STUDIES ON THE EPIDEMIOLOGY OF *CAMPYLOBACTER JEJUNI*

ONDERZOEKINGEN NAAR DE EPIDEMIOLOGIE VAN *CAMPYLOBACTER JEJUNI*

PROEFSCHRIFT

ter verkrijging van de graad van doctor in de geneeskunde
aan de Erasmus Universiteit Rotterdam
op gezag van de Rector Magnificus
Prof. Dr. M.W. van Hof
en volgens besluit van het college van dekanen

De openbare verdediging zal plaats vinden
op woensdag 16 januari 1985 te 15.45 uur

door

JOHANNES OOSTEROM

geboren te Rotterdam

DRUKKERIJ ELINKWIJK B.V. — UTRECHT

Begeleidingscommissie:

Promotoren:	Prof.Dr. M.F. Michel
	Prof.Dr. E.H. Kampelmacher
Overige leden:	Prof.Dr. J. Huisman
	Prof.Dr. J.-P. Butzler (Brussel)

The investigations described in this thesis were carried out at the former National Institute of Public Health, now National Institute of Public Health and Environmental Hygiene, at Bilthoven, the Netherlands, in close collaboration with a number of scientific workers located elsewhere in the country.

To Els

Something long
is a long body of Buddha
Something short
is a short body of Buddha

Zenrin-poems,
compiled by Toyo Eicho
(1429-1504)

CONTENTS

CHAPTER I

GENERAL INTRODUCTION

CHAPTER I

GENERAL INTRODUCTION

In July 1977, Skirrow demonstrated in an article published in the British Medical Journal that Campylobacter jejuni, a bacterial species that could grow only in a micro-aerobic atmosphere, was an important cause of acute enteritis in man (Skirrow, 1977). Although Butzler and co-workers in Belgium had already shown some years earlier that this microorganism was associated with the occurrence of human enteritis (Butzler et al., 1973; Butzler, 1974), Skirrow's publication was the first that drew international attention. Since it also described for the first time a convenient method for the isolation of C.jejuni from human stools, it activated many other laboratories to seek this enteropathogen. Over a short period of time it became clear that C.jejuni was occurring in many different countries and that between 4 and 14 % of patients with acute enteritis were infected with the organism, whereas the prevalence of C.jejuni in the stools of normal controls, at least in industrialized countries, usually was negligible (Butzler et al., 1973; Skirrow, 1977; Severin, 1978; Steele and McDermott, 1978; Pai et al., 1979). In some investigations it was shown that campylobacter infections occurred more frequently than those caused by Salmonella spp. (Bruce et al., 1977; Telfer Brunton and Heggie, 1977; Pearson et al., 1977).

From the beginning it was suspected that campylobacteriosis was mainly a food-borne infection. Also, it was found that many animal species, both wild and domesticated, were healthy intestinal carriers of

C.jejuni (Elazhary, 1968; Smibert, 1969; Devriese and Devos, 1971; Clark and Monsbourgh, 1979; Oosterom, 1980; Bruce et al., 1980; Luechtefeld et al., 1980). In consequence, special attention was paid to foodstuffs of animal origin as a possible source of human infection. Although there remained many gaps in existing knowledge of the epidemiology of C.jejuni, it was found that poultry meat and raw milk in particular were transmitting the organism to the consumer (Grant et al., 1980; Robinson and Jones, 1981). In addition, contaminated drinking water was also reported to be the cause of a few large campylobacter outbreaks (Mentzing, 1981; Vogt et al., 1982; Palmer et al., 1983). Apart from foods and water, dogs seemed to be responsible for the direct transmission to man, especially in the case of puppies suffering from diarrhoea (Blaser et al., 1978).

C.jejuni seemed also to be an important enteropathogenic organism in the Netherlands, as was indicated by Severin (1978). For this reason investigations were initiated at the former National Institute of Public Health, now the National Institute of Public Health and Environmental Hygiene, in order to gather more information about the epidemiology and related public health aspects of campylobacter infections in this country. In view of the prevalence of C.jejuni in healthy animals, in foods of animal origin and in patients with enteritis, it was suspected that many epidemiological characteristics of C.jejuni would resemble those of Salmonella spp. It was with existing knowledge of salmonella epidemiology in mind that many of the investigations described here were carried out. The aim of these present studies was to find an answer to each of the following questions:

1. What are, in the Dutch situation, the sources of C.jejuni occurring in animals and the environment that might result in public health

hazards?

2. From which of these sources does C.jejuni effectively reach man and how is the organism transmitted?
3. What are the effects of campylobacter infection in man?
4. What preventive measures might be considered in order to reduce the incidence of these infections?

Following an account of the historical and taxonomic aspects of C.jejuni in Chapter II, clinical features associated with C.jejuni infection are described in Chapter III. A survey of laboratory techniques for the isolation, identification, transport and storage of strains is given in Chapter IV. Attempts are made in Chapter V to answer the first question regarding possible sources of campylobacter infection. Part of Chapter V also deals with the second question concerning the actual transmission of the organism to man. More information on this subject is presented in Chapter VI (epidemiological surveys in households) and Chapter VIII (typing of campylobacter strains of different origin). Data regarding the third question on the effects on human health are given in Chapter VI and also in Chapter VII, which deals with serological responses after infection. Possible preventive measures are considered in the general discussion presented in Chapter IX. The work is summarized in Chapter X.

References

Blaser, M.; Cravens, J.; Powers, B.W. and Wang, W.L. Campylobacter enteritis associated with canine infection. Lancet 1978. ii, 979-981.

Bruce, D.; Zochowski, W. and Ferguson, I.R. Campylobacter enteritis. Br. Med. J. 1977. ii, 1219.

Bruce, D.; Zochowski, W. and Fleming, G.A. Campylobacter infections in dogs and cats. Vet. Rec. 1980. 107, 200-201.

Butzler, J.P. Campylobacter, een miskende enteropathogene kiem. Thesis Vrije Universiteit Brussel 1974.

Butzler, J.P.; Dekeyser, P.; Detrain, M. and Dehaen, F. Related vibrio in stools. J. Pediatr. 1973. 82, 493-495.

Clark, B.L. and Monsbourgh, M.J. The prevalence of Campylobacter fetus in the gall bladder of sheep. Aust. Vet. J. 1979. 55, 42-43.

Devriese, L. and Devos, A. Vibrio spp. bij pluimvee: isolatie, identificatie en experimentele infectie. Tijdschr. Diergeneeskd. 1971. 96, 193-201.

Elazhary, M.A.S.Y. An assay of isolation and differential identification of some animal Vibrios and of elucidation of their pathological significance. Meded. Veeartsenijschool Univ. Gent 1968. 12.

Grant, I.H.; Richardson, N.J. and Bokkenheuser, V.D. Broiler chickens as a potential source of Campylobacter infections in humans. J. Clin. Microbiol. 1980. 11, 508-510.

Luechtefeld, N.A.W.; Blaser, M.J.; Reiller, L.B. and Wang, W.-L.L. Isolation of Campylobacter fetus subsp. jejuni from migratory waterfowl. J. Clin. Microbiol. 1980. 12, 406-408.

Mentzing, L.O. Waterborne outbreaks of Campylobacter enteritis in central Sweden. Lancet 1981. ii, 352-354.

Oosterom, J. Het voorkomen van Campylobacter fetus subspecies jejuni bij normale slachtvarkens. Tijdschr. Diergeneeskd. 1980. 105, 49-50.

Pai, C.H.; Sorger, S.; Lackman, L.; Sinai, R.E. and Marks, M.I. Campylobacter gastroenteritis in children. J. Pediatr. 1979. 94, 589-591.

Palmer, S.R.; Gully, P.R.; White, J.M.; Pearson, A.D.; Suckling, W.G.; Jones, D.M.; Rawes, J.C.L. and Penner, J.L. Water-borne outbreak of Campylobacter gastroenteritis. Lancet 1983. i, 287-290.

Pearson, A.D.; Suckling, W.G.; Ricciardi, I.D.; Knill, M. and Ware, E. Campylobacter-associated diarrhoea in Southampton. Br. Med. J. 1977. ii, 955-956.

Robinson, D.A. and Jones, D.M. Milk-borne campylobacter infection. Br. Med. J. 1981. i, 1374-1376.

Severin, W.P.J. Campylobacter en enteritis. Ned. Tijdschr. Geneeskd. 1978. 122, 499-504.

Skirrow, M.B. Campylobacter enteritis: a "new" disease. Br. Med. J. 1977. ii, 9-11.

Smibert, R.M. Vibrio fetus var. intestinalis isolated from the intestinal content of birds. Am. J. Vet. Res. 1969. 30, 1437-1442.

Steele, T.W. and McDermott, S. Campylobacter enteritis in South Australia. Med. J. Aust. 1978. 2, 404-406.

Telfer Brunton, W.A. and Heggie, D. Campylobacter-associated diarrhoea in Edinburgh. Br. Med. J. 1977. 2, 956.

Vogt, R.L.; Sours, H.E.; Barrett, T.; Feldman, R.A.; Dickinson, R.J. and Witherell, L. Campylobacter enteritis associated with contaminated water. Ann. Intern. Med. 1982. 96, 292-296.

CHAPTER II

HISTORY AND TAXONOMY

CHAPTER II

HISTORY AND TAXONOMY

Many decades ago the first investigations concerning the organism that is now called Campylobacter jejuni were carried out. In 1931, the bacterium was found in cows and calves (Jones et al., 1931) and in 1944 it was detected in pigs by Doyle (Doyle, 1944). Because of the resemblance of the isolates to Vibrio fetus, a well-known cause of abortion and sterility in cattle, they were named Vibrio jejuni and Vibrio coli respectively. These two new members of the genus Vibrio were considered responsible for the occurrence of enteritis in animals. For many years Vibrio coli was even thought to be the causative agent of the so-called "Vibrio dysentery" or "Dysentery Doyle", a haemorrhagic enteritis in pigs (Doyle, 1944; 1948), until it was shown that Treponema hyodysenteriae was associated with this disease, and not Vibro coli (Akkermans and Pomper, 1973). A similar vibrio-like organism was also found in poultry, in which it would be likely to cause hepatitis (Hofstad et al., 1958).

In 1959 the "real" Vibrio fetus was divided into two new subspecies (Florent, 1959). These were Vibrio fetus subsp. venerialis, the causative agent of abortion and sterility in cattle which does not occur in other animal species or in man, and Vibrio fetus subsp. intestinalis, which is associated with abortion in sheep, occurs in many animal species and is an opportunist pathogen in man in which it may cause septicaemia, meningitis and abortion (Table II.1).

Table II.1 Nomenclature of campylobacter species and subspecies

Initial name	King 1957	Florent 1959	Véron and Chatelain 1973	Smibert (Bergey) 1974	Smibert (Bergey) 1984
V. foetus		V. fetus subsp. venerialis	C. fetus subsp. venerealis	C. fetus subsp. fetus	C. fetus subsp. venerealis
		V. fetus subsp. intestinalis	C. fetus subsp. fetus	C. fetus subsp. intestinalis	C. fetus subsp. fetus
V. jejuni	related vibrio		C. jejuni	C. fetus subsp. jejuni	C. jejuni
V. coli			C. coli		C. coli

In a search for these opportunist pathogens in the blood of debilitated people, Elisabeth King isolated vibrios of a different kind; they could for instance grow at 42°C, whilst Vibrio fetus subsp. intestinalis could not. King observed that these "related vibrios" were identical with Vibrio jejuni found in cattle (King, 1957; 1962). For the first time in the study of this organism the epidemiological link was made between animals and the occurrence of enteritis in man.

In 1973, the group of vibrios discussed here was transferred to a new genus, Campylobacter, belonging to the family Spirillaceae (Vèron and Chatelain, 1973). Vibrio fetus subsp. venerialis became Campylobacyter fetus subsp. venerealis, Vibrio fetus subsp. intestinalis became Campylobacter fetus subsp. fetus and the "related vibrio" or Vibrio jejuni was renamed Campylobacter jejuni. One year later, a divergent nomenclature was published in the eighth edition of Bergey's Manual of Determinative Bacteriology (Smibert, 1974), according to which the last three species were respectively named Campylobacter fetus subsp. fetus, Campylobacter fetus subsp. intestinalis and Campylobacter fetus subsp. jejuni (Table II.1). For some time, many authors have adopted the latter nomenclature, and considerable confusion has resulted. In the latest edition of Bergey's Manual of Systematic Bacteriology, however, the author has returned to the officially accepted nomenclature of Vèron and Chatelain (Smibert, 1984). Further complications arise from the fact that some investigators tend to use the name Campylobacter coli for those strains of Campylobacter jejuni that show a negative reaction for hippurate hydrolysis.

In the early seventies, the group of Butzler in Brussels demonstrated that C.jejuni could be isolated from the stools of many patients with diarrhoea by means of a filtration technique (Butzler et

al., 1973) and for the first time the prevalence of the disease could be assessed. Later, Skirrow developed a more convenient method for the isolation of C.jejuni (Skirrow, 1977) and the results of epidemiological surveys that followed led to a worldwide recognition that C.jejuni is a major cause of enteritis in man.

References

Akkermans, J.P.W.M. and Pomper, W. Aethiology and diagnosis of swine dysentery (Doyle). Tijdschr. Diergeneeskd. 1973. 98, 649-654.

Butzler, J.P.; Dekeyser, P.; Detrain, M. and Dehaen, F. Related vibrio in stools. J. Pediatr. 1973. 82, 493-495.

Doyle, L.P. A Vibrio associated with swine dysentery. Am. J. Vet. Res. 1944. 5, 3-5.

Doyle, L.P. The ethiology of swine dysentery. Am. J. Vet. Res. 1948. 9, 50-51.

Florent, A. Les deux vibrioses génitales. La vibriose vénérienne due à V. fetus venerialis et la vibriose d'origine intestinale due à V. fetus intestinalis. Meded. Veeartsenijsch. Rijksuniv. Gent 1959. 3, 1-60.

Hofstad, M.S.; McGehee, E.H. and Bennett, P.C. Avian infectious hepatitis. Avian Dis. 1958. 2, 358-364.

Jones, F.S.; Orcutt, M. and Little, R.B. Vibrios (Vibrio jejuni N. Sp.) associated with intestinal disorders of cows and calves. J. Exp. Med. 1931. 53, 853-864.

King, E.O. Human infections with Vibrio fetus and a closely related Vibrio. J. Inf. Dis. 1957. 101, 119-128.

King, E.O. The laboratory recognition of Vibrio fetus and a closely related Vibrio isolated from cases of human vibriosis. Ann. N. Y. Acad. Sci. 1962. 98, 700-711.

Skirrow, M.B. Campylobacter enteritis: a "new" disease. Br. Med. J. 1977. ii, 9-11.

Smibert, R.M. Campylobacter. In: Buchanan, R.E. and Gibbons, N.E. (eds.). Bergey's Manual of Determinative Bacteriology, 8th edition. Williams and Wilkins, Baltimore 1974. 204-212.

Smibert, R.M. Campylobacter. In: Tansil, B. (ed.). Bergey's manual of systematic bacteriology. William and Wilkins, Baltimore 1984. 1, 111-118.

Véron, M. and Chatelain, R. Taxonomic study of the genus Campylobacter Sebald and Véron and designation of the neotype strain for the type species, Campylobacter fetus (Smith and Taylor) Sebald and Véron. Int.J.Syst.Bacteriol. 1973. 23, 122-134.

CHAPTER III

CLINICAL FEATURES OF *CAMPYLOBACTER JEJUNI* INFECTION IN MAN

CHAPTER III

CLINICAL FEATURES OF *CAMPYLOBACTER JEJUNI* INFECTION IN MAN

In essence, human campylobacter enteritis is similar to other enteric infections like salmonellosis or shigellosis. However, symptoms caused by campylobacteriosis are usually considered to be somewhat more distressing than those caused by most other enteropathogens.

In general, two distinct syndromes associated with C.jejuni infection are discerned. In one case, influenza-like symptoms occur initially and may include malaise, headache, myalgia, dizziness, abdominal pain and fever with body temperatures up to 40°C (Butzler and Skirrow, 1979). This stage may last for one to two days before the diarrhoea becomes manifest. C.jejuni is an invasive organism (Duffy et al., 1980), and these prodromal symptoms could be ascribed to the occurrence of bacteraemia. The organism is, indeed, isolated from the blood of campylobacter patients (King, 1957; Wheeler and Borchers, 1961; Muytjens and van Dis, 1978).

In other cases, diarrhoea occurs from the onset of disease. Stools may be watery or slimy, and after a few days may contain fresh blood, particularly when young children are affected (Karmali and Fleming, 1979; Blaser et al., 1979). Generally, diarrhoea is observed for about two to three days, although in severe cases it may last for several weeks (Skirrow, 1977; Svedhem and Kaijser, 1980). The most prominent symptom during campylobacter enteritis is abdominal cramps. These may persist

for many weeks, even when other symptoms have long subsided. The occurrence of severe abdominal pain, together with the presence of fresh blood in the stools may sometimes lead to acute hospitalization on suspicion of intussusception, particularly in the case of young children (Butzler and Skirrow, 1979). In adults, inflammation of the jejunum and ileum and the presence of mesenteric adenitis may mimic an "acute abdomen" (Skirrow, 1977). The appendix itself, however, is seldom affected.

In some patients the infection is not limited to the small intestine, but may affect the colon as well. Such cases of enterocolitis may show a very slow recovery, and may even present as presumptive Crohn's disease or chronic ulcerative colitis (Lambert et al., 1979; Colgan et al., 1980). In some instances, C.jejuni even seems to be responsible for the flare-up of established, chronic colitis (Newman and Lambert, 1980). The occurrence of campylobacter proctitis has been demonstrated in homosexual men (Carey and Wright, 1979; Quinn et al., 1980).

Skirrow reported that the average, adult patient is not able to perform his daily duties for about 10 to 14 days (Skirrow, 1977), but probably the duration and severity of campylobacter-associated symptoms can vary as widely as in other bacterial enteric infections, and it may be assumed that a number of campylobacter infections occur unnoticed.

Males and females are about equally affected by the disease. Regarding age distribution, campylobacter infections seem to be predominant in older children and young adults (Butzler and Skirrow, 1979; Severin, 1978). Average incubation time ranges from three to five days, with a maximum of seven to 10 days (Butzler and Skirrow, 1979; Duffell and Skirrow, 1978). Excretion of the organism may occur for a

period of two to five weeks, but sometimes a period of several months is involved (Butzler and Skirrow, 1979).

Although most campylobacter infections recover without the need for specific therapy, antibiotics are sometimes used with the intention of shortening the period of illness. The drug of choice seems to be erythromycin, because of its rapid intestinal absorption and its narrow spectrum of activity (which means that the normal gut flora is little changed) (Butzler and Skirrow, 1979). However, it has been shown that the administration of erythromycin immediately interrupts the faecal excretion of campylobacters, but medication usually starts too late to shorten the duration of clinical symptoms (Anders et al., 1982; Mandal et al., 1984).

In some sporadic cases campylobacter infection was found to be associated with disease patterns other than enteritis; these included reactive arthritis (Berden et al., 1979; Weir et al., 1979), cystitis (Davies and Penfold, 1979), meningitis (Thomas et al., 1980; Norrby et al., 1980), carditis (Pönkä et al., 1980), abortion (Gilbert et al., 1981), appendicitis (Megraud et al., 1982), erythema (Ellis et al., 1982) and urticaria (Pitkänen et al., 1981).

References

Anders, B.J.; Lauer, B.A.; Paisley, J.W. and Reller, L.B. Double-blind placebo controlled trial of erythromycin for treatment of campylobacter enteritis. Lancet 1982. i, 131-132.

Berden, J.H.M.; Muytjens, H.L. and Putte L.B.A. van de. Reactive arthritis associated with Campylobacter jejuni enteritis. Br. Med. J. 1979. i, 380-381.

Blaser, M.J.; Berkowitz, I.D.; LaForce, F.M.; Cravens, J.; Reller, L.B. and Wang, W.-L.L. Campylobacter enteritis: clinical and epidemiological features. Ann. Intern. Med. 1979. 91, 179-185.

Butzler, J.P. and Skirrow, M.B. Campylobacter enteritis. Clin. Gastroenterol. 1979. 8, 737-765.

Carey, P.B. and Wright, E.P. Campylobacter jejuni in a male homosexual. Br. J. Vener. Dis. 1979. 55, 380.

Colgan, T.; Lambert, J.R.; Newman, A. and Luk, S.C. Campylobacter jejuni enterocolitis. Arch. Pathol. Lab. Med. 1980. 104, 571-574.

Davies, J.S. and Penfold, J.B. Campylobacter urinary infection. Lancet 1979. i, 1091-1092.

Duffell, S.J. and Skirrow, M.B. Shepherd's scours and ovine campylobacter abortion. A "new" zoonosis? Vet. Rec. 1978. 103, 144.

Duffy, M.C.; Benson, J.B. and Rubin, S.J. Mucosal invasion in Campylobacter enteritis. Am. J. Clin. Pathol. 1980. 73, 706-708.

Ellis, M.E.; Pope, J.; Mokashi, A. and Dunbar, E. Campylobacter colitis associated with erythema nodosum. Br. Med. J. 1982. ii, 937.

Gilbert, G.L.; Davoren, R.A.; Cole, M.E. and Radford, N.J. Midtrimester abortion associated with septicaemia caused by Campylobacter jejuni. Med. J. Aust. 1981. 1, 585-586.

Karmali, M.A. and Fleming, P.C. Campylobacter enteritis in children. J. Pedriatr. 1979. 94, 527-533.

King, E.O. Human infections with Vibrio fetus and a closely related Vibrio. J. Inf. Dis. 1957. 101, 119-128.

Lambert, M.E.; Schofield, P.F.; Ironside, A.G. and Mandal, B.K. Campylobacter colitis. Br. Med. J. 1979. i, 857-859.

Mandal, B.K.; Ellis, M.E.; Dunbar, E.M. and Whale, K. Double-blind placebo-controlled trial of erythromycin in the treatment of clinical campylobacter infection. J. Antimicrob. Chemoth. 1984. 13, 619-623.

Megraud, F.; Tachoire, C.; Latrille, J. and Bondonny, J.M. Appendictis due to Campylobacter jejuni. Br. Med. J. 1982. ii, 1165-1166.

Muytjens, H.L. en van Dis, P. Campylobacter fetus subspecies jejuni als verwekker van diarree. Ned. Tijdschr. Geneeskd. 1978. 122, 504-506.

Newman, A. and Lambert, J.R. Campylobacter jejuni causing flare-up in inflammatory bowel disease. Lancet 1980. ii, 919.

Norrby, R.; McCloskey, R.V.; Zackrisson, G. and Falsen, E. Meningitis caused by Camplobacter fetus ssp jejuni. Br. Med. J. 1980. i, 1164.

Pitkänen, T.; Pettersson, T.; Pönkä, A. and Kosunen, T.U. Clinical and serological studies in patients with Campylobacter fetus ssp. jejuni infection. I. Clinical findings. Infection 1981. 9, 274-277.

Pönkä, A.; Pitkänen, T.; Pettersson, T.; Aittoniemi, S. and Kosunen, T.U. Carditis and arthritis associated with Campylobacter jejuni infection. Acta Med. Scand. 1980. 208, 495-496.

Quinn, T.C.; Corey, L.; Chaffee, R.G.; Schuffler, M.D. and Holmes, K.K. Campylobacter proctitis in a homosexual man. Ann. Intern. Med. 1980. 93, 458-459.

Severin, W.P.J. Campylobacter en enteritis. Ned. Tijdschr. Geneeskd. 1978. 122, 499-504.

Skirrow, M.B. Campylobacter enteritis: a "new" disease. Br. Med. J. 1977. ii, 9-11.

Svedhem, A. and Kaijser, B. Campylobacter fetus subsp. jejuni: A common cause of diarrhea in Sweden. J. Inf. Dis. 1980. 142, 353-359.

Thomas, K.; Chan, K.N. and Ribeiro, C.D. Campylobacter jejuni/coli meningitis in a neonate. Br. Med. J. 1980. i, 1301-1302.

Weir, W.; Keat, A.C.; Welsby, P.D. and Brear, G. Reactive arthritis associated with Campylobacter infection of the bowel. J. Infect. 1979. 1, 281-284.

Wheeler, W.E. and Borchers, J. Vibrionic enteritis in infants. Am. J. Dis. Child. 1961. 101, 60-66.

CHAPTER IV

ISOLATION, BIOCHEMICAL IDENTIFICATION, TRANSPORT AND STORAGE OF *CAMPYLOBACTER JEJUNI* STRAINS

Most investigations presented in this Chapter are also described in the following publications:

Oosterom, J. Het voorkomen van Campylobacter fetus subspecies jejuni bij normale slachtvarkens. Tijdschr.Diergeneeskd. 1980. 105, 49-50.

Oosterom, J.; Vereijken, M.J.G.M. and Engels, G.B. Campylobacter isolation. Vet.Quart. 1981. 3, 104.

Oosterom, J. and Beckers, H.J. Campylobacter outbreak in a military camp: investigations, results and further epidemiological studies. In: Newell, D.G. (ed.). Campylobacter. Epidemiology, pathogenesis and biochemistry. MTP Press Ltd., Lancaster 1982. 288-289.

CHAPTER IV

ISOLATION, BIOCHEMICAL IDENTIFICATION, TRANSPORT AND STORAGE OF *CAMPYLOBACTER JEJUNI* STRAINS

Introduction

The first human strains of C.jejuni were isolated from blood samples (King 1957, 1962) by means of a method initially intended for the isolation of Vibrio fetus (now called Campylobacter fetus subsp. venerealis) from cattle. Isolations were made in a micro-aerobic atmosphere since earlier veterinary investigations had shown that this bacterial species grew best under conditions of reduced oxygen and increased carbon dioxide concentration (Stegenga and Terpstra, 1949). However, attempts at that time to isolate the organism from human faecal flora still involved so much difficulty, that only bacteraemia could be recognized as a sign of infection in man.

