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ISOLATION OF THERMOPHILIC CAMPYLO-BACTERS FROM NORWEGIAN DOGS AND CATS

By

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GONDROSEN, BJØRN, TORUNN KNÆVELSRUD and KARI DOMMARSNES: *Isolation of thermophilic campylobacters from Norwegian dogs and cats.* Acta vet. scand. 1985, 26, 81—90. — A total of 147 dogs and 85 cats was surveyed for faecal carriage of thermophilic campylobacters. Isolates were obtained from 33 (22.4 %) of the dogs and from 10 (11.8 %) of the cats investigated. The isolation rates recorded for diarrhoeic and non-diarrhoeic dogs and cats were not significantly different. Likewise, campylobacters were isolated with about equal frequencies from puppies and mature dogs as well as from bitches and male dogs. Nineteen canine and 7 feline strains were biotyped and serotyped. A large majority (88.5 %) of these 26 strains belonged to serotypes previously recovered from human C. coli and C. laridis constituted 7.7 % and 3.8 %, respectively. Three strains belonged to serotypes previously recovered from human patients in Norway.

C a m p y l o b a c t e r j e j u n i; C. c o l i; C. l a r i d i s; b i o t y p i n g; s e r o t y p i n g; c a n i n e; f e l i n e; N o r w a y; d i a r r h e a; f a e c a l c u l t u r e s.

Thermophilic campylobacters (synonyms Campylobacter fetus subsp. jejuni, C. jejuni, C. jejuni/coli) have been frequently isolated from intestinal contents in many animal species, in most cases without apparent morbidity (*Butzler & Skirrow* 1979, *Prescott & Munroe* 1982, *Skirrow* 1982, *Kist* 1983, *Rosef et al.* 1983). The occurrence of these bacteria in faeces from dogs and cats has been amply documented (*Skirrow* 1977, *Hosie et al.* 1979, *Bruce et al.* 1980, *Svedhem & Nordkrans* 1980, *Schifferli et al.* 1982, *Fleming* 1983).

There are two aspects concerning Campylobacter infection in dogs and cats which deserve attention: The prevalence and pa-

thogenicity of campylobacters in the pets themselves, and the role of these animals as transmitters of infection to man (*Skirrow* 1981). The isolation frequencies reported from pets vary widely, and have been stated to depend upon clinical status (diarrhoea or not) and type of housing (stray, kennel, or household dogs). Likewise, some studies have indicated a predominance among puppies and kittens relative to mature dogs and cats (*Blaser et al.* 1980, *Hosie et al.* 1979, *Fleming* 1983, *Fox et al.* 1983). It has also been suggested that campylobacters may act synergistically with canine parvovirus (*Skirrow* 1981, *Schifferli et al.* 1982, *Simpson & Burnie* 1983). Epidemiological evidence has indicated that dogs and cats may transmit campylobacters to man, leading to gastroenteritis (*Bruce et al.* 1980, *Hay & Ganguli* 1980, *Skirrow et al.* 1980, *Svedhem & Norkrans* 1980, *Blaser et al.* 1982). These bacteria have been shown to be among the most important aetiological agents of acute enteritis in man (*Prescott & Munroe* 1982, *Skirrow* 1982).

The purpose of the present investigation was to survey the occurrence of thermophilic campylobacters among dogs and cats from an urban region in Norway. In addition, biotyping and serotyping were used in order to recognize epidemiologically related strains.

MATERIALS AND METHODS

Isolation and identification

Stool specimens from 147 dogs and rectal swabs from 85 cats were examined. All animals were patients admitted to the Policlinic for Small Animals, Department of Obstetrics, Norwegian College of Veterinary Medicine. The pets under study were household animals, originating from the city of Oslo and its suburbs. Rectal swabs and stool specimens were plated out onto colistin-amphotericin-keflin (CAK) agar (*Rosef et al.* 1983) and Skirrows' medium (*Skirrow* 1977) within 6 h of collection. Plates were incubated at 42°C in anaerobic jars without catalysts, using gas generating sachets (no. BR 38, Oxoid Ltd., Basingstoke, Hampshire, England) to achieve the proper microaerobic atmosphere. Plates were read after 24 and 38 h. All colonies showing a morphology similar to Campylobacter spp. were examined by phase contrast microscopy (1000 ×). Bacteria exhibiting the typical motility and cell morphology suggestive of Campylobacter spp.,

