

MED

STUDIES ON ENTERIC CAMPYLOBACTERIOSIS

AND

OTHER ENTERIC BACTERIA

IN

TIKUR ANBASSA HOSPITAL

ADDIS ABABA, ETHIOPIA.

DEPARTMENT OF MEDICAL MICROBIOLOGY AND PARASITOLOGY

FACULTY OF MEDICINE, ADDIS ABABA UNIVERSITY.



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AUGUST, 1993.



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TIKUR ANBASSA HOSPITAL ADDIS ABABA, ETHIOPIA.**

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**Studies on Enteric Campylobacteriosis in Tikur Anbessa Hospital,
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ABBREVIATIONS

ATCC	American Type Culture Collection, Rockville, Maryland
CCDA	Charcoal-Cefoperazone-Desoxycholate-Agar
<i>C. jejuni/coli</i>	<i>Campylobacter jejuni</i> AND <i>Campylobacter coli</i>
CRP	C-reactive protein
DIG	Diffusion-in-gel
<i>E. coli</i>	<i>Escherichia coli</i>
ELISA	Enzyme-linked-immunosorbent-assay
<i>EPEC</i>	<i>Enteropathogenic Escherichia coli</i>
ESR	Erythrocyte sedimentation rate
FBP	Ferrous sulphite, sodium bisulphite and sodium pyruvate
GM ₁	Membrane bound ganglioside ₁
HLA	Human leucocyte antigen
HL	Heat labile
HS	Heat stable
Ig	Immunoglobulin
LPS	Lipopolysaccharide
LMG	Culture Collection, Laboratorium Microbiologie Ghent, Belgium
NARTC	Nalidixic acid resistant thermophilic <i>campylobacter</i>
NBL	National Bacteriological Laboratory, Stockholm, Sweden
NCTC	National Collection of Type Cultures, Colindale, London
ssp	Subspecies

ABSTRACT

Between February 1992 and January 1993 *Campylobacter* species were isolated from 86 (13.6%) of 630 patients with diarrhoea at Tikur Anbassa Hospital, Addis Ababa, Ethiopia. In the same study population, *shigellae* were found in 11.7% and *salmonellae* in 3.8%. Of the 345 paediatric patients, 72 (20.8%) and 13 (17.1%) of the 76 controls less than 2 years of age had *EPEC* in their stools. No *Yersinia enterocolitica* was isolated. *Campylobacter* species were found in all age groups, but the majority were isolated from children less than five years of age (68.6%). Only 2 (0.9%) of 220 controls had *campylobacter* in their stools. Of the *campylobacters* that were differentiated at species level, *C. jejuni* accounted for 82.4% and *C. coli* for 17.6% of the isolates. Serotyping of each strain was done, according to the methods of Lior, using 16 antisera against heat labile antigens; 89.3% of the *C. jejuni* and 75% of the *C. coli* were typeable. Lior serotypes 1, 2, 4, 5, 6 and 7 were the most common among the *C. jejuni*, while Lior serotypes 1 and 2 were dominant among the *C. coli* isolates. These serotypes accounted for 63.2% of all isolates. More patients (53.1%) presented for investigation of diarrhoea during the months of April through July but there was no significant difference in the isolation frequency of the enteric pathogens studied throughout the year. Concomitant isolation of *Shigella* or *Salmonella* species was achieved in 12 of the *Campylobacter* infected patients. In general, *campylobacter* diarrhoea is not a severe disease. The most common symptoms and signs were watery diarrhoea in 82.4%, low grade fever in 78.4% and frequent vomiting in 45.9%. Dehydration ranging from mild to severe was observed in 25.4% of the 55 children with *campylobacter* infections.

Of the 55 children 67.2% had signs of malnutrition and most of them (47.2%) were underweight. The antibiogram for 85 strains of the *campylobacters* isolated showed that all strains were sensitive to chloramphenicol, erythromycin, gentamicin, nalidixic acid, norfloxacin, sulphonamide and tetracycline. All of the strains were resistant to cephalothin, while 51 (60.0%) and 50 (58.8%) strains were resistant to ampicillin and trimethoprim-sulphamethoxazole, respectively. This study indicates that *campylobacters* are an important cause of diarrhoea both in adults and children in Addis Ababa, and should be considered routinely in the diagnosis of patients with diarrhoea.

1.0 INTRODUCTION

New bacterial species are still being discovered. Other infectious agents, previously thought of as being opportunists or non pathogenic are increasingly being recognized as pathogens. Current examples are *Clostridium difficile*, *Legionella pneumophila* and *Campylobacter* species, particularly *C. jejuni*, *C. coli* and *C. laridis*.

The genus *Campylobacter* (Greek. *campylo*, curved; *bacter*, rod) are gram-negative, non spore-forming, slender, spirally, S- or V-shaped rods. They are motile by means of a single, polar, unsheathed flagellum at one or both ends of the cell with a characteristic cork-screw kind of movement. Metabolically they are microaerophilic to anaerobic with an oxidative type of metabolism, but they are unable to break down carbohydrates. *Campylobacter* species are found in the intestinal tract, reproductive organs and oral cavity of humans and animals. Some species are pathogenic. These species were undetected for many years because the techniques traditionally used in clinical laboratories did not permit the growth of these fastidious bacteria. Initially *Campylobacter* species were only associated with systemic infections in debilitated individuals. Systemic infections are still uncommon, and they have been shown to be caused by another *campylobacter*, *Campylobacter fetus ssp. fetus*. However, in 1977 Skirrow developed a selective medium for the isolation of *campylobacter* species from patients with enteritis and published his paper entitled, "*Campylobacter* enteritis: a 'new' disease" (Skirrow, 1977).

This was a milestone, after which, the significance of these bacteria in human diarrhoea became apparent and generally accepted. These *Campylobacter* species were subsequently identified as being *Campylobacter jejuni*, *C. coli* and *C. laridis*. In fact it is now recognized that these species are some of the most ubiquitous agents responsible for

bacterial diarrhoea throughout the world.

As *campylobacter* enteritis is a relatively "new" human disease, it is necessary to have a clear understanding of the role played by *Campylobacter* species as causative agents of diarrhoea in developing countries, such as Ethiopia, where diarrhoea is a common cause of malnutrition and death especially in children less than five years of age. It was for this reason that the present study was undertaken.

1.1 REVIEW OF LITERATURE

1.1.1 HISTORICAL BACKGROUND

Bacteria today referred to as *campylobacter*, were mentioned in the literature more than 100 years ago. Escherich (1886) observed *campylobacter* shaped organisms in faeces from patients with diarrhoea. His observations and similar findings by other investigators at that time, however, sank into oblivion for a century. In 1909 a British report was published: "Report of the Departmental Committee Appointed by the Board of Agriculture and Fisheries to inquire into Epizootic Abortion. Presented to Both Houses of Parliament by Command of His Majesty". The report concerned cattle but a brief comment about isolation of *vibrios* from ewes was made and it was followed by another report dealing with abortions in sheep where the microorganisms were described (McFadyean et al., 1913). Smith (1918) isolated similar *vibrios* from aborted calf fetuses. He also described and named the organisms "*Vibrio fetus*" (Smith and Taylor, 1919; Smith 1919). These bacteria caused economic loss to farmers because they impaired the fertility of the cattle. The disease was considered venereal, carried by the bull in the testis for life, where it caused no signs of disease.