Cells of C.jejuni are somewhat smaller than those of most other bacterial species (0.2 to 0.5 µm in width and 0.5 to 0.8 µm in length; Smibert, 1974), and it is therefore not surprising that the first improvements in isolation were obtained when initial suspensions were filtered through 0.65 µm membrane filters, after which each filtrate was streaked on a solid medium containing antibiotics (Dekeyser et al., 1972). With this technique, it became possible for the first time to isolate C.jejuni from stools and other materials contaminated with a variety of microorganisms.

The most important achievements followed the discovery by Skirrow

(1977) that incorporation of certain other antibiotics and chemotherapeutic agents in the plating medium eliminated the need for prior filtration of the test material. From then on, the isolation of C.jejuni became a relatively simple matter which could be carried out on a large scale, and the importance of C.jejuni as a human pathogen was soon recognized in many different laboratories.

Until now, the method described by Skirrow has been generally employed, be it that various mixtures of antimicrobial compounds have been suggested for the purpose. Skirrow himself used vancomycin, polymyxin B-sulphate and trimethoprim (Skirrow, 1977). Blaser et al. (1979) recommended the addition of cephalothin to the compounds used by Skirrow, while Butzler suggested a mixture containing bacitracin, novobiocin, colistin and cephalothin (Lauwers et al., 1978). Recently, it was found that cefoperazone, a third generation cephalosporin, was one of the most suitable antibiotics for the isolation of C.jejuni (Goossens et al., 1983). In addition, cycloheximide or amphotericin B are often added to media containing the above compounds in order to suppress the growth of moulds and yeasts.

After inoculation of the media they should be incubated under micro-aerobic conditions, that is, in an atmosphere containing approximately 6 % O_2 and 10 % CO_2. This can be achieved by replacing 2/3 of the air in an anaerobic jar by a mixture containing 15 % CO_2, or by the use of special envelopes in anaerobic jars without catalysts (Wang et al., 1982; de Boer and Hartog, 1982). Growth does not occur in an aerobic or anaerobic atmosphere and generally is poor in the so-called candle jar (Smibert, 1978; George et al., 1978).

Some years ago it was reported by George et al. (1978) that aerotolerance of C.jejuni could be enhanced by the addition of three

compounds (ferrous sulphate, sodium metabisulphite and sodium pyruvate) to the medium. It was subsequently observed that the use of these chemicals did not entirely obviate the need for a micro-aerobic atmosphere, but had some advantages when a candle jar was employed. Today, the compounds listed above are widely used, even when incubation is under micro-aerobic conditions, but there seems to be little point in this practice (Severin, personal communication, 1979).

Incubation of solid media is usually for two or three days at a temperature of 37°C or 42°C. Incubation at 42°C is to be preferred since it provides an additional selective factor in the isolation of C.jejuni.

In the course of time, several enrichment media have been proposed. Most of them were based on the composition of the solid media described by Skirrow or Butzler (Park et al., 1981; Lander, 1982; Bolton and Robertson, 1982; Doyle and Roman, 1982), but so far, little information is available regarding their efficiency. A similar enrichment medium was produced in our laboratory and its development, composition and efficiency will be described below.

Development of an enrichment medium

The bacteriological examination of materials from patients using the solid media and incubation conditions described above does not provide any problem for well-equipped laboratories. The situation is rather different, however, when epidemiologial investigations are to be carried out. Material from animals or from the environment may have a quite different flora from that of human stools. In addition, C.jejuni may be present in very diverse physiological conditions, for instance on frozen poultry or in prepared meat. For this reason, there was a need for a fluid medium to enrich samples before plating on one of the available

solid media. There was also a further reason for developing an enrichment medium. In epidemiological surveys there are many types of material that are unsuitable for direct plating, e.g. pieces of meat, swabs taken from surfaces, litter from poultry houses, contaminated water, but these can be conveniently incubated in a fluid medium. The two considerations led to our search for a suitable enrichment method.

In an initial survey, four liquid media were tested. Three of them are known as transport media (Cary-Blair medium, Stuart transport medium and Amies transport medium) and had been tested already for their ability to maintain strains of C.jejuni (Lior and Krol, 1979). The fourth was described by Waterman (Autumn Meeting of the Society for Applied Bacteriology, London, 1980) and consisted of thioglycollate broth, lysed horse blood and the Skirrow mixture of antimicrobial compounds, supplemented with a fungicide. The latter medium was somewhat modified (THA broth) before it was tested in our laboratory. The exact composition of all four media is given in the Annex to this Chapter. Because C.jejuni appears to be capable of growth in an intestinal milieu, the addition of bile as a means of improving the isolation of this organism was also evaluated. Hence, Cary-Blair transport medium and the THA enrichment medium were tested with and without the addition of 1 % ox bile.

Campylobacter-contaminated minced pork was used to evaluate the media. Fifty gram quantities of the pork were each inoculated with 5 ml of an appropriate dilution of a fully-grown culture of C.jejuni in Brain Heart Infusion broth. The number of organisms introduced was assessed by inoculating duplicate plates of plain sheep blood agar with 0.1 ml inocula of each dilution used. Because C.jejuni cannot grow at temperatures below 30°C, it was possible to store the contaminated

samples of minced pork overnight at room temperature or in a refrigerator before they were examined. In this way, it was assumed that natural conditions were simulated as closely as possible. The same minced pork, without added C.jejuni, was used as control sample. Mean counts of other bacterial groups in the samples of minced meat were: total aerobes 10^6/g; psychrophiles 10^5/g and Enterobacteriaceae 10^3/g.

For enrichment purposes, 2 g of each sample were transferred to 20 ml of liquid medium. To inoculate the semi-solid medium (Stuart, Amies) the minced meat was stirred with small swabs which were added respectively to 8 and 4 ml of the medium. All media were incubated at 37°C for 24 h, the liquid media under rotation in a micro-aerobic atmosphere. After incubation, one loopful of each liquid medium was streaked on Skirrow's agar, sometimes Butzler's agar (see Annex). Swabs removed from Stuart medium and Amies medium were streaked on the same solid media. The plates were incubated for 48 h at 37°C in a micro-aerobic atmosphere and afterwards examined for C.jejuni. The results of three of these experiments are recorded in Table IV.1. The conclusion from the data thus listed was that THA enrichment broth with the addition of 1% bile gave the best results regarding isolation of C.jejuni and suppression of the associated flora.

Next, an attempt was made to determine the optimum proportion of ox bile for use in THA enrichment broth. For that purpose, essentially the same experiments as those described above were carried out. It was found that the proportion of ox bile present was not critical for the isolation of C.jejuni but that a concentration of about 1.5 % was slightly to be preferred. In another study, it was shown that the growth of pure cultures of C.jejuni was not inhibited by bile concentrations of about 1-4%, so that the THA-enrichment broth with 1.5 % ox bile appeared to be

Table IV.1 Comparison of different enrichment media for the isolation of C. jejuni from artificially contaminated minced pork

	Enrichment media																	
	THA						Cary-Blair						Stuart			Amies		
Number of	I*)			II			I			II			I			I		
C. jejuni per gram of pork	1	2	3a)	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
4.2×10^7	+b)	+	+	+	+	+	+	+	v	+	+	−	+	+	+	+	+	+
4.2×10^5	+	+	+	+	+	+	+	+	v	+	v	v	+	+	−	+	+	+
4.2×10^3	+	+	+	+	+	+	+	+	v	+	v	v	−	+	−	−	+	+
42	−	−	−	+	+	+	−	+	v	−	v	v	−	−	−	−	−	−
control	−	−	−	−	−	−	−	−	v	−	v	v	−	−	−	−	−	−

*) I = without bile
II = with 1 % ox-bile

a) results of three experiments

b) + = growth of C. jejuni after subsequent plating on a solid medium
− = no growth of C. jejuni on solid medium
v = solid medium overgrown by associated flora

Table IV.2 The effect of adding ox-bile to THA enrichment medium for campylobacter isolation

Examined materials	No. examined	Medium without bile no. positive	Medium with 1.5 % bile no. positive
Chicken carcasses	40	15	37
Chicken livers	22	6	19
Human faeces	176	11	12
Dog faeces	23	1	2
River water and sewage	29	0	4

the most suitable combination. The improved efficiency of the medium by the addition of ox bile was further evaluated by examining several other naturally contaminated materials like chicken carcasses, chicken livers, human stools, dog faeces, river water and sewage. The chicken carcasses were shaken in a sterile plastic bag which contained 500 ml thioglycollate broth. Two millilitres of this broth and 2 g or 2 ml of each of the other materials were incubated in 20 ml of the enrichment broth. Further isolation methods, as described previously, were followed and the solid medium of Skirrow was used for plating. The results are listed in Table IV.2. It may be concluded from these data that ox bile has a clear, positive effect in the isolation of C.jejuni, except when bile is already present in the sample material, i.e. in faeces.

As ox bile, whether collected at the slaughterhouse or obtained in dry form from the industry, is a substance that is difficult to standardize, it was substituted, after several experiments, by sodium lauryl sulphate which is chemically defined. A concentration of 0.1 % of this substance gave optimal results, comparable to those obtained with the medium containing ox bile. Using this liquid medium with sodium lauryl sulphate (termed THAL enrichment medium) further investigations were carried out on minced pork, artificially contaminated with campylobacters, to assess its efficiency in isolating C.jejuni from among the other organisms present. The results of these investigations showed that three to 10 campylobacter cells per gram of heavily contaminated minced meat can be detected by means of THAL enrichment broth with subsequent plating on Skirrow's agar.

Isolation methods used in epidemiological investigations

During the period of our epidemiological investigations slight modifications were made in the campylobacter isolation methods used. In this section the different media and methods employed will be described. The exact composition of each medium is given in the Annex.

Media, both liquid and solid, were always incubated under micro-aerobic conditions. In most cases, the required atmosphere was established by using anaerobic jars (without catalysts) and replacing 2/3 of the air present by a mixture of 15 % CO_2 and 85% N_2. Sometimes, anaerobic envelopes (BBL) were used, again in anaerobic jars without catalysts. It was shown that this latter technique was just as effective as the former (de Boer and Hartog, 1982).

The first samples collected during the investigations were plated directly on Butzler's agar (Lauwers et al., 1978) and incubated at 42°C for two days. Similar samples were also plated on Skirrow's agar (Skirrow, 1977). Later, the growth promoting chemicals described by George et al. (1978) and marketed by Oxoid as supplement SR 84 were added to Skirrow's agar. As indicated before, this supplement has only minimal advantages with micro-aerobic incubation, but may be of value if the right atmosphere is not maintained, for instance by leakage of the jars.

Enrichment was carried out at 37°C for 16-20 h in 20 ml or 100 ml quantities of enrichment broth. It always took place under rotation, while tubes and glass jars containing the inoculated broth were not made air-tight, so that an adequate exchange of gases between the medium and the micro-aerobic atmosphere was ensured. Initially, THA-broth was used and this contained vancomycin, polymyxin B-sulphate, trimethoprim and, as a fungicide, cycloheximide. Later, sodium lauryl sulphate was added to the medium (THAL-broth). During studies on the antimicrobial sensitivity

of C.jejuni, it was confirmed that this organism is very resistant to cephalothin, while most other bacterial species are very sensitive to this agent (Muytjens et al., 1981). Therefore, cephalothin was added to the antimicrobial mixture used in THAL, thus, in fact, establishing the combination of compounds suggested by Blaser for a solid selective campylobacter medium (Blaser et al., 1979).

Confirmation of isolates

After incubation for 48 h C.jejuni can be seen growing on the solid selective medium (and on plain blood agar) either as small, round, gleaming colonies or as flat and somewhat spreading ones. Both types are easily detected among other colonies by their greyish-brown colour. A first and most important stage in identification is by way of phase-contrast microscopy of the live organism: C.jejuni appears in a hanging droplet as a small, spiral-shaped (one to three windings), highly motile bacterium. Preliminary biochemical examination of cultures checked for purity on plain sheep blood agar shows that the organism is positive for both catalase and oxidase.

If the isolate is obtained from one of the special selective media, in particular when incubated at 42°C, and the above observations are made, it is almost certain that the organism in question belongs to the species C.jejuni. However, if incubation is at 37°C, it is necessary to differentiate C.jejuni from C.fetus subsp. fetus. The two species can be distinguished by growth tests in Brain Heart Infusion broth, involving incubation at 25°C (for five days) and at 42°C (for two days). C.jejuni grows at 42°C, but not at 25°C; C.fetus subsp. fetus grows at 25°C, but not at 42°C. C.fetus subsp. venerealis, which does not cause disease in man, has the same temperature characteristics as C.fetus subsp. fetus

(Table IV.3). Only one other species, C.fecalis, could be confused with C.jejuni. However, C.fecalis produces sufficient H_2S to blacken Triple Sugar Iron agar, while the production of H_2S by C.jejuni can only be demonstrated by means of lead acetate paper. Hence, inoculation and incubation of Triple Sugar Iron agar may be used to distinguish between the two species (Table IV.3).

Other tests that may be done are utilisation of glucose and growth in 3.5 % NaCl. Both tests are usually negative for Campylobacter spp., but positive for the organisms now classified as vibrios. Furthermore, campylobacters reduce nitrate into nitrite (although Doyle (1944; 1948) described the same organisms as nitrate negative) and can grow in 1% glycine medium, with the exception of C.fetus subsp. venerealis (Table IV.3).

In our investigations, isolates were confirmed as C.jejuni by the results of the following tests (characteristics of C.jejuni in parenthesis): catalase (+), oxidase (+), growth in Brain Heart Infusion broth at 25°C after five days (-) and at 42°C after two days (+), reduction of nitrate to nitrite in a liquid medium (+), production of H_2S in Triple Sugar Iron agar (-) and utilisation of 0,5 % glucose in peptone water (-). Media were incubated in a micro-aerobic atmosphere, if necessary under rotation. The last three media were incubated at 37°C for two days.

The name C.jejuni is used for all isolates described in this study. Some investigators adopted the name C.coli for isolates that show a negative result in the test for hippurate hydrolysis (Skirrow and Benjamin, 1980), but it does not seem advisable to the present author to base species differentiation on the result of only one biochemical reaction.

Table IV.3 Biochemical differentiation of some campylobacter species and subspecies

	Catalase	Oxidase	H_2S (TSI-agar)	Growth in: 1 % glycine	Growth in: 3.5 % NaCl	Growth at: 25°C	Growth at: 42°C
C. fetus subsp. venerealis	+*)	+	-	-	-	+	-
C. fetus subsp. fetus	+	+	-	+	-	+	-
C. jejuni	+	+	-	+	-	-	+
C. fecalis	+	+	+	+	v	-	+

*) + = positive reaction
- = negative reaction
v = variable reaction

Transport and storage of isolates

C. jejuni does not readily survive transportation. It has been found that even fully-grown cultures degenerate rapidly when on solid media. However, cultures sometimes survive for weeks in Stuart's medium when held at room temperature, whereas at other times, for reasons unknown, they die within a number of days.

Satisfactory and consistent results were finally obtained with the preservation medium described by Balakrish Nair and Pal (1982). Although this medium (Brain Heart Infusion agar overlaid with thioglycollate broth) was intended for storage of cultures under refrigeration, it was found in our laboratory that it can keep C. jejuni strains viable for more than three weeks at room temperature, which means that in this medium strains can practially be sent all over the world.

In our laboratory, 30 ml glass bottles with screw-caps are used for transportation purposes. Each bottle contains 20 ml of Brain Heart Infusion agar, and after cooling, thioglycollate broth is added until the bottle is completely full (for the exact composition of these media see the Annex to this Chapter). Inoculation of the final medium is done by stabbing into the agar, after which the bottles are closed to make them air-tight. Recovery of the inoculated strains is achieved by transferring the thioglycollate broth into, for instance, Brain Heart Infusion broth and incubating appropriately. The original site of inoculation in the agar is also transferred to Brain Heart Infusion broth by means of a wire-loop, and incubated as before.

Long-time preservation is achieved by holding cultures at $-70^{\circ}C$. For this purpose, strains are again cultured in Brain Heart Infusion broth. After incubation at $37^{\circ}C$ for two days in a micro-aerobic

atmosphere under rotation, 1 ml of this broth is transferred to 2 ml polypropylene tubes containing about 14 sterile glass beads of 5 mm diameter (Nagel and Kunz, 1972). After adding 0.15 ml glycerol, the tubes are sealed, shaken and frozen at -70°C. Retrieval of strains can be carried out by removing one glass bead from the tube by means of forceps, without the necessity to thaw the medium. Each glass bead is first rolled over the surface of a plate containing plain sheep blood agar and then transferred to a test tube containing Brain Heart Infusion broth. Thus, following incubation, both a liquid and solid culture are available and purity of the isolate can be checked anew.

References

Balakrish Nair, G. and Pal, S.C. Short-term preservation medium for Campylobacter fetus subspecies jejuni. Indian J. Med. Res. 1982. 76, 692-695.

Blaser, M.J.; Berkowitz, I.D.; LaForce, F.M.; Cravens, J.; Reller, L.B. and Wang, W.-L.L. Campylobacter enteritis: clinical and epidemiological features. Ann. Intern. Med. 1979. 91, 179-185.

Boer, E. de and Hartog, B.J. De isolatie van Campylobacter jejuni uit voedingsmiddelen - Spreidplaatmethode. Ware(n) Chemicus 1982. 12, 14-23.

Bolton, F.J. and Robertson, L. A selective medium for isolating Campylobacter jejuni/coli. J. Clin. Pathol. 1982. 35, 462-467.

Dekeyser, P.; Gossuin-Detrain, M.; Butzler, J.P. and Sternon, J. Acute enteritis due to related Vibrio: First positive stool cultures. J. Infect. Dis. 1972. 125, 390-392.

Doyle, L.P. A Vibrio associated with swine dysentery. Am. J. Vet. Res. 1944. 5, 3-5.

Doyle, L.P. The ethiology of swine dysentery. Am. J. Vet. Res. 1948. 9, 50-51.

Doyle, M.P. and Roman, D.J. Recovery of Campylobacter jejuni and Campylobacter coli from inoculated foods by selective enrichment. Appl. Environ. Microbiol. 1982. 43, 1343-1353.

George, H.A.; Hoffman, P.S.; Smibert, R.M. and Krieg, N.R. Improved media for growth and aerotolerance of Campylobacter fetus. J. Clin. Microbiol. 1978. 8, 36-41.

Goossens, H.; Boeck, M. de and Butzler, J.P. A new selective medium for the isolation of Campylobacter jejuni from human faeces. Eur. J. Clin. Microbiol. 1983. 2, 389-394.

King, E.O. Human infections with Vibrio fetus and a closely related Vibrio. J. Inf. Dis. 1957. 101, 119-128.

King, E.O. The laboratory recognition of Vibrio fetus and a closely related Vibrio isolated from cases of human vibriosis. Ann. N. Y. Acad. Sci. 1962. 98, 700-711.

Lander, K.P. A selective, enrichment and transport medium for campylobacters. In: Newell, D.C. (ed.). Campylobacter. Epidemiology, pathogenesis and biochemistry. MTP Press Ltd., Lancaster 1982. 77-78.

Lauwers, S.; de Boeck, M. and Butzler, J.P. Campylobacter enteritis in Brussels. Lancet 1978. i, 604-605.

Lior, M. and Krol, P. Evaluation of transport media for the recovery of Campylobacter fetus subsp. jejuni. Can. Dis. Wkly. Rep. 1979. 5, 114-116.

Muytjens, H.; Heessen, F.; Ros, J.v.d. and Oosterom, J. In vitro activity of 13 beta-lactam antibiotics. Abstr.12th Intern.Congress of Chemotherapy, Florence, Italy 1981. no. 945.

Nagel, J.G. and Kunz, L.J. Simplified storage and retrieval of stock cultures. Appl. Microbiol. 1972. 23, 837-838.

Park, C.E.; Stankiewicz, Z.K.; Lovett, J. and Hunt, J. Incidence of Campylobacter jejuni in fresh eviscerated whole market chickens. Can. J. Microbiol. 1981. 27, 841-842.

Skirrow, M.B. Campylobacter enteritis: a "new" disease. Br. Med. J. 1977. ii, 9-11.

Skirrow, M.B. and Benjamin, J. "1001" Campylobacters: cultural characteristics of intestinal campylobacters from man and animals. J. Hyg. 1980. 85, 427-442.

Smibert, R.M. Campylobacter. In: Buchanan, R.E. and Gibbons, N.E. (eds.). Bergey's Manual of Determinative Bacteriology, 8th edition. Williams and Wilkins, Baltimore 1974. 204-212.

Smibert, R.M. The genus Campylobacter. Ann. Rev. Microbiol. 1978. 32, 673-709.

Stegenga, T. and Terpstra, J.I. Over Vibrio fetus-infecties bij het rund en "enzoötische" steriliteit. Tijdschr. Diergeneeskd. 1949. 74, 293-296.

Wang, W.-L.L.; Luechtefeld, N.W.; Blaser, M.J. and Reller, L.B. Comparison of Campypak II with standard 5% oxygen and candle jars for growth of Campylobacter jejuni from human feces. J. Clin. Microbiol. 1982. 16, 291-294.

Annex

Composition of media

1. Cary Blair medium

sodium thioglycollate	1.5
disodium hydrogen phosphate	1.1
sodium chloride	5.0
calcium chloride	0.1
distilled water	1000 ml

2. Stuart transport medium

sodium glycerophosphate	10.0
sodium thioglycollate	0.5
cysteine hydrochloride	0.5
calcium chloride	0.1
methylene blue	0.001
agar no. 1 (Oxoid)	5.0
distilled water	1000 ml

3. Amies transport medium

charcoal pharmaceutical neutal	10.0
sodium chloride	3.0
sodium hydrogen phosphate	1.15
potassium dihydrogen phosphate	0.2
potassium chloride	0.2
sodium thioglycollate	1.0
calcium chloride	0.1
magnesium chloride	0.1

agar no. 1 (Oxoid)		4.0
distilled water		1000 ml

4. THA enrichment medium

thioglycollate broth (BBL)		930 ml
trypticase	15.0	
L-cystine	0.5	
dextrose	5.0	
yeast extract	5.0	
sodium chloride	2.5	
sodium thioglycollate	0.5	
resazurin	0.001	
agar	0.75	
distilled water	1000 ml	
lysed horse blood		70.0 ml
vancomycin		0.04
polymyxin B-sulphate		10,000 IU
trimethoprim		0.02
cycloheximide		0.10

4a. THA enrichment medium with ox bile

See under 4., plus	
ox bile	15.0 ml

4b. THAL enrichment medium

see under 4., plus	
sodium lauryl sulphate	1.0

4c. THAL enrichment medium with cephalothin

see under 4., plus	
sodium lauryl sulphate	1.0
cephalothin	0.10

5. Butzler's agar

Oxoid blood agar base no. 2		930 ml
proteose peptone	15.0	
liver digest	2.5	
yeast extract	5.0	
sodium chloride	5.0	
agar	12.0	
distilled water	1000 ml	
pH approx. 7.4		
defibrinated sheep blood		70 ml
bacitracin		25,000 IU
novobiocin		0.005
colistin		10,000 IU
cephalothin		0.015
cycloheximide		0.05

5a. Butzler's agar with growth promoting supplement

see under 5., plus	
ferrous sulphate	0.25
sodium metabisulphite	0.25
sodium pyruvate	0.25

6. Skirrow's agar

Oxoid blood agar base no. 2		930 ml
proteose peptone	15.0	
liver digest	2.5	
yeast extract	5.0	
sodium chloride	5.0	
agar	12.0	
distilled water	1000 ml	
pH approx. 7.4		
lysed defibrinated horse blood		70 ml
vancomycin		0.01
polymyxin B-sulphate		2,500 IU
trimethoprim		0.005

6a. Skirrow's agar with growth promoting supplement

see under 6., plus	
ferrous sulphate	0.25
sodium metabisulphite	0.25
sodium pyruvate	0.25

7. Preservation medium according to Balakrish Nair and Pal

Brain Heart Infusion agar		20 ml
brain heart infusion (Difco)	37.0	
agar (So-bi-gel)	15.0	
distilled water	1000 ml	
Thioglycollate broth (BBL)		10 ml
trypticase	15.0	
L-cystine	0.5	

dextrose	5.0
yeast extract	5.0
sodium chloride	2.5
sodium thioglycollate	0.5
resazurin	0.001
agar	0.75
distilled water	1000 ml

CHAPTER V

THE PREVALENCE OF *CAMPYLOBACTER JEJUNI* IN FARM ANIMALS AND DOMESTIC PETS, IN SLAUGHTERHOUSES AND IN FOODS OF ANIMAL ORIGIN

Most investigations presented in this Chapter are also described in the following publications:

Oosterom, J.; Notermans, S.; Karman, H. and Engels, G.B. Origin and prevalence of Campylobacter jejuni in poultry processing. J.Food Prot. 1983. 46, 339-344.

Oosterom, J.; Dekker, R.; Wilde, G.J.A. de; Kempen-de Troye, F. van and Engels, G.B. Prevalence of Campylobacter jejuni and Salmonella during pig slaughtering. Vet.Quart., accepted.

Oosterom, J.; Wilde, G.J.A. de; Boer, E. de; Blaauw, L.H. de and Karman, H. Survival of Campylobacter jejuni during poultry processing and pig slaughtering. J.Food Prot. 1983. 46, 702-706.

Oosterom, J.; Engels, G.B.; Peters, R. and Pot, R. Campylobacter jejuni in cattle and raw milk in The Netherlands.
J.Food Prot. 1982. 45, 1212-1213.

CHAPTER V

THE PREVALENCE OF *CAMPYLOBACTER JEJUNI* IN FARM ANIMALS AND DOMESTIC PETS, IN SLAUGHTERHOUSES AND IN FOODS OF ANIMAL ORIGIN

Introduction

Even from the earliest investigations it was suspected that domesticated animals play an important role in the epidemiology of C.jejuni (King 1957, 1962). The organism was found not only in poultry (Devriese and Devos, 1971), but also in pigs (Doyle, 1944) and cattle (Jones et al., 1931). Since it had become apparent that C.jejuni was one of the major causes of acute enteritis in man, it was also suspected that campylobacteriosis was mainly a food-borne infection, and that foods of animal origin were mostly to blame (Butzler et al., 1977). A number of studies provided further information about the campylobacter sources among animals and the exact transmission of C.jejuni to man.

