野鳥からのカンピロバクター検出状況および分離菌株の血清型

誌名	Japanese journal of veterinary science
ISSN	00215295
巻/号	483
掲載ページ	p. 487-493
発行年月	1986年6月

Distribution of *Campylobacter jejuni* in Wild Birds and Serogroup of Isolates by Slide Agglutination Technique

Masafumi FUKUYAMA, Tomoo KAMIMURA, Takeshi ITOH[3], Kahiko SAITO[3], Masaki TAKAHASHI[3], Senzo SAKAI[3], Motohide MURATA[1], Ken-ichi KOHZAKI[1] Motonobu HARA[2], Takehiko SHIMIZU[2], and Kiyoshi TABUCHI[2]

Department of Environmental Microbiology, [1]Veterinary Public Health, [2]Veterinary Microbiology, Azabu University. 1-17-71, Fuchinobe, Sagamihara-shi, Kanagawa 229, and [3]Department of Microbiology, Tokyo Metropolitan Research Laboratory of Public Health. 3-24-1, Hyakunin-cho, Shinjuku-ku, Tokyo 160, Japan

(Received 13 June 1985/Accepted 12 February 1986)

ABSTRACT. Distribution of thermophilic campylobacter was investigated in the total of 700 wild birds living in urban areas of Japan and mountainous environments around the base of Mt. Fuji. Thermophilic campylobacters were isolated from 55 (7.9%) of the 700 birds. By species, they were detected in 51 (13.5%) of 378 feral pigeons, 2 (2.4%) of 82 pintails and 3 (60.0%) of 5 sparrows. However, campylobacters were not isolated from other 235 wild birds consisting of swallows, sparrows caught at Sagamihara, black-tailed gulls, pintails caught at Tokyo, eastern turthledoves caught at Ebina and grey starling, brown thrush, narcissus flycatcher, etc. caught at Mt. Fuji. In pigeons, large differences were demonstrated in the detection rates of this bacterium depending on the site of capture and even among the groups captured in the same area, varying from zero to 50.0%. All the strains isolated from pigeons and sparrows were *C. jejuni*. Two strains isolated from pintails were identified to be *C. coli*, while no *C. laridis* was detected. Seventeen (34.7%) of 49 strains of *C. jejuni* isolated from feral pigeons and 2 strains (100.0%) from sparrows were serotyped into some of the author's serogroup system (TCK 1-TCK 32) by typing with slide agglutination. Especially TCK 20 was frequently found in feral pigeons. Two strains of *C. coli* isolated from pintails were not serotyped by any of the antisera to *C. jejuni*.—KEY WORDS: *Campylobacter jejuni*, pigeon, distribution, serogroup, wild bird.

Jpn. J. Vet. Sci. 48(3): 487–493, 1986

Campylobacter jejuni (*C. jejuni*), known to cause gastroenteritis in man, colonizes in the intestinal tracts of mammals such as cattle, sheep, dogs, cats and monkeys [1, 2, 10, 17, 19, 24]. This bacterium is also isolated at a high rate from domestic fowls including chickens and quails. A previous study has shown that 38% of chickens and 45% of quails carried *C. jejuni* in their intestinal contents [11]. Pigs also carry *Campylobacter coli* (*C. coli*) and sometimes *C. jejuni* is considered as intestinal commensals. *Campylobacter jejuni* and *C. coli* in mammals and birds have recently attracted much attention as sources of campylobacter enteritis in humans [1, 7, 19, 22].

The purpose of this study was to investigate the distribution of thermophilic campylobacters in various wild birds including pigeons and sparrows living in urban areas, and grey starlings (*Sturnus cineraceus*) and brown thrushes (*Turdus chrysolaus*) living in mountainous regions of Japan. The strains isolated were serotyped by the slide agglutination technique [8] and examined for their relationship to human gastroenteritis.

MATERIALS AND METHODS

Materials: During the period from October, 1981 to December, 1982, a total of 700 wild birds of 20 species were captured in Kanagawa and Tokyo areas and around the base of Mt. Fuji. They consisted of 378 feral pigeons, 82 pintails, 5 sparrows, 134 swallows and 101 others (Tables 1 and 2). For

pigeons, the intestinal contents were used as test materials. For other birds, the fresh stool specimens were collected into 5 m*l* of Cary and Blair transport medium and transported at 7°C to the laboratory as soon as possible.