The written history of enteropathogenic *campylobacters* proceeded with a series of papers concerning the etiology of infectious diarrhoea in cattle (Jones and Little, 1931). Diseased animals in different herds were autopsied and samples for bacteriological examination were taken from the jejunum and ileum. The vibronic bacteria found differed antigenically from "*Vibrio fetus*" by agglutination tests.

They seemed to make up a related group and were given the name "*Vibrio jejuni*". Some years later, an outbreak of acute gastroenteritis occurred in two American institutions, both getting their food from the same kitchen. For a modern reader of the report (Levy, 1946)

clinical, epidemiological and microbiological findings point to campylobacteriosis. This disease was characterized by nausea, abdominal cramps, diarrhoea, fever, general malaise and head-ache. Small coiled bacteria were observed in 31 of 73 mucoid stools and in 13 of 39 blood cultures and the author mentioned the similarity of the organisms to "*Vibrio jejuni*" and "*Vibrio fetus*". Vibrios were also discovered in pigs (Doyle, 1944) and it was suggested that they were the cause of swine dysentery. They were designated "*Vibrio coli*" (Doyle, 1948). Poultry were also reported to carry microaerophilic vibrios which could be isolated from cases of avian hepatitis (Hofstad, 1956).

V. fetus was not suspected to be pathogenic for humans until 1947, when Vinzent et al. reported two cases of abortion in women caused by *V. fetus* that were isolated from blood (Vinzent et al., 1947). Since then, a number of human cases of infections with *V. fetus* have been described, the most common form being a pure septicemia, or splenomegally but with fever as a constant feature (Butzler, 1978). This *V. fetus*, now called *Campylobacter fetus ssp. fetus*, (Sebald and Véron, 1963) is recognized as an opportunist that mainly attacks debilitated individuals with impaired defence mechanisms; it is occasionally isolated from blood, cerebrospinal fluid (CSF) and abscesses (Bokkenheuser, 1970).

It was not until 1957, that King recognized that *V. fetus* could be associated with enteric disease (King, 1957). She studied isolates from human infections with "*Vibrio fetus*" and a similar vibrio. The latter was called "*related vibrio*" and it differed from the former in antigenic properties and by its ability to grow in candle jars at 42°C but not at 25°C. The "*Vibrio fetus*" infections showed a clinical picture consisting of diverse symptoms. The disease associated with *related vibrios* was manifested by diarrhoea or loose stools. King (1962) postulated that "*Vibrio jejuni*", the causative agent of avian infectious hepatitis, and

related vibrios were the same and that they were pathogenic for humans causing a characteristic syndrome, distinct from the "*Vibrio fetus*" infections which were characterized by septicemia. She also called attention to the need for selective media for cultivation.

Except for the odd chance isolation, "*related vibrios*" as a cause of enteritis were for many years undetected because the techniques traditionally used in clinical laboratories did not fulfill the growth requirements of these fastidious bacteria. The few successful isolations were all obtained from blood or other normally sterile sites free from competing organisms (Darrel et al., 1967). In these reports it was suggested that the organisms were present in the gut and that the infection was not as rare as the few reports suggested, but attempts to prove this were frustrated by the lack of suitable selective culture techniques.

In order to verify that these organisms isolated from blood were correlated with a preceding diarrhoeal disease, the development of a selective method for isolation of these fastidious bacteria from stool samples was necessary.

Dekeyser et al. (1972) in Brussels made the first isolation of *Campylobacter* species from the stools of two patients with enteritis by using a 0.65 μm millipore filter technique developed by Plumer et al. 1962.

Suspensions of stool samples were passed through a 0.65 μm membrane filter which retained most faecal bacteria. *Campylobacters*, however, could, because they were very slender, pass through these filters and appear in the filtrates which was subsequently inoculated on culture media for growth. Incubation was made at 37°C in a microaerophilic atmosphere (two-thirds of the air volume replaced by a mixture of 95% nitrogen and 5% carbon dioxide). By this method Dekeyser et al. isolated the bacteria, serologically identical to *related vibrios* from stool specimens of two patients with acute enteritis. One

year later the same team (Butzler et al., 1973) presented a large study concerning the association between acute enteritis and related vibrios. Stools from 900 patients (800 children and 100 adults) with, and 1000 patients without diarrhoea were examined with the filtration technique in order to find *campylobacters*. The samples were also cultivated for other enteropathogenic bacteria. *Related vibrios* identified as *C. jejuni* and *C. coli* were isolated from 5% (41) of children and 4% (100) of adult diarrhoeic patients and from 1.3% (13 patients) of the control group.

The *campylobacters* were catalase and H₂S positive, tolerant to 1% glycine, able to reduce sodium selenite and to grow at 42°C (thermotolerant). Although the results reported were noteworthy, a reaction to the study was four years in coming.

In 1977 Skirrow confirmed Butzler's discovery and introduced a selective medium which replaced the filtration technique and thus made the isolation procedure easier (Skirrow, 1977). Out of 803 patients with diarrhoea *Campylobacter jejuni/coli* (the two species were not distinguished in the report) was found in faecal samples from 57 patients (7.1%). *Campylobacters* were not found at all in 194 non diarrhoeic controls. The agar medium used was made selective by the addition of 10 mg/l vancomycin, 2.5 IU/ml polymyxin B sulphate and 5 mg/l trimethoprim lactate. Incubations were made overnight in an atmosphere of 5% oxygen, 10% carbon dioxide and 85% hydrogen at 43°C. The sources of infection were in three cases apparently chickens. In each case isolation of *C.jejuni/C. coli* was made from the bird concerned. The clinical picture reported consisted of fever, abdominal pain and offensive, often watery and blood stained diarrhoea. The disease was self limiting and lasted from three days up to more than a week. Thirty-eight patients with *campylobacter* enteritis were tested for serum antibodies using whole inactivated bacteria (formolinized) as an antigen and 31 had titers to their own strains of

campylobacter.

The article of Skirrow (1977) started a world-wide search for *Campylobacter* species. It became evident that *C. jejuni/coli* was one of the most important causes of acute bacterial enteritis in all parts of the world and the prevalence of *campylobacter* in the fauna was investigated too, in order to find sources for human infections on one hand and to clarify the importance of *campylobacter* in veterinary medicine on the other.