In one study in England it was found that more than 60 % of chickens were infected with Campylobacter spp. (Bruce et al., 1977), while in another survey 83 % of live chickens at a market were found to be positive (Grant et al., 1980). Apparently, the animals were healthy intestinal carriers of the organism. Campylobacter spp. were also isolated from poultry products. Simmons and Gibbs (1979) isolated C.jejuni from 48 % of poultry carcasses purchased from shops in England. Similar results were obtained by Park et al. (1981) in Canada. Finally, it was demonstrated in the U.S.A. that there was a high prevalence of the

organism in a turkey processing plant and on turkey end-products (Luechtefeld and Wang, 1981).

With regard to pigs, it was found that these animals too are frequently healthy intestinal carriers of C.jejuni. Initial surveys showed that 72 % and 61 % of pigs respectively harboured Campylobacter spp. (Jørgensen, 1979; Oosterom, 1980). In contrast to poultry meat, pork appeared to be only sporadically contaminated with C.jejuni (Teufel, 1982; Turnbull and Rose, 1982).

Little information is available concerning the prevalence of C.jejuni in cattle and sheep. In one survey Stern (1981) found 2 % of beef and 24 % of lamb carcasses to be positive. On only one occasion was a herd examined extensively; about 50 % of the cattle on a farm in the U.S.A. were found to be excreting campylobacters (Doyle and Roman, 1982a). Apart from an outbreak in the Netherlands, probably caused by eating raw hamburger (Oosterom et al., 1980), no substantial data are available concerning the role of mutton or beef in campylobacter epidemiology. Yet, the prevalence of C.jejuni in cattle must be high in certain countries, considering the numerous and extensive milk-borne campylobacter outbreaks that are reported from countries where pasteurisation of milk is not always customary, such as the U.S.A., Canada and the U.K. (Blaser et al., 1979; Robinson and Jones, 1981; McNaughton et al., 1982). These outbreaks sometimes affect hundreds or even thousands of people, depending on the quantity and distribution of the contaminated milk.

Among pet animals, dogs are frequently found to be carriers of C.jejuni. In one survey, the organism was isolated from 50 % of adult diarrhoeic animals, and from 75 % of puppies (Hastings, 1978), but in a number of other studies the prevalence was much lower, being between 4

and 30 % (Hosie et al., 1979; Fleming, 1980; Svedhem and Kaijser, 1981), and the presumed association between Campylobacter spp. and enteritis in dogs was not always clear (Bruce et al., 1980). As far as public health is concerned, it has been demonstrated that the presence in a family of a puppy with campylobacter enteritis can lead to campylobacteriosis in man (Blaser et al., 1978; Bruce et al., 1980).

C.jejuni has also been isolated from wild animals such as birds, rodents and insects (Smibert, 1969; Fernie and Park, 1977; Rosef and Kapperud, 1983). In addition, pigeons may be highly contaminated (Weber et al., 1981). It must be assumed that these animals may spread such contamination throughout the environment, including surface water. Faecal pollution of surface water or inadequate treatment of drinking water led, in the last few years, to three extensive water-borne campylobacter outbreaks, in which hundreds of people were involved (Mentzing, 1981; Vogt et al., 1982; Palmer et al., 1983). In one outbreak in Vermont (U.S.A.) about 10,000 people were exposed and about 3,000 were affected.

Finally, man himself must be regarded as a possible source of human campylobacteriosis. Although person-to-person spread has been examined in several investigations, it seldom appeared to be important (Butzler and Skirrow, 1979). Only Butzler (1974) found considerable spread among young children in several infant nurseries.

In this Chapter some original investigations concerning possible campylobacter sources in the Netherlands are described, as well as the possibility of resultant contamination of food products. Only drinking water has not been examined, since in the Netherlands this is subject to strict rules concerning hygiene and safety, and treatment procedures are

checked regularly by bacteriological testing of the end-product. It is assumed that this is adequate to ensure the absence of C.jejuni (Anonymous, 1983).

CHAPTER Va

ORIGIN AND PREVALENCE OF *CAMPYLOBACTER JEJUNI* IN POULTRY PROCESSING AND IN POULTRY END-PRODUCTS

Materials and methods

Processing plants

Investigations were made in collaboration with two chicken processing plants in the centre of the Netherlands. One plant (plant A) produced deep-frozen broilers and the other (plant B) fresh broilers. In both cases, the birds were processed at five to six weeks of age, having body weights of approximately 1.3 kg.

Plant A

At this plant, the processing capacity was 5,300 carcasses per hour and the broilers were water-scalded at 58°C for 120 s. Two successive defeathering machines were used in conjuction with 0.5 to 0.7 l of water per carcass. After defeathering, the carcasses entered one of two evisceration lines, were opened and the intestines were exposed by mechanical means. Following veterinary inspection, intestines and other organs were removed manually. The carcasses were spray-cleaned and chilled in a counter-flow spinchiller. After chilling and packing, the carcasses were frozen at -40°C for 20 h and stored at -20°C.

Plant B

The processing capacity at this plant was 9,000 broilers per hour. The carcasses were water-scalded at 51.8°C for 170 s. Feathers were

removed by passing the birds through a series of five successive defeathering machines. During defeathering 0.2 to 0.4 l of water per carcass was used, mainly in the first machine. Evisceration was similar to that used at plant A. After evisceration, the carcasses were spray-cleaned before being air-chilled at 0°C for 55 min.

Samples

Internal organs and intestinal contents of killed, defeathered but unopened broilers

Broilers that were scalded and defeathered in plant A were taken from the conveyer belt and brought to the laboratory under chill conditions (approximately -10°C) within 1 h. Five birds from one flock were collected per investigation. In the laboratory, the animals were opened aseptically and parts of the lungs and liver and the contents of the gall bladder were collected. Samples were also taken from the contents of the duodenum, ileum, caecum and colon. The presence or absence of C.jejuni was determined in all samples. The samples of intestinal contents were also used to assess the number of C.jejuni per gram of material.

Swabs from the processing line

Cotton swabs of 5 cm diameter were moistened with physiological saline and used during processing to sample surfaces of equipment that directly or indirectly came in contact with the birds. After sampling, the swabs were put into glass jars, transported immediately to the laboratory and examined for the presence of C.jejuni.

Processing water

Samples of water were collected from scalding tanks, spray washers, equipment in which the internal organs were washed, and the water-inlet and water-outlet of the spinchiller. Sampling took place at the same

time as the collection of skin portions (see below). The water samples were examined to assess the numbers of C.jejuni and Enterobacteriaceae per ml.

Air samples

Samples of air were collected by means of a Biotest air sampler (Biotest-Serum-Institut GmbH, Frankfurt, Federal Republic of Germany). The strips used for this purpose were filled with Skirrow's agar containing the growth promoting supplement. Samples were collected in plant B at definite sites along the processing line and were used to determine the numbers of C.jejuni per m^3 of air.

Pericloacal skin portions from broilers on the processing line

Pericloacal skin portions, each of approximately 5 g, were collected from the birds after different stages of processing: after bleeding, scalding, defeathering, evisceration and chilling in the spinchiller or chill room. Sampling times were arranged in such a way that during one investigation all skin portions were collected from the same flock of birds (Notermans et al., 1977). Eight skin portions from eight different birds were collected at each stage. They were transferred in pairs to plastic bags, cooled immediately (approximately $-10^{\circ}C$), and examined for the numbers of C.jejuni and Enterobacteriaceae per gram.

Hands of processing line workers

People working at different stages of processing in plant B were requested to put on a sterile plastic glove which contained 15 ml of thioglycollate broth and to knead the glove for 1 min (de Wit and Kampelmacher, 1981). The gloves were tied, put into a cold box (approximately $-10^{\circ}C$) for transportation to the laboratory, and examined to determine the numbers of C.jejuni and Enterobacteriaceae in the fluid.

End-products

Various end-products (whole carcasses, livers, hearts and gizzards) were collected at plant A after final washing but before freezing. These products were brought to the laboratory under chill conditions (approximately $-10^{o}C$) and analyzed for the presence of C.jejuni. Broilers were shaken together with 500 ml of thioglycollate broth in a plastic bag. In some samples, the numbers of C.jejuni were assessed.

Examination for the presence or absence of C.jejuni

Tests for the presence of C.jejuni were carried out with either 2 g or 2 ml of material in 20 ml THAL enrichment broth with cephalothin. Swabs from surfaces were incubated in 100 ml of this broth. After enrichment, one loopful of the broth was streaked on supplemented Skirrow's agar (Chapter IV).

Enumeration of C.jejuni

Bacterial counts from intestinal contents and from water were made by adding 2 g or 2 ml of these materials to 20 ml of THAL enrichment broth plus cephalothin. The tests were made in triplicate. Water samples were also examined in triplicate by adding 10 ml to 10 ml of double strength THAL enrichment broth with cephalothin. In addition, either 1 g or 1 ml of these materials was blended in 10 ml of physiological saline, and 2 ml of this macerate were added to 20 ml of enrichment broth, again in triplicate. Two millilitre amounts of the next ten-fold serial dilution were added to triplicate amounts of this broth. Also, from this and from further serial dilutions, 0.1 ml was plated in duplicate on supplemented Skirrow's agar. This procedure, i.e. the determination of the Most Probable Number (MPN, de Man, 1975) together with plating on the agar medium was adopted, because, in the first dilution, particles of organ tissues tended to be present that

would have interfered with counts of C.jejuni made by direct plating on solid media.

Pericloacal skin samples were blended in physiological saline to give a ten-fold dilution. These macerates, as well as the thioglycollate broths from the hand rinsing samples and from the sampling of whole carcasses, were treated as described above.

The strips from the Biotest air sampler were incubated for 48 h at 42°C under micro-aerobic conditions. Typical colonies were enumerated with the aid of a binocular microscope.

Enumeration of Enterobacteriaceae

Both the dilutions made in thioglycollate broth to assess the presence of C.jejuni on the hands of workers and on whole carcasses and the dilutions of skin portions in physiological saline were used in addition to determine the numbers of Enterobacteriaceae per ml of fluid. For this purpose, 1 ml of each dilution was mixed in a petri dish of 9 cm diameter with 10 ml of violet red-bile-glucose agar at 45°C. After allowing the agar to set, a second layer of the same medium was poured over the surface. The plates were incubated at 37°C for 24 h. Colonies having a violet-red halo were enumerated.

Results

Internal organs and intestinal contents of killed, defeathered but unopened broilers

The intestinal tract, especially the lower parts of the intestines (ileum, caecum and colon) of birds brought to processing plant A, were frequently contaminated with C.jejuni. A total of 23 of 25 birds was positive (Table V.1). Large numbers were found in the ileum of 10 of these birds (mean $\log_{10}$ per g was 5.26, maximum $\log_{10}$=7.04, minimum

Table V.1 C. jejuni in one gram samples of internal organs and intestinal contents of five killed, defeathered but unopened chickens from different flocks collected at processing plant A

Flocks	Number positive						
	Lung	Liver	Gall bladder	Duodenum	Ileum	Caecum	Colon
1	NE[a)]	1	NE	4	5	5	5
2	1	0	0	1	1	4	2
3	0	1	0	3	5	4	3
4	3	1	0	4	5	5	5
5	5	0	0	5	5	5	5
	9/20[b)]	3/25	0/20	17/25	21/25	23/25	20/25

a) NE = not examined

b) Number positive/number examined

$\log_{10}$=4.00). In the caeca of the same birds, the counts varied from $\log_{10}$=7.08 to $\log_{10}$ < 0.30, with the mean being $\log_{10}$=4.11. The lungs were contaminated in nine of 20 birds, whereas the contents of all 20 gall bladders were negative. C.jejuni was in or on the liver of three of 25 birds. In these three cases, only one or two colonies grew on the agar plates.

Swabs from the processing line

A total of 36 of 40 (90 %) swab samples taken from surfaces of equipment along the processing line of plant A during four visits was positive for C.jejuni. The four negative samples were divided over three visits and were not obtained from one particular surface.

Processing water

C.jejuni was not detected in the scalding water (58°C) of plant A (Table V.2); low numbers of C.jejuni (maximum 10 CFU/ml) were isolated from the counter-flow spinchiller near the water-inlet, and higher numbers (maximum 10^3 CFU/ml) near the water outlet. In two instances, 10 C.jejuni per ml and, in one instance, 5.0×10^3 C.jejuni per ml were found in the scalding water in plant B (51.8°C). In total, 22 samples of water with which carcasses or organs had been washed were collected in both plants. C.jejuni was isolated from 15 such samples, with the mean count of the positive samples being 3.0×10^2 CFU/ml and the highest number being 2.5×10^4 CFU/ml.

Air samples

Air samples taken in plant B (Table V.3) showed that C.jejuni was present near the defeathering machine and evisceration apparatus on four of eight occasions. The organism could not be detected in any sample of air from the cooling room. Positive samples contained 10^3 to 10^4 CFU/m^3, and, in one instance, 50 CFU/m^3.

Table V.2 Prevalence of C. jejuni in scald water (poultry processing plants A and B) and in spinchiller water (plant A)*)

Sample	Sampling no. 1	2	3
	(number of C. jejuni/ml)		
Plant A			
Scald water (58°C)	< 0.1	< 0.1	< 0.1
Water of counter-flow spinchiller near water-inlet	0.5	1.0	10
Water of counter-flow spinchiller near water-outlet	900	1000	1000
Plant B			
Scald water (51.8°C)	5000	10	10

*) Samples collected at the same time as the skin portions (sampling no. 1,2 and 3, see Table V.4 and V.5)

Table V.3 Prevalence of C. jejuni in air samples taken on eight different occasions at poultry processing plant B

Sampling site	Sampling no. 1	2	3	4	5	6	7	8
	($\log_{10}$ C. jejuni/m^3)							
Near defeathering machine	3.34	-a)	3.26	3.23	-	-	-	4.19
Near evisceration equipment	3.04	-	3.81	1.70	-	-	-	4.20
In chill room	-	-	-	-	-	-	-	-

a) - = no C. jejuni found ($\log_{10}$ < 1.00 CFU/m^3)

Pericloacal skin portions from broilers on the processing line

The numbers of C.jejuni on portions of pericloacal skin from plant A (Table V.4) initially amounted to approximately $5x10^2$ to $5x10^3$ CFU/g, but these were reduced after scalding at 58°C to, at most, 15 CFU/g, and returned to $5x10^2$ CFU/g after defeathering and evisceration. Reduction of C.jejuni also occurred during spinchilling (maximum 70 CFU/g). A similar pattern was observed for Enterobacteriaceae contamination at the same stages of processing, although at a level one to three log cycles higher. Scalding and evisceration most frequently resulted in marked changes in contamination.

The same initial level of C.jejuni contamination was observed in plant B (Table V.5). In two cases, a marked reduction in contamination occurred during scalding (51.8°C), but was not observed during the third visit. Contamination returned to levels comparable with those in plant A after defeathering, evisceration and washing (maximum $5x10^2$ CFU/g). After air-chilling, the numbers of C.jejuni on skin portions were reduced to less than detectable levels (<2 CFU/g) in two instances and amounted to $5x10^3$ CFU/g in one instance. The prevalence of Enterobacteriaceae showed approximately the same pattern, with only a few divergences. In two cases marked reductions in the numbers of Enterobacteriaceae were found after defeathering, whereas C.jejuni possibly increased in number. Moreover, in two instances, Enterobacteriaceae counts increased after chilling, whereas C.jejuni was reduced to <2 CFU/g. Because of the varying results obtained before and after chilling, three additional samplings were made in plant B. The results of these investigations showed no consistent pattern, neither with respect to C.jejuni, nor to Enterobacteriaceae.

Table V.4 Prevalence of C. jejuni and Enterobacteriaceae in one gram samples of pericloacal skin portions from chickens at different stages of processing at plant A

Stage of processing	Sampling no. 1 C[a)]	1 E[b)]	2 C	2 E	3 C	3 E
After: bleeding	2.39 ± 1.08	6.57 ± 0.39	3.42 ± 1.65	5.79 ± 0.65	3.44 ± 1.92	4.90 ± 0.85
scalding (58°C)	[c]0.61 ± 0.06	[c]4.10 ± 0.25	[c]1.25 ± 0.35	[c]3.20 ± 0.25	1.26 ± 0.35	3.67 ± 0.26
defeathering	1.07 ± 0.76	3.97 ± 0.24	1.99 ± 0.73	2.90 ± 0.71	[c]2.85 ± 0.70	3.69 ± 0.69
evisceration	[c]2.58 ± 0.68	[c]5.73 ± 0.19	2.44 ± 0.53	3.60 ± 0.34	2.60 ± 1.56	[c]4.86 ± 0.35
spinchilling	[c]0.98 ± 0.61	4.25 ± 1.36	1.24 ± 0.89	3.17 ± 0.38	1.83 ± 0.21	[c]4.04 ± 0.30

a) $\log_{10}$ mean number of C. jejuni/g ($\bar{x}$) with standard deviation (s) per 4 x 2 skin portions

b) $\log_{10}$ mean number of Enterobacteriaceae/g with standard deviation per 4 x 2 skin portions

c) marked increase or decrease compared with mean number at previous stage ($\bar{x}_a + s_a < \bar{x}_b - s_b$)

Table V.5 Prevalence of C. jejuni and Enterobacteriaceae in one gram samples of pericloacal skin portions from chickens at different stages of processing at plant B

Stage of processing	Sampling no. 1 C[a]	1 E[b]	2 C	2 E	3 C	3 E
After: bleeding	3.99 ± 1.00	6.15 ± 0.44	3.30 ± 0.92	6.64 ± 0.31	2.18 ± 0.67	5.39 ± 1.03
scalding (51.8°C)	[c]1.37 ± 1.44	[c]4.69 ± 0.22	[c]1.68 ± 0.44	[c]4.42 ± 0.29	2.40 ± 0.80	5.03 ± 0.72
defeathering	2.46 ± 0.81	[c]2.94 ± 1.08	2.09 ± 0.44	[c]3.69 ± 0.43	2.18 ± 0.35	4.16 ± 0.48
evisceration and washing	2.24 ± 1.18	3.86 ± 0.83	2.62 ± 1.24	3.60 ± 0.98	2.50 ± 0.63	3.87 ± 0.28
air-chilling	3.73 ± 0.56	4.89 ± 0.55	[c]< 0.30	[c]5.26 ± 0.45	[c]< 0.30	[c]4.50 ± 0.18

a) $\log_{10}$ mean number of C. jejuni/g ($\bar{x}$) with standard deviation (s) per 4 x 2 skin portions

b) $\log_{10}$ mean number of Enterobacteriaceae/g with standard deviation per 4 x 2 skin portions

c) marked increase or decrease compared with mean number at previous stage ($\bar{x}_a + s_a < \bar{x}_b - s_b$)

Hands of processing-line workers

Hands of processing-line workers were contaminated with C.jejuni in 19 of 24 cases (Table V.6). In 13 cases, about 10 to 15 C.jejuni were isolated from the total amount of washing fluid. Highest numbers (10^4 CFU from one hand) were found at defeathering (twice) and at veterinary inspection. The five negative samples (<2 C.jejuni/ hand) were collected from hands of individuals working at dressing and portioning. All hands of people working at defeathering, evisceration, handling of internal organs and veterinary inspection contained more than 10^4 CFU of Enterobacteriaceae, sometimes more than 10^6 CFU per hand. Seven of 11 hands of people working at dressing and portioning contained less than 10^4 Enterobacteriaceae per hand; in two cases these organisms were not detected at all (<1500 CFU per hand). The hands of these two workers were also negative for C.jejuni.

End-products

From the 120 carcasses that were investigated, 59 (49 %) yielded C.jejuni, wheras 50 to 73 % of internal organs (livers, gizzards and hearts) were contaminated (Table V.7). The numbers of C.jejuni on six carcasses examined ranged from $2x10^3$ to 10^5 CFU per carcass.

Discussion

Two major sources of contamination must be considered with respect to the bacteriological aspects of poultry processing. First, it is possible that certain bacterial species are indigenous to a processing plant and cause a constant level of contamination of the products being processed. Such a mechanism has recently been demonstrated for Staphylococcus aureus (Notermans et al., 1982). The other possibility is that contamination is brought in with the chickens and is of faecal

Table V.6 Prevalence of C. jejuni and Enterobacteriaceae on hands of 24 individual workers at processing plant B

Sampling site	Worker no.	Log_{10} C. jejuni per hand	Log_{10} Enterobacteriaceae per hand
At defeathering	1	0.70	5.86
	2	4.08	5.18
	3	5.26	4.88
At evisceration	4	1.04	5.18
	5	1.04	6.08
	6	0.70	6.04
At handling of internal organs	7	0.35	4.58
	8	0.70	4.34
	9	1.20	4.43
	10	2.18	4.15
	11	1.24	5.82
At veterinary inspection	12	4.26	4.26
	13	1.24	5.59
At dressing and portioning	14	1.04	6.04
	15	0.85	4.80
	16	< 0.35	3.48
	17	1.24	3.65
	18	0.74	3.88
	19	0.48	3.65
	20	< 0.35	< 3.18
	21	< 0.35	< 3.18
	22	1.24	3.18
	23	< 0.35	4.43
	24	< 0.35	4.68

Table V.7 Prevalence of C. jejuni on poultry end-products (before freezing) at processing plant A

Product	No. examined	No. positive	% positive
Chicken carcasses	120	59	49
Livers	40	29	73
Gizzards	20	10	50
Hearts	20	13	65

origin.

The results of the present investigations indicate that large numbers of C.jejuni may enter poultry processing plants with the birds. If chickens that are transported to plants generally carry the numbers of C.jejuni that were observed in these studies, then contamination of birds, processing lines, hands of workers and end-products can be completely attributed to intestinal sources.

The numbers of C.jejuni intitally present on the skin of poultry were reduced by scalding at 58°C. This reduction was not always observed if scalding was done at 51.8°C. The possibility of survival of C.jejuni within this temperature range has been reported earlier (Doyle and Roman, 1981). If scalding results in a low level of contamination, defeathering and evisceration subsequently bring about an increase in the numbers of C.jejuni on the skin. During defeathering, rubber fingers that are used to remove the feathers evoke some pressure on the carcasses so that faecal material is pressed out. Therefore, the observed increase in the numbers of C.jejuni were likely to be due to contamination by campylobacters of intestinal origin.

A similar increase in contamination by C.jejuni after scalding at 51.8°C could not be determined accurately in all cases because the initial counts were sometimes too high to demonstrate additional contamination. However, in view of the results from air sampling, it can be concluded that large numbers of C.jejuni may be released during defeathering and evisceration so that an additional contamination of the carcasses is likely.

Considering the numbers of C.jejuni in the counter-flow spinchiller, it appears that this apparatus washes a large number of bacteria off the carcasses. This is reflected in the decrease in the numbers of C.jejuni

on portions of pericloacal skin after spinchilling, as illustrated in Table V.4.

The chilling in plant B was done by air that was drawn from the processing hall. This may explain the increase in numbers of Enterobacteriaceae on the skin of the chickens. However, the air used for chilling was mechanically dried. Results of related studies, both from our own laboratory and from those of other investigators (Doyle and Roman, 1982b), suggest that C.jejuni is very sensitive to drying conditions, whereas it is known that Enterobacteriaceae are less sensitive. This may explain why C.jejuni died off during chilling on two occasions. In one case, however, for some unexplainable reason, there appeared to be a noticeable increase in C.jejuni contamination during chilling. Additional investigations in plant B did not provide an answer to this question. Perhaps the occurrence of unpredictable cross-contamination from the processing hall was responsible for the increase in C.jejuni contamination during chilling.

The high prevalence of C.jejuni in poultry processing plants, as shown in this study, ultimately results in contamination of end-products. C.jejuni - contaminated poultry products are undoubtedly serious hazards to public health, especially if these products are improperly handled (Holl et al., 1982; Mouton et al., 1982; Severin, 1982). Hazards for processing-line workers also exist within the processing plants themselves, as demonstrated by the presence of C.jejuni in the air and on the hands of the workers. Contamination of hands generally decreased when carcasses were handled after they had been washed and chilled, i.e. at dressing and portioning. Greatest risks were in the areas of defeathering and evisceration. Serological investigations have demonstrated that poultry processing workers are indeed affected by

increased exposure to C.jejuni (Jones and Robinson, 1981), but so far this has not been supported by further case studies.

In conclusion, it seems that C.jejuni contamination in chicken processing plants is almost exclusively of intestinal origin. This contamination is not eliminated during processing (except in some cases during scalding and air-chilling) and results in contamination of poultry end-products. Both the process and end-products resulting from processing may be hazards to public health.

CHAPTER Vb

COMPARATIVE STUDIES ON THE PREVALENCE OF *CAMPYLOBACTER JEJUNI* AND *SALMONELLA* SPP. DURING PIG SLAUGHTERING AND IN MINCED PORK

Materials and methods

Sampling

In three different slaughterhouses in the Netherlands a total of 210 pigs was examined during slaughter. At every visit to one of the slaughterhouses a maximum of five pigs were sampled. Four samples were collected from each animal. These were:

a) Intestinal contents

Immediately after evisceration of the animal the last part of the caecum was opened aseptically and about 10 g of its contents were collected in a sterile plastic jar.

b) Surface swab

A swab was taken from each animal just after slaughter but before meat inspection. An area on the left side of the ventral incision was swabbed, 10 cm in breadth and running from pelvis to jaw, thus covering an area of about 1,000 cm^2. The cotton swab of 10 cm diameter was moistened with sterile physiological saline before use and afterwards put into a sterile glass jar.

c) Lymph nodes

The left superficial inguinal, prescapular and submaxillary lymph nodes were excised, together with some of the surrounding tissue, from

each of 62 carcasses after meat inspection, and collected in a sterile plastic bag. From the remaining 148 carcasses the medial iliac and lumbar aortic lymph nodes were collected in the same way.

d) Surface swab after chilling

A swab was taken from each carcass as described above, but at this stage the sample was taken from the right side of the ventral incision after 18 to 24 h in the abattoir's chilling room.