Isolation and identification of campylobacteris: The intestinal contents and stool specimens were plated on a modified Skirrow's agar, nutrient agar (Nissui) containing 5% horse defibrinated blood and antibiotics (Oxoid: trimethoprim 5 mg/*l*, vancomycin 10 mg/*l* and polymyxin B 2500 IU/*l*). The plates were incubated under microaerophilic conditions by the Gas Pak method (BBL) without catalysts at 37°C or 43°C for 2 to 3 days. The suspicious colonies on the Skirrow's blood agar were gram-stained and examined for motility by phase-contrast microscopy. Organisms having a curved or spiral rod in gram-staining and corkscrew-like motion were examined further by biochemical tests using the methods of Skirrow and Benjamin [20]. The oxidase activity was determined using a cytochrome-oxidase test strip (Nissui). The catalase activity was determined with H_2O_2. Glucose utilization was examined using a PYP medium base (Nissui). The H_2S production was tested using a sensitive medium described by Véron and Chatelain [25]. Growth at 25°C or 43°C on the blood agar was examined in the microaerophilic condition. Brucella broth (Difco) containing 1% glycine was used for the glycine tolerance test under microaerophilic conditions. Hippurate hydrolysis was tested by the method described by Hwang and Ederer [5]. The nalidixic acid sensitivity was determined using a commercial antibiotic disk (Showa Yakuhin Kako) containing 30 μg of nalidixic acid.

Serological typing of Campylobacter jejuni: Antisera were prepared in rabbits against 32 serotype reference strains of the scheme developed by Itoh *et al.* [8] and absorbed with heterologous cross reactive antigens. Fifty-one isolates for serological typing were cultured on nutrient agar at 37°C for 2 days under the microaerophilic condition, and the cells were suspended in physiological saline containing 0.3% formalin a slide agglutination test was performed on glass slide with absorbed antisera [8].

RESULTS

Thermophilic campylobacters were isolated from the intestinal contents of 51 (13.5%) of 378 feral pigeons captured from March, 1981 to November, 1982 in the Tokyo and Kanagawa areas (Table 1). The isolation rate of thermophilic campylobacters varied markedly according to the time of the captures even within a defined small area. Feral pigeons at Shrine A in Tokyo were captured 6 times during the period. Those captured in March, April, May and June were found campylobacter-positive, while those captured in July and November were found all negative. Campylobacters were positive in 25.0% and 9.7% of feral pigeons captured at Shrines B and D, respectively, but were negative in those captured at Shrine C. Of 7 feral pigeons caught at Park E in Tokyo and 122 at Park F in Kanagawa, 1 (14.3%) and 7 (5.7%), respectively, were campylobacter-positive. Of 3 groups captured in different months at Park G in Kanagawa, campylobacters were positive in 2 groups and negative in 1. No strains were isolated from all 21 feral pigeons captured at Airport H in Tokyo.

Of 30 campylobacter-positive pigeons from Shrine A, 15 (50.0%) carried the organisms in both the large and small intestines and 10 (33.3%) in the large intestine alone. Thus, the detection rates did not significantly differ by the anatomical site.

Table 2 shows the results of theromphilic campylobacters isolated from the stools of 322 wild birds including pintails, sparrows and swallows. Two of 82 pintails caught at parks in Tokyo and 3 of 5 sparrows in Sagamihara were found to carry these or-

Table 1. Detection of thermophilic campylobacter in feral pigeons by the capture area

Capture area	Month of capturing (1981–82)	Number of samples examined	Number of positive(%) birds	Serogroups of isolated strains
Shrine				
A (Tokyo)	March	27	13(48.1)	TCK 20(3)[a] TCK 25/26(1) TCK 2(1) TCK 16(1) UT[b](6) NT[c](1)
	April	15	5(33.3)	TCK 24(1) UT(4)
	May	20	10(50.0)	UT(10)
	June	7	3(42.9)	TCK 20(1) UT(2)
	July	15	0(0.0)	
	November	15	0(0.0)	
B (Tokyo)	May	12	3(25.0)	TCK 20(1) TCK 14(1) UT(1)
C (Tokyo)	May	7	0(0.0)	
D (Kanagawa)	July	8	1(12.5)	TCK 20(1)
	August	23	2(8.7)	TCK 21(2)
Park				
E (Tokyo)	July	7	1(14.3)	TCK 20(1)
F (Kanagawa)	October	122	7(5.7)	TCK 20(1) TCK 14/21(1) UT(5)
G (Kanagawa)	July	20	2(10.0)	TCK 21(1) NT(1)
	October	22	0(0.0)	
	November	37	4(10.8)	UT(4)
Airport (Sea side)				
H (Tokyo)	August	9	0(0.0)	
	October	12	0(0.0)	

a) Number of strains.
b) Untypable.
c) Not tested.