The differentiation among different species of *campylobacter* mainly between *C. jejuni* and *C. coli* afforded difficulties until Skirrow and Benjamin (1980a) suggested application of the hippurate test. This test had been used by Harvey (1980) to distinguish between *C. fetus* and thermotolerant *campylobacter*. Skirrow and Benjamin tested 126 *campylobacter* strains and found that *C. jejuni*, but not *C. coli* had hippurate activity. *C. jejuni* appeared to be the species most frequently associated with human *campylobacter* infections. *C. coli* was isolated in just about 5% of the cases (Skirrow and Benjamin, 1982). DNA-DNA hybridization studies (Owen and Leaper, 1981) supported the classification based on biochemical tests and confirmed that *C. jejuni*, *C. coli* and *C. fetus* were distinct species. The cellular fatty acid pattern of different *campylobacter* species also added credence to earlier observations, but did not give more information than did the biochemical tests (Owen and Leaper, 1981).

Since the demonstration of *C. jejuni/coli* as a common cause of bacterial diarrhoea, different serotyping systems have been developed. Many efforts have been made to standardize and simplify the serotyping systems for *campylobacter*. Finally it was decided by the International Committee on Serotyping *Campylobacter* at the IIIrd International Workshop on *Campylobacter* Infections in Ottawa 1985, to use only two serotyping systems.

One was based on the heat labile antigens (HL) using slide agglutination with live bacteria and adsorbed antisera as described by Lior et al. (1982).

The other method was based on heat stable antigens (HS) using passive haemagglutination as described by Penner et al. (1983). With the heat stable systems, the lipopolysaccharide (LPS) was determined and approximately 70 serotypes have been designated. With heat labile typing systems, surface protein antigens were determined and approximately 90 different serotypes were described.

Studies by Kaijser and Sjögren (1985) have shown that there is a great variability of the antigens that appear on the different *campylobacter* strains causing enterocolitis. However, among the very different antigens about 20 HL antigens appear on approximately 90% of the strains causing enterocolitis and about 20 HS antigens appear on approximately 80% of the strains causing enterocolitis. Several epidemiological studies have shown the value of serotyping, and in most cases only one of the two systems is necessary. For its greater simplicity, direct agglutination for HL antigen has been the most preferred. However, in some cases a double serotyping using the two systems contributes epidemiological information.

As a result of the intensified work in the field of *campylobacter* studies new species were described besides *C. jejuni/coli*. One group of *campylobacters* was isolated from seagulls and occasionally from diseased humans (Skirrow and Benjamin, 1980b). It resembled *C. coli* but differed in sensitivity to nalidixic acid. The group was called NARTC (*nalidixic acid resistant thermophilic campylobacter*) until the name *C. laridis* was proposed (Benjamin et al., 1983).

Lawson et al. (1981) isolated *campylobacters* from lesions of intestinal adenomatosis in swine. The organisms had many characteristics in common with

C. sputorum and were classified as *C. sputorum ssp. mucosalis*. A *campylobacter* associated with periodontal disease in humans was described by Tanner et al. (1981) and designated *C. concisus*. Later on a new, extended biotyping scheme based on hippurate hydrolysis, rapid H₂S production and DNA hydrolysis tests were developed by Lior (1984). This scheme allows the separation of the three species of enteric *campylobacter* (*C. jejuni*, *coli* and *laridis*) into biotypes and, when used in conjunction with the serotyping scheme described earlier (Lior et al., 1982), provides additional significant markers in the study of the epidemiology of these organisms. Four biotypes among *C. jejuni* and two biotypes among *C. coli* and *C. laridis* were identified by this method. Even though, biotyping and serotyping have been used to characterize enteric *campylobacter* strains, it is also important to study the genetic relationships between animal and human strains. It would be interesting to determine whether the human pathogens constitute a subpopulation of the animal pathogens.

Aeschbacher and Piffaretti (1989) studied the population of enteric *campylobacter* strains isolated from human diarrhoeic patients by the multilocus enzyme electrophoresis method (MEE) and concluded that enteric *campylobacter* strains are genetically indistinct from the population of isolates of animal origin.

This finding, supports the hypothesis that *campylobacter* infection is a zoonosis (i.e., an infection naturally transmitted from vertebrate animals to humans) and that each animal strain of enteric *campylobacter* is a potential human pathogen.

MEE is a particularly useful technique in epidemiological surveys performed over large geographic regions, because it allows the clustering of closely related but not identical isolates that would otherwise be considered different and unrelated strains.

1.1.2 TAXONOMY

From the brief historical presentation above, it is obvious that the taxonomy of *Campylobacter* species can be somewhat confusing.

Since Smith and Taylor in 1919 isolated an oxidase positive, curved, motile, gram-negative rod associated with bovine abortion and named it "*Vibrio fetus*", there have appeared descriptions of several other species or subspecies of microaerophilic curved bacteria which should also be included in the genus *Vibrio* (Figure 1).

These taxa were the following:

- 1) *V. fetus venerealis* (Florent, 1959), the causative agent of enzootic abortions in cattle, a venereally transmitted bacterium.
- 2) *V. fetus intestinalis* (Florent, 1959), found in the intestinal tract of cattle, sheep and pigs. This *vibrio* was associated with enzootic abortion in ewes and sporadic cases of abortion in cattle.

Vibrio fetus intestinalis, in contrast to *Vibrio fetus venerealis*, was found to grow in the presence of 1% glycine. By this test the two taxa could be differentiated.
- 3) *V. fetus ssp. intermedius* (Elazhary, 1968), first described by Florent (1963) as an "*intermediate group*", adapted, in cattle, to the genital tract as well as to the intestines. Strains of this subspecies could be transferred by the venereal route.
- 4) *V. coli* (Doyle, 1948), a normal inhabitant of the intestine of swine, poultry and (occasionally) man, was also present in sheep and cattle. It caused dysentery in swine, hepatitis in birds and bloody diarrhoea in man.
- 5) *V. jejuni* (Jones and Little, 1931) frequently occurred in the intestinal tract of cattle and sheep.

6) *V. sputorum* (Prévot, 1940) and *V. bubulus* (Florent, 1953) have very similar characteristics and their consolidation as two separate subspecies in a single species, as proposed by Loesche et al. (1965), appears desirable. On the basis of priority, the specific epithet to be used in the name of the species is *sputorum*.

V. sputorum ssp. sputorum an occasionally pathogenic human parasite has been principally recovered from the oral cavity in gingivitis or from sputum in bronchiolitis. *V. sputorum ssp. bubulus* appears to be non pathogenic, it has been isolated from vaginal and preputial secretions, sperm, fetuses and fetal membranes, and from stools of sheep, horses and cattle. The name "*Vibrio fetus*" given by Smith and Taylor (1919) was mainly based on morphological criteria. Subsequently, other tools for taxonomic work became available and in 1963 Sebald and Véron determined the G+C (guanine plus cytosine) content of the DNA of different vibrios (Sebald and Véron, 1963). They found *Vibrio fetus* and a saprophytic vibrio called *Vibrio bubulus* (Florent, 1953) to have 33.1-35.4 mol% G+C and 29.5 mol% G+C, respectively.

