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ISOLATION OF CAMPYLOBACTER FETUS SUBSP. JEJUNI FROM FAECES OF NORWEGIAN POULTRY

By

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ROSEF, O. and G. KAPPERUD: *Isolation of Campylobacter fetus subsp. jejuni from faeces of Norwegian poultry.* Acta vet. scand. 1982, *23*, 128—134. — Pooled faeces samples from 106 poultry flocks in Norway were examined. Campylobacter fetus subsp. jejuni was isolated from 10 of 100 chicken flocks and from 4 of 6 turkey flocks. Eight of the 14 isolates were classified as biotype C. jejuni, which is frequently associated with human campylobacteriosis. Five strains belonged to the biotype C. coli. One strain was resistant to nalidixic acid but differed from the biotype NARTC in its ability to hydrolyse hippurate. The results indicate that C. fetus subsp. jejuni is widespread among Norwegian poultry.

Campylobacter fetus subsp. jejuni; poultry; Norway.

Bacteria belonging to the genus Campylobacter have attracted considerable interest during the past decade due to an increasing frequency of isolations from animal and human sources (*Skirrow* 1977, *Smibert* 1978, *Butzler & Skirrow* 1979). Campylobacter fetus subsp. jejuni is now recognized as an important causal agent in human enteritis. This bacterium is frequently encountered in the intestinal tract of both domestic and wild-living animal species, most of which seem to be healthy carriers. Nevertheless, the epidemiology of human campylobacteriosis is incompletely understood. The significance of these bacteria in veterinary medicine and in food hygiene requires further evaluation.

King (1962) was the first to suggest an epidemiological link between humans and poultry, and since then, poultry has been implicated in several cases of human campylobacteriosis (*Hayek*

& Cruickshank 1977, *Skirrow, Butzler & Skirrow*). An antigenic relationship between human and poultry isolates has recently been indicated (*Lior* 1981).

The present study was undertaken in order to assess the occurrence of C. fetus subsp. jejuni in pooled faeces samples from Norwegian poultry.

MATERIALS AND METHODS

Collection

Pooled faeces samples from a total of 106 poultry flocks were examined. Of these 106 flocks, 100 consisted of hens and broiler chickens, and 6 were turkeys. Faeces were collected from the cages in which the birds were transported to the abattoir. Each cage contained 15 chickens or 3—5 turkeys, and 10—15 cages were sampled per flock. Faeces samples were collected in sterile glass jars which were submitted to the Institute of Food Hygiene, Veterinary College of Norway, for microbiological investigation. This was performed within 3 days, the samples meanwhile being stored at room temperature.

Isolation

Each sample was blended with a sterile glass rod. One loopful was streaked out onto each of 2 agar plates containing Skirrow's selective medium (*Skirrow* 1977). Incubation was performed at 42 °C for 48 h in a microaerobic atmosphere using the GasPak system (BBL Microbiological Systems 60626) without catalyst. All colonies morphologically similar to Campylobacter spp. were subjected to phase contrast microscopy (1000 ×). Bacteria showing the typical motility and cell morphology suggestive of Campylobacter were subjected to further cultural-biochemical examination.

Characterization

The ability to grow under aerobic or anaerobic conditions was assessed after incubation at 42 °C for 48 h. Growth at 25 °C was tested in a microaerobic atmosphere. Catalase activity was tested on microscopic slides by addition of 1 drop of 3 % H_2O_2. Oxidase activity was examined on filter paper with 1 % aqueous solution of tetramethyl-*p*-phenylene-diamine dihydrochloride. The ability to grow in a 3.5 % NaCl solution was determined

using the broth described by *Rosef* (1981), modified by omitting the antibiotic components and adjusting the concentration of NaCl. H_2S production was recorded in the TSI medium (Triple Sugar Iron). The strains were tested for growth on blood agar containing 2,3,5-triphenyl-tetrazolium chloride (TTC) at a concentration of 400 µg/ml.

Hydrolysis of hippurate was tested by the rapid method described by *Hwang & Ederer* (1975) as modified by *Skirrow & Benjamin* (1980 b). Antibiotic susceptibility was evaluated on blood agar by means of commercial antibiotic discs (Neo-Sensitabs Rosco) containing 130 µg nalidixic acid, 78 µg erythromycin, 100 µg streptomycin, or 16 µg metronidazole. Bacteria showing inhibition zones of $\geq$ 28 mm after incubation at 37 °C for 24 h, were considered sensitive.

RESULTS

Campylobacter fetus subsp. jejuni was isolated from 14 (13.2 %) of 106 poultry flocks represented by pooled faeces samples. Only 1 strain was obtained from each sample. C. fetus subsp. jejuni was recovered from 10 (10.0 %) of 100 chicken flocks and from 4 of 6 turkey flocks (Table 1).

Table 1. Isolation of Campylobacter fetus subsp. jejuni from pooled faeces samples from Norwegian poultry flocks.

Source	Number of samples*	Samples with Campylobacter	
		number	%
Hens/chickens	100	10	10.0
Turkeys	6	4	66.7
Total	106	14	13.2

* Each sample represents 1 poultry flock.