Table 2. Detection of thermophilic campylobacter in wild birds

Kind of wild birds	Capture area	Number of samples examined	Number of positive (%) birds
Pintail (*Anas acuta*)	Tokyo	82	2(2.4)
Sparrow (*Passer montanus*)	Kanagawa (Sagamihara)	5	3(60.0)
Swallow (*Hirundo rustica*)	Kanagawa (Sagamihara)	134	0
Black-tailed gull (*Larus crassirostris*)	Tokyo	36	0
Eastern turtledove (*Streptopelia orientalis*)	Kanagawa (Ebina)	17	0
Grey starling (*Sturnus cineraceus*)	Mt. Fuji	12	0
Brown thrush (*Turdus chrysolaus*)	Mt. Fuji	6	0
Narcissus flycatcher (*Muscicapa narcissina*)	Mt. Fuji	6	0
Black-faced bunting (*Emberiza spodocephala*)	Mt. Fuji	4	0
Rustic bunting (*Emberiza rustica*)	Mt. Fuji	4	0
Coal-tit (*Parus ater*)	Mt. Fuji	4	0
Other wild birds (8 species)	Mt. Fuji	12	0
Total		322	5(1.6)

Table 3. Biochemical characteristics of strains isolated from wild birds

Characteristics	Feral pigeon (51 strains)		Pintail (2 strains)		Sparrow (3 strains)	
	+	−	+	−	+	−
Oxidase	51	0	2	0	3	0
Catalase	51	0	2	0	3	0
Fermentation of glucose	0	51	0	2	0	3
Growth at 25°C	0	51	0	2	0	3
43°C	51	0	2	0	3	0
H_2S production	51	0	2	0	3	0
Growth in 1% glycine	51	0	2	0	3	0
Hippurate hydrolysis	51	0	0	2	3	0
Nalidixic acid sensitivity (30 μg)	51	0	2	0	3	0

Table 4. Serogroups of *Campylobacter jejuni* isolated from wild birds

Serogroups (TCK)	No. of strains	
	Feral pigeon	Sparrow
2	1	—
14	1	—
16	1	—
20	8	—
21	3	—
24	1	—
27	—	2
14/21	1	—
25/26	1	—
Total typable	17(34.7%)	2
Untypable	32(65.3%)	0
Total	49(100.0%)	2

ganisms.

No campylobacter organisms were detected in wild birds cuaght around the base of Mt. Fuji, including brown thrushes, narcissus flycatchers (*Muscicapa narcissina*), black-faced buntings (*Emberiza spodocephala*), etc.

Table 3 shows the biochemical characteristics of 56 strains of thermophilic campylobacters isolated from pigeons, pintails and sparrows. Fifty-one strains isolated from pigeons and 3 from sparrows were identified to be *C. jejuni*, since they hydrolyzed hippurate and demonstrated sensitivity to nalidixic acid (30 μg).

Two strains isolated from pintails did not hydrolyze hippurate and were identified to be *C. coli*.

The serogroups of 51 strains (49 from feral pigeons and 2 from sparrows) are shown in Table 4. Seventeen (34.7%) of the 49 strains from feral pigeons and both (100.0%) of the 2 strains from sparrows were serotyped by slide agglutination. Of the pigeon isolates, 15 fitted in 6 serogroups and 2 revealed the presence of 2 antigenic factors. Among the serogroups, TCK 20 was the most frequently identified serogroups, followed by TCK 21.

Table 1 also shows the places where pigeons were caught and the serotypes of the isolated strains. Serotype TCK 20, which was isolated most frequently was distributed in almost all the areas of capture. This serogroup was identified in 4 pigeons from Shrine A, in 1 each from Shrines B and D, in 1 from Park E and in 1 from Park F.

Two strains isolated from 2 sparrows were serotyped as TCK 27. These sparrows were caught in the same area of Sagamihara. Two strains of *C. coli* isolated from pintails were not typed by any of the antisera (TCK 1-TCK 32) to *C. jejuni*.

DISCUSSION

In 1969 when campylobacter enteritis in human was not known, Smibert [23] reported the isolation of campylobacters from pigeons,

gray starlings and sparrows.

Later, Skirrow and Benjamin [21] and Luectefeld *et al.* [14, 15] found that wild animals carried campylobacter organisms which cause enteritis in humans.

In the present study, the distribution of campylobacter organisms in wild birds was investigated using the intestinal contents or feces from various bird species such as pigeons and sparrows living in urban areas and brown thrushes, flycatchers and buntings living in mountainous regions in Japan. The organisms were very frequently detected in the intestinal contents of feral pigeons living in urban areas such as shrines and parks.

In USA, Smibert [23] found campylobacter organisms in 20% of feral pigeons. Rosef [18] and Fenlon [3] in Norway found them in 42% and 41% of feral pigeons, respectively. In Japan, Matsuzaki *et al.* [16] and Kinjo *et al.* [13] found them in 7.9% and 24.0% respectively. This indicates that feral pigeons distributed over various parts of the world are infected with *C. jejuni* at high frequency.

