Epidemiological investigations on *Campylobacter jejuni* in households with a primary infection

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SUMMARY

Fifty-four Rotterdam patients in which a primary infection with *Campylobacter jejuni* had been detected (index patients) were compared with 54 control subjects with regard to the consumption and preparation of foods 7 days before onset of illness and the keeping of pet animals. Significantly more index patients than controls had eaten chicken meat (47 *v.* 29; $P = 0.0002$), particularly at barbecues (14 *v.* 2; $P = 0.0015$). Marginally more index patients had eaten pork (47 *v.* 39; $P = 0.048$) or inadequately heated meat (13 *v.* 8), though in the last case numbers were too small to be statistically significant. The consumption of beef or mutton and outdoor eating (other than at barbecues) were essentially the same in both groups. There was no significant association with the keeping of pet animals, although a few more index patients had cage birds than controls (18 *v.* 12).

Twenty-one (15 %) of 136 household contacts of index patients also suffered from diarrhoea during the same period. Circumstantial evidence pointed to a common source of infection with the index patient in 13 instances (nine households) and probable intrafamilial spread of infection in six instances.

Campylobacters were isolated from one of 110 swabs of kitchen work surfaces and eight of 107 swabs taken from lavatory bowls in index households.

INTRODUCTION

In recent years *Campylobacter jejuni* has been recognized as an important cause of acute enteritis in man (Skirrow, 1977; Anonymous, 1981). In The Netherlands about 10 % of all patients with acute diarrhoea have campylobacter infections (Severin, 1978).

It is generally accepted that campylobacter enteritis is a food-borne infection, although the epidemiology of *C. jejuni* is not completely clear. Chickens, pigs, sheep, cattle, dogs, cats and wild birds have been found to be carriers of this organism, in most cases without showing any symptoms of disease (Devriese & Devos, 1971; Oosterom, 1980; Clark & Monsbourgh, 1979; Al-Mashat & Taylor, 1980; Bruce, Zochowski & Fleming, 1980; Smibert, 1969; Luechtefeld *et al.* 1980). The foods that are most often implicated are, as a consequence, those of animal origin, particularly poultry and unpasteurized milk (Severin, 1982; Robinson & Jones, 1981). Apart from foods, dogs are a possible direct source of infection to man – in particular young ones with diarrhoea (Blaser *et al.* 1978).

The object of this study was to identify significant epidemiological factors for the acquisition of campylobacter enteritis by means of a case control study.

MATERIALS AND METHODS

Selection of patients

The laboratory of the Municipal Public Health Service in Rotterdam provides for the microbiological examination of stools for general practitioners, outpatient clinics, and a number of hospitals in the Rotterdam region. It serves a population of about 1100000 people. In 1982 the laboratory received some 11000 primary faecal samples and isolated *C. jejuni* from 8·5 %. The equivalent isolation rates of other bacteria were: *Salmonella* spp., 5·1 %; *Shigella* spp., 1·4 %; *Yersinia enterocolitica*, 1·0 % (Bänffer, unpublished data).

Patients with proven campylobacter infection were selected at random on those days that one of us (C. H. U.) was available to visit them. The investigations were carried out from June to September 1982. Fifty-four patients with a primary infection with *C. jejuni* (index patients) living in 54 households (index households) were studied.

Household enquiry

Visits were paid to index households as soon as possible after the first isolation of *C. jejuni* from a stool sample, usually 5–8 days after the onset of the index patient's symptoms. During these visits an enquiry was made according to the following protocol:

(*a*) *Index patients*

    (1) Name, sex, age, occupation.

    (2) Date of onset, nature and duration (follow up by telephone) of symptoms.

    (3) Consumption of chicken meat, pork, beef or mutton during the 7 days before onset of disease, and whether eaten raw or undercooked. Modes of food preparation (including barbecues) and eating outdoors.

(4) Travel abroad in the two weeks before onset of disease.

(5) Presence of pet animals: species, numbers, degree of contact, any illness, whether given the same food as consumed by human patients.

(b) Household contacts

As for index patients, but in addition: relationship to index patient and foods consumed in common with index patient and other household members.

(c) Control subjects

After every visit to an index patient an enquiry was made regarding another person living in the same street as the index patient. These controls were also selected at random. The only criterion was that they had not experienced symptoms of enteritis for 2 weeks before the enquiry. The enquiry for controls was the same as that for index patients except that item (2) was not applicable and the questions listed under items (3) and (4) related respectively to the 7 or 14 days before the day of enquiry.

All enquiries, both for index cases and controls, were made by one and the same investigator.

Bacteriological sampling of households

After the questioning of the index patients, arrangements were made for the collection of stool samples from index patients and, as far as possible, from household members with symptoms of enteritis. In addition, faecal specimens were collected from pet animals.

