequently.

Very large numbers of contaminant microorganisms were observed on selective media onto which samples from caecum had been inoculated. This is not surprising since it has been shown by Salanitro et al (14) that lower parts of chicken intestine ,particularly caecum, contain large numbers of bacteria as comparing with the upper parts. For this reason, ileum can be considered as an alternative sampling site for the isolation of *Campylobacter sp.*, since its contaminant bacteria content is small relative to the bacterial content of caecum.

In this study, four chickens carrying *Campylobacter sp.* in their small intestine but not in caecum have been determined. Examination of individual cloacal swabs or caecal content from a random sample of birds is considered as an effective method by several authors (6, 18). This is true for surveys with large sample size, but when sampling an individual chicken for culturing *Campylobacter sp.*, it must be kept in mind that campylobacters, even in small numbers, may be colonize only in smal intestine.

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