were subjected to cultural and biochemical examination as follows: The ability to grow under aerobic or anaerobic conditions was assessed after incubation at 37°C for 48 h. Growth at 25°C was tested in a microaerobic atmosphere. Catalase activity was tested on microscopic slides by addition of one drop of H_2O_2. Oxidase activity was examined on filter paper with 1 % aqueous solution of tetramethyl-*p*-phenylenediamine dihydrochloride. The parameters listed above formed the basis for identification of the isolated strains according to established criteria (*Smibert* 1974).

Biotyping

Biotyping was based on 3 tests: hippurate hydrolysis, H_2S production, and susceptibility to nalidixic acid. This enabled allocation to Campylobacter jejuni biotype 1 or 2, C. coli, or C. laridis (synonym nalidixic-acid-resistant thermophilic campylobacters (NARTC)), as proposed by *Skirrow & Benjamin* (1980). Prior to biochemical examination, all strains were grown on blood agar plates for 18—24 h in a microaerobic atmosphere. Hydrolysis of hippurate was tested by the method of *Hwang & Ederer* (1975). H_2S production was examined using the iron-containing FBP-medium described by *Skirrow & Benjamin* (1980). Susceptibility to nalidixic acid was evaluated on blood agar plates by means of commercial antibiotic disks (Neo-Sensitabs; A/S Rosco, Taastrup, Denmark) containing 130 μg of nalidixic acid. Bacteria showing inhibition zones of ≥ 28 mm after incubation at 37°C for 24 h in a microaerobic atmosphere, were considered sensitive.

Serotyping

Serotyping was accomplished on the basis of heat-stable antigens identified by means of the passive haemagglutination technique, as described by *Lauwers & Penner* (1984). The procedure followed has been detailed previously (*Kapperud et al.* 1984). The serotyping was carried out using 50 unabsorbed rabbit antisera, which were prepared at the University Hospital of St. Pierre, Microbiological Laboratory, Brussels, (courtesy of Dr. S. Lauwers).

RESULTS

Isolation frequencies

Thermophilic campylobacters were isolated from 33 (22.4 %) of the 147 dogs and from 10 (11.8 %) of the 85 cats examined. Forty-seven dogs and 13 cats were suffering from diarrhoea. The isolation frequencies from diarrhoeic (23.0 %) and non-diarrhoeic dogs (21.3 %) were not significantly different ($\chi^2 = 0.05$, $P > 0.05$) (Table 1). Likewise, no significant difference was observed between cats with (7.7 %) and without (12.5 %)

Table 1. Faecal carriage rates of thermophilic campylobacters in dogs and cats related to age and clinical history.

Age (months)	Non-Diarrhoeic			Diarrhoeic		
	Total	With camp. No.	%	Total	With camp. No.	%
Dogs						
≤ 12	21	5	23.8	14	5	35.7
> 12	79	18	22.8	33	5	15.2
Total	100	23	23.0	47	10	21.3
Cats						
≤ 6	7	0	0	4	1	25.0
> 6	62	9	14.5	9	0	0
Not known	3	—	—	—	—	—
Total	72	9	12.5	13	1	7.7

Table 2. Faecal carriage rates of thermophilic campylobacters in dogs and cats related to sex and clinical history.

	Non-Diarrhoeic			Diarrhoeic		
	Total	With camp. No.	%	Total	With camp. No.	%
Dogs						
Male	44	14	31.8	26	5	19.23
Female	56	9	16.1	17	4	23.5
Not known	—	—	—	4	1	—
Total	100	23	23.0	47	10	21.27
Cats						
Male	43	6	14.0	9	1	11.1
Female	29	3	10.3	4	0	0
Total	72	9	12.5	13	1	7.7

diarrhoea ($\chi^2 = 0.25$, $P > 0.05$). The number of diarrhoeic cats was, however, insufficient to justify definite conclusions.