In one of the slaughterhouses swabs were also taken from organs (livers, hearts, tongues) just after slaughter and from surfaces and equipment in the slaughter hall just before and during slaughter. In addition, minced pork was bought in butchers' shops and examined in the slaughterhouse laboratories.

Isolation methods

In the laboratory, the lymph nodes were immersed in boiling water for about 10 s and then peeled (Kampelmacher et al., 1965). Next, all the lymph nodes from one animal were bulked and blended for 2 min in a ten-fold quantity (w/w) of thioglycollate broth. Intestinal contents and the macerate of the lymph nodes were examined for Campylobacter spp. by employing a combined Most Probable Number/direct plating method involving the incubation of 2 ml amounts of appropriate serial dilutions in THAL enrichment broth with cephalothin; 0.1 ml of the same dilutions was plated directly on Skirrow's agar. Counts of Salmonella spp. were determined by means of the Most Probable Number method. Serial dilutions were incubated in buffered peptone water (BPW) for 16 h at 37°C, then 1 ml of this pre-enrichment broth was transferred to 10 ml of tetrathionate broth. After incubation at 42°C, one loopful of this broth was streaked, after 24 and 48 h respectively, on brilliant green phenol red agar, the

plates being incubated at 37°C for 24 h.

All swabs were blended for 2 min in 100 ml thioglycollate broth by means of a Stomacher and serial dilutions of this broth were used to inoculate THAL and BPW. Ten grams of each sample of minced pork were incubated in 100 ml THAL and 100 ml BPW respectively. Further isolation procedures were carried out as described previously.

Results

Table V.8 lists the results obtained in the three different slaughterhouses. In all, 165 of the 210 pigs (78.6 %) carried C. jejuni in the intestinal tract, and 44 of them (21.0 %) were contaminated with Salmonella spp. Mean $\log_{10}$ numbers were 3.56 (SD 1.44) and 1.05 (SD 1.05) respectively. Swabs taken immediately after evisceration were positive for Campylobacter spp. in 19 instances (9.1 %) and for Salmonella spp. in 27 (12.9 %). It was calculated that the numbers of campylobacters removed by swabbing were < 100 CFU per carcass, except in one case where 460 CFU were found to be present. In three instances counts of salmonellas were > 100 CFU per carcass (but < 1,000); in another four the dilutions examined only permitted the results to be described as > 110 CFU.

C. jejuni was found in two samples of the medial iliac and lumbar aortic lymph nodes, but only in very small numbers (2.3 and 4 CFU per gram respectively). Salmonella spp. were isolated from the lymph nodes of seven carcasses; in three instances from a sample of superficial inguinal, prescapular and submaxillary lymph nodes (< 1, 1.5 and > 1100 CFU per gram respectively) and in four instances from medial iliac and lumbar aortic lymph nodes (maximum 120 CFU per gram).

The swabs taken after the carcasses had been chilled overnight were

Table V.8 Prevalence of C. jejuni and Salmonella spp. in intestinal contents, on the skin and in lymph nodes of 210 pigs slaughtered at three different slaughterhouses

Slaughter house	Total number of pigs	Number of pigs positive							
		Intestinal contents		Swabs after evisceration		Lymph nodes		Swabs after cooling	
		C	S[o)]	C	S	C	S	C	S
I	64	52	21	8	19	0	0	0	9
II	71	68	19	4	4	0	3	0	2
III	75	45	4	7	4	2	4	0	1
Total	210	165	44	19	27	2	7	0	12
% positive		78.6	21.0	9.1	12.9	1.0	3.3	0	5.7

o) C = C. jejuni
S = Salmonella spp.

Table V.9 Prevalence of C. jejuni and Salmonella spp. on surfaces and equipment in the hall of slaughterhouse I

	Number of samples	Campylobacter positive	Salmonella positive
Before slaughtering	80	2 (2.5%)	13 (16%)
During slaughtering	80	26 (32.5%)	17 (21%)

all negative for Campylobacter spp. However, Salmonella spp. were isolated from 12 of the carcasses (5.7 %). In 10 instances the total count per carcass was $\leq$ 110, and in two instances > 110 CFU. These last two carcasses also yielded large numbers of salmonellas before chilling.

Twenty-four swabs taken from organs were examined. Only one swab yielded Campylobacter spp., but salmonellas were isolated from 14 swabs (58.3 %).

Swabs from slaughter-hall surfaces and equipment, collected during slaughter, were positive for Campylobacter spp. in 26 instances (32.5 %) and in 17 instances (21 %) for Salmonella spp. When swabs were taken from surfaces and equipment that had been allowed to dry overnight (after the usual cleaning and disinfection), two swabs yielded Campylobacter spp. (2.5 %) and 13 Salmonella spp. (16 %) (Table V.9).

Table V.10 gives the results of the examination of minced pork. Campylobacters were not isolated from any of the 248 10 g samples, while Salmonella spp. were found in 33 of them (13.3 %).

Discussion

In these studies it was shown that a large proportion (78.6 %) of healthy pigs ready for slaughter is intestinal carrier of campylobacters and that a considerable proportion (21.0 %) harbours Salmonella spp. in the intestines. The mean number of campylobacters in the intestinal tract is also much higher than the mean number of salmonellas. It is, therefore, surprising that campylobacter contamination of carcasses immediately after slaughtering does not differ significantly from that due to salmonellas, both with regard to the number of carcasses involved and the numbers of organisms found on those carcasses. Thus, the proportions of salmonellas and campylobacters on carcasses do not reflect

Table V.10 Prevalence of C. jejuni and Salmonella spp. in 248 ten grams samples of minced pork

Examined by laboratory of slaughterhouse	Number of samples	Campylobacter positive*)	Salmonella positive°)
I	94	0	9
II	98	0	18
III	56	0	6
Total	248	0	33
% positive			13.3

*) detection limit: 3 - 10 campylobacters/g

°) detection limit: about one salmonella/g

the proportions of these species in the intestinal tract (Table V.8). It must be concluded therefore, that much of the carcass contamination does not originate directly from the intestinal material of the animals in question.

The only other means by which carcasses (and organs) may become contaminated during slaughtering is by contact with surfaces, equipment and utensils in the slaughter-hall. It has been shown in this study that campylobacters survive poorly in the slaughterhouse environment; it is suspected that the organisms die very rapidly under dry conditions, which may occur overnight. Moreover, C. jejuni does not grow at temperatures below 30°C (Skirrow and Benjamin, 1980), so that a possible campylobacter contamination on the carcass does not grow out. These supposed disadvantages do not apply to Salmonella spp., so that salmonellas may persist in such an environment. This hypothesis was confirmed by examining surfaces and equipment in the slaughter-hall early in the morning before slaughtering started. Campylobacters were found only sporadically in the samples collected on these occasions, but were frequently present in the samples collected during slaughtering. No such significant difference in prevalence was observed for Salmonella spp. isolated on the same occasions (Table V.9).

These studies demonstrate that contamination of carcasses with campylobacters is eliminated in the slaughterhouse chill rooms. To some degree this is also true for Salmonella spp.

Lymph nodes are sometimes considered to be important sources of salmonella contamination of pork, because they may be cut during portioning of carcasses and thus may contaminate knives and meat. This possibility might also apply to campylobacters. However, in our studies there were no indications to support this hypothesis. Lymph nodes were

only sporadically contaminated, both with regard to campylobacters (1.0 %) or salmonellas (3.3 %) (Table V.8).

The examination of minced meat finally confirmed that campylobacters were no longer present (or occurred below the level of detection) on pork that had left the slaughterhouse. Salmonellas were still isolated from 13 % of these samples. It must be noted however that the level of salmonella detection in our studies was about ten times lower than that for Campylobacter spp., so that results obtained by the two isolation methods are not fully comparable.

CHAPTER Vc

SURVIVAL EXPERIMENTS WITH *CAMPYLOBACTER JEJUNI* IN RELATION TO POULTRY PROCESSING AND PIG SLAUGHTERING

Materials and methods

In this study the survival of C.jejuni during some of the critical stages of poultry processing and pig slaughtering was examined. In addition, the ability of the organism to survive on surfaces was determined. In all experiments, including those carried out in the laboratory, materials were contaminated by natural sources of C.jejuni. These sources were:

a) spinchiller water from poultry processing plants;

b) intestinal contents of broiler chickens, and

c) pig faeces.

Spinchiller water was collected in three different poultry processing plants from the most polluted part of the system and transported to the laboratory within 2 h under chill conditions (approximately $0^{\circ}C$). The three different samples were mixed in the laboratory before use during the tests.

To obtain samples of intestinal contents of broiler chickens, freshly killed and defeathered, but unopened chickens were collected at a poultry processing plant and brought to the laboratory within 2 h under chill conditions (approximately $0^{\circ}C$). The birds were opened aseptically and the contents of the caecum and colon were collected and mixed. A

suspension of this intestinal material was first examined by phase-contrast microscopy for the presence of C.jejuni.

Faeces of pigs were collected from the rectum of pigs just before slaughter. These faeces were also examined by phase-contrast microscopy to determine the presence of Campylobacter spp.

Heating experiments

For these experiments, a vessel containing approximately 2 l of water was heated on an electric heater with a magnetic stirrer. A glass jar containing 100 ml of peptone-saline (0.1 % peptone, 0.85 % sodium chloride) with a magnetic stirring rod and a thermometer was placed in this water. When the peptone-saline slightly exceeded the desired temperature, 1 g of chicken intestinal content was added. As a consequence, the temperature of the mixture dropped 1 to 1.5°C and thereafter could be kept constant (± 0.2°C) throughout the experiment. The mixture was examined at regular intervals to determine the numbers of C.jejuni per ml, as described below.

Chilling experiments

The first chilling experiments were done in the chill room of a pig slaughterhouse. A temperature of 0 to 4°C and forced ventilation were used to chill the carcasses in this room. The ventral side of a fresh pig carcass was inoculated with a 1 : 10 dilution of campylobacter-containing pig faeces in physiological saline and the carcass was brought to the chill room. Samples were taken at different times to examine the rate of contamination on the skin during chilling. The excision method was used for this purpose (Snijders and Gerats, 1977). Three round portions of skin were collected (total 15 cm^2) by means of a sterile cork borer (2.5 cm diameter) and a sterile scalpel and put into a plastic bag for immediate bacteriological investigation in the laboratory.

In additional experiments, the effect of drying during the chilling of pig carcasses was studied. Portions of pig skin were contaminated as described above and subjected to gradually decreasing temperatures in two cooling incubators, simulating the temperature course of normal carcass chilling in the slaughterhouse. The air in one of the incubators was mechanically ventilated during chilling (relative humidity 60 to 70 %), in the other incubator no ventilation was used (relative humidity 95 %). Skin portions from both treatments were examined at fixed intervals for the numbers of campylobacters per cm^2.

Freezing experiments

Pericloacal skin portions were collected from broiler chickens after processing and final washing at a poultry processing plant. Chickens from one particular flock were examined in each experiment. Skin portions from birds of the same flock were taken after overnight freezing at $-45^{\circ}C$ and further storage at $-20^{\circ}C$ (maximum 4 h). The portions were examined to determine the numbers of C.jejuni and Enterobacteriaceae per gram. Similar studies were done with chicken livers. Samples from a single batch were collected after washing and further samples from the same batch were obtained after overnight freezing at $-45^{\circ}C$ and storage at $-20^{\circ}C$ (maximum 4 h).

Storage experiments

The effect of prolonged storage at $+4^{\circ}C$, $-20^{\circ}C$ and $-70^{\circ}C$ was studied in the laboratory. A number of broiler carcasses originating from one particular flock and several portions of liver from a single batch were washed in contaminated spinchiller water for 2 min, put in plastic bags and stored at the required temperature. After fixed periods of time, the number of C.jejuni per carcass or per gram of liver was determined in each case.

Drying experiments

Small tiles (approximately 4 x 4 cm) of different materials (aluminium, stainless steel, Formica and ceramic) were used for these experiments (van Klingeren, 1983). A number of tiles of each material was moistened with 0.1 ml of contaminated spinchiller water and put in large petri dishes which were held at ambient temperatures (20 to 24°C) in the laboratory. Drying of the tiles was regulated by closing the lids of the petri dishes or by leaving them open. Two tiles of each material were examined at fixed intervals for the numbers of surviving campylobacter cells.

Bacteriological examination

The prevalence of C.jejuni in mixed spinchiller water, intestinal contents of broilers and pig faeces respectively was determined by plating in duplicate 0.1 ml amounts of serial decimal dilutions in peptone-saline on Skirrow's agar containing the growth promoting supplement. The same method was used to determine the numbers of campylobacters in the heating experiments.

The prevalence of C.jejuni on pig skin was assessed by bulking three skin portions in 45 ml of thioglycollate broth and blending them for 2 min in a Stomacher. The homogenate was examined by a combined MPN/direct plating method. This involved adding 10 ml of homogenate to 10 ml of double strength THAL-enrichment broth with cephalothin in triplicate and 2 ml of homogenate to 20 ml of normal concentration of this medium, again in triplicate. In addition, 0.1 ml amounts of serial decimal dilutions were plated in quintuplicate on Skirrow's agar plus supplement.

Chicken skin portions were blended for 1 min in physiological saline and the macerate was again examined using a combined MPN/direct plating method. Two millilitre amounts from ten-fold serial dilutions of the

macerate were added in triplicate to 20 ml of THAL-enrichment broth with cephalothin. In addition, 0.1 ml amounts of ten-fold serial dilutions were plated in duplicate on Skirrow's agar plus supplement. All liver samples were examined by macerating 2 g in 20 ml of peptone-saline and by plating 0.1 ml amounts of serial decimal dilutions of the macerate in duplicate on Skirrow's agar plus supplement. Whole carcasses were examined by the rinsing method. Carcasses were put in sterile plastic bags with 500 ml of thioglycollate broth and shaken for 2 min. The prevalence of C.jejuni in this broth was determined as for the macerates of chicken skin portions.

In the drying experiments, two tiles of each material were examined simultaneously; one by the use of contact agar plates (2.8 cm diameter) filled with Skirrow's agar plus supplement and the other by immersion in 20 ml of THAL-enrichment broth with cephalothin, which was subsequently shaken for 2 min. Further isolation procedures were carried out as described previously.

To determine the prevalence of Enterobacteriaceae, 1 ml of each appropriate decimal dilution was mixed with violet red-bile-glucose agar at 45°C and, after allowing the agar to set, plates were overlaid with the same medium. The plates were incubated at 37°C for 24 h. Colonies having a violet-red halo were enumerated.

Results

Heating experiments

Decimal reduction values for C.jejuni in chicken intestinal contents diluted in peptone-saline are presented in Table V.11. D-values at 58 and 60°C were between 0.39 and 0.18 min, whereas D-values at 55°C were approximately 1.5 to 2 min. In one instance at 52°C a D-value of more

Table V.11 Decimal reduction times at different temperatures for C. jejuni in chicken intestinal contents*)

Temperature	Experiment no.	D-values (min)	95% confidence interval	Number of observations
60°C	1	0.23	0.17-0.34	3
	2	0.39	0.32-0.50	4
	3	0.18	0.10-0.73	4
58°C	4	0.37	0.33-0.43	7
	5	0.36	0.24-0.67	5
	6	0.27	0.21-0.38	5
55°C	7	1.75	1.69-1.81	9
	8	1.45	1.38-1.51	9
	9	2.13	2.09-2.21	7
52°C	10	10.82	10.76-10.88	11
	11	4.35	4.24-4.41	10
	12	1.96	1.88-2.05	10

*) chicken intestinal contents mixed 1:100 with peptone-physiological saline

than 10 min was obtained. First order kinetics were observed in most instances: in only three experiments was the correlation coefficient (r) <0.98 (but $\geq$ 0.96). In one experiment at 55°C (not presented in the Table V.11), no regression line could be calculated because of irregular results.

Chilling experiments

Data obtained during the exposure of contaminated pig carcasses to normal chill conditions in a slaughterhouse (with forced ventilation of air) are presented in Table V.12. Initial contamination varied between $\log_{10}$=4.60 and $\log_{10}$=2.00 CFU per cm^2 of skin. After 3 h of chilling, no campylobacters could be isolated by direct plating ($\log_{10}$ < 0.78 CFU per cm^2) but the organisms were recovered by enrichment ($\log_{10} \geq -0.52$ CFU per cm^2) in all experiments. Enrichments were negative in three experiments after further chilling ($\log_{10}$ < -0.52 per cm^2, i.e., less than 50 campylobacter cells per 15 cm^2 of skin).

Ventilation of air during the chilling of pig skin portions in a laboratory incubator resulted in a more than hundred-fold reduction of campylobacter counts in 24 h, whereas chilling without ventilation did not appreciably reduce the campylobacter contamination (Table V.13). This experiment was carried out twice with similar results.

Freezing experiments

Results of investigations of the freezing of carcasses and livers (overnight freezing at -45°C and further storage at -20°C for up to 4 h) are shown in Table V.14. In general, unlike the situation with campylobacters, there was no clear reduction in the level of Enterobacteriaceae. After freezing, C.jejuni was recovered from only one carcass and from one sample of liver.

Table V.12 Prevalence of C. jejuni on pig carcasses during chilling (0-4°C) with forced ventilation in a pig slaughterhouse (carcasses inoculated on surface with contaminated pig faeces)

	Experiment no.			
	1	2	3	4
	Log_{10} campylobacters per cm^2			
Initial contamination	4.60	2.00	3.30	3.28
After: 1 h	2.48	< 0.78	< 0.78	< 0.78
3 h	< 0.78 o)	< 0.78	< 0.78	< 0.78
19 h	< 0.78	< -0.52 *)	< -0.52	< 0.78
27 h	< 0.78	< -0.52	< -0.52	< -0.52

o) direct plating method negative, enrichment method positive ($log_{10} \geq -0.52$)

*) direct plating as well as enrichment method negative

Table V.13 Prevalence of C. jejuni on pig skin during chilling with and without forced ventilation

Time (h)	Chilling with ventilation (r.h. 60-70%) *)	Chilling without ventilation (r.h. 95%)	Skin temperature (°C)
	Log_{10} number of campylobacters per cm^2		
0	3.51	3.52	30.5
1	3.11	3.57	19.5
3	2.40	3.11	12.5
6	2.36	3.15	9.5
24	1.00	3.18	4.0

*) r.h. = relative humidity

Table V.14 Prevalence of C. jejuni and Enterobacteriaceae on pericloacal skin portions and livers of broiler chickens before and after freezing[o)]

Pericloacal skin portions	C. jejuni		Enterobacteriaceae	
Flock no.	before	after	before	after
1	2.32 ± 0.29	< 1.70	4.14 ± 0.33	3.30 ± 0.32
2	2.73 ± 1.21	< 1.70	3.89 ± 0.30	2.29 ± 0.27
3	3.29 ± 0.19	< 1.70[x)]	2.52 ± 0.23	2.66 ± 0.42

Livers	C. jejuni		Enterobacteriaceae	
Batch no.	before	after	before	after
1	2.32 ± 0.29	< 1.70	3.73 ± 0.82	2.89 ± 0.58
2	2.82 ± 0.40	< 1.70	3.18 ± 0.55	1.25 ± 0.19
3	2.27 ± 0.43	< 1.70[x)]	2.52 ± 0.23	2.66 ± 0.42

o) mean $\log_{10}$ number per gram and standard deviation for five samples from each flock or batch; overnight freezing at -45°C, further storage at -20°C, maximum 4 h

x) only one sample contained $\log_{10}$=1.70 per g

Storage experiments

Chicken carcasses and livers which had been contaminated with spinchiller water and stored at different temperatures remained positive for campylobacters throughout the experiments. Chickens stored at $4^{o}C$ had an initial campylobacter count of $\log_{10}$=5.00 per carcass. After seven and eight days of storage respectively, at which time complete spoilage had occurred, $\log_{10}$=4.00 campylobacters per carcass were recovered. At $-20^{o}C$, the initial campylobacter level was $\log_{10}$=3.70 CFU per carcass, of which $\log_{10}$=3.24 were still viable after 36 days and $\log_{10}$=2.35 after 64 days. Initial contamination of carcasses held at $-70^{o}C$ was $\log_{10}$=6.70 CFU. This level of contamination dropped within two days to $\log_{10}$=3.72, and thereafter remained approximately at the same level until examination at 21 days ($\log_{10}$=3.70 CFU per carcass).

The same results were obtained with chicken livers. At $4^{o}C$, the initial campylobacter count was $\log_{10}$=2.00 per g, and after eight days $\log_{10}$=1.65 organisms were recovered. The livers were also spoiled at that time. During storage at -20 and $-70^{o}C$, an initial reduction in the level of contamination was observed after the first few days (from $\log_{10}$=3.30 CFU to $\log_{10}$=2.30 per g and from $\log_{10}$=4.74 CFU to $\log_{10}$=3.65 per g, respectively) after which campylobacters were still detected following 84 days at $-20^{o}C$ ($\log_{10}$=1.74 CFU per g) and after 29 days at $-70^{o}C$ ($\log_{10}$=4.40 CFU per g, but the average number during the former period was $\log_{10}$=3.65 CFU per g).

Drying experiments

Results of the drying experiments (Table V.15) show that Campylobacter spp. remained viable as long as the surfaces of the different tiles were moist. No significant variation in results was observed between the different materials or between the two methods of

Table V.15 Survival of C. jejuni on tiles of different materials during rapid and slow drying*)

Time (h)	Rapid drying					Slow drying			
		A	S	F	C x)	A	S	F	C
0		+o)	+	+	+	+	+	+	+
0.25		+	+	+	+				
0.50	(D)•)	-	v	v	-				
0.75	D	-	-	-	-				
1.00		-	-	-	-				
1.50						+	+	+	+
3.00						v	v	+	+
4.00						+	+	+	+
5.50						v	v	v	+
7.00						v	v	+	+
24.00						D -	-	-	-

*) tiles (4 x 4 cm) moistened with campylobacter-contaminated spinchiller water. Bacteriological examination of the tiles by contact agar plates and by immersion in enrichment broth

x) A = aluminium; S = stainless steel; F = Formica^{R}; C = ceramic

o) + = C. jejuni isolated
- = no C. jejuni isolated
v = differing results obtained by the two isolation methods employed

•) (D) = tiles almost dry
D = tiles visually dry

examination. These experiments were repeated twice with similar results.

Discussion

Heating experiments

More than one strain of campylobacter was probably present in some of our heating experiments because we used mixed intestinal contents of chickens. Nevertheless, we found very clear regression lines in most of the tests. Only one test at 55°C, in which an irregular decrease in the number of campylobacters was found, suggested that two or more strains of differing heat resistances were involved, or that some of the cells were better protected than others. In general, D-values from the heating experiments were in accordance with the ranges reported in other experiments carried out with laboratory strains in chicken meat, milk or beef. Others have also found great differences in D-values at temperatures near 50°C (Doyle and Roman, 1981; Blankenship and Craven, 1982; Christopher et al., 1982).

The intestinal contents of chickens may contain 10^7 campylobacter cells per gram. Considering that scalding times in poultry processing normally range between 120 and 170 s, even a temperature of 58 or 60°C would not be enough to eliminate all campylobacters on chicken carcasses. At 52°C, the reduction may be minimal in some instances. These conclusions are in agreement with the earlier results of investigations at poultry processing plants (Subchapter Va).

In pig slaughtering, scalding is done at 60°C for several minutes, which is probably sufficient to kill any campylobacters on the skin, although some cells may remain viable when present in deeper hair follicles. Moreover, the maximum number of campylobacters likely to be found in porcine intestinal contents is 10^4 cells per gram, which is much

lower than the levels which can occur in chickens.

Chilling experiments

Death of C.jejuni on pig carcasses during chilling in a slaughterhouse is likely to be an important factor in the epidemiology of campylobacteriosis. It was found in this study that about 80 % of pigs harbour C.jejuni in their intestinal tract. Similar percentages have been reported in earlier studies (Jørgensen, 1979; Oosterom, 1980; Sticht-Groh, 1982). However, very low isolation rates were obtained from minced pork (Teufel, 1982; Turnbull and Rose, 1982). In our laboratory, C.jejuni was not detected in 248 samples (10 g each) of minced pork and in an earlier investigation it was found absent in 300 samples of 2 g each. Reduction in the numbers of C.jejuni during chilling might explain these discrepancies. It was suspected that decreasing temperatures alone did not cause this loss of viability, but that the drying effect of forced ventilation in the chill rooms was the most important factor. Some evidence for this hypothesis has already been given by Hudson and Roberts (1982), who isolated C.jejuni from moist but not from dry parts of pig carcasses. This study confirms the claim that drying is the decisive factor. That the sensitivity of campylobacters to oxygen plays a role, as has been suggested by others (Bolton et al., 1982), seems unlikely.

It should be noted that the foregoing comments are only relevant to the chilling of pig carcasses. Other pig products, such as livers, hearts and intestines, are treated in a different manner and may remain contaminated with campylobacters.

The drying of poultry carcasses during air chilling appears to be less effective in destroying campylobacters than is the case with pigs. This may be explained by the fact that in poultry processing chilling

times are shorter (approximately 1 h) than those used for pigs (16 to 24 h); that sometimes heavily contaminated air is used to chill the birds and, most important, that because of the shape and surface texture of the skin and body cavity of a poultry carcass, campylobacters may be protected from the adverse effect of drying. Pig carcasses are relatively smooth and clean and probably provide much less protection.

Freezing and storage experiments

From our experiments, it may be concluded that the freezing of broiler carcasses and livers, as is done in commercial processing plants, reduces campylobacter contamination, as was indicated previously (Hartog et al., 1981).

Storage experiments in the laboratory show that following an initial reduction, C.jejuni can survive on chilled or frozen chicken products in such numbers and for such periods of time that after storage these products may still establish potential hazards to public health. This conclusion is supported by information on the possible infective dose for man, which may be as low as four C.jejuni - cells per gram of meat (Park et al., 1982).

On the other hand, it has been found that during chilling and freezing campylobacter contamination may be reduced below detectable levels. Factors that promote this reduction during the chilling and freezing of poultry meat remain to be identified.