As the G+C of other vibrios was around 47.2% mol% they proposed a new genus with the name *Campylobacter* and with *Campylobacter fetus* as the type species in the family *Spirillaceae*.

In 1973 Véron and Chatelain published a taxonomic study and, as a consequence, a reclassification of the genus *Campylobacter* based on serological typing of O-antigens, morphological investigations, biochemical tests, tolerance tests to nalidixic acid, 2,3,5-triphenyltetrazolium chloride (TTC), sodium chloride and DNA base ratio was done (Véron and Chatelain, 1973). All *campylobacters* investigated were small, gram-negative, motile, curved rods with a flagellum at one or both ends of the cell. They were oxidase positive and produced no acid from carbohydrates. They were microaerophilic and the G+C

contents of DNA ranged from 30.1 mol% to 34.4 mol%. The following species were suggested: *C. fetus.ssp. venerealis* ("*V. fetus venerealis*" of Florent, 1959), *C. fetus ssp. fetus* ("*V. fetus intestinalis*" of Florent, 1959), *C. jejuni* ("*V. jejuni*" of Jones and Little, 1931), *C. coli* ("*V. coli*" of Doyle, 1948), *C. sputorum* ("*V. sputorum*" of Prévot, 1940) and *C. sputorum ssp. bubulus* ("*V. bubulus*" of Florent, 1953).

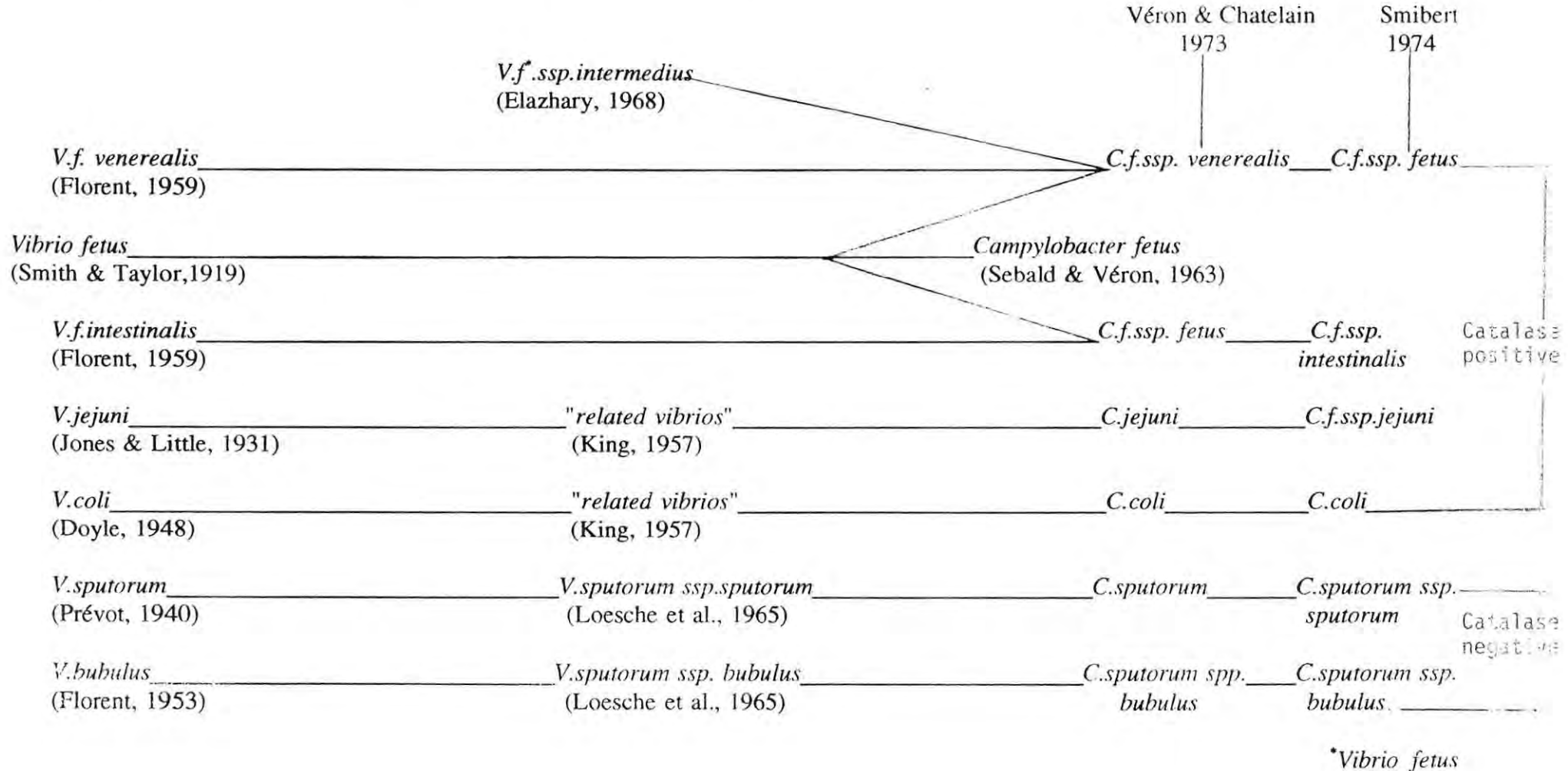
C. jejuni, *C. coli* and *C. fetus* were catalase positive while *C. sputorum* was catalase negative and the only species to be strongly H₂S positive.

The two subspecies of *C. fetus* were differentiated by glycine tolerance. *C. fetus ssp. venerealis* was inhibited by 1% glycine in contrast to other *campylobacters*. *C. fetus* was able to grow at 25°C but not at 42°C. *C. jejuni* and *C. coli*, on the contrary were able to grow at 42°C but not at 25°C. *C. fetus* was resistant to nalidixic acid at a concentration of 40µg/ml and *C. coli* and *C. jejuni* were sensitive.

On the approved lists of bacterial names (Skerman et al, 1980) the classification of Véron and Chatelain was followed in spite of Smibert's alternative in Bergey's Manual (8th edition, 1974) where *C. fetus ssp. fetus* was listed as "*C. fetus ssp. intestinalis*", *C. fetus ssp. venerealis* as "*C. fetus ssp. fetus*" and *C. jejuni* as "*C. fetus ssp. jejuni*". The identity of *C. coli* was considered doubtful, because the original *C. coli* strain was poorly characterized and representative strains were no longer available for the study.