The influence of age and sex on the recovery rates are given in Tables 1 and 2. No statistically significant differences were found in this investigation ($P > 0.05$).

T a b l e 3. Biochemical and serological classification of 26 thermophilic campylobacters isolated from dogs and cats.

Animal[1]	Isolation medium		Biotype[2]	Serotype[3]
	CAK	Skirrow's		
Healthy dogs				
A		+	*C. jejuni* biot. 1	LAU 3/16
B_1		+	„ „	LAU 3
B_2	+		„ „	NT
C_1	+		„ „	NT
C_2		+	„ „	NT
D	+		*C. jejuni* biot. 2	NT
E		+	„ „	NT
Diarrhoeic dogs				
F_1		+	*C. jejuni* biot. 2	PEN 27
F_2		+	„ „	PEN 27
F_3		+	„ „	PEN 27
F_4	+		„ „	PEN 27
G_1		+	„ „	PEN 21
G_2	+		„ „	PEN 21
G_3		+	„ „	PEN 21
G_4	+		„ „	PEN 21
G_5	+		„ „	PEN 21
H		+	*C. coli*	NT
I		+	*C. coli*	NT
J		+	*C. laridis*	NT
Healthy cats				
K		+	*C. jejuni* biot. 1	NT
L_1	+		„ „	LAU 16
L_2		+	„ „	NT
M_1		+	„ „	NT
M_2	+		„ „	NT
N		+	„ „	PEN 19
O	+		„ „	LAU 14/PEN 19

[1] The letters A through O refer to 15 different individuals.

[2] Biotypes were defined by the criteria of *Skirrow & Benjamin* (1980).

[3] The LAU prefix refers to serotypes of *Lauwers* and the PEN prefix refers to serotypes of *Penner* (*Lauwers & Penner* 1984). NT; not typable with available antisera.

Biochemical and serological characterization

Out of 68 strains recovered in this study, 19 canine and 7 feline isolates from 10 dogs and 5 cats, respectively, were subjected to further biochemical and serological characterization. Twelve (46.2 %) of these 26 strains belonged to Campylobacter jejuni biotype 1, 11 (42.3 %) to C. jejuni biotype 2, and 2 (7.7 %) were classified as C. coli. One strain (3.8 %) belonged to the nalidixic-acid-resistant group of thermophilic campylobacters, C. laridis (Table 3). Of the 26 strains tested, 14 (53.8 %) fell into 7 different serotypes, while the remaining 12 strains were not typable (NT) with the available antisera (Table 3).

One dog and 1 cat harboured 2 different serotypes (Table 3). In each case, the biotype was identical, thus emphasizing the need for serotyping to discriminate strains isolated from the same individual. This study was too limited to justify any definite conclusions as regard the relative distribution of individual serotypes among cats and dogs. Three of the serotypes encountered in this study have previously been associated with human Campylobacter enteritis in Norway. One of these was obtained from a diarrhoeic dog, the other two being isolated from healthy individuals. The serotypes concerned were PEN 27, LAU 3/16, and LAU 16, respectively.

Comparison of selective media

The relative efficacy of CAK versus Skirrow's agar is presented in Table 4. Out of 43 positive samples encountered, 34 (79.1 %) were detected by Skirrow's medium and 24 (55.8 %) by CAK. This difference was statistically significant ($\chi^2 = 5.29$, $P < 0.025$). Hence, Skirrow's medium proved superior to CAK agar for isolation of thermophilic campylobacters from pets.

T a b l e 4.   Comparison of 2 selective agar media for recovery of thermophilic campylobacters from pets.

| Selective media | | No. of positive samples | | |
CAK	Skirrow's	Dogs	Cats	Total (%)
+	+	12	3	15 (34.9)
+	—	7	2	9 (20.9)
—	+	14	5	19 (44.2)
Total		33	10	43 (100.0)

+ = isolation of thermophilic campylobacters.
— = no isolation of thermophilic campylobacters.