Drying experiments

Results of experiments with moistened tiles indicate that C.jejuni is very sensitive to drying, as was shown previously with pure cultures (Doyle and Roman, 1982b). This sensitivity may be an important factor in the epidemiology of C.jejuni. We know that in the epidemiology of Salmonella spp. the occurrence of contaminated surfaces and cross-

contamination between food products play an important part in transmitting the disease (Smeltzer et al., 1980). These factors are probably less important with respect to C.jejuni because (a) cross-contamination is seldom followed by growth of C.jejuni, which does not propagate below 30°C (Skirrow and Benjamin, 1980) and (b) surfaces do not appear to carry viable C.jejuni after they become dry. The inability of C.jejuni to survive on dry surfaces may also be important with respect to the cleaning and disinfection of poultry processing plants and pig slaughterhouses. Campylobacters may survive on dry surfaces if the relative humidity is $\leq$ 14 % (Doyle and Roman, 1982b), but this condition is not likely to occur in commercial plants.

CHAPTER Vd

CAMPYLOBACTER JEJUNI IN CATTLE AND IN RAW MILK

Materials and methods

Two hundred samples of caecal contents from individual cattle were collected during three visits to seven different slaughterhouses. After opening the intestines in a sterile manner, the contents were sampled in each case with a cotton swab which was next transported to the laboratory in Amies transport medium. Transit times varied from 0.5 to 2 h. Milk samples were collected from the milk tanks of 200 different dairy farms around Gouda, the Netherlands. These samples were refrigerated (approximately $0^{\circ}C$) during transport to the laboratory. The swabs and 0.1 ml of the milk samples were used to inoculate both Skirrow's medium and Butzler's medium.

The investigations were repeated after an enrichment procedure had been developed. Two enrichment broths were used in parallel: THA and THAL (see the Annex to Chapter IV).

Again, 200 samples of both caecal contents and milk were collected and transported as described above. Swabs from caecal contents of cattle were collected in triplicate. One swab was enriched in 20 ml of THA and one in 20 ml of THAL. After enrichment, cultures were plated on Skirrow's agar. The third swab was streaked directly on Skirrow's agar as in the initial investigation. For each milk sample, 2 ml were incubated in both THA and THAL, then streaked on Skirrow's agar.

Modern milking techniques involve intensive mechanical mixing of the milk, which causes aeration; this is followed by cold storage in large tanks at the farm (usually two to five days at $4^{o}C$). In order to determine whether this procedure promotes the death of C.jejuni, sterile milk was inoculated with 10^{7} CFU per ml of a human pathogenic strain of the organism. The milk was then divided into two portions: one portion was agitated vigorously for 2 min by means of a Whirl-mix and the other was left unshaken. Both portions were stored at $4^{o}C$ and at regular intervals the numbers of campylobacters were determined by direct plating.

Finally, milk samples from 50 mastitic cows taken at slaughter were examined for C.jejuni. For that purpose, 0.1 ml of the milk was plated directly on Skirrow's agar and 1 ml and 0.1 ml amounts were enriched in THAL enrichment broth with cephalothin. The milk samples were also examined for the presence of antibiotics by means of a Micrococcus luteus growth test.

Results

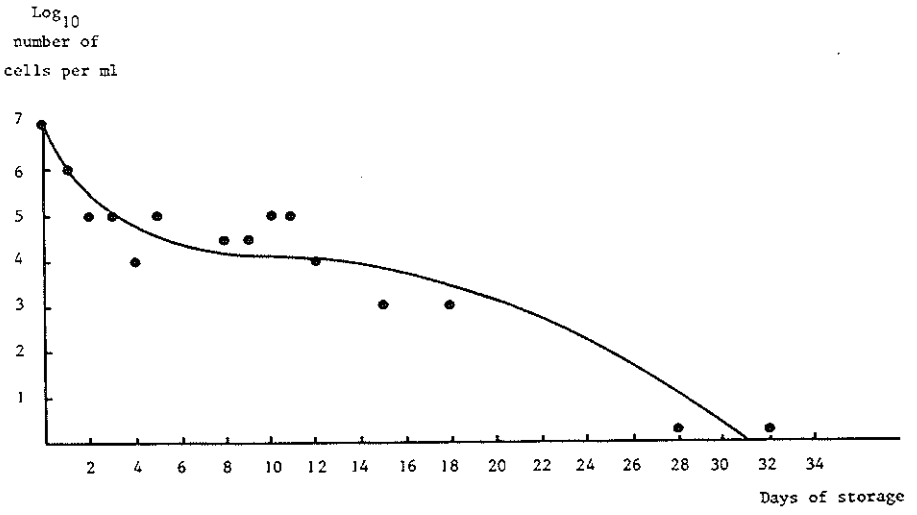
In the first series of investigations, C.jejuni was not detected in any of the cattle or milk samples. Results for the examination of caecal contents in the second series of investigations are shown in Table V.16. In total, 5.5 % of these samples were positive for C.jejuni; however, in most of the positive samples the organism appeared to be present in low numbers because direct plating resulted in only three isolations.

C.jejuni was not isolated from the second series of milk samples. In the survival experiment, C.jejuni could be isolated from both milk portions for about one month (Figure V.1). No significant differences in counts were observed between the two inoculated portions.

Table V.16 Isolation of C. jejuni from caecal contents of slaughtered cattle using direct plating and enrichment (THA and THAL) techniques

Number of samples	Numbers campylobacter positive			
	direct plating	THA	THAL	total
200	3	4	11	11

Figure V.1 Survival of C. jejuni in milk at 4°C



Campylobacters were not detected in any of the mastitic udders, nor was the presence of antibiotics demonstrated in any of the respective milk samples.

Discussion

The results of these investigations show that in the Netherlands neither cattle nor milk seem to be important sources of campylobacter infection in man. It was found that the organism occurs only sporadically in the intestinal contents of cattle, so that contamination of beef must be generally very low. In addition, cattle carcasses are chilled in the slaughterhouse in a manner similar to that used for pig carcasses, so it may be assumed that during chilling any contamination of the meat is reduced even further. Therefore, the reported campylobacter outbreak in a Dutch military camp that was probably caused by eating raw hamburger, must be considered as a rather exceptional event (Oosterom et al., 1980).

The role of milk seems to be negligible in the epidemiology of C.jejuni in the Netherlands, not only because campylobacter contamination of milk appears to be absent, but also because almost all Dutch milk is pasteurized, if not sterilized, before it reaches the consumer. In this country only about 1 % of the total milk production is retained on dairy farms for direct sale to the public (Dutch Dairy Products Marketing Board, 1982) and part of this milk possibly is consumed raw.

It was found in our studies that campylobacters in milk were not susceptible to contact with oxygen, and were able to survive in sterilized refrigerated milk for several weeks. Only during the first few hours of storage a ten- to one hundred-fold reduction might take place. Hence, it was concluded that milking techniques did not in themselves

contribute to the elimination of C.jejuni from milk. The milk samples examined were kept cool, even during transport to the laboratory, so that the killing effect of raw milk on bacterial organisms (Doyle and Roman, 1982a; Koidis and Doyle, 1984) did not play a role either. Thus, it may be assumed that the milk samples examined were not, in fact, contaminated with C.jejuni.

It is still uncertain whether contamination of milk is normally due to faecal pollution during milking or to campylobacter mastitis. Lander and Gill (1980) demonstrated that experimental inoculation of the udder with C.jejuni can lead to mastitis and that this infection may cause excretion of large numbers of campylobacter cells with the milk. However, so far, no naturally acquired campylobacter mastitis has been properly reported and our studies have confirmed that the organism is unlikely to be associated with mastitis.

CHAPTER Ve

PREVALENCE OF *CAMPYLOBACTER JEJUNI* IN DOGS

Materials and methods

In an initial survey 123 dogs were examined for the presence of C.jejuni during normal consultation hours at a small animal clinic in the Netherlands. The animals in question were brought to the clinic because of common minor afflictions or otherwise for routine vaccination. During sampling, information was collected regarding the age of the animal and the occurrence of diarrhoea during the previous seven days.

In a second survey investigations were primarily concerned with the prevalence and enteropathogenic effects of Canine Parvo Virus (CPV) in dogs. In order to assess the exact role of CPV in enteritis, the animals were also investigated for the presence of other enteropathogenic organisms. This meant that methods for the isolation of C.jejuni were also included. Two groups of animals were examined in this second survey. The first group consisted of 89 dogs from 15 different dog breeders and the second of 50 other, unrelated dogs for which veterinary help had been sought because of enteritis. For the breeders' dogs, health records were kept for the seven days preceding the survey.

Faecal material was collected from all dogs by means of rectal swabs. The swabs were transported in Amies medium and on arrival at the laboratory were streaked directly on Skirrow's agar. Further isolation methods were as described in Chapter IV.

Results

In the initial survey C.jejuni was isolated from the faeces of five out of 123 (4 %) animals. The age of the positive dogs ranged from six weeks to 1.5 years, and only one (a six week old puppy) actually had diarrhoea at the time of the examination. The other four dogs showed no symptoms of enteritis in the week before the examination.

In three out of 15 kennels of dog breeders, the organism was demonstrated in a total of five out of 89 (5.5 %) dogs. In one kennel three out of nine dogs were positive, and one also yielded a Salmonella sp., but no diarrhoea was observed in any of the animals. In two other kennels only a single puppy was found to excrete C.jejuni. Both animals showed enteric symptoms. One of the puppies also gave serological evidence of recent CPV-infection.

Among the 50 individual dogs seen by veterinarians because of enteritis four (8.0 %) were positive for Campylobacter. spp. All four were older than six months. Apparently, three of them had also been recently infected by CPV.

Discussion

From the results of these investigations, it is concluded that C.jejuni is rarely present in Dutch dogs. Even in kennels, where contaminated food like chicken offal is sometimes fed, and where the spread of C.jejuni among the animals is most likely to occur, the organism was found only sporadically. In general, there seems to be no clear relationship between the presence of C.jejuni in dog faeces and the occurrence of enteritis. Taken together, these considerations show clearly that campylobacter infections in dogs do not amount to a serious hazard to public health in the Netherlands.

References

Anonymous. Public Water Supply Act. Nederlandse Staatscourant, Staatsuitgeverij, the Hague, the Netherlands 1983. no. 41 suppl.

Blankenship, L.C. and Craven, S.E. Survival response of Campylobacter fetus s.s. jejuni to temperatures between 4 C and 57 C in chicken meat. Abstr. Ann. Meeting, Am. Soc. Microbiol. 1981. P12, 197.

Blaser, M.; Cravens, J.; Powers, B.W. and Wang, W.L. Campylobacter enteritis associated with canine infection. Lancet 1978. ii, 979-981.

Blaser, M.J.; Cravens, J.; Powers, B.W.; LaForce, F.M. and Wang, W-L.L. Campylobacter enteritis associated with unpasteurized milk. Am. J. Med. 1979. 67, 715-18.

Bolton, F.J.; Dawkins, H.C. and Robertson, L. Campylobacter jejuni/coli in abattoirs and butchers shops. J. Infect. 1982. 4, 243-245.

Bruce, D.; Zochowski, W. and Ferguson, I.R. Campylobacter enteritis. Br. Med. J. 1977. ii, 1219.

Bruce, D.; Zochowski, W. and Fleming, G.A. Campylobacter infections in dogs and cats. Vet. Rec. 1980. 107, 200-201.

Butzler, J.-P.; Dereume, J.-P.; Barbier, P.; Smekens, L. and Dekeyser, J. L'origine digestive des septicémies à Campylobacter. Nouv. Presse Med. 1977. 6, 1033-1035.

Butzler, J.P. Campylobacter, een miskende enteropathogene kiem. Thesis Vrije Universiteit Brussel 1974.

Butzler, J.P. and Skirrow, M.B. Campylobacter enteritis. Clin. Gastroenterol. 1979. 8, 737-765.

Christopher, F.M.; Smith, G.C. and Vanderzant, C. Effect of temperature and pH on the survival of Campylobacter fetus. J. Food Prot. 1982. 45, 253-259.

Devriese, L. and Devos, A. Vibrio spp. bij pluimvee: isolatie, identificatie en experimentele infectie. Tijdschr. Diergeneeskd. 1971. 96, 193-201.

Doyle, L.P. A Vibrio associated with swine dysentery. Am. J. Vet. Res. 1944. 5, 3-5.

Doyle, M.P. and Roman, D.J. Growth and survival of Campylobacter fetus subsp. jejuni as a function of temperature and pH. J. Food Prot. 1981. 44, 596-601.

Doyle, M.P. and Roman, D.J. Prevalence and survival of Campylobacter jejuni in unpasteurized milk. Appl. Environ. Microbiol. 1982a. 44, 1154-1158.

Doyle, M.P. and Roman, D.J. Sensitivity of Campylobacter jejuni to drying. J. Food Prot. 1982b. 45, 507-510.

Dutch Dairy Products Marketing Board. Statistical Annual Review. Rijswijk, the Netherlands 1982.

Fernie, D.S. and Park, R.W.A. The isolation and nature of Campylobacters (microaerophilic Vibrios) from laboratory and wild rodents. J. Med. Microbiol. 1977. 10, 325-329.

Fleming, M.P. Incidence of campylobacter infection in dogs. Vet. Rec. 1980. 107, 202.

Grant, I.H.; Richardson, N.J. and Bokkenheuser, V.D. Broiler chickens as a potential source of Campylobacter infections in humans. J. Clin. Microbiol. 1980. 11, 508-510.

Hartog, B.J.; Severin, W.P.J. and Boer, E. de. Epidemiology of campylobacteriosis and occurrence of Campylobacter fetus subsp. jejuni in poultry products. Proc. 5th Eur. Fed. World Poultry Sci. Ass., Apeldoorn, the Netherlands 1981. 425-433.

Hastings, D.H. Campylobacter enteritis in pets. Lancet 1978. ii, 1249-1250.

Holl, H.; Boer, E. de and Stigter, H.H. Campylobacter-enterocolitis na consumptie van kip. Ned. Tijdschr. Geneeskd. 1982. 126, 949-951.

Hosie, B.D.; Nicolson, T.B. and Henderson, D.B. Campylobacter infections in normal and diarrhoeic dogs. Vet. Rec. 1979. 105, 180.

Hudson, W.R. and Roberts, T.A. The occurrence of Campylobacter on commercial red-meat carcasses from one abattoir. In: Newell, D.C. (ed.). Campylobacter. Epidemiology, pathogenesis and biochemistry. MTP Press Ltd., Lancaster 1982. 273.

Jones, D.M. and Robinson, D.A. Occupational exposure to Campylobacter jejuni infection. Lancet 1981. i, 440-441.

Jones, F.S.; Orcutt, M. and Little, R.B. Vibrios (Vibrio jejuni N. Sp.) associated with intestinal disorders of cows and calves. J. Exp. Med. 1931. 53, 853-864.

Jørgensen, K. Forekomst af Campylobacter hos svin. Nord. Vet. Med. 1979. 31, 534.

Kampelmacher, E.H.; Guinée, P.A.M. and Noorle Jansen, L.M. van. Studies on the use of surface decontamination of Salmonella-contaminated mesenteric lymph nodes by boiling water. Zbl. Bakt., I. Abt. Orig. 1965. 195, 34-52.

King, E.O. Human infections with Vibrio fetus and a closely related Vibrio. J. Inf. Dis. 1957. 101, 119-128.

King, E.O. The laboratory recognition of Vibrio fetus and a closely related Vibrio isolated from cases of human vibriosis. Ann. N. Y. Acad. Sci. 1962. 98, 700-711.

Klingeren, B. van. A two-tier system for the evaluation of disinfectants. Thesis. University of Utrecht, the Netherlands 1983.

Koidis, P. and Doyle, M.P. Procedure for increased recovery of Campylobacter jejuni from inoculated unpasteurized milk. Appl. Environ. Microbiol. 1984. 47, 445-460.

Lander, K.P. and Gill, K.P.W. Experimental infection of the bovine udder with Campylobacter coli/jejuni. J. Hyg. 1980. 84, 421-428.

Luechtefeld, N.W. and Wang, W.-L.L. Campylobacter fetus subsp. jejuni in a turkey processing plant. J. Clin. Microbiol. 1981. 13, 266-268.

Man, J.C. de. The probability of most probable numbers. Eur. J. Appl. Microbiol. 1975. 1, 67-78.

McNaughton, R.D.; Leyland, R. and Mueller, L. Outbreak of Campylobacter enteritis due to consumption of raw milk. Can. Med. Assoc. J. 1982. 126, 657-658.

Mentzing, L.O. Waterborne outbreaks of Campylobacter enteritis in central Sweden. Lancet 1981. ii, 352-354.

Mouton, R.P.; Veltkamp, J.J.; Lauwers, S. and Butzler, J.P. Analysis of a small outbreak of campylobacter infections with high morbidity. In: Newell, D.C. (ed.). Campylobacter. Epidemiology, pathogenesis and biochemistry. MTP Press Ltd., Lancaster 1982. 129-134.

Notermans, S.; Dufrenne, J. and Leeuwen, W.J. van. Contamination of broiler chickens by Staphylococcus aureus during processing; incidence and origin. J. Appl. Bacteriol. 1982. 52, 275-280.

Notermans, S.; Leusden, F.M. van and Schothorst, M. van. Suitability of different bacterial groups for determining faecal contamination during post scalding stages in the processing of broiler chickens. J. Appl. Bacteriol. 1977. 43, 383-389.

Oosterom, J. Het voorkomen van Campylobacter fetus subspecies jejuni bij normale slachtvarkens. Tijdschr. Diergeneeskd. 1980. 105, 49-50.

Oosterom, J.; Beckers, H.J.; Noorle-Jansen, L.M. van and Schothorst, M. van. Een explosie van Campylobacter-infectie in een kazerne, waarschijnlijk veroorzaakt door rauwe tartaar. Ned. Tijdschr. Geneeskd. 1980. 124, 1631-1634.

Palmer, S.R.; Gully, P.R.; White, J.M.; Pearson, A.D.; Suckling, W.G.; Jones, D.M.; Rawes, J.C.L. and Penner, J.L. Water-borne outbreak of Campylobacter gastroenteritis. Lancet 1983. i, 287-290.

Park, C.E.; Lior, H.; Pauker, P. and Purvis, U. Campylobacter food poisoning from steaks - Ontario. Can. Dis. Wkly. Rep. 1982. 8, 177.

Park, C.E.; Stankiewicz, Z.K.; Lovett, J. and Hunt, J. Incidence of Campylobacter jejuni in fresh eviscerated whole market chickens. Can. J. Microbiol. 1981. 27, 841-842.

Robinson, D.A. and Jones, D.M. Milk-borne campylobacter infection. Br. Med. J. 1981. i, 1374-1376.

Rosef, O. and Kapperud, G. House flies (Musca domestica) as possible vectors of Campylobacter fetus subsp. jejuni. Appl. Environ. Microbiol. 1983. 45, 381-383.

Severin, W.P.J. Epidemiology of campylobacter infection. In: Newell, D.C. (ed.). Campylobacter. Epidemiology, pathogenesis and biochemistry. MTP Press Ltd., Lancaster 1982. 285-287.

Simmons, N.A. and Gibbs, F.J. Campylobacter spp. in oven-ready poultry. J. Infect. 1979. 1, 159-162.

Skirrow, M.B. and Benjamin, J. "1001" Campylobacters: cultural characteristics of intestinal campylobacters from man and animals. J. Hyg. 1980. 85, 427-442.

Smeltzer, T.; Thomas, R. and Collins, G. The role of equipment having accidental or indirect contact with the carcass in the spread of Salmonella in an abattoir. Aust. Vet. J. 1980. 56, 14-17.

Smibert, R.M. Vibrio fetus var. intestinalis isolated from the intestinal content of birds. Am. J. Vet. Res. 1969. 30, 1437-1442.

Snijders, J.M.A. and Gerats, G.E. Hygiene bei der Schlachtung von Schweinen. VI. Die Verwendung eines Infrarottunnels in der Schlachtstrasse. Fleischwirtschaft 1977. 57, 2216-2219.

Stern, N.J. Recovery rate of Campylobacter fetus ssp. jejuni on eviscerated pork, lamb and beef carcasses. J. Food Sci. 1981. 46, 1291.

Sticht-Groh, V. Campylobacter in healthy slaughter pigs: a possible source of infection for man. Vet. Rec. 1982. 110, 104-106.

Svedhem, A. and Kaijser, B. Isolation of Campylobacter jejuni from domestic animals and pets: probable origin of human infection. J. Infect. 1981. 3, 37-40.

Teufel, P. Campylobacter fetus ss. jejuni - Ausscheidungsraten beim Schwein und Überleben in Leitungswasser und Hackfleisch. Fleischwirtschaft 1982. 62, 1344-1345.

Turnbull, P.C.B. and Rose, P. Campylobacter jejuni and salmonella in raw red meats. J. Hyg. 1982. 88, 29-37.

Vogt, R.L.; Sours, H.E.; Barrett, T.; Feldman, R.A.; Dickinson, R.J. and Witherell, L. Campylobacter enteritis associated with contaminated water. Ann. Intern. Med. 1982. 96, 292-296.

Weber, A.; Lembke, C. and Kettner, A. Nachweis von Campylobacter jejuni in Kotproben von klinisch gesunden Brieftauben. Berl. Münch. Tierärztl. Wochenschr. 1981. 94, 449-451.

Wit, J.C. de and Kampelmacher, E.H. Some aspects of microbial contamination of hands of workers in food industries. Zbl. Bakt. Hyg. I Abt. Orig. B 1981. 172, 390-400.

CHAPTER VI

EPIDEMIOLOGICAL INVESTIGATIONS ON *CAMPYLOBACTER JEJUNI* IN HOUSEHOLDS WITH A PRIMARY INFECTION

Most investigations presented in this Chapter are also described in the following publication:

Oosterom, J.; Uyl, C.H. den; Bänffer, J.R.J. and Huisman, J. Epidemiological investigations on Campylobacter jejuni in households with a primary infection. J.Hyg. 1984. 92, 325-332.

CHAPTER VI

EPIDEMIOLOGICAL INVESTIGATIONS ON *CAMPYLOBACTER JEJUNI* IN HOUSEHOLDS WITH A PRIMARY INFECTION

Introduction

In recent years C.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 enteritis who consult their physician have campylobacter infections (Severin, 1978), a percentage that is comparable with those found in other countries (Telfer Brunton and Heggie, 1977; de Mol and Bosmans, 1978; Bengtsson and Uhnoo, 1978). In general, surveys have shown that the prevalence of campylobacter infections exceeds that of human salmonellosis.

Campylobacter enteritis is mainly a food-borne infection. Chickens, pigs, sheep, cattle, dogs, cats and wild birds have been found to be carriers of the organism, in most cases without showing any symptoms of disease (Devriese and Devos, 1971; Oosterom, 1980; Clark and Monsbourgh, 1979; Elazhary, 1968; Bruce et al., 1980; Smibert, 1969; Luechtefeld et al., 1980). In consequence, the foods most often implicated are those of animal origin, particularly poultry (Severin, 1982). Apart from foods, dogs may sometimes be possible direct sources of infection for man, in particular puppies with diarrhoea (Blaser et al., 1978). Person-to-person spread also occurs, mostly among young children (Butzler and Skirrow, 1979). In some countries like Sweden, the U.K., Australia and Finland, it is assumed that a high proportion of campylobacter infections

are acquired abroad (Falsen et al., 1978; Anonymous, 1978; Cavanagh et al., 1980; Pitkänen, 1982).

The object of this investigation was to identify, by means of a case control study, significant epidemiological factors such as foods eaten, methods of food preparation and the possible involvement of pet animals in the acquisition of campylobacter enteritis in Rotterdam households. In addition, the symptomatology of campylobacter infection was studied.

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, out-patient clinics and a number of hospitals in the Rotterdam region. It serves a population of about 1,100,000 people. In 1982, the laboratory received some 11,000 primary faecal samples and isolated C.jejuni from 8.5 % of them. The corresponding isolation rates for other bacteria were: Salmonella spp., 5.1 %; Shigella spp., 1.4 %; Yersinia enterocolitica 1.0 % (Bänffer, personal communication).

The investigations described here were carried out from June to September 1982. Fifty-four patients with primary C.jejuni infections (index patients), selected at random, were studied; each patient was living in a separate household (index household).

Household enquiries

Visits were paid to index households as soon as possible after the first isolation of C.jejuni from a stool sample, usually five to eight 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 seven days before the onset of disease, and whether the food had been eaten raw or undercooked. Modes for food preparation (including barbecues) and eating outdoors.
4. Travel abroad in the two weeks before the onset of disease.
5. Possession of pet animals: species, numbers, degree of contact, any illness, whether given the same food as that 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 two weeks before the enquiry. The enquiry procedure 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 seven and 14 days before the day of enquiry.

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

Bacteriological sampling of households

After the questioning of index patients, arrangements were made for the collection of stool samples from these individuals and, as far as possible, from household members with symptoms of enteritis. In a number of cases patients were requested to submit stool samples daily in order to investigate the duration of campylobacter excretion. 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. Immediately before use, the swabs were moistened with sterile physiological saline. The kitchen work-surfaces were mostly dry, the lavatory-bowl surfaces were mostly wet.

Isolation methods

First stool samples from index patients were cultured on campylobacter selective agar made according to the Butzler formula and incubated under micro-aerobic conditions for 72 hours at 42°C.

Subsequent stool samples from index patients and household contacts (human and animal) were transported (usually within 24 hours) to the Laboratory for Water and Food Microbiology at Bilthoven, where they were cultured in THAL enrichment broth. After incubation cultures were streaked onto Skirrow's agar with growth promoting supplement (Chapter IV). Surface swabs were put into 100 ml of THAL broth and treated in the same way.

For the collection of stool samples to assess the duration of campylobacter excretion, a modification of the method described by Lie Kian Joe (1950) was employed. Patients were supplied with a number of normal mailing envelopes, each containing a round sheet of filter paper

with a diameter of 8.5 cm, moistened with physiological saline in a small plastic sachet which could be made air-tight by means of adhesive plaster. The envelopes further contained a small paper folder for the sachet and a spoon. The entire pack was sterilised by irradiation (Gammaster, Wageningen, The Netherlands). Patients were requested to transfer daily some faeces to the filter paper by means of the spoon and, after closing the plastic sachet, to put it in the folder and subsequently into the envelope, and to send the envelope to the laboratory by regular mail. In the laboratory, a suspension was made from the faecal material present on each filter paper. This suspension was then examined as described above.