The carrier rate of campylobacters varied markedly among the captured groups of pigeons and by the capture site. The campylobacter-positive ratio in pigeons caught at shrines ranged from 40% to 50%, while all pigeons caught at an airport, where they seem to have little contact with humans, proved to be campylobacter-negative. The positive ratio reported by Kinjo *et al.* [13] ranged from 0 to 50% depending on the site of capture. Ito *et al.* [6] also reported a large variation in the carrier rates depending on the site of capture.

The organisms of the same serotype were found to be excreted from pigeons caught at the same areas. This suggests that direct contact with pigeons themselves or feces may play an important role in transmission of campylobacters in these birds. Luechtefeld *et al.* [14, 15] detected campylobacters in many wild animals including birds in a zoo. This seems to indicate the favorable environment for campylobacter infection among various species of animals in a crowded zoo. In addition to pigeons, *C. jejuni* was detected in sparrows. The organisms isolated from 2 sparrows caught from the same flock were found to belong to one serogroup, suggesting a contact infection within the flock.

Luechtefeld *et al.* [14, 15] isolated campylobacter organisms from 34% to 60% of migrating birds such as wild ducks. However, in the present study, the isolation rate in the wild ducks showed only 2%. The present results showed the absence of *C. jejuni* in all the 48 wild birds (brown thrushes, flycatchers, buntings, etc.) that lived in mountainous regions, in contrast to a high carrier rate in urban pigeons. This finding suggested a marked variation in the carrier rates of the bacterium in wild birds according to living environments. Kapperud and Rosef [12] also reported a high carrier rate in wild birds caught in a city and a low rate in those caught in suburban areas.

Skirrow and Benjamin [21], Rosef [18], Kapperud and Rosef [12], and Fricker *et al.* [4] showed high frequencies of campylobacter carriers in sea gulls such as sea mews (laridas). None of the sea gulls examined in this study carried the organisms.

In various foreign countries, *C. laridis* is isolated in most cases from sea gulls, while this bacterium has not been isolated from sea gulls in Japan. This suggests geographical differences in the carrier rates and distributions of campylobacters in wild birds.

The carrier rate of campylobacters in wild birds seems to be greatly affected by food in addition to living environments. Matsuzaki *et al.* [16] reported a high rate of campylobacters in polyphagous crows. Luechtefeld *et al.* [14, 15] found that the campylobacter carrier rate was low in ducks fond of vegetable diet and high in those eating small animals in pond and river mud. This study showed that brown thrushes, flycatchers and buntings that chiefly eat nuts carried no campylobacters.

These findings may support the relationship between the diet style and carrier rate of this bacterium in wild birds.

Domestic animals and fowls are considered to play an important role as a source of campylobacter enteritis in humans [9]. Environmental contamination of rivers and ponds with campylobacters may also be mediated by wild birds. Therefore, involvement of wild birds in waterborne infection with campylobacters cannot be neglected. In the present study, approximately 34.7% of the pigeon strains were identified into 8 serogroups, including high frequencies of TCK 20 and TCK 21. These serogroups are the same strains that are isolated from human enteritis. The presence of the same types of strain supports an association between wild birds campylobacters and human campylobacter enteritis.

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要　　約

野鳥からのカンピロバクター検出状況および分離菌株の血清型：福山正文・上村知雄・伊藤　武[3]・斉藤香彦[3]・高橋正樹[3]・坂井千三[3]・村田元秀[1]・光崎研一[1]原　元宣[2]・清水武彦[2]・田渕　清[2](麻布大学環境保健学部環境微生物学教室，[1]獣医公衆衛生学教室，[2]獣医微生物学教室，[3]東京都立衛生研究所微生物部)——都市環境に生息する野鳥および富士山麓の自然環境下に生息する野鳥総計700例についてカンピロバクターの検索を行った結果，以下の成績が得られた。1) 野鳥700例中55例（7.9%）より Thermophilic campylobacter が検出された。その内訳は，ドバト378例中51例（13.5%），オナガガモ82例中2例（2.4%）スズメ5例中3例(60%)から本菌が検出された。しかし，ツバメやキジバトなどの野鳥235例からは本菌は検出されなかった。2) ハトおよびスズメ由来株はすべて，*C. jejuni* であった。オナガガモ由来の2株は *C. coli* と同定された。諸外国で野鳥に広く分布する *C. laridis* はまったく検出されなかった。3) スライド凝集反応によって分離菌株の血清型別を行った結果，ドバト由来の *C. jejuni* 49株中17株(34.7%)，スズメ由来2株中2株(100%)が著者らの血清型（TCK 1～TCK 32）のいずれかに型別された。ドバトでは特に TCK 20 に該当する菌株が多く認められた。オナガガモ由来の *C. coli* 2株は著者らの血清型別には該当しなかった。