All strains showed catalase and oxidase activity. Growth under aerobic conditions was not observed. Under anaerobic conditions, very small colonies were produced. None of the isolates was able to grow in 3.5 % NaCl solution. H_2S production was not detected in the TSI medium. All strains grew on TTC agar. Nine of the 14 isolates were able to hydrolyse hippurate. No strain was resistant to erythromycin, 1 was resistant to nalidixic acid, 2 to streptomycin, and 3 to metronidazole (Table 2).

Table 2. Cultural-biochemical characteristics and antimicrobial susceptibility of 14 strains of Campylobacter fetus subsp. jejuni isolated from Norwegian poultry.

Parameter	Source of isolation	
	Hens/chickens (10)*	Turkeys (4)*
Cultural-biochemical characters		
Catalase activity	10	4
Oxidase activity	10	4
Growth on TTC agar	10	4
Growth in 3.5 % NaCl	0	0
H_2S production (TSI)	0	0
Hippurate hydrolysis	8	1
Sensitivity to antibiotics		
Erythromycin	10	4
Streptomycin	10	2
Nalidixic acid	9	4
Metronidazole	7	4

* Figures in parentheses represent the total number of strains examined.

DISCUSSION

Campylobacter fetus subsp. jejuni is frequently isolated from the caecal contents or faeces of healthy poultry (*Smibert* 1978, *Butzler & Skirrow* 1979). The bacteria have also been isolated from commercially processed chickens (*Simmons & Gibbs* 1979). C. fetus subsp. jejuni was recovered from 83 % of rectal swabs from broiler chickens in the USA (*Grant et al.* 1980). In Sweden, 6.6 % of hens and 1 % of chickens examined were intestinal carriers of C. fetus subsp. jejuni (*Persson* 1981). Likewise, this microbe was recovered from 100 % of the turkeys, 98 % of the ducks, and 8 % of the hens in a survey conducted in Denmark (*Jørgensen* 1980). In Norway, C. fetus subsp. jejuni has previously been isolated from frozen chickens and ducks imported from Denmark (*Rosef & Bjorland* 1981). The present results indicate that C. fetus subsp. jejuni is also widespread among Norwegian poultry (Table 1). Ten per cent of the chicken flocks and 4 of 6 turkey flocks were infected. It is possible that an even higher isolation rate might have been obtained if the samples had been subjected to more favourable storage conditions. Only

pooled faeces samples were investigated. Hence, no conclusion can be made as to the exact prevalence of C. fetus subsp. jejuni among the birds examined.

The antibiotic susceptibility and the cultural-biochemical characteristics of the strains reported in this work, have been compared with the properties of porcine and human isolates from Norway in a concurrent publication (*Rosef & Yndestad* 1982).

Skirrow & Benjamin (1980 b) recognized 4 biotypes among C. fetus subsp. jejuni, which they named: C. jejuni biotype 1 and 2, C. coli, and NARTC (Nalidixic Acid Resistant Thermophilic Campylobacters). C. jejuni is prevalent in the avian fauna including both wild and domestic species, whereas C. coli predominates among swine (*Skirrow & Benjamin* 1980 a). Furthermore, C. jejuni is the most frequently encountered biotype in human campylobacteriosis, while C. coli is less commonly involved in human infections. The majority of the NARTC strains has been isolated from gulls. Although this biotype is recovered very occasionally from human clinical specimens, its clinical significance is dubious.

In the present investigation, 7 of the 10 strains isolated from hens and chickens were classified as C. jejuni and 2 as C. coli. The remaining strain was resistant to nalidixic acid, but differed from NARTC by its ability to hydrolyse hippurate and by sensitivity to metronidazole. Three of the 4 isolates from turkeys belonged to C. coli, and 1 was C. jejuni.

In conclusion, Norwegian poultry harbour C. fetus subsp. jejuni belonging to the same biotype as the bacteria associated with human enteritis. Furthermore, evidence has been presented relating Campylobacter enteritis in an abattoir worker to contamination from chickens (*Ertzaas,* personal communication). Thus, more work will be needed to further elucidate the role of poultry as a reservoir of human campylobacteriosis in Norway.

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SAMMENDRAG

Campylobacter fetus subsp. jejuni i faeces fra norske fjørfe.

Samleprøver av faeces fra 106 fjørfebesetninger ble undersøkt. Campylobacter fetus subsp. jejuni ble isolert fra 10 av 100 besetninger med høns og broilere, og fra 4 av 6 kalkunbesetninger. Åtte av de 14 stammene som ble funnet tilhørte biotype C. jejuni som hyppig isoleres i forbindelse med Campylobacter-enteritt hos mennesker. Fem stammer tilhørte biotypen C. coli. En stamme var Nalidixan-resistent, men adskilte seg fra biotypen NARTC ved evnen til å hydrolysere hippurat. Resultatene indikerer at C. fetus subsp. jejuni er vanlig blant norske fjørfe.

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