Figure 1

HISTORICAL DEVELOPMENT OF THE TAXONOMIC NOMENCLATURE FOR THE CLASSIFICATION OF CATALASE POSITIVE AND CATALASE NEGATIVE *CAMPYLOBACTERS*



1.1.3 RECENT DEVELOPMENTS WITHIN THE GENUS *CAMPYLOBACTER*

The genus *campylobacter* has expanded during the last decade and consists, at present of 13 well defined species: *C. fetus ssp. fetus*, *C. fetus ssp. venerealis*, *C. fetus ssp. jejuni*, *C. coli*, *C. sputorum biovar. sputorum*, *C. sputotum biovar. bubulus*, *C. sputorum biovar. faecalis*, *C. rectus* (Tanner et al., 1981), *C. concisus* (Tanner et al., 1981), *C. mucosalis* (Lawson et al., 1981), *C. laridis* (Benjamin et al., 1983), *C. curvus* (Tanner et al., 1984), *C. hyointestinalis* (Gebhart et al., 1985), *C. fetus ssp. doylei* (Steele and Owen, 1988) and *C. upsaliensis* (Sandstedt et al., 1983). *C. curvus* and *C. rectus* have recently been transferred from genus *Wolinella* on the basis of DNA-rRNA hybridization (Vandamme et al., 1991b). *C. mucosalis* is the previous *C. sputorum ssp. mucosalis*. *C. concisus*, *C. curvus* and *C. rectus* are of human origin and are found in periodontal lesions. *C. sputorum biovar. bubulus* and *C. sputorum biovar. faecalis* are animal commensals. Until lately, morphological criteria have been important in *campylobacter* classification. Studies of the genotype have, in some instances, given rise to alternative opinions. Two new genera have been created for a number of species originally described as *campylobacters*. The former *C. nitrofigilis* (McClung et al., 1983), a nitrogen-fixing bacterium cultivated from plants, and *C. cryaerophila* (Neill et al., 1985), an organism associated with abortion in animals and enteritis in man, have, in line with DNA-rRNA hybridization results, been included in the proposed genus *Arcobacter* (Vandamme et al., 1991b). "*C. butzleri*" will also be placed in genus *Arcobacter* (Kiehlbauch et al., 1991). Likewise, gastritis-causing bacteria, previously named *C. pylori* (Marshall et al., 1984) and *C. mustelae* (Fox et al, 1988) now constitute genus *Helicobacter* (Goodwin et al., 1989) together with the former *C. cinaedi* (Totten et al., 1985) and *C. fennelliae* (Totten et al., 1985). *H. cinaedi* and *H. fennelliae* act as opportunistic pathogens in humans.

A separate family, *Campylobacteriaceae*, including the genera *Campylobacter* and *Arcobacter* has been proposed (Vandamme and DeLey, 1991a).

In man *C. jejuni/coli* has clinical implications and occasionally *Campylobacter fetus ssp. fetus*, *C. laridis* and *C. upsaliensis*. *C. jejuni* is more often isolated from patients with enteritis than is *C. coli*. The isolation rates of *C. jejuni* and *C. coli*, respectively, show geographical variations. Studies in Great Britain have revealed a percentage of 97% *C. jejuni* isolation versus 3% *C. coli* isolation (Skirrow and Benjamin, 1980b). In some countries, for example, the Central African Republic (Martin et al., 1988) and Poland (Dzieranowska and Rozynek, 1987) about 40% of *campylobacter* isolates from patients with enteritis were *C. coli*.

The importance of *C. upsaliensis* to human and animal health is not completely clarified. It has been isolated from humans and animals with enteritis (Sandstedt et al., 1983). It has also been associated with septicemia in ectopic pregnancy, fever, respiratory tract symptoms and immunodeficiency (Patton et al., 1989). *C. fetus ssp. fetus* is occasionally an invasive opportunistic pathogen in immunocompromised individuals.

1.1.4 CULTURAL TECHNIQUES AND MICROBIOLOGIC CHARACTERISTICS

a) CULTURE MEDIA

The isolation of *Campylobacter* species from faeces requires special selective techniques which depend either on differential filtration or direct inoculation on agar containing selective antibiotics. The filtration method first used by Dekeyser et al. (1972) is too tedious for routine use and it is less sensitive than selective agar methods. In brief, faecal material was diluted with nutrient broth. After homogenization and sedimentation the supernatant was centrifuged. The surface liquid (4ml) was sucked into a syringe for subsequent filtration through a 0.65µm millipore filter. The first 3 ml was discarded, and

0.3 ml of the remaining liquid was filtered directly onto blood-thioglycolate agar medium containing 25 IU of bacitracin, 10 IU of polymyxin B sulphate, 0.0005 mg of novobiocin, and 0.005 mg of acti-dione (cycloheximide) per ml.

The selective agar methods now widely used, depend on the use of several combinations of antibiotic supplements to the agar. They are called Skirrow's medium (1977), Butzler's medium (1979), Blaser's medium (1979), Preston's medium (Bolton and Robertson, 1982), and Modified CCDA-Preston's medium (Bolton et al., 1984). All contain 7-15% horse or sheep blood except Modified CCDA-Preston's medium.

Enrichment methods are also essential, especially for detection of a small number of *campylobacter*, as for example from specimens originating from asymptomatic carriers, patients treated with antibiotics, or from sources like water or food (Sjögren et al., 1987).

However, if the sampling technique is adequate and the individual is heavily infected, the choice of medium or the number of samples taken are not of crucial importance.

b) THE GASEOUS ENVIRONMENT REQUIRED BY *CAMPYLOBACTER*

McFadyean and Stockman discovered *campylobacter* when they tried to isolate *brucellae* from aborted sheep fetuses (Mc Fadyean et al, 1913). They inoculated samples into agar-gelatin-serum and, after three days of incubation, growth appeared below the surface of the medium. This was consistent with the growth of a microaerophilic organism. *Brucellae* can grow in a similar way but microscopic examination showed vibrio-like rods. Subsequently the media have been modified, always considering the microaerophilic character of *campylobacter*. An optimal composition of the gaseous atmosphere for the multiplication of *campylobacter* was studied by Kiggins and Plastridge (1956), and their results still form the basis for the method used in *campylobacter* cultivation. The gaseous environment providing a good growth of culture contains 5% oxygen, 10-30% carbon

dioxide and 65-85% nitrogen or hydrogen. These conditions can be achieved by evacuating anaerobic jars and filling them with the gas mixture stated. They can also be generated in jars equipped with commercially available gas producing envelopes. If the means by which this microaerophilic atmosphere can be attained are not available (for example, in field laboratories in the tropics) there are acceptable alternatives such as candle extinction jars (Smibert, 1978).

c) **INCUBATION TEMPERATURE AND TIME**

An incubation temperature of 37°C is satisfactory, but selectivity increases and quicker results are obtained by incubating at 42°C-43°C for 48 hours. Incubation at the higher temperature means that *C. fetus ssp. fetus* is excluded, and also suppresses the normal competing faecal flora. As a rule, growth is visible after overnight incubation, but when a few organisms are present growth may not be seen until the second day.

d) **NUTRITIONAL REQUIREMENTS FOR GROWTH OF CAMPYLOBACTER**

SPECIES

Campylobacters do not metabolize carbohydrates and they can neither phosphorylate nor transport glucose. They derive their energy from the tricarboxylic acid cycle. A few amino acids such as glutamic acid and aspartic acid are deaminated to α -ketoglutamic acid and oxaloacetic acid (Smibert, 1978).