Antibody detection

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

Results

Index and secondary patients

During the investigations in the 54 index households, 21 additional people obviously suffering from campylobacter enteritis were found. Therefore, some of the data to be presented subsequently refer to 75 patients, in stead of to the initial 54.

Age and sex distribution

The age and sex distribution of the 54 initial and 21 additional patients are recorded in Figure VI.1. Campylobacter infection was most frequently demonstrated in children from birth to nine years of age (12 patients, of whom four were under one year old), in men and women of the 20-29 year age group (10 males and 10 females) and in men of 30-39 years (11 patients). Women between 30 and 39 were less frequently affected (five cases). In total, 57.3 % of the patients were men and 42.7 % women.

Symptoms

In Table VI.1, the different symptoms experienced during campylobacter infection are listed for the group of 75 patients. Abdominal cramps and diarrhoea occurred in more than 90 % of the cases, and fever (with a temperature ranging from 38.0 to 40.2°C) was noted in more than 70 %. Other symptoms were less frequently observed. The occurrence of overt blood in the stools was reported by 19 % of the patients.

The median duration of abdominal cramps and diarrhoea was seven and six days respectively, and for fever one day. In the most extreme case, cramps occurred for 40 days, and in another diarrhoea was observed for 23 days. In general, people felt ill for eight days and were unfit to perform their daily duties for six days. One patient suffered from general weakness for 65 days after a campylobacter infection.

Concurrent infections

The routine bacteriological examination of stool samples at the Laboratory of the Municipal Public Health Service at Rotterdam revealed that three of 54 initial patients were also infected with Salmonella spp., but no shigella or Yersinia enterocolitica strains were isolated.

Figure VI.1 Differentiation of 54 initial and 21 additional patients with campylobacter enteritis according to age and sex

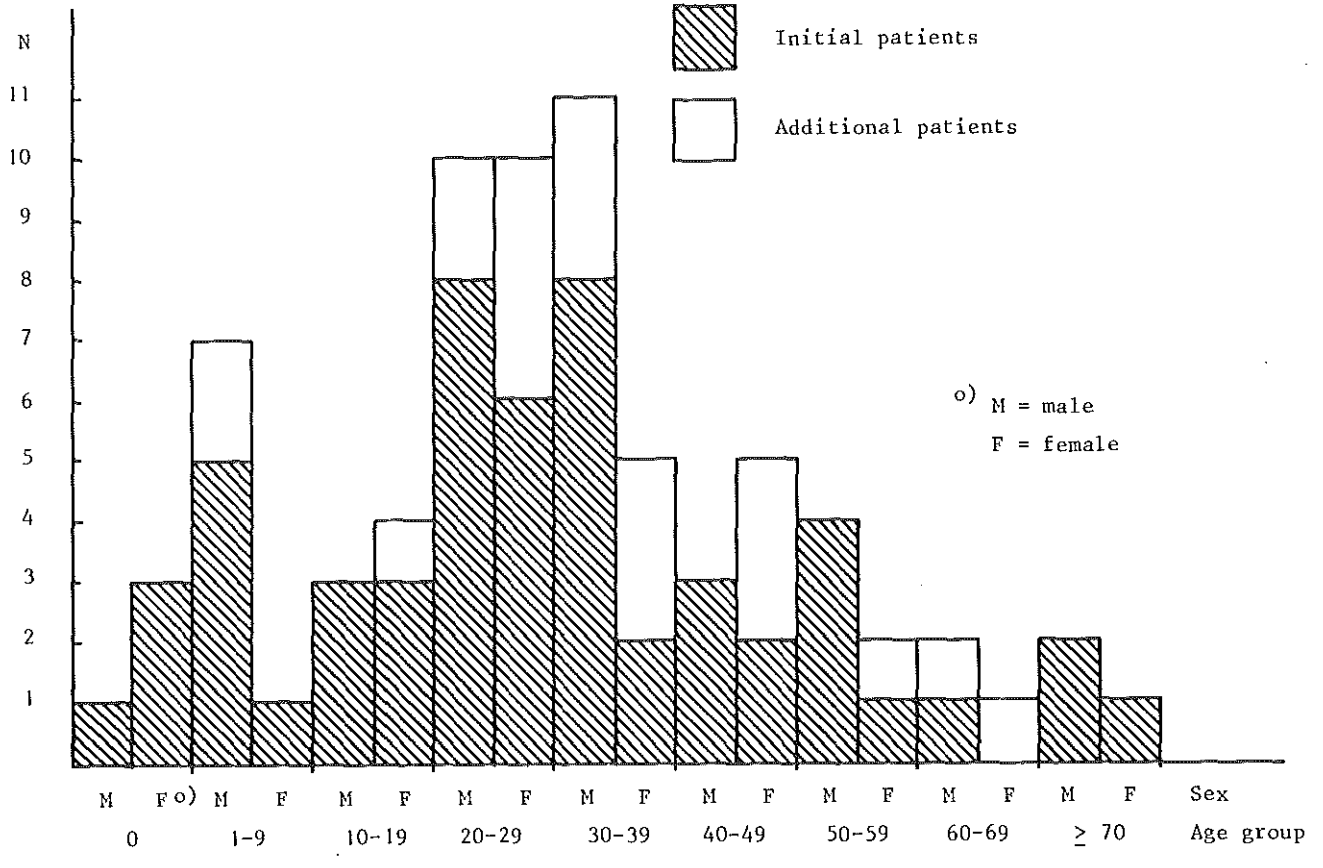


Table VI.1 The occurrence of different symptoms, together with some of the median and maximum duration times, reported for 75 patients suffering from campylobacter enteritis

Symptoms	Number of patients involved	Percentage of total number	Median duration (days)	Maximum duration (days)
Abdominal cramps	72	96	7	40
Diarrhoea	70	93	6	23
Fever	54	72	1	5
Blood in stools	14	19		
Nausea	29	39		
Vomiting	13	17		
Headache	9	12		
Myalgia	4	5		
Dizziness	4	5		
Total period of illness			8	65
Total period unable to work			6	65

All three patients with combined salmonella/campylobacter infection experienced illness of about average severity and average duration.

Use of medicines

In total, 43 of 54 initial patients (79.6 %) used medicines at some time during their campylobacter enteritis. Drugs for alleviating the immediate effects of the enteritis were prescribed in 31 instances (loperamide and papaverin, 23; tanalbumin, 6; oral rehydration salts; 2) and in 19 instances antibiotics were given (erythromycin, 16; sulphonamide, 2 and tetracycline, 1). One of the 21 additional patients took loperamide.

Duration of carrier state

Thirty-one patients sent envelopes to the laboratory at Bilthoven to enable the duration of their carrier state to be assessed. Excretion periods were calculated from the first day of illness to the last day on which a campylobacter positive stool sample was collected. In 19 patients the carrier state lasted less than three weeks (this group comprised all 11 of the patients who both received antibiotic treatment and were examined for the duration of excretion). Nine patients had a carrier state which lasted from three to six weeks, two patients had excretion times lasting between six and eight weeks and one person still had campylobacters in his stools in the ninth week.

Evaluation of risk factors

Food consumption and preparation

Consumption of relevant foods by both index patients and controls are listed in Table VI.2. 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

Table VI.2 Foods consumed and cooking and eating habits in relation to the occurrence of campylobacter infection

Foods/means of preparation or consumption	Index patients (n = 54)	Controls (n = 54)	Significance (p value)
Chicken meat	47	29	0.0002
Pork	47	39	0.048
Beef	43	44	n.s.*)
Mutton	2	4	n.s.
Raw or inadequately heated meat	13	8	n.s.
Barbecue	14	2	0.0015
Eating outdoors	17	17	n.s.

*) n.s. = not significant ($p > 0.05$)

Table VI.3 The possession 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	n.s.*)
Dogs	13	13	n.s.
Cats	12	12	n.s.
Cage birds	18	12	n.s.
Chickens	1	1	n.s.
Rodents	4	5	n.s.

*) n.s. = not significant ($p > 0.05$)

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 VI.3). More index than control households kept cage birds, but the numbers recorded were too low to show a significant difference.

Travelling abroad

Numbers of index patients and controls that had travelled abroad were almost the same (five and six respectively). All but one had travelled in Southern or Eastern Europe. One patient had been infected in Pakistan.

Infection in household contacts

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

Of the 21 contacts showing symptoms, 13 (in nine households) had eaten the same suspect food as their respective index patients and were considered to have been infected from this common source; the onset of illness in these patients was generally within two days of the index patient. In four cases the timing of the illness (onset more than four days later than in the index patient) and details of the foods eaten

suggested that they had been secondarily infected from their respective index patients. In the remaining four contacts the source of infection was uncertain, but apparently two of them had been the source of infection for two of the index patients.

Bacteriological examination of 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, 5 rodents, 15 chickens and one goat). One dog and one cat had campylobacters in their faeces but neither showed signs of illness. The chickens also had C.jejuni in their droppings.

Estimation of incubation times

Identification of the most likely source of infection has been used in assessing the incubation time for campylobacter infection in 40 separate cases. In Table VI.4 a list has been made of incubation times determined in this way. In most instances, the incubation time seems to be about two to four days (22 cases or 55 %), but in two cases only a few hours of incubation was observed. In two other cases an incubation time of one week or more must be accepted.

Discussion

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

Table VI.4 Estimation of incubation times for campylobacter infections in 40 patients

Incubation times	Number of patients
Several hours	2
1 day	3
1.5 days	4
2 days	6
3 days	8
4 days	8
5 days	5
6 days	2
7 days	1
8 days	1
	median : 3 days

Earlier investigations in the Netherlands also indicated that poultry was a possible source of campylobacter infection (Severin, 1982). Other studies in this country showed that poultry and pigs were frequently infected with campylobacters (Hartog and de Boer, 1982; Oosterom, 1980), whereas only 5.5 % of cattle carried these organisms, mostly in low numbers. Campylobacters were isolated from 50 to 75 % of poultry products (Chapter V). 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 and Rose, 1982) and is seldom reported as a source of human infection (Anonymous, 1981).

The transmission of campylobacters from chicken carcasses to the consumer may be via undercooked meat, but it seems more likely that the handling of raw poultry (Norkrans and Svedhem, 1982) and subsequent immediate 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 K 12, causes extensive contamination of surfaces, utensils and hands (de Wit et al., 1979).

Poultry is also frequently contaminated by 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. As the investigations described here were carried out between June and September they may have accentuated the contribution of barbecueing to infection.

It was reported earlier that eating at restaurants and the possession of cage birds (Severin, 1982) also were significant factors in acquiring campylobacter enteritis. These findings were not confirmed by our study, although we found that more patients than controls had cage birds. 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, firstly because almost all milk is pasteurised in the Netherlands and secondly because in this country raw milk did not appear to be contaminated by campylobacters (Chapter V).

In complete accordance with a study in Sweden, in which 55 cases of campylobacter infection were investigated (Norkrans and 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 and Skirrow (1979).

Concerning the distribution of patients according to age and sex, it must be accepted that numbers are too small to draw definite conclusions, but there seems to be little difference from the overall pattern described by others. Both Skirrow (1977) and Severin (1978) observed that the young adult in particular is affected by campylobacteriosis. Severin noted that young children form a second group with a high incidence, but suggested that this age group is also the one most frequently examined. Comparison of our data with those for faecal samples from the different age groups in Rotterdam for the year 1982 suggests that this assumption is correct.

Data concerning symptoms, probable incubation periods and periods of carriage are well within the limits given by others (Butzler and Skirrow, 1979). Only a few minor differences are worth to be mentioned. In this

study, and in an earlier one involving Dutch soldiers (Oosterom et al., 1980), only 19 % of patients were found to have overt blood in their stools. However, higher percentages (40-90 %) are sometimes recorded in the literature (Blaser et al., 1979; Karmali and Fleming, 1979). Secondly, people were usually unfit to perform their daily duties for about 7.5 days (median six days), while Skirrow reported that people were unable to work for 10 to 14 days (Skirrow, 1977). We observed that many patients felt ill for longer periods (median eight days) than their inability to return to work lasted (Table VI.1).

As far as incubation periods are concerned, we noted in two instances that the incriminated food was consumed five to six hours prior to the onset of symptoms. So far, no such observations have been found in the literature.

Because the number of observations was too small, we had no opportunity to evaluate the effect of antibiotics on either symptoms or excretion periods during campylobacter enteritis.

Campylobacters were cultured 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 et al., 1978). The low prevalence of campylobacters on kitchen surfaces can be explained by their extreme sensitivity to drying (Chapter V). This finding, together with the fact that C.jejuni does not grow at temperatures below 30°C (Skirrow and Benjamin, 1980), means that the mechanism of cross-contamination is generally of less consequence in the epidemiology of campylobacters than of salmonellae, unless infection takes place immediately after cross-contamination.

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.

References

Anonymous. Campylobacter infections in Britain, 1977. Br. Med. J. 1978. i, 1357.

Anonymous. Campylobacter infections, 1977-1980. Br. Med. J. 1981. i, 1484.

Bengtsson, S. and Uhnoo, I. Campylobacter som orsak till enterit. Lakartidningen 1978. 75, 4703-4706.

Blaser, M.; Cravens, J.; Powers, B.W. and Wang, W.L. Campylobacter enteritis associated with canine infection. Lancet 1978. ii, 979-981.

Blaser, M.J.; Berkowitz, I.D.; LaForce, F.M.; Cravens, J.; Reller, L.B. and Wang, W.-L.L. Campylobacter enteritis: clinical and epidemiological features. Ann. Intern. Med. 1979. 91, 179-185.

Brouwer, R.; Mertens, M.J.A.; Siem, T.H. and Katchaki, J. An explosive outbreak of Campylobacter enteritis in soldiers. Antonie Leeuwenhoek Microbiol. 1979. 45, 517-519.

Bruce, D.; Zochowski, W. and Fleming, G.A. Campylobacter infections in dogs and cats. Vet. Rec. 1980. 107, 200-201.

Butzler, J.P. and Skirrow, M.B. Campylobacter enteritis. Clin. Gastroenterol. 1979. 8, 737-765.

Cavanagh, P.; Ryden, A.; Pritchard, H. and Kaldor, J. The public-health significance of campylobacter enteritis. Food Technol. Aust. 1980. 32, 98-100.

Clark, B.L. and Monsbourgh, M.J. The prevalence of Campylobacter fetus in the gall bladder of sheep. Aust. Vet. J. 1979. 55, 42-43.

De Mol, P. and Bosmans, E. Campylobacter enteritis in Central Africa. Lancet 1978. i, 604.

Devriese, L. and Devos, A. Vibrio spp. bij pluimvee: isolatie, identificatie en experimentele infectie. Tijdschr. Diergeneeskd. 1971. 96, 193-201.

Elazhary, M.A.S.Y. An assay of isolation and differential identification of some animal Yibrios and of elucidation of their pathological significance. Meded. Veeartsenijschool Univ. Gent 1968. 12.

Falsen, E.; Kaijser, B.; Nehls, L.; Nygren, B. and Svedhem, A. Campylobacter coli – en nyupptäckt, vanlig orsak till diarré hos människa. Lakartidningen 1978. 75, 4697-4699.

Hartog, B.J. and Boer, E. de. Campylobacter jejuni in poultry products from retail outlets and in poultry slaughterhouses. In: Newell, D.G. (ed.). Campylobacter. Epidemiology, pathogenesis and biochemistry. MTP Press Ltd., Lancaster 1982. 270.

Karmali, M.A. and Fleming, P.C. Campylobacter enteritis in children. J. Pedriatr. 1979. 94, 527-533.

Kist, M. Campylobacter enteritis: epidemiological and clinical data from recent isolations in the region of Freiburg, West Germany. In: Newell, D.G. (ed.). Campylobacter. Epidemiology, pathogenesis and biochemistry. MTP Press Ltd., Lancaster 1982. 138-143.

Lie Kian Joe. A simple method of sending dysenteric faeces to a laboratory. Docum. neerl. indones. Morbis trop. 1950. 2, 97-112.

Luechtefeld, N.A.W.; Blaser, M.J.; Reiller, L.B. and Wang, W.-L.L. Isolation of Campylobacter fetus subsp. jejuni from migratory waterfowl. J. Clin. Microbiol. 1980. 12, 406-408.

Mouton, R.P.; Veltkamp, J.J.; Lauwers, S. and Butzler, J.-P. Analysis of a small outbreak of campylobacter infections with high morbidity. In: Newell, D.G. (ed.). Campylobacter. Epidemiology, pathogenesis and biochemistry. MTP Press Ltd., Lancaster 1982. 129-134.

Norkrans, G. and Svedhem, A. Epidemiological aspects of Campylobacter jejuni enteritis. J. Hyg. 1982. 89, 163-170.

Oosterom, J. Het voorkomen van Campylobacter fetus subspecies jejuni bij normale slachtvarkens. Tijdschr. Diergeneeskd. 1980. 105, 49-50.

Oosterom, J.; Beckers, H.J.; Noorle-Jansen, L.M. van and Schothorst, M. van. Een explosie van Campylobacter-infectie in een kazerne, waarschijnlijk veroorzaakt door rauwe tartaar. Ned. Tijdschr. Geneeskd. 1980. 124, 1631-1634.

Pitkänen, T. Travellers' diarrhoea caused by Campylobacter jejuni. Ann. Clin. Res. 1982. 14, 111-113.

Schothorst, M. van; Huisman, J. and Os, M. van. Search for Salmonellae in homes with salmonellosis in infants. Ned. Tijdschr. Geneeskd. 1978. 122, 1121-1125.

Severin, W.P.J. Campylobacter en enteritis. Ned. Tijdschr. Geneeskd. 1978. 122, 499-504.

Severin, W.P.J. Epidemiology of campylobacter infection. In: Newell, D.C. (ed.). Campylobacter. Epidemiology, pathogenesis and biochemistry. MTP Press Ltd., Lancaster 1982. 285-287.

Skirrow, M.B. Campylobacter enteritis: a "new" disease. Br. Med. J. 1977. ii, 9-11.

Skirrow, M.B. and Benjamin, J. Differentation of enteropathogenic campylobacter. J. Clin. Pathol. 1980. 33, 1122.

Smibert, R.M. Vibrio fetus var. intestinalis isolated from the intestinal content of birds. Am. J. Vet. Res. 1969. 30, 1437-1442.

Telfer Brunton, W.A. and Heggie, D. Campylobacter-associated diarrhoea in Edinburgh. Br. Med. J. 1977. 2, 956.

Teufel, P. Campylobacter fetus ss. jejuni - Ausscheidungsraten beim Schwein und Überleben in Leitungswasser und Hackfleisch. Fleischwirtschaft 1982. 62, 1344-1345.

Turnbull, P.C.B. and Rose, P. Campylobacter jejuni and salmonella in raw red meats. J. Hyg. 1982. 88, 29-37.

Wit, J.C. de; Broekhuizen, G. and Kampelmacher, E.H. Cross-contamination during the preparation of frozen chickens in the kitchen. J. Hyg. 1979. 83, 27-32.

CHAPTER VII

EVALUATION OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR THE DETECTION OF *CAMPYLOBACTER JEJUNI* ANTIBODIES, AND COMPARISON WITH A COMPLEMENT FIXATION TEST (CFT)

The investigations presented in this Chapter are also described in the following publication:

Oosterom, J.; Uyl, C.H. den; Bänffer, J.R.J.; Lauwers, S.; Huisman, J.; Busschbach, A.E.; Poelma, F.G.J. and Bellemans, R. Evaluation of an enzyme-linked immunosorbent assay (ELISA) for the detection of Campylobacter jejuni antibodies, and comparison with a complement fixation test (CFT). Antonie Leeuwenhoek Microbiol., accepted.

CHAPTER VII

EVALUATION OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR THE DETECTION OF *CAMPYLOBACTER JEJUNI* ANTIBODIES, AND COMPARISON WITH A COMPLEMENT FIXATION TEST (CFT)

Introduction

Since the discovery that C.jejuni is an important enteropathogen in man, efficient isolation methods have been developed for the bacteriological examination of campylobacter infections (Skirrow, 1977; Lauwers et al., 1978; Goossens et al., 1983). In addition, attempts were made to develop appropriate serological methods for the detection of campylobacter antibodies in infected individuals. In certain cases serological diagnosis of the infection may have some advantages over bacteriological procedures.

In the first place, serological tests usually can be performed within 24 h, whereas culturing of the organism generally takes two or three days. Serological examination may even detect campylobacter infections when, for some reason, the organism can no longer be isolated from the patient's stools. This may happen when the examination is carried out some time after the onset of disease (for instance, in the case of persistent abdominal cramps) or when antibiotics have been used. Finally, campylobacter serology may also be of help in the diagnosis of reactive arthritis. For some of the above reasons, serological tests are also very suitable for use in epidemiological surveys.

During the early years, many different techniques such as complement fixation, agglutination, immunofluorescence and one involving the bactericidal effects of antibodies have been assessed (Butzler, 1974; Skirrow, 1977; Prescott and Karmali, 1978; Watson et al., 1979; Jones et al., 1980), but apparently none was fully satisfactory. In recent years, attention has been focused on the complement fixation test (CFT) and the enzyme-linked immunosorbent assay (ELISA) (Mosimann et al., 1981; Walder and Forsgren, 1982; Svedhem et al., 1983). Some years ago, we tried a tube agglutination test in our laboratory, but this test gave variable results, mainly because it was difficult to read (Oosterom et al., 1980). Later we developed an ELISA system. After preliminary evaluation, the opportunity occurred to use this ELISA to examine a large number of sera, collected during epidemiological investigations of Rotterdam households with campylobacter infections. Over the same period, a complement fixation test became commercially available. For further evaluation of the ELISA system we also used this CFT to test the Rotterdam sera, and compared the results of both methods.

Materials and methods

The ELISA system

Antigen preparation.

Antigen suspensions were prepared from strains C 235 and C 283, isolated from the stools of patients following a campylobacter outbreak in a military camp (Oosterom et al., 1980). Both strains showed all the reactions characteristic of C.jejuni (Chapter IV), also, both were able to hydrolyse sodium hippurate. According to the serotyping system of Lauwers and Penner (Penner and Hennessy, 1980; Lauwers et al., 1981), strain C 235 belonged to serotype LAU 5,8, while strain C 283 was

untypable.

The strains were cultured on Skirrow's agar for 24 h at 42°C in a micro-aerobic atmosphere (Chapter IV). The cells were harvested, washed twice in physiological saline containing 0.01 % merthiolate and resuspended 1 : 40 in the same solution. Suspensions were then mixed 1 : 1 with sterile, fine-grained sand and shaken in a Mickle disintegrator for 30 min. Disintegration of cells was checked by microscopy. After disintegration, the antigen suspension was separated from the sand grains and brought to a protein concentration of 450 µg/ml. Antigen suspensions derived from the two strains were mixed in equal proportions. Stock suspensions thus produced were stored at 4°C until required for use.

Preliminary investigations had demonstrated that these antigen suspensions were preferable to those containing whole cells or extracts of the same cells made by treatment with phenol-water or glycine-HCl.

The assay.

Prior to use, antigen suspensions were diluted 1 : 200 in phosphate buffered saline (PBS) with pH 7.2. Of this dilution, 100 µl were transferred by pipette to each well of a microtitre plate (Dynatech, Microelisa). Adsorption was allowed to take place overnight at room temperature with rotation of the plates. The plates were then washed with tap water containing 0.05 % Tween 20. After washing, any remaining water was carefully shaken off.

Serial two-fold dilutions of sera were made in PBS containing 0.05 % Tween 20 and 100 µl of each dilution were transferred to the wells as before. The plates were incubated with rotation at room temperature for 1.5 h; then they were washed again.

Horse-radish peroxidase-labelled sheep-anti-human total immuno-

globulins (Dr.J.Nagel, National Institute of Public Health and Environmental Hygiene, Bilthoven, the Netherlands) were used as a conjugate at a working dilution of about 1 : 1000 in PBS containing 0.05 % Tween 20, the exact concentration depending on the activity of each separate batch. Next, 100 µl of this conjugate dilution were transferred to each well and again incubation was for 1.5 h at room temperature, followed by washing.

The substrate was prepared just before use. A quantity of 80 mg of 5-amino 2-hydroxy benzoic acid (Merck) was dissolved in 100 ml of distilled water and the pH value was adjusted to 6.0. Then 0.05 % H_2O_2 was added in the proportion of 1 : 9. To each well were added 100 µl of substrate solution and after 1 h of incubation with rotation, extinction at 450 nm was measured by means of a Titertek Multiscan (Flow Laboratories).

Campylobacter antibody titres were determined according to the highest serum dilution that showed an extinction of $\geq$ 0.200. Slight variations in this limit were accepted according to the extinction values obtained with a known positive and negative control serum examined at the same time.

Evaluation of sensitivity and specificity of the ELISA system

Tests for possible cross-reactivity of campylobacter antibodies with antigens of other possibly enteropathogenic bacterial species were carried out with campylobacter-positive human sera, that is, sera with a total Ig antibody titre of $\geq$ 1 : 2560 as determined by the ELISA method. These sera were absorbed with disintegrated cells of respectively Yersinia enterocolitica, Salmonella typhi murium, Escherichia coli and a mixture of the strains C 235 and C 283 themselves. Extinction values for control and absorbed sera were measured in the ELISA system. This test

was also carried out in reverse. Six Y.enterocolitica - positive human sera (three against serotype 0:3 and three against serotype 0:9) were absorbed with broken cells of C.jejuni strains C 235 and C 283, after which the yersinia antibody titre was reassessed by means of a tube agglutination test.

Further tests were made to determine whether ELISA was suitable to detect antibodies produced against different serotypes of C.jejuni. For that purpose 44 hyper-immune sera were examined from rabbits which had been inoculated with different campylobacter serotypes as distinguished in the Lauwers and Penner typing system (Penner and Hennessy, 1980; Lauwers et al., 1981). Pre-immune sera were available from 21 of the rabbits and these were also tested in the ELISA system.