In the index households, swabs were taken from surfaces in kitchens (working surfaces, sinks, refrigerators) and from lavatory bowls. Swabs consisted of several layers of cotton, bound together to form a ball of about 5 cm diameter. Directly before use, the swabs were moistened with sterile physiological saline. The kitchen work surfaces were mostly dry, but the lavatory bowl surfaces were mostly wet.

Cultural methods

First stool samples from index patients were cultured on campylobacter selective agar made to the Butzler formula (sheep blood agar containing the Oxoid SR 85 supplement) and incubated under microaerobic conditions (6% O_2, 10% CO_2 and 84% N_2) for 72 h at 42 °C.

Subsequent stool samples from index patients and household contacts (human and animal) were transported (usually within 24 h) to the Laboratory for Water and Food Microbiology at Bilthoven, where they were cultured in THAL enrichment broth (thioglycollate broth BBL 20 ml, 7 % lysed horse blood, vancomycin 40 mg/l, trimethoprim 20 mg/l, polymyxin B-sulphate 10000 i.u./l, cefalothin 100 mg/l, actidione 100 mg/l and sodium lauryl sulphate 1 g/l: Oosterom, Vereijken & Engels, 1981). After incubation for 18 h at 37 °C they were subcultured on Skirrow's agar (Skirrow, 1977), to which the growth-promoting supplement Oxoid SR 84 had been added. This agar was incubated microaerobically at 42 °C for 48 h. Surface swabs were put into 100 ml of THAL broth and treated in the same way.

Table 1. *The consumption of foods and methods of food preparation in relation to the occurrence of campylobacter infection*

Food/method of preparation	Index patients ($n = 54$)	Controls ($n = 54$)	Significance (P value)
Chicken meat	47	29	0·0002
Pork	47	39	0·048
Beef	43	44	NS
Mutton	2	4	NS
Raw or inadequately heated meat	13	8	NS
Barbecue	14	2	0·0015
Eating outdoors	17	17	NS

NS = not significant ($P > 0.05$).

Antibody detection

Attempts were made to collect three serum samples from each patient and each other member of index households. These serum samples were tested in parallel for campylobacter antibodies using an ELISA-technique developed in our laboratory. In this test campylobacter cells disrupted in a Mickle disintegrator were used as antigen. Total (IgG plus IgM) antibodies were measured. An antibody titre of 1:640 (or a fourfold drop in antibody titre from first to third serum sample) was considered to be indicative of current campylobacter infection. An exact description of this technique and the results obtained from it will be presented elsewhere (Oosterom *et al.* 1984).

RESULTS

Index patients

The age distribution of index patients was typical of campylobacter enteritis in Western Europe. Thirty of the 54 patients were aged between 10 and 40 years; only four were less than 1 year old. The median duration of diarrhoea was 6 days. Fever ranging from 38·0 to 40·2 °C was noted in 39 (72 %) patients. Three patients were also infected with salmonellae but suffered illness of no more than average severity or duration.

Evaluation of risk factors

Food consumption and preparation

Consumption of relevant foods in the index patients and controls is listed in Table 1. Chicken meat, particularly when eaten at barbecues, was the only food strongly associated with the index cases. There was a small but marginally significant association with pork consumption, but none with beef or mutton. Raw or inadequately heated meat was consumed more frequently by the index patients, but the numbers were too low to show a significant difference.

Pet animals

The number of households keeping pet animals was essentially the same in both groups (Table 2). More index than control households kept cage birds, but the numbers recorded were too low to show a significant difference.

Table 2. *The presence of pet animals in relation to the occurrence of
campylobacter infection*

Presence of animals	Index households (n = 54)	Control households (n = 54)	Significance (P value)
Pet animals in general	33	27	NS
Dogs	13	13	NS
Cats	12	12	NS
Cage birds	18	12	NS
Chickens	1	1	NS
Rodents	4	5	NS

NS = not significant ($P > 0.05$).

Travelling abroad

The numbers of index patients and controls that had travelled abroad were almost the same (five and six respectively).

Infection in household contacts

In the 54 index households there were 136 members additional to the index patients (0–6 per household). Of these 136 contacts, 21 (15%) living in 15 (28%) households also suffered from diarrhoea during the same period. Eleven of them had one or more campylobacter-positive stools (six of 10 tested were serologically positive) and five of those with negative cultures had serological evidence of infection. The remaining five contacts had typical symptoms but with negative laboratory tests. Six out of 27 symptomless contacts tested had serological evidence of infection.