High energy radicals such as superoxide are produced in culture media when exposed to light and air. The aerotolerance of *campylobacter* was reported to be increased by the addition of ferrous sulphate, sodium bi-sulphite and sodium pyruvate (FBP) (George et al., 1978). Iron and bisulphite together act non enzymatically, to destroy superoxide radicals whereas pyruvate can destroy H₂O₂ (hydrogen peroxide).

e) **IDENTIFICATION METHODS**

Colonies of the enteric *campylobacter* group are typically flat, becoming low convex, glossy, and effuse. Many strains show frank swarming on moist agar resembling droplets of fluid that have splattered on the agar to produce a transparent carpet (Skirrow and Benjamin, 1980b). In later mature cultures, the transparent colonies develop a reflective glistening surface, but the early carpet may escape detection. Some strains, however, particularly those commonly found in pigs, form discrete colonies.

Any colonies suspected to be *C. jejuni/coli* should be checked for oxidase and catalase activity. If positive, gram staining should be performed.

Microscopically, *campylobacters* are gram negative, short, spirally curved, thin, motile rods, 1.5-3.5 μm long and 0.2-0.4 μm wide and the ends of the cell are pointed. S-shaped or gull winged chains are seen in young cultures. After 4-6 days in culture the cells may become coccoid. Generally these coccoid forms have lost their viability and fail to grow after subculture. There is a single flagellum at one or both poles of the bacterium. After microscopy a preliminary diagnosis of *C. jejuni/coli* can be made; to confirm the diagnosis the susceptibility to nalidixic acid should be performed. Sensitivity to nalidixic acid is a simple verification for *C. jejuni/coli*. There are a few resistant strains both in the *C. jejuni* group and among *C. coli*. The latter were designated as NARTC (*nalidixic acid resistant group of thermophilic campylobacter*) (Skirrow and Benjamin, 1980b) until the name *C. laridis* was proposed (Benjamin et al., 1983). For further identification, a number of tests may be performed for differentiating *Campylobacter* species after isolation from human faeces as shown in Table 1.

Table 1. Laboratory tests for differentiating *Campylobacter* species after isolation from human faeces^a

	Test ^b							Growth at ^f	
	Cate- lase	Hippuricase	Nitrate reduction	Urease	H ₂ S ^c	Susceptibility to: NA ^d KF ^e		25°C	42°C
<i>C.f.*ssp. jejuni</i>	+	+	+	-	-	S	R	-	+
<i>C.f.ssp. doylei</i>	d	+	-	-	-	S	S	-	(+)
<i>C.coli</i>	+	-	+	-	-	S	R	-	+
<i>C.laridis</i> ^g	+	-	+	-	-	R	R	-	+
<i>C.upsaliensis</i>	(-)	-	+	-	-	S	S	-	+
<i>C.f.ssp.fetus</i>	+	-	+	-	-	R	S	+	(-)
<i>C.hyointestinalis</i>	+	-	+	-	+	R	S	+	+
<i>C.cinaedi</i>	+	-	+	-	-	S	I	-	-
<i>C.fennelliae</i>	+	-	-	-	-	S	S	-	-
<i>C.cryaerophila</i>	+	-	+	-	-	d	R	+	-

^aData were obtained from Manual of Clinical Microbiology fifth edition (404). ASM, Washington, D.C. 1991.

^b+, More than 90% positive; -, more than 90% negative; (+), most strains positive but a low percentage negative; (-), most strains negative but a low percentage positive or weakly positive; d, 10 to 90% positive; S, susceptible; R, resistant; I, intermediate zones of inhibition.

^cOn triple sugar iron.

^dNalidixic acid (30µg).

^eCephalothin (30µg).

^fAll species grow at 35 to 37°C

^gThe urease-positive thermophilic campylobacter variants of *C. laridis* are urease positive and susceptible to nalidixic acid.

**Campylobacter fetus*.

f) SURVIVAL IN BIOLOGICAL MILIEUS

Luechtefeld et al. found in 1981 that *C. jejuni/coli* survived in faecal specimens for an average of 4 days at 25°C, 9 days at 4°C and over 24 days in transport medium at 4°C. However, after freezing at -20°C for 24 hours, 80% of faecal specimens were negative and similar results were obtained with specimens frozen at -70°C.

The survival of *campylobacters* in fresh food and biological milieus has been investigated by Blaser et al. (1980b) and Svedhem et al. (1981b). They all found that maximal viability of *C. jejuni/coli* was obtained if strains were refrigerated at 4°C. It made no difference if the bacteria were kept in intestinal contents, food, milk, water, or urine. Heat treatment at 60°C for 15 minutes rapidly killed *C. jejuni/coli* strains. In conclusion, it is advisable to deliver specimens to the laboratory within a few hours of collection or if delay is unavoidable, to refrigerate them (2-8°C) or they should be conveyed in a transport medium, preferably semi-solid Cary-Blair medium.

g) ANTIBIOTIC SUSCEPTIBILITY

In vitro *C. jejuni/coli* is susceptible to a wide variety of antimicrobial agents including erythromycin, nalidixic acid, doxycycline, the tetracyclines, the aminoglycosides, chloramphenicol, fluoroquinolones, nitrofurans and clindamycin.

Vanhoof et al. (1978) reported 8% *C. jejuni/coli* strains resistant to erythromycin and later this figure altered to 2.3% (Vanhoof et al., 1980). From Canada (Karamali et al., 1981) and England (Brunton et al., 1978) 0.5%-1% of the strains have been reported to be erythromycin resistant. In these series, the antimicrobial sensitivity testing was done by the agar dilution method on Mueller-Hinton medium.

Analyzing the sensitivity to tetracycline Vanhoof et al. (1978) found 5% and Karamali et al. (1981) found 10% of the strains to be resistant to this drug. Resistance to tetracyclines

has been shown to be transmitted by plasmids (Taylor et al., 1981).

In 1979, Walder studied the susceptibility of 100 *C. jejuni/coli* strains to 20 antimicrobial agents by agar dilution tests and found 8 strains resistant to erythromycin (8%). Later on when 435 strains of *C. jejuni/coli* were assayed by the disc diffusion method 11 strains were resistant to erythromycin, constituting a total of 2.6% of the strains (Walder, 1982). In the same publication Walder reported that 2% of the strains were resistant to doxycycline and chloramphenicol and all strains were resistant to most of the beta-lactam antibiotics tested (benzylpenicillin, ampicillin, carbenicillin and cefuroxime). However, all were sensitive to cefotaxime. Ampicillin resistance is probably associated with beta-lactamase production (Wright and Knowles, 1980). Analyzing the sensitivity to aminoglycosides, Walder (1982) found that all strains were sensitive to gentamicin.