For further assessment of the sensitivity and specificity of the ELISA method, 121 sera were examined from patients with a bacteriologically proven campylobacter infection, samples having been collected at the acute or early convalescent stage. A limited proportion of these sera belonged to the 258 serum samples collected in the Rotterdam survey (see below). In parallel, 112 sera were tested from individuals not suspected of enteric disease (reference sera).

Examination of sera

Two hundred and fifty eight serum samples were collected from index cases and from household members with or without symptoms in an epidemiological survey of campylobacter infections in Rotterdam households (Chapter VI). In general, these serum samples were collected approximately 8, 22 and 110 days respectively after the onset of enteritis in each index patient. Three serum samples were obtained from each of 76 individuals, two from each of 14 and twice a single sample was received from separate individuals. These samples were examined by means

of the ELISA method described above and also by means of a CFT method (Mosimann et al., 1981) developed earlier. It may be assumed that the antigen suspension for CFT (which is now commercially produced by Institute Virion Ltd., Zürich, Switzerland) was different from that used in the ELISA test. The antigen for CFT consisted of several campylobacter strains, the cells of which, among other treatments, were broken by sonification. An antibody titre of 1 : 10 was considered positive in the CFT. The results of testing the sera by both ELISA and CFT were compared statistically.

Evaluation of antibody response in patients

Titre values determined by means of the ELISA method for sera collected in the Rotterdam investigations were related to the severity of campylobacter-associated symptoms in the respective patients. It was also determined whether titre values increased or decreased between days 8 and 22. Finally, the persistence of campylobacter antibodies in the blood of these patients after 110 days was assessed.

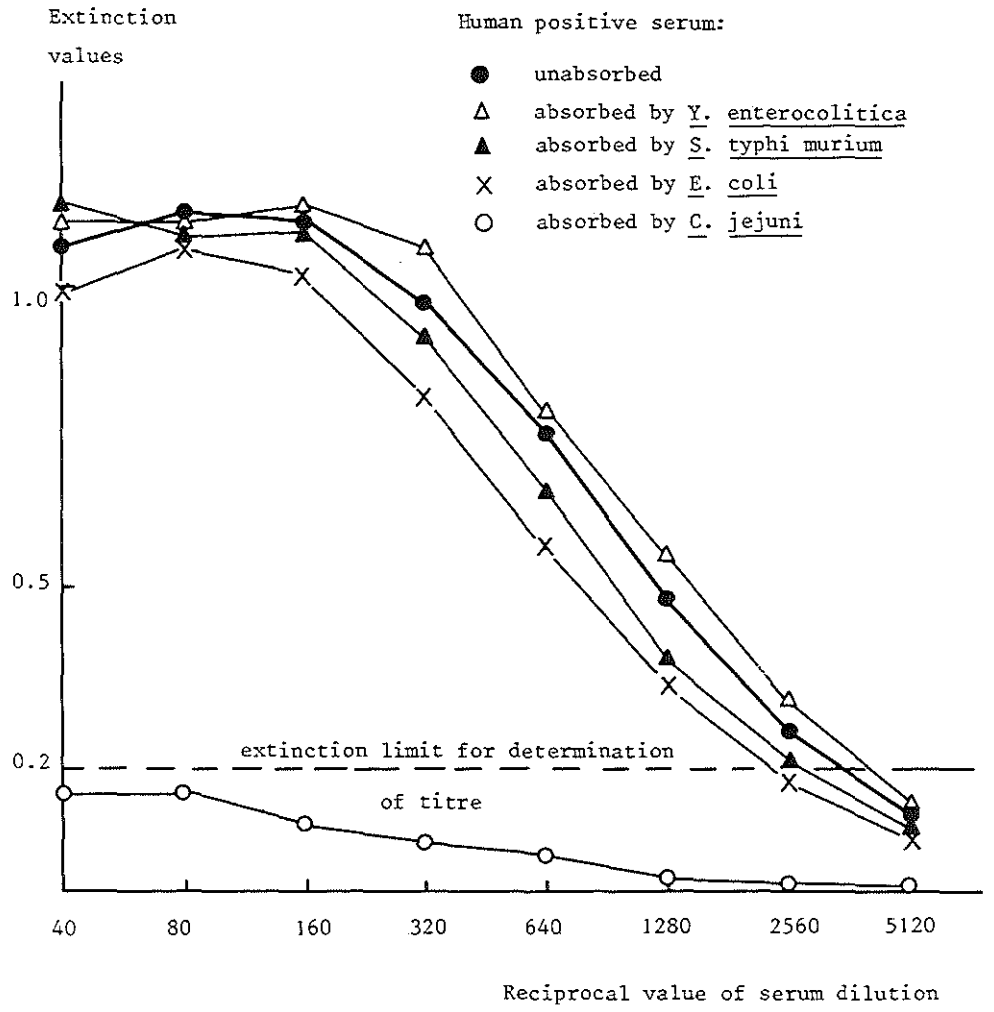
Results

Evaluation of the ELISA system

In Figure VII.1, the specificity of the ELISA test is demonstrated by the results of absorption of a positive human serum with different possibly enteropathogenic bacterial species. Extinction values were unaffected by absorption with Y.enterocolitica, S.typhi murium or E.coli, but fell below 0.2 when the serum was absorbed by C.jejuni. Similar tests were carried out with other campylobacter-positive human sera. Results were always identical with those presented in the Figure. In all six Y.enterocolitica sera the yersinia antibody titre remained unchanged after absorption with campylobacter antigens.

Figure VII.1

Determination of C. jejuni antibody titres by means of ELISA in a human positive serum with and without absorption by different possibly enteropathogenic bacterial species



The hyper-immune sera from rabbits examined by ELISA with disintegrated C 235 and C 283 strains as the antigen showed a titre of 1 :1280 in five instances and a titre of 1 : 2560 in 11, while the remaining 28 sera had titres of $\geq$ 1 : 5120. All available pre-immune sera from these rabbits showed a titre of $\leq$ 1 : 80, in most instances $\leq$ 1 : 40.

Table VII.1 gives antibody titres determined by means of ELISA for 51 human sera collected approximately eight days after the onset of disease, and for 70 human sera collected after approximately 22 days. All these sera were from cases of bacteriologically confirmed campylobacter infection. Also listed are the results of examining the 112 reference sera, obtained from healthy individuals. In the first group, 88 % of the sera showed an antibody titre of $\geq$ 1 : 320 and 65 % $\geq$ 1 : 640. For the second group, these figures were 86 % and 77 % respectively. Among the reference sera 31 % had a titre of $\geq$ 1 : 320 and 5 % $\geq$ 1 : 640.

Examination of sera

Examined by ELISA, 158 of 258 Rotterdam sera had a titre of $\leq$ 1 : 320, 49 sera had a titre of 1 : 640 whilst 51 serum samples showed higher titres ($\geq$ 1 : 1280). In the CFT, 166 sera were negative, 24 had a titre of 1 : 10 and 65 a titre of $\geq$ 1 : 20. Three sera showed anticomplementary reactions in this test, so statistical comparison of results obtained by ELISA and CFT was restricted to 255 sera (Table VII.2). This comparison revealed a very significant correlation ($p < 0.0001$).

Evaluation of antibody response in patients

Sera were collected both after eight and 22 days from 87 individuals with or without symptoms in the Rotterdam survey. Association of

Table VII.1 Examination of serum samples from patients with proven campylobacter infections and of reference samples for determining campylobacter antibody titres by means of ELISA

Reciprocal of antibody titre	Sera from patients collected at 8 days	Sera from patients collected at 22 days	Reference sera
< 80	0	1 (1.4%)	20 (17.9%)
80	2 (3.9%)	0	25 (22.3%)
160	4 (7.9%)	9 (12.9%)	32 (28.6%)
320	12 (23.5%)	6 (8.6%)	29 (25.9%)
640	9 (17.6%)	14 (20.0%)	3 (2.6%)
1280	12 (23.5%)	23 (32.9%)	3 (2.6%)
2560	8 (15.7%)	15 (21.4%)	0
5120	2 (3.9%)	1 (1.4%)	0
> 5120	2 (3.9%)	1 (1.4%)	0
Total	51 (100%)	70 (100%)	112 (100%)

Table VII.2 Cross tabulation of campylobacter antibody titres for 255 human sera, collected in an epidemiological survey, determined by means of ELISA and CFT methods

		Reciprocal values for antibody titres determined by ELISA					
		≤ 320	640	1280	2560	≥ 5120	Total
Reciprocal values for antibody titres determined by CFT	0	141	20	5	0	0	166
	10	9	11	4	0	0	24
	20	6	13	16	4	0	39
	40	0	4	7	3	0	14
	≥ 80	0	1	1	6	4	12
	Total	156	49	33	13	4	255

Statistical analysis for positive correlation: Spearman's rank correlation test (with correction for ties, normal approximation) : $p < 0.0001$
Estimated rank correlation coefficient : 0.81

antibody titres in these sera and the severity of symptoms experienced during campylobacter enteritis is presented in the Tables VII.3 and VII.4. In Table VII.3 data are given for sera collected after eight days. Statistical analysis showed a good association ($p = 0.004$). In Table VII.4 data are given regarding sera collected after 22 days. Again the association was strongly significant ($p < 0.001$).

Comparison of titre values determined by ELISA for 60 pairs of sera collected after eight and 22 days showed that these titres increased in 23 patients, remained the same in 22 patients and decreased in 15. No association was observed with the severity of symptoms experienced by the respective patients.

Examination of serum samples collected after approximately 110 days from 58 patients with bacteriologically proven campylobacter infection demonstrated that in 16 cases antibody titres of $\geq$ 1 : 640 were still present. Highest titres (1 : 2560) were associated with the most severe cases of enteritis experienced about four months earlier. In 22 cases (41 %) a four-fold decline in antibody titre was observed by comparison with that for the serum sample collected after eight or 22 days.

Discussion

In the preliminary tests of our study, it has been shown that, when cells are disintegrated, certain strains of C.jejuni possess common antigens that react with antibodies elicited by a great variety of campylobacter serotypes. However, there were no cross-reactions between these antibodies and antigens of certain other, possibly enteropathogenic bacterial species, so that the ELISA system developed seems to have a good immunological specificity.

Tests with sera from proven campylobacter cases demonstrated that

Table VII.3 Campylobacter antibody titres determined by ELISA in serum samples from 87 individuals with or without symptoms, collected approximately eight days after the onset of disease in the respective index patients, in relation to severity of enteritis-associated symptoms

		Reciprocal values for antibody titres							
		< 80	80	160	320	640	1280	2560	≥ 5120
Symptoms o)	-	1	7	8	5	6	0	0	0
	+	3	4	5	9	10	4	1	1
	++	0	0	0	6	4	9	1	3

o) - = no symptoms observed, bacteriological tests not done
+ = mild to average severity, bacteriological tests positive
++ = more than average severity (fever > 40°C, prostration or more than six bowel movements a day), bacteriological tests positive

Statistical analysis for association: chi square (after combination of columns < 80 and 80, columns 160 and 320 and columns ≥ 640): 15.43 (4 df); p = 0.004

Table VII.4 Campylobacter antibody titres determined by ELISA in serum samples from 87 individuals with or without symptoms, collected approximately 22 days after the onset of disease in the respective index patients, in relation to severity of enteritis-associated symptoms

		Reciprocal values for antibody titres						
		< 80	80	160	320	640	1280	2560
Symptoms o)	-	1	8	6	10	2	0	0
	+	2	1	7	9	5	12	1
	++	0	0	1	1	8	7	6

o) - = no symptoms observed, bacteriological tests not done
+ = mild to average severity, bacteriological tests positive
++ = more than average severity (fever > 40°C, prostration or more than six bowel movements a day), bacteriological tests positive

Statistical analysis for association: chi square (after combination of columns < 80 and 80, columns 160 and 320 and columns ≥ 640): 38.50 (4 df); p = < 0.001

not all patients develop large amounts of specific antibodies. In our study, positive campylobacter antibody titres were found in 77 % or 86 % of patients examined approximately 22 days after infection, and in 5 % or 31 % of controls, depending on the (arbitrary) choice of the lowest titre (1 : 640 or 1 : 320) indicative of campylobacter infection (Table VII.1). If we decide to choose a titre of 1 : 640 as the marginal value, statistical sensitivity is 77 % and specificity 95 %. These results are in accordance with data published by others. In general, positive antibody titres are found in 49 to 79 % of patients examined by means of a single test (Kosunen et al., 1981; Walder and Forsgren, 1982; Holländer, 1983; Kaldor et al., 1983). When more tests are employed, these percentages are higher. Detection of 95 % of patients by one test is only claimed by Svedhem et al. (1983), who used an ELISA method in gel (DIG-ELISA). Campylobacter antibodies in healthy individuals were generally found in 2 to 21 % of cases.

Some investigators make a distinction between different types of campylobacter enteritis and suspect that there is a relationship with variable behaviour of the organism (invasiveness, pathogenicity, toxin production). It is possible that in some cases antibody production is stimulated more than in others. In our study, the numbers of patients were too small to allow conclusions of this kind to be drawn.

Another problem with campylobacter serology is that elevated serum titres can exist in healthy individuals. Several factors may contribute to this phenomenon. First, it can be demonstrated that after infection campylobacter antibodies may persist for many months (Walder and Forsgren, 1982; Holländer, 1983). Secondly, the incidence of campylobacter enteritis in the population is high; in developed countries the organism is responsible for about 5 to 10 % of cases of acute

enteritis (Bruce et al., 1977; Steele and McDermott, 1978; Severin, 1978; Blaser et al., 1979). The first finding was confirmed by our study. The two factors (previous infections and persisting antibodies) might even play a role in the varying antibody responses that are observed after recent infection.

There appeared to be a good association between antibody titres and the severity of symptoms experienced after campylobacter infection (Tables VII.3 and VII.4). Considering these Tables in particular, the sensitivity of the ELISA system seems to be rather low (55 % and 65 % respectively of the examined individuals with symptoms showed a titre of $\geq$ 1 : 640). This must be ascribed to the fact that this group comprized mildly infected household members of index patients, who found no reason to consult a physician and were detected by chance during the epidemiological survey.

In general, campylobacter antibody titres are already at a high level on the eighth day after infection, but some further increase may be observed after 22 days (Tables VII.1, VII.3 and VII.4). However, in most cases this increase is not enough to yield a four-fold rise in titre. On the other hand, due to persisting antibodies, a four-fold decline in titre values was seen after 110 days in only 22 of 54 patients.

All investigations carried out were concerned with total antibody immunoglobulins. Preliminary investigations concerning the levels of anti- campylobacter IgM in the sera of patients did not appear to provide additional information.

A significant correlation was observed between results obtained by means of ELISA and those from CFT. This probably implies that all conclusions drawn concerning serology by ELISA (sensitivity, association with symptoms, persistence of antibodies) also apply to CFT.

In conclusion, it may be stated that ELISA is a good method for the detection of campylobacter antibodies in serum, especially in relation to epidemiological surveys. Due to the possible absence of detectable antibody response or the presence of persisting antibodies the test is less suitable for serological diagnosis in the individual patient.

References

Blaser, M.J.; Berkowitz, I.D.; LaForce, F.M.; Cravens, J.; Reller, L.B. and Wang, W.-L.L. Campylobacter enteritis: clinical and epidemiological features. Ann. Intern. Med. 1979. 91, 179-185.

Bruce, D.; Zochowski, W. and Ferguson, I.R. Campylobacter enteritis. Br. Med. J. 1977. ii, 1219.

Butzler, J.P. Campylobacter, een miskende enteropathogene kiem. Thesis Vrije Universiteit Brussel 1974.

Goossens, H.; Boeck, M. de and Butzler, J.P. A new selective medium for the isolation of Campylobacter jejuni from human faeces. Eur. J. Clin. Microbiol. 1983. 2, 389-394.

Holländer, R. Serologischer Nachweis von Antikörpern gegen Campylobacter jejuni/coli beim Menschen. Immun. Infekt. 1983. 11, 193-196.

Jones, D.M.; Eldridge, J. and Dale, B. Serological response to Campylobacter jejuni/coli infection. J. Clin. Pathol. 1980. 33, 767-769.

Kaldor, J.; Pritchard, H.; Serpell, A. and Metcalf, W. Serum antibodies in Campylobacter enteritis. J. Clin. Microbiol. 1983. 18, 1-4.

Kosunen, T.U.; Pitkänen, T.; Pettersson, T. and Pönkä, A. Clinical and serological studies in patients with Campylobacter fetus ssp. jejuni infection. II. Serological findings. Infection 1981. 9, 279-282.

Lauwers, S.; Vlaes, L. and Butzler, J.P. Campylobacter serotyping and epidemiology. Lancet 1981. i, 158-159.

Lauwers, S.; de Boeck, M. and Butzler, J.P. Campylobacter enteritis in Brussels. Lancet 1978. i, 604-605.

Mosimann, J.; Jung, M.; Schär, G.; Bonifas, V.; Heinzer, I.; Brunner, S.; Hermann, G. and Lambert, R.A. Serologische Diagnose menschlicher Campylobacter-Infectionen. Schweiz. Med. Wochenschr. 1981. 111, 846-853.

Oosterom, J.; Beckers, H.J.; Noorle-Jansen, L.M. van and Schothorst, M. van. Een explosie van Campylobacter-infectie in een kazerne, waarschijnlijk veroorzaakt door rauwe tartaar. Ned. Tijdschr. Geneeskd. 1980. 124, 1631-1634.

Penner, J.L. and Hennessy, J.N. Passive haemagglutiuation technique for serotyping Campylobacter fetus subsp. jejuni on the basis of soluble heat-stable antigens. J. Clin. Microbiol. 1980. 12, 732-737.

Prescott, J.F. and Karmali, M.A. Attempts to transmit Campylobacter eteritis to dogs and cats. Can. Med. Assoc. J. 1978. 119, 1001-1002.

Severin, W.P.J. Campylobacter en enteritis. Ned. Tijdschr. Geneeskd. 1978. 122, 499-504.

Skirrow, M.B. Campylobacter enteritis: a "new" disease. Br. Med. J. 1977. ii, 9-11.

Steele, T.W. and McDermott, S. Campylobacter enteritis in South Australia. Med. J. Aust. 1978. 2, 404-406.

Svedhem, A.; Gunnarsson, M. and Kaijser, B. Diffusion-in-gel enzyme-linked immunosorbent assay for routine detection of IgG and IgM antibodies to Campylobacter jejuni. J. Infect. Dis. 1983. 148, 82-92.

Walder, M. and Forsgren, A. Enzyme-linked immunosorbent assay (Elisa) for antibodies against Campylobacter jejuni, and its clinical application. Acta Pathol. Microbiol. Immunol. Scand. 1982. 90, 423-433.

Watson, K.C.; Kerr, E.J.C. and Mc Fadzean S.M. Serology of human campylobacter infections. J. Infect. 1979. 1, 151-158.

CHAPTER VIII

DETERMINATION OF SEROTYPE AND HIPPURATE HYDROLYSIS FOR *CAMPYLOBACTER JEJUNI* ISOLATES FROM HUMAN PATIENTS, POULTRY AND PIGS IN THE NETHERLANDS

The investigations presented in this Chapter are also described in the following publication:

Oosterom, J.; Bänffer, J.R.J.; Lauwers, S. and Busschbach, A.E. Determination of serotype and hippurate hydrolysis for Campylobacter jejuni isolates from human patients, poultry and pigs in The Netherlands. Antonie Leeuwenhoek Microbiol., accepted.

CHAPTER VIII

DETERMINATION OF SEROTYPE AND HIPPURATE HYDROLYSIS FOR *CAMPYLOBACTER JEJUNI* ISOLATES FROM HUMAN PATIENTS, POULTRY AND PIGS IN THE NETHERLANDS

Introduction

Epidemiological studies have shown that many animal species, including farm animals, may be healthy intestinal carriers of C.jejuni (Smibert, 1969; Fernie and Park, 1977; Hastings, 1978; Smibert, 1965; Doyle and Roman, 1982). In particular poultry and pigs are frequently colonised by large numbers of campylobacters as was also shown in Chapter V. By analogy with the results of epidemiological studies on Salmonella spp. (Edel et al., 1977), it may be assumed that this campylobacter contamination reaches the consumer by way of meats and meat products. It has been demonstrated in Chapter V and VI that this assumption is valid for poultry, but hardly for pork, and additional data have been presented to explain the factors that are responsible for this difference.

By means of typing of strains originating from human patients, poultry and pigs, this study has attempted to provide further information concerning the relative importance of chickens and pigs in the epidemiology of human campylobacteriosis in the Netherlands.

Materials and methods

Strains for typing

One hundred strains of human origin were obtained in our laboratory from the examination of stools from patients with diarrhoea. An equal number of strains was isolated from live poultry and poultry products during epidemiological investigations on poultry farms and in poultry processing plants, situated in different regions in the Netherlands. In addition, one hundred strains were isolated from pig carcasses from three slaughterhouses situated in different areas in this country.

The strains were selected to avoid including more than one isolate from each source. All strains were identified biochemically according to generally accepted criteria (Chapter IV). After identification, strains were grown and stored at -70°C in Brain Heart Infusion broth plus 15 % glycerol in plastic tubes containing glass beads.

Serotyping

For serotyping, the scheme used by Lauwers et al. (1981) and Penner and Hennessy (1980) was adopted. In order to obtain hyperimmune sera, the 36 type strains of Lauwers and an additional 10 from Penner were used to inoculate rabbits. The strains were grown on sheep blood agar plates which were incubated at 42°C for 48 h in a micro-aerobic atmosphere. After incubation, cells were harvested in physiological saline and the density of each suspension was adjusted to that of a McFarland no. 10 tube. For the first animal inoculation, an equal amount of each live bacterial suspension and complete Freund's adjuvant were mixed, and 0.3 ml amounts of the mixture were injected subcutaneously at five separate sites in the abdominal wall of a rabbit. One week later 0.7 ml of the same bacterial suspension without adjuvant was injected intravenously, and this injection was repeated three and seven days later. One week

after the last injection, the antibody titre of the serum against the autologous strain was assessed by the haemagglutination test. If an antibody titre of $\geq$ 1 : 1280 was found, the animal was bled. If not, intravenous injections were repeated.

Serum was obtained by centrifuging the blood at 2,500 r.p.m. for 10 min, and then diluted with phosphate buffered saline (PBS), pH 7.2 until an antibody titre of 1 : 2560 (or at least 1 : 1280) was attained. Diluted sera were transferred to small plastic tubes and frozen at -70°C for storage. For immediate use, they were kept at +4°C.

Serotyping was carried out by means of a passive haemagglutination test. Cells of strains to be typed were again harvested from sheep blood agar and thick suspensions made of them were subsequently boiled for 2 h. Fresh human O rhesus-negative red blood cells were washed three times in PBS (centrifugation at 2,500 r.p.m. for 5 min), after which a 10 % suspension was made in PBS. Equal amounts of this suspension and of the boiled antigen suspensions were mixed and incubated under repeated agitation for 2 h at 37°C in a water bath. After adsorption, the red blood cells were again washed three times. After final centrifugation, 250 µl of the red blood cells were suspended in 50 ml of PBS. Fifty µl amounts of this suspension were mixed with 75 µl of serial dilutions of the type-specific hyperimmune sera in PBS using microtitre plates (Dynatech, Microelisa). Following incubation for 1 h at 37°C and subsequently for 18 h at 4°C, the haemagglutination reaction was recorded.

Hippurate hydrolysis

For the determination of hippurate hydrolysis one loopful of a young culture of each strain under investigation was suspended in 2 ml of PBS (pH 7.0). Then 0.5 ml of 5 % sodium hippurate solution was added and the

suspension incubated for 2 h at 37°C in a water bath. After incubation, 1 ml of 3.5 % ninhydrin solution was added without mixing and the reaction was recorded as positive if a purple colour appeared within 10 min. (Skirrow and Benjamin, 1980).

Results

Sixty-five of 100 human strains, 79 of 100 poultry strains and 67 of 100 strains from pigs were typable by means of the hyperimmune sera which were available. Regarding the test for hippurate hydrolysis, 86 human strains were positive, as were 94 poultry strains, but only 6 pig strains gave a positive result. Two strains from human patients and three from pigs showed a weak reaction (Table VIII.1).

Four of the six most common serotypes in chickens also belonged to the group of the five most frequently prevailing serotypes in human patients. The six most frequently prevailing serotypes in pigs established a group quite distinct from those in humans or in poultry (Table VIII.2). All specific antigenic determinants occurring in the different serotypes are represented by Arabic numbers. The serotypes originally established by Lauwers carry the prefix "LAU", those from Penner the prefix "PEN". Because of the variable expression of determinants, all strains reacting with hyperimmune serum 3, 16 or 25, or with combinations of these are included in serotype 3, 16, 25.

Further evaluation of the similarity of strains in order to determine the serotypes occurring most frequently in the different hosts was done by statistical analysis using the chi-square test. This showed no significant difference in distribution between serotypes from human patients and those from poultry ($p > 0.10$), but a highly significant difference in distribution existed for serotypes from humans and from

Table VIII.1 Typability by specific antisera of and hippurate hydrolysis for C. jejuni strains from human patients, poultry and pigs

Origin	Humans				Poultry				Pigs			
	Hippurate hydrolysis[o)]			Total	Hippurate hydrolysis			Total	Hippurate hydrolysis			Total
	+	±	−		+	±	−		+	±	−	
Typable	59	1	5	65	73	0	6	79	6	1	60	67
Untypable	27	1	7	35	21	0	0	21	0	2	31	33
Total	86	2	12	100	94	0	6	100	6	3	91	100

o) + = positive reaction
± = weak reaction
− = negative reaction

Table VIII.2 Distribution of isolates among the most frequently prevailing serotypes of C. jejuni from human patients, poultry and pigs

Serotypes	Human strains	Poultry strains	Pig strains
LAU 3,16,25[o)]	13	9	
LAU 1	12	9	
LAU 2,36	11	10	
LAU 17,46	6	11	
PEN 25	4		
LAU 5,8		12	
LAU 7		9	
LAU 14			16
LAU 11			9
LAU 6,32			6
LAU 6			5
LAU 43			5
LAU 37			5

o) including the "incomplete" serotypes 3,16; 3,25; 16 and 25

pigs ($p < 0.001$).