DISCUSSION

More than 30 years ago, the isolation of so called "spirochetal organisms'" from the faeces of both healthy and diarrhoeic dogs was resported (*Blaser et al.* 1984). The bacteria concerned were later identified as C. jejuni and other Campylobacter spp. (*Blaser et al.* 1984). Pets were early recognized as a potential reservoir for human campylobacteriosis (*Skirrow* 1977). Moreover, some recent investigations have indicated that thermophilic campylobacters may act as potential enteric pathogens in pets (*Fleming* 1983, *Fox et al.* 1983, *Davies et al.* 1984, *Fox et al.* 1984), although absolute proof is still lacking. A causal relationship to canine abortion has also been suggested (*Bulgin et al.* 1984).

The isolation frequencies reported from dogs and cats have varied widely, from approximately 0—50 % (*Bruce et al.* 1980, *Fleming* 1980). The faecal carriage rates recorded in this study are markedly higher than those reported from Denmark and Sweden (*Jørgensen* 1981, *Svedhem & Kayser* 1981). However, the use of different isolation techniques and other variable factors, like clinical status, environment of the pets, may have influenced results, thus making comparisons difficult.

Although no statistically significant relationship between Campylobacter infection and clinical status was found in this study, the possibility cannot be excluded that these bacteria may cause enteritis in dogs and cats. Young pets may be infected early in life, leading to serological immunity and a subsequent healthy carrier state. Such circumstances make it difficult to implicate the organism as a cause of diarrhoea (*Prescott & Munroe* 1982). Demonstration of a rising antibiody titre, together with specific clinical symptoms, and positive stool cultures, would be required to establish a causal relationship to canine or feline gastrointestinal disease. Valuable information may also be provided by experimental infections. However, oral challenge of kittens and puppies has so far given conflicting results (*Prescott & Karmali* 1978, *Prescott & Barker* 1980, *Fox et al.* 1983).

In this investigation, Skirrow's selective agar medium recovered more positive samples than did the CAK agar (Table 4). In contrast, CAK proved superior to Skirrow's medium for isolation of thermophilic campylobacters from poultry carcasses in a previous study (*Rosef et al.* 1984). Differences in the background flora and in the number and type of campylobacters present may explain this discrepancy. In both studies, best recovery was

achieved when both media were used in combination. The patterns of biotypes and serotypes recovered by the 2 media were not notably different, though this study was too limited to justify firm conclusions.

A few cases of human Campylobacter enteritis have been described in which dogs or cats have been implicated as the source of infection (*Bruce et al.* 1980, *Hay & Ganguli* 1980, *Skirrow et al.* 1980, *Svedhem & Norkrans* 1980, *Blaser et al.* 1982). Most of these cases involved children, who had been in close contact with a pet animal suffering from diarrhoea (*Skirrow* 1981). According to *Skirrow* (1981), probably no more than 5 % of the human cases in Britain have been associated with dogs or cats (*Prescott & Munroe* 1982). The present results indicate that Norwegian pets may also constitute a reservoir for human infection. Three of the serotypes recovered in this study have previously been implicated in human campylobacteriosis in our country (*Kapperud et al.* 1984). However, since the factors responsible for virulence are unknown, it is quite possible that isolates from dogs and cats may not be pathogenic for man, even though they are serologically identical to human isolates. This circumstance emphasizes the need for effective pathogenicity models capable of screening Campylobacter isolates for potential virulence.

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SAMMENDRAG

Isolasjon av termofile Campylobacter fra norske hunder og katter.

Fæces fra 147 hunder og 85 katter ble undersøkt for forekomst av termofile Campylobacter. Bakteriene ble isolert fra 33 (22,4 %) av hundene og 10 (11,8 %) av kattene. Forekomsten hos hunder og katter med og uten diaré var ikke signifikant forskjellig. Hos unge dyr kontra voksne, og hos hanndyr kontra hunndyr, fant en heller ingen signifikant forskjell i bærerfrekvens. Nitten stammer isolert fra hunder og 7 stammer fra katter ble underkastet biotyping og serotyping. 88,5 % ble klassifisert som C. jejuni biotype 1 og 2, 7,7 % som C. coli og 3,8 % som C. laridis. Tre stammer tilhørte serotyper som tidligere har vært isolert i forbindelse med human campylobacteriose i Norge.