Svedhem et al. (1981c) reported 17% of *C. jejuni* strains, isolated from people with diarrhoea and from healthy chickens resistant to erythromycin and 25% of them were resistant to doxycycline; all strains were resistant to sulphonamide and trimethoprim. There were no significant differences between the results obtained with the strains isolated from people and those isolated from chickens.

In 1991, Lind and Kaijser studied the sensitivity pattern of 88 *C. jejuni/coli* strains isolated from patients with enteritis in three different countries (Kuwait n=33; Mexico n=23; Tanzania n=32) to 15 antimicrobial agents by agar dilution tests and found 4 strains isolated in Mexico strains resistant to erythromycin (17.4%), but all of the isolates from Kuwait and Tanzania were sensitive to this drug. In the same study, all strains were sensitive to cefotaxime, ciprofloxacin, chloramphenicol, gentamicin and norfloxacin.

A number of studies have demonstrated that fluoroquinolones are effective as prophylactic agents against travellers' diarrhoea caused by *C. jejuni/coli* or that treatment can shorten

the duration of the illness by 1-2 days. Originally, it was thought that the risk for the development of antibiotic resistance was small. However, reports have indicated that *C.jejuni/coli* may become resistant to quinolones both in vitro, and in vivo during therapy (Endtz et al., 1991).

Wretling et al. (1992) isolated norfloxacin sensitive *C. jejuni* strains from stool specimens of 15 patients with *campylobacter* enteritis before treatment. Seven of these patients received placebo, and norfloxacin sensitive strains were found in follow-up specimens from 3 of 4 patients examined. On the other hand, follow-up specimens from 3 of the 8 patients that received the active drug, showed norfloxacin resistant isolates of the same serotype as the strains isolated before treatment. The norfloxacin resistant strains were also resistant to ciprofloxacin and nalidixic acid.

Since person-to-person transmission of *campylobacter* is rare and the treatment of *campylobacter* enteritis does not often require the use of norfloxacin, it is unlikely that the introduction of norfloxacin in humans has contributed significantly to the emergence of quinolone resistance. It seems more probable that the veterinary use of quinolones for treatment and prophylaxis of *E. coli* and *mycoplasma* infections in the poultry industry contributed significantly to the development of resistance in the *campylobacter* strains isolated from man and poultry (Endtz et al., 1991). Quinolone resistance is probably associated with a single mutation in the DNA gyrase gene (Gootz and Martin, 1991).

h) SERODIAGNOSIS

Access to sensitive and reliable diagnostic serological tests are of importance in many clinical situations. This can also be the case in *Campylobacter jejuni/coli* infections. Detection of antibodies to *C. jejuni/coli* might be useful in the diagnosis of extra-intestinal reactive complications such as arthritis and glomerulonephritis in patients with *C.jejuni/coli*

infections. A number of different serodiagnostic techniques for the diagnosis of *campylobacter* infections have been developed (Svedhem et al., 1983; Oosterom et al., 1985). The most commonly used are enzyme linked immunosorbent assays (ELISA), DIG-ELISA, indirect haemagglutination and direct agglutination tests.

The techniques differ in specificity and sensitivity. In the procedure introduced by Svedhem et al. (1983) a common glycoprotein antigen was used in the DIG-ELISA technique and it was found that 90-100% of patients and 0-3% of healthy controls were positive. This assay gives good specificity as well as sensitivity for *C. jejuni*. Similar results were reported by Melby et al. (1986).

It has also been shown, from studies of an epidemic outbreak of *campylobacter* enterocolitis, that antibody determination is a valuable diagnostic tool in the diagnosis of campylobacteriosis (Bremell et al., 1991). Antibody determination was also valuable in the late follow-up of patients with reactive arthritis.

1.1.5 EPIDEMIOLOGY

a) SOURCE OF INFECTION AND MODE OF TRANSMISSION

The prevalence of *campylobacters* in the fauna was investigated extensively after the discovery of a selective medium by Skirrow in 1977, in order to find sources for human infection on one hand and to clarify the importance of *campylobacter* in veterinary medicine on the other.

Poultry appeared to be a significant source of *campylobacter*, and chickens were found to be healthy carriers of *campylobacters*, mostly *C. jejuni* (Svedhem & Kaijser, 1981a; Lindblom et al., 1986). *C. jejuni/coli* was also found in wild birds (Rosef et al., 1983), zoo animals (Luechtefeld et al., 1981a), laboratory animals (Fox et al., 1982), pets (Svedhem and Kaijser, 1981a), sheep (Svedhem and Kaijser, 1981a), horses (Atherton and Ricketts,

1980), and cattle (Elegbe, 1983). *C. coli* was found to be very common in the normal porcine intestinal flora, with isolation rates near 100% reported (Rosef et al., 1983). Faecal contamination of milk contributed to the spread of human infections (Robinson and Jones, 1981). Pasteurization, if correctly carried out, will effectively eliminate *campylobacter* from milk. *Campylobacter* contamination in drinking water systems was associated with water borne outbreaks of enteritis (Vogt et al., 1982). Commercial pork, lamb, and beef carcasses may be contaminated with *C. jejuni* (Stern, 1981).

Spread of the infection through person to person contact has been described in institutions exclusively from infants and small children, who were incontinent of faeces, to their tenders, for example, in day care nurseries (Blaser et al., 1981b). Transmission of *C.jejuni* from symptomatic or asymptomatic mothers to their neonates has also been reported (Vesikari et al., 1981; Kapperud et al., 1992). Laboratory-acquired infection (Prescott and Karamali, 1978) and infection resulting from a contaminated blood transfusion (Peppersack, 1979) have also been reported.

In conclusion, the ingestion of infected or contaminated food, such as poultry and milk, probably accounts for most cases of *C. jejuni/coli* infections in humans; thus only a minor part is due to ingestion of pork, close contact with domestic animals and pets or person to person transmission.

b) INFECTIOUS DOSE

There is some controversy concerning the infectious dose for *C. jejuni/coli*. Probably the infectivity of *C. jejuni* lies between that of *shigellae* (about 10^2 - 10^8 CFU/ml) and *salmonellae* (10^5 - 10^8 CFU/ml), but it must be emphasized that in addition to possible strain variations, such variables as the type of food or drink containing the organisms, and the contents of the stomach may greatly influence the size of the infectious dose.

c) **INCUBATION PERIOD**

Information on the incubation period is fragmentary and difficult to evaluate. In symptomatic patients the average incubation period is about 2-5 days (Korlath et al., 1985), but it may range from 2-11 days (Skirrow, 1977).

d) **PREVALENCE OF *CAMPYLOBACTER* INFECTIONS**

1) **DEVELOPED COUNTRIES**

It is well established that *campylobacters* are very common causes of bacterial diarrhoea in both developed and developing countries. In developed countries the average isolation rate from patients with enteritis and healthy carriers is in the region of 5-15% and 0.5-1% respectively (Blaser and Reller, 1981a).