Discussion

Epidemiological studies carried out in the Netherlands have shown that poultry products play an important role for the acquisition of human campylobacter enteritis, whilst pork is only marginally involved (Chapters V an VI). These findings are fully confirmed by the results of our present study on the typing of campylobacter isolates. There appears to be a clear relationship between the serotypes isolated from poultry and those from human patients. In contrast, the serotypes prevailing in pigs form a distinct group and seem to have little or no relationship to campylobacters in man or poultry.

The same is true regarding the ability of strains to hydrolyse sodium hippurate. The majority of strains from man and poultry are hippurate positive, whereas almost all pig isolates are negative.

In a few instances it was observed that, within the same serotype, human or poultry strains gave a positive result in the test for hippurate hydrolysis, whereas the pigs strains were negative. In these cases it is pertinent to ask whether the ability to hydrolyse sodium hippurate is really such a stable characteristic among strains of C.jejuni as is stated by others (Penner et al., 1983), or whether sometimes it can be influenced by biological factors, such as different intestinal environments.

References

Doyle, M.P. and Roman, D.J. Prevalence and survival of Campylobacter jejuni in unpasteurized milk. Appl. Environ. Microbiol. 1982. 44, 1154-1158.

Edel, W.; Schothorst, M. van and Kampelmacher, E.H. Salmonella and Salmonellosis - The present situation. Proc. Int. Symp. on Salmonella and Prospects for Control, Guelph, Canada 1977. 1-26

Fernie, D.S. and Park, R.W.A. The isolation and nature of Campylobacters (microaerophilic Vibrios) from laboratory and wild rodents. J. Med. Microbiol. 1977. 10, 325-329.

Hastings, D.H. Campylobacter enteritis in pets. Lancet 1978. ii, 1249-1250.

Lauwers, S.; Vlaes, L. and Butzler, J.P. Campylobacter serotyping and epidemiology. Lancet 1981. i, 158-159.

Penner, J.L. and Hennessy, J.N. Passive haemagglutiuation technique for serotyping Campylobacter fetus subsp. jejuni on the basis of soluble heat-stable antigens. J. Clin. Microbiol. 1980. 12, 732-737.

Penner, J.L.; Hennessy, J.N. and Congi, R.V. Serotyping of Campylobacter jejuni and Campylobacter coli on the basis of thermostable antigens. Eur. J. Clin. Microbiol. 1983. 2, 378-383.

Skirrow, M.B. and Benjamin, J. Differentation of enteropathogenic campylobacter. J. Clin. Pathol. 1980. 33, 1122.

Smibert, R.M. Vibrio fetus var. instestinalis isolated from fecal and intestinal contents of clinically normal sheep: Biochemical and cultural characteristics of microaerophilic Vibrios isolated from the intestinal contents of sheep. Am. J. Vet. Res. 1965. 26, 320-327.

Smibert, R.M. Vibrio fetus var. intestinalis isolated from the intestinal content of birds. Am. J. Vet. Res. 1969. 30, 1437-1442.

CHAPTER IX

GENERAL DISCUSSION

CHAPTER IX

GENERAL DISCUSSION

In the introduction to this work, four questions were raised regarding sources and transmission ways of C.jejuni, the effects of campylobacter infection in man and possible preventive measures to reduce the incidence of human infection. Having dealt with the various investigations that followed from these questions, this Chapter will attempt to summarize the results obtained and to formulate the answers.

It has been shown that among the farm animals reared in the Netherlands, poultry and pigs in particular may be healthy intestinal carriers of large numbers of C.jejuni. Average numbers in poultry even reached 10^5 organisms per gram of intestinal material. Particularly during the processing of poultry there is an extensive dissemination of C.jejuni in all parts of the processing hall. Consequently, poultry end-products are frequently contaminated with campylobacters.

Because of differences in slaughter techniques and the fact that the intestinal prevalence of campylobacters in pigs in general is much lower (about 10^3 per gram) than in poultry, a much lower level of contamination is found on pig carcasses than on poultry meat. Moreover, a considerable reduction occurs in campylobacyter contamination of these carcasses during chilling in slaughterhouse chill-rooms. This reduction is brought about by the drying effect of forced ventilation, and not by the chilling itself. It was shown that C.jejuni is very sensitive to dry conditions. As a result, C.jejuni is almost absent from pork. Poultry carcasses are

mostly chilled in a quite different manner, that is, by cold water or by air without the possibility of drying, and hence little or no reduction is observed in campylobacter contamination of poultry meat.

Only a moderate colonization was demonstrated in Dutch cattle and raw milk was found to be free from campylobacter contamination. Moreover, cattle carcasses are chilled in much the same way as pig carcasses, so that any possible contamination of beef with campylobacters will be similarly reduced during the chilling process.

Taken together, these observations lead to the conclusion that of the four most important foods of animal origin, namely poultry meat, pork, beef and milk, only poultry meat plays an important role in the transmission of C.jejuni to man in the Netherlands.

In the literature two other important sources of campylobacter infection have been identified, namely drinking water and pet dogs. Considering the stringent processing and process control which is applied to the production of drinking water in the Netherlands, water-borne campylobacter outbreaks are very unlikely to occur. Concerning Dutch dogs, only a small proportion of the animals was found to be carriers of campylobacters and any association with diarrhoea was rare. This means that dogs, too, pose little hazard to human health in this respect.

It may be assumed from the data obtained that poultry meat is the major source of human campylobacteriosis in the Netherlands. This hypothesis was confirmed by the use of typing procedures for campylobacter isolates, which indicated a very strong relationship between isolates from human patients and poultry, whereas strains from pigs formed a quite distinct group. The results of an epidemiological survey of Rotterdam households gave supporting evidence. It was found that the consumption of poultry meat was a highly significant factor in

the acquisition of campylobacter enteritis, whereas pork was only marginally implicated. Other foods or the possession of pet animals by the families concerned were not significant. In the same survey it was discovered that barbecues in particular greatly increased the chances of campylobacter infection. This may be ascribed to the frequent handling and insufficient heating of raw meat, especially poultry meat, on these occasions. Cross-contamination in kitchens and in other places where foods are handled seems in general to be less important in campylobacter infection than it would be for salmonellosis, because, as stated previously, C.jejuni is very susceptible to drying and, moreover, the organism does not grow at temperatures below 30°C. The examination of surfaces in a slaughterhouse showed this very clearly.

Compared with data from other countries, the overall clinical effect of campylobacter enteritis in our survey appeared to be rather mild. Nevertheless, the vast majority of patients in our study experienced diarrhoea and abdominal cramps, most of them also had fever, but the occurrence of fresh blood in the stools was only observed in a minority of cases. The average duration of illness was about eight days, and excretion of the organism in general lasted for two to four weeks. In a number of households, outbreaks occurred which were evidently food-borne in nature. Person-to-person spread was also observed in some families.

By means of an ELISA, developed in our laboratory, it was found that in about 80 % of campylobacter patients clear amounts of serum antibodies were produced, in most cases within eight days of infection. These antibodies persisted for at least four months. Examination of sera from the epidemiological survey of different households showed a good relationship between the severity of campylobacter-related symptoms and antibody titre values.

It is generally accepted that the epidemiology of campylobacter enteritis is very similar to that of salmonellas. Therefore, preventive measures, suggested to combat the problem of human salmonellosis, might also be applicable in reducing the incidence of human campylobacteriosis (and, most probably, of many other zoonoses of bacterial or parasitic nature). Preventive measures for Salmonella spp. are described in detail in the WHO Guidelines on Prevention and Control of Salmonellosis (1983). These preventive measures, together with those that might be taken specifically to control campylobacter enteritis, can be summarized as follows:

a) At the level of animal production one should aim for the raising and fattening of campylobacter free slaughter animals. For this purpose, it is necessary to have campylobacter-free parent animals, campylobacter-free animal feed and quarters that can be isolated hygienically from the environment. Strict hygiene rules must also apply to the transportation of the animals. Some experiments in the Netherlands have shown that the realization of this kind of preventive measures is very difficult, if not impossible (Oosterom et al., 1982; Oosterom and Notermans, 1983).

b) During slaughter of the animals and further processing of the meat it is necessary to apply strictly the rules of hygiene, so that cross-contamination is prevented as far as possible. To achieve this, new slaughter techniques and new equipment should be developed. Moreover, studies are needed to determine how far the drying of surfaces, utensils and equipment can be exploited as a means of eliminating campylobacters from the slaughterhouse. Apart from hygiene measures, decontamination treatments for end-products might be worthwhile. At the moment, treatment with lactic acid and irradiation of meat seem to

be the most promising methods for this purpose. As mentioned before, it might be possible to eliminate campylobacter contamination of carcasses or meat by air chilling using forced ventilation.

c) At the level of the consumer, adequate information should be given to all those concerned with the preparation of meat and meat products: housewives, kitchen staff in restaurants, hospitals, geriatric homes, etc. Attention should be paid to proper heating and/or cooling of foods, in particular those of animal origin. The hazards of cross-contamination in the kitchen, especially from raw foods to hands and surfaces and to other foods ready for consumption, should be emphasized.

d) At the level of patient care, it is important to know that the excretion of campylobacters in the faeces can be stopped by the administration of appropriate antibiotics. This approach could be used, in addition to strict personal hygiene, for individuals that are associated with higher risks of person-to-person spread or are more likely to contaminate foods, like young children in kindergartens, nurses, cooks, etc. With the same intention, one might consider treating contaminated pet animals, especially those with diarrhoea. For these purposes, erythromycin is the antibiotic to be preferred, because it is non-toxic and has little effect on the normal intestinal flora. However, in view of the possible development of antibiotic resistance, the cases to be treated should be selected with the utmost reserve.

References

Oosterom, J.; Erne, E.H.W. van and Schothorst, M. van. Epidemiological studies on Salmonella in a certain area ("Walcheren Project"). V. Studies into the possibility of fattening pigs free from Salmonella. Zbl. Bakt. Hyg. I. Abt. Orig. A. 1982. 252, 490-506.

Oosterom, J. and Notermans, S. Further research into the possibility of salmonella-free fattening and slaughter of pigs. J. Hyg. 1983. 91, 59-69.

World Health Organization. Guidelines on prevention and control of salmonellosis. WHO Report VPH/83.42. Linton, A.H. (ed.). Geneva, Switzerland 1983.

CHAPTER X

SUMMARY

SAMENVATTING

CHAPTER X

SUMMARY

Over the last few years the bacterial species Campylobacter jejuni has been recognized as an important cause of acute enteritis in man. Investigations in several countries have shown that infections caused by C.jejuni may be as serious as those due to Salmonella spp., both in prevalence and in the severity of symptoms. In the Netherlands, campylobacters and salmonellae are each responsible for about 10 % of all cases of acute enteritis.

Campylobacteriosis is mostly a food-borne infection. In the epidemiological studies described here it was found that in the Netherlands poultry and pigs in particular are healthy intestinal carriers of large numbers of C.jejuni. These intestinal organisms readily contaminate the meat during slaughter. As a consequence, C.jejuni was frequently isolated from poultry carcasses and from other poultry end-products at the processing plants. However, pork in butchers' shops appeared to be free from such contamination. It was found that this was due to the inability of campylobacters to survive the drying effect of the chilling process used for pig carcasses in the slaughterhouse. Poultry products are mostly chilled in another manner, without the possibility of drying, so that campylobacters on poultry meat, in contrast to those on pork, in fact reach the consumer.

In the literature, apart from poultry, three other important vectors of C.jejuni infection are discussed, namely unpasteurized milk,

unadequately treated drinking water and pet dogs. It was demonstrated in this study that all three did not play a role in the Dutch situation.

In an epidemiological survey of households in Rotterdam, it was confirmed that only the handling and consumption of poultry meat were significant factors for the acquisition of campylobacter enteritis. The attending of a barbecue in particular appeared to be hazardous from this point of view. Pork was only marginally involved, other significant factors were not identified.

The conclusions drawn from the examination of pork and poultry meat and of the household survey were futher confirmed by the typing of campylobacter isolates from different sources. Strains from human patients and from poultry showed a good relationship, whereas pig strains appeared to form a quite different group.

During the Rotterdam survey, it was found that campylobacter enteritis was, in general, not as dramatic as is sometimes described in the literature. Nevertheless, in most cases diarrhoea occurred for several days, often accompanied by fever. Prolonged abdominal cramps appeared to be a rather characteristic symptom. In a number of cases overt blood was noted in the stools. Some household members had laboratory-confirmed infections without symptoms.

Serological testing by means of an ELISA technique showed a good association between serum titre values and the severity of symptoms. However, it was found that antibodies were not formed in all cases but, if produced, could persist for several months. Comparison of results obtained by ELISA with those of a CFT developed elsewhere showed a good correlation.

Concerning the prevention of human campylobacteriosis, a number of possible measures are discussed. In the first place, it is theoretically

feasible to produce slaughter animals that are free from pathogenic organisms, including C.jejuni. Studies in the Netherlands have demonstrated that this approach is very likely to fail. Secondly, improved hygiene during slaughter may further prevent the transmission of C.jejuni from the intestinal tract of the animals to meat surfaces. In connection herewith, it might be possible to make use of the specific susceptibility of campylobacters to drying conditions, perhaps even during the chilling of poultry carcasses. Thirdly, all those involved in the handling and final preparation of meat products should be made aware of proper heating and cooling procedures and of the hazards of cross-contamination. Lastly, campylobacter carriers which may establish exceptional hazards of spreading the infection, whether they are humans or pet-animals, could be treated with erythromycin to stop faecal excretion of the organism. In view of the possible development of antibiotic resistance, this should be restricted to carefully selected cases.

SAMENVATTING

Sinds enkele jaren wordt de bacteriesoort Campylobacter jejuni tot één van de belangrijkste verwekkers van acute enteritis bij de mens gerekend. Onderzoekingen in verschillende landen hebben duidelijk gemaakt dat Campylobacter-infecties een even groot probleem vormen als die veroorzaakt door Salmonella, zowel voor wat betreft het voorkomen als de ernst van de ziekteverschijnselen. In Nederland zijn Campylobacter en Salmonella elk verantwoordelijk voor ongeveer 10 % van alle gevallen van acute enteritis.

Campylobacteriosis is meestal een voedselinfectie. In de hier beschreven epidemiologische onderzoekingen werd aangetoond dat in Nederland speciaal pluimvee en varkens gezonde dragers zijn van grote aantallen C.jejuni in het darmkanaal. Tijdens het slachten van deze dieren treedt vanuit het darmkanaal een besmetting van het vlees op. In pluimveeslachterijen werd C.jejuni danook frequent op de geslachte kuikens en op andere pluimveeproducten aangetroffen. Bij onderzoek in slagerijen bleek echter, dat varkensvlees in het geheel niet met Campylobacter was besmet. Gevonden werd dat C.jejuni niet bestand was tegen het uitdrogend effect dat bij het koelen van varkenskarkassen in het slachthuis optreedt. Hiermede kon de afwezigheid van de kiem op varkensvlees worden verklaard. Geslacht pluimvee wordt meestal op andere wijze dan varkens gekoeld, zonder de mogelijkheid dat uitdroging optreedt, zodat Campylobacter via pluimveeproducten de consument wèl kan bereiken.

In de literatuur worden naast pluimveevlees drie andere belangrijke

vectoren van Campylobacter-besmetting genoemd, en wel ongepasteuriseerde melk, onvoldoende gezuiverd drinkwater en honden. Aangetoond kon worden dat geen van deze drie in de Nederlandse situatie een rol van betekenis speelde.

Bij een epidemiologisch onderzoek in huishoudens te Rotterdam werd bevestigd dat alleen het hanteren en het eten van pluimveevlees significante factoren waren bij het ontstaan van Campylobacter-infecties. Speciaal het deelnemen aan een barbecue bleek in dit opzicht riskant te zijn. Varkensvlees speelde slechts een marginale rol. Andere factoren bleken niet van belang.

De conclusies uit het onderzoek van varkens- en pluimveevlees en uit het onderzoek in huishoudens werden verder bevestigd door het typeren van Campylobacter-stammen. Isolaten van patiënten en van pluimvee kwamen zeer goed overeen, terwijl stammen van varkens een aparte groep vormden.

Bij het epidemiologisch onderzoek in huishoudens te Rotterdam werd verder gevonden dat een Campylobacter-enteritis over het algemeen niet zo ernstig verliep als in de literatuur wel wordt aangegeven. Niettemin trad in de meeste gevallen gedurende meerdere dagen diarrhee op, vaak gepaard met koorts. Als meest karakteristieke symptoom werden lang aanhoudende buikkrampen aangetroffen. In een gering aantal gevallen kwam bloed in de ontlasting voor. Soms werden in het laboratorium infecties aangetoond, die voor de patiënt zonder symptomen verliepen.

Bij onderzoek van sera door middel van een ELISA-techniek bleek een verband te bestaan tussen de hoogte van de antistoftiters en de ernst van de symptomen. Tevens werd duidelijk dat niet in alle gevallen antistoffen werden gevormd, doch dat eenmaal geproduceerde antistoffen enkele maanden aantoonbaar bleven. Vergelijking van de resultaten van onderzoek van de ELISA met die van een elders ontwikkelde CFT gaf een

goede correlatie te zien.

Voor wat betreft de eventuele preventie van humane Campylobacter - infecties worden een aantal maatregelen besproken. In de eerste plaats is het theoretisch mogelijk om slachtdieren te produceren die vrij zijn van pathogene micro-organismen, dus ook van C.jejuni. Onderzoek in ons land heeft aangetoond dat deze benadering op welhaast onoverkomelijke moeilijkheden stuit. Ten tweede kan een verbeterde hygiëne tijdens het slachten de besmetting met C.jejuni vanuit de darminhoud van de dieren naar vleesoppervlakken beperken. Wellicht kan daarnaast tevens gebruik worden gemaakt van de speciale gevoeligheid van C.jejuni voor droge omstandigheden, misschien zelfs in pluimveeslachterijen. Ten derde dient eenieder die vlees hanteert of met de uiteindelijke bereiding van vleesproducten te maken heeft, op de hoogte te zijn van het op de juiste wijze toepassen van verhitting en koeling en bewust te zijn van de gevaren van kruisbesmetting. Tenslotte zouden Campylobacter-dragers, hetzij mensen, hetzij gezelschapsdieren, die extra risico opleveren voor de verspreiding van de infectie, behandeld kunnen worden met erythromycine om de uitscheiding van C.jejuni te beëindigen. In verband met het gevaar van resistentievorming moet hierbij echter de uiterste terughoudendheid in acht worden genomen.

DANKWOORD

Eén van de leukste bijkomstigheden van epidemiologisch onderzoek is, dat je het niet in je eentje kunt doen. Wie een bepaalde bacteriesoort, zoals hier Campylobacter jejuni, langs zijn transmissiewegen van dier naar mens probeert te volgen, komt in de meest uiteenlopende omstandigheden terecht en heeft verschillende technieken nodig om zich staande te houden. En niet zelden blijkt de hulp van anderen daarbij onontbeerlijk. Bij mijn onderzoekingen heb ik het geluk gehad inderdaad een aantal mensen te ontmoeten die bereid waren hun specifieke kennis, hun ervaring en hun werkkracht te mijnen gunste beschikbaar te stellen. Het aandeel van "buitenstaanders" is daarbij waarlijk niet gering geweest: in de artikelen die de basis van dit proefschrift vormen worden 19 mede-auteurs genoemd; 13 van hen waren niet, of niet permanent, aan het RIVM verbonden. Naast al deze werkers in het veld hebben anderen geholpen door met mij over opzet, uitvoering, verslaggeving en verdere voortgang van het onderzoek te overleggen. Alles bijeen hebben deze activiteiten geleid tot het verschijnen van dit proefschrift. Ik kan bij deze gelegenheid alleen maar recht doen door het aandeel van een ieder die heeft meegewerkt zo nauwkeurig mogelijk te vermelden.

In de eerste plaats ben ik veel dank verschuldigd aan mijn promotor, Prof.Dr.M.F.Michel, voor zijn kritische belangstelling en zijn nauwgezette begeleiding. Zijn adviezen hebben in grote mate bijgedragen aan de uiteindelijke vorm van dit werk.

Gedurende de jaren dat ik bij het RIVM werkzaam ben geweest, heb ik vele facetten van de epidemiologie van bacteriële zoönosen leren kennen.

Mijn co-promotor, Prof.Dr.E.H.Kampelmacher, heeft daarbij een belangrijke rol gespeeld. Enkele, door hem aangegeven, epidemiologische patronen zijn in dit proefschrift terug te vinden.

Prof.Dr.J.Huisman dank ik voor het vele overleg betreffende de verschillende onderzoekingen, voor zijn enthousiaste medewerking aan het gezinsonderzoek te Rotterdam en voor zijn bereidheid dit proefschrift mede te beoordelen.

Vanaf mijn eerste kennismaking met Campylobacter jejuni heb ik vaak gebruik gemaakt van de goede raadgevingen van Prof. Dr. J.-P. Butzler. Voor zijn hulpvaardigheid, zijn gastvrijheid te Brussel en zijn welwillendheid over dit proefschrift zijn oordeel uit te spreken, ben ik hem zeer erkentelijk.

De samenwerking met John Bänffer bestaat al vele jaren. Het is moeilijk na te gaan waar zijn aandeel in dit werk ophoudt en het mijne begint.

Sabine Lauwers ben ik zeer erkentelijk voor de onbaatzuchtige wijze, waarop zij haar Campylobacter-serotyperingssysteem aan ons ter beschikking heeft gesteld en tevens voor haar medewerking bij het evalueren van de door ons ontwikkelde ELISA-techniek.

Zoals vermeld vonden een aantal van de onderzoekingen elders in het land plaats. Ik dank Enne de Boer en Bob Hartog voor hun aandeel bij de Keuringsdiensten van Waren en Gerrit de Wilde, Fransje van Kempen - de Troye en Ruud de Blaauw voor hun deelonderzoekingen bij de diverse Vleeskeuringsdiensten.

Facetten van de Campylobacter - epidemiologie werden verder belicht door Marijn Vereijken, Hetty Karman, Kees den Uyl en Rob Dekker, die in het kader van hun doctoraalstudie aan de Landbouwhogeschool te Wageningen (Laboratorium voor Levensmiddelenmicrobiologie en -hygiëne) bij het RIVM

een stage vervulden, alsook door Fred Poelma, die zijn militaire diensttijd met immunologisch onderzoek bij het RIVM doorbracht.

Minstens even belangrijk was de inbreng van de medewerkers van mijn "eigen" afdeling. Ton Busschbach, George Engels en Mevrouw Esendam - Zonnevylle hebben alle ontwikkelingen vanaf het eerste begin meegemaakt en kunnen terugzien op vele jaren Campylobacter - onderzoek, vaak met stukjes en beetjes naast het andere werk opgebouwd. Aan enkele onderzoekingen hebben ook Roel Peters en Roel Pot deelgenomen.

Servè Notermans is in het laboratorium altijd aanspreekbaar geweest als het eens niet wilde lukken. Speciaal zijn mening over pluimveeslacht-hygiëne en het nut van promoveren hebben mij dikwijls zeer geholpen. Daarnaast heb ik vele goede raadgevingen ontvangen van Boudewijn Engel, Arie Havelaar en Harry Beckers.

Elk epidemiologisch onderzoek staat of valt met de wijze, waarop de te onderzoeken materialen worden verzameld en vervoerd. Wat dat betreft heb ik altijd kunnen vertrouwen op de goede zorgen van de heer Sint, en later van de heer Hout.

Wat de tekstverwerking betreft: voor Loes van Dijk was dit het tweede proefschrift binnen een jaar. Speciaal door haar ervaring en inzicht liep alles op rolletjes. Natuurlijk leek het soms onmogelijk de gestelde tijdslimieten te halen, maar allengs leerde ik op haar werkexplosies te vertrouwen. Haar aandeel heb ik zeer op prijs gesteld.

Particularly I wish to thank Dr.Geoff Mead, Food Research Institute, Norwich, U.K., for his willingness to correct the English text of this work. It took a long time before I realised how much I had asked from him. Nevertheless, he remained as kind and helpful as ever. All faults and errors that may have persisted in this thesis are entirely due to my stubbornness.

Wie in een groot instituut werkt wordt, vaak zonder het duidelijk te beseffen, gesteund door een aantal centrale diensten. Ik dank allen die via deze weg een bijdrage hebben geleverd, in het bijzonder Wachtel Sekhuis en Erik van Erne voor de statistische berekeningen, de heer Kruizinga en Ben van Rheenen voor het beschikbaar stellen en verzorgen van de proefdieren, de heren Weiss en Verschraagen voor het verzamelen van de relevante literatuur, de tekenafdeling voor het verzorgen van tabellen en grafieken en de foto-afdeling, tesamen met Peter Tips voor het verzorgen van de omslag.

Tenslotte wil ik de Directie van het Rijksinstituut voor Volksgezondheid en Milieuhygiëne danken voor de gelegenheid die mij is geboden dit Campylobacter - onderzoek uit te voeren en op deze wijze te rapporteren.

CURRICULUM VITAE

De schrijver van dit proefschrift werd in 1945 te Rotterdam geboren. In deze stad behaalde hij in 1962 aan het Libanon Lyceum het HBS-b diploma. In 1963 werd de studie begonnen aan de Faculteit der Diergeneeskunde van de Rijksuniversiteit te Utrecht, welke studie in 1971 werd voltooid.

Na vervulling van de militaire dienstplicht was hij van 1973 tot eind 1976 werkzaam in de algemene diergeneeskundige praktijk. Vanaf december 1976 is hij verbonden aan het Laboratorium voor Zoönosen en Levensmiddelenmicrobiologie, thans Laboratorium voor Water- en Levensmiddelenmicrobiologie (tijdelijk hoofd: Prof.Dr.E.J.Ruitenberg) van het Rijksinstituut voor de Volksgezondheid, thans Rijksinstituut voor Volksgezondheid en Milieuhygiëne, waar hij zich bezighoudt met de epidemiologie, diagnose en preventie van bacteriële zoönosen.