Of the 21 symptomatic contacts, 13 (in nine households) had eaten the same suspected food as their index patients and were considered to have been infected from this common source; the onset of illness in these patients was generally within 2 days of the index patient. In four patients the timing of illness (onset more than 4 days later than in the index patient), and details of the foods eaten suggested that they had been secondarily infected from their index cases. In the remaining four contacts the source of infection was uncertain, but two of them had apparently been the source of infection for two of the index patients.

Bacteriological examination of surface swabs

Campylobacters were isolated from eight of 107 lavatory-bowl swabs (7·5%) and from one of 110 swabs from kitchen surfaces (0·9%) in the 54 index households.

Bacteriological examination of household animals

Thirty-three index households (61%) kept 92 animals (14 dogs, 16 cats, 41 cage birds, five rodents, 15 chickens and one goat). One dog and one cat had campylobacters in their faeces but neither showed signs of illness. Also the chickens had *C. jejuni* in their droppings.

DISCUSSION

The results of our case control study showed that in The Netherlands the consumption of chickens prepared in the home, and particularly at barbecues, is an important factor in the epidemiology of campylobacter enteritis. There was also evidence that pork can be a source of infection.

Earlier investigations in The Netherlands also indicated poultry as a source of campylobacter infection (Severin, 1982). Other studies in this country showed that poultry and pigs were frequently infected with campylobacters (Hartog & de Boer, 1982; Oosterom, 1980), whereas only 5·5 % of cattle carried these organisms, mostly in low numbers (Oosterom et al. 1982). Campylobacters were isolated from 50–75 % of poultry products (Oosterom et al. 1983a). These products have repeatedly been incriminated as a source of campylobacter enteritis (Skirrow, 1977; Brouwer et al. 1979; Severin, 1982; Mouton et al. 1982; Kist, 1982). In contrast, pork generally shows a low contamination rate (Teufel, 1982; Turnbull & Rose, 1982) and is seldom reported as a source of human campylobacter infection (Anonymous, 1981). An explanation for this difference was found in our laboratory when it was shown that campylobacters were killed by the drying effect of forced ventilation used for cooling the carcasses of slaughtered pigs; C. jejuni appeared very sensitive to drying (Oosterom et al. 1983b). Cattle and lamb carcasses are usually cooled in the same way, so that campylobacter contamination of beef or mutton might also be expected to be low. The cooling of chicken carcasses is carried out differently, and in many instances there is little or no reduction in the numbers of campylobacters (Oosterom et al. 1983a).

The transmission of campylobacters from chicken carcasses to the consumer may be by way of undercooked meat, but it seems more likely that the handling of raw poultry (Norkrans & Svedhem, 1982) and subsequent cross-contamination to hands, surfaces, and other foods, whether in the kitchen or at a barbecue, is a more important factor. Studies in family kitchens have shown that the handling of frozen poultry inoculated with Escherichia coli K12 causes extensive contamination of surfaces, utensils and hands (de Wit, Broekhuizen & Kampelmacher, 1979).

Poultry is also frequently contaminated with salmonellae and it is therefore not surprising that three cases of combined salmonella and campylobacter infection were found.

Although exact data are lacking, it is common knowledge that barbecues are popular in The Netherlands, particularly in the summer months, so the fact that the investigations described here were carried out between June and September could have accentuated the contribution made by barbecueing to infection.

Other investigators reported that eating at restaurants (Severin, 1982) and the presence of pet animals (Severin, 1982; Norkrans & Svedhem, 1982) were significant factors for acquiring campylobacter enteritis. These findings were not confirmed by our study. Moreover, in only three instances did we find pet animals carrying campylobacters, and none had obvious clinical symptoms. The role of milk consumption was not evaluated in our studies, first because almost all milk is pasteurized in The Netherlands, and secondly because in this country raw milk did not appear to be contaminated with campylobacters (Oosterom et al. 1982).

In complete accordance with a study from Sweden in which 55 cases were

investigated (Norkrans & Svedhem, 1982), we found six cases of presumptive person-to-person spread. In only one of these was a baby implicated, which contrasts with the experience of Butzler & Skirrow (1979).

We cultured campylobacters from only 0·9 % of kitchen surfaces and 7·5 % of lavatory bowls in the households of infected patients. Similar studies in households with salmonella infections showed that 18 % of kitchen surfaces were contaminated, but in these studies only households with infected babies were selected (van Schothorst, Huisman & Van Os, 1978). The scarcity of campylobacters on kitchen surfaces can be explained by their extreme sensitivity to drying (Oosterom *et al.* 1983 *b*). This finding, together with the fact that *C. jejuni* does not grow below 30 °C (Skirrow & Benjamin, 1980), means that cross-contamination of foods is probably of less consequence in the case of campylobacters than with salmonellae.

We conclude that the most significant risk factor for the acquisition of campylobacter enteritis in a typical urban area of The Netherlands is the handling and consumption of chickens in the house, particularly at barbecues.

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