In Britain, *C. jejuni/coli* was isolated from faeces of 57 (7.1%) out of 803 unselected patients with diarrhoea, but in none of the samples from 194 non diarrhoeic controls (Skirrow, 1977). In Sweden *C. jejuni/coli* was isolated in 10.9% of the 2550 (Svedhem and Kaijser, 1980) and 6.9% of the 5571 patients with enteritis (Walder, 1982).

2) **DEVELOPING COUNTRIES**

The prevalence of *C. jejuni/coli* infections in some developing countries is probably much higher than in the industrialized countries and seems directly related to socioeconomic factors. It is well known that children in developing countries are often asymptomatic excretors of *C. jejuni/coli*. Studies in South Africa (Bokkenheuser et al., 1979) and Bangladesh (Blaser et al., 1980a), showed that 16-39% of children less than 2 years of age hospitalized with diarrhoea excreted *C. jejuni/coli* and that 16-17% of the controls also excreted the bacteria. Studies in Ethiopia (Thorén et al., 1982) showed that 13% of the children less than 2 years of age with diarrhoea excreted *C. jejuni* compared with 3% for matched controls. The high percentage of excretion of *campylobacter* among

healthy children makes the interpretation of isolation of these bacteria from children with diarrhoea difficult in developing countries.

e) **AGE AND SEX DISTRIBUTION**

Information on age-specific and sex-specific rates of infection is based largely on culture surveys of patients with diarrhoea (Butzler and Skirrow, 1979). A population-based study in England showed that the highest age-specific isolation rates were in children less than five years of age when the denominator used was the number of diarrhoeal infections in each age group. However, when the denominator used was the total number of faecal specimens submitted for culture in each age group, the highest proportion of positive stools were in specimens from persons 5-34 years old. The most accurate presentation would be by the percentage of positive faecal samples within each age group, even if there is a disproportion in faecal samples submitted for culture. Differences in the likelihood of adults and children having cultures performed because of illness may in part explain these results (Butzler and Skirrow, 1979). In Bangladesh, the highest proportion of positive tests for *C. jejuni* in stools from patients with diarrhoea was in children less than one year old (Blaser et al., 1980a). These age-specific differences in the rates of *C. jejuni* suggest that the epidemiology of infection is quite different in the developed and developing countries and that the infection occurs very early in life in the developing countries. On the whole *campylobacter* enteritis is more or less equally common in both sexes.

f) **SEASONAL DISTRIBUTION**

In developed countries most patients with *C. jejuni/coli* enteritis are infected during the warm months of the year between July and September, e.g in Belgium (Lauwers et al., 1978), England (Weekly Epidemiol, Rec, 1979), USA (Blaser et al., 1979) and in Sweden (Walder, 1982). Most patients in these studies acquired the infection outside their countries

after they travelled to African-Mediterranean countries or to Asia for a short period of time during the summer.

In developing countries the isolation frequency of *C. jejuni/coli* from patients with diarrhoea is much the same during the rainy season as in the dry season (Demol and Bosmans, 1978; Bokkenheuser et al., 1979; Blaser et al., 1980a).

g) **GEOGRAPHICAL DISTRIBUTION**

Since the report by Skirrow approximately fifteen years ago (Skirrow, 1977), the results of several investigations of *C. jejuni/coli* have been reported from many countries in Europe (Svedhem and Kaijser, 1980; Skirrow, 1977; Kapperud et al., 1992), Asia (Blaser et al., 1980a), Africa (Demol and Bosmans, 1978; Thorén et al., 1982; Wamola et al., 1983), Canada (Karamali and Fleming, 1979), U.S.A (Blaser et al., 1979), Latin America (Riccardi and Ferreira, 1980), Australia (Steele and McDermot, 1978) and New Zealand (MMWR., 1990).

h) **PERSISTENCE OF THE ORGANISMS IN STOOLS**

There is a period of convalescent excretion of *C. jejuni/coli* in stools after diarrhoea has stopped (Karamali and Fleming, 1979). Svedhem and Kaijser (1980) reported that after two weeks of infection 50% of 55 patients had no *campylobacter*, and after 5 weeks 90% of the patients had no *campylobacter* in their stools. Walder (1982) reported that, for most patients, disappearance of the organisms from stools was rapid, 80% being culture negative within one month, but some patients are carriers for many months. The median period of convalescent excretion of *C. jejuni/coli* was reported to be 2-3 weeks in untreated subjects (Blaser et al., 1980c). There have been no reports as to whether these convalescent and asymptomatic excretors constitute a public health risk or not.

1.1.6 PATHOGENESIS AND PATHOLOGIC CHARACTERISTICS

Campylobacter organisms are ingested with food or water. They survive the gastric acid barrier and make their way to reach the bile-rich, microaerophilic part of the upper small intestine, a milieu favourable for their survival and multiplication. Adherence of the bacteria to mucosal surfaces appears to be an initial step in the pathogenesis and a prerequisite for colonization. A number of recent studies have been focused on the ability of *C. jejuni/coli* strains to adhere to enterocytes (Ruiz Pallacios and Crevantes, 1987). The sites of tissue injury include the jejunum, ileum and colon, with similar pathological features in each. On gross examination, a diffuse bloody oedematous and exudative enteritis has been observed (King, 1962; Lambert et al., 1979). Microscopic examination of rectal biopsy specimens from infected persons has shown a non specific colitis with an inflammatory infiltrate of neutrophils, mononuclear cells and eosinophils in the lamina propria. In addition there may be mucus depletion, and crypt abscess formation in the epithelial glands and ulceration of the mucosal epithelium (Blaser et al., 1980d). These histological appearances are not specific for *C. jejuni/coli* enterocolitis and can not be reliably distinguished from other infections caused by *salmonellae* or *shigellae* or from acute ulcerative colitis or Crohn's disease (Lambert et al., 1979; Blaser et al., 1980d). The detailed mechanism for *C. jejuni/coli* diarrhoea has not been clarified. *C. jejuni/coli* strains are known to produce a variety of toxins. Best described is an enterotoxin which causes elongation of chinese hamster ovary cells and fluid accumulation in rat intestinal loops; it is heat labile, and binds to GM₁ gangliosides. Functionally, this toxin resembles cholera toxin and the heat labile toxin of *E. coli* (Klipstein and Engert, 1985). The amount of toxin produced varies considerably from strain to strain but usually is not nearly as great as from toxigenic *E. coli* or *V. cholerae* strains. The proportion of strains producing this toxin

varies from study to study but ranges from about 30-100% in reports from Belgium (Goosens et al., 1985), India (Mathan et al., 1984), Mexico (Ruiz-Pallacios et al., 1983) and Sweden (Lindblom et al., 1989).

Cytotoxic activity by *C. jejuni/coli* strains has been described (Johnson and Lior, 1984).

This activity has been demonstrated using the Vero and HeLa tissue culture cell lines.

The presence of bacteraemia in some patients (Butzler and Skirrow, 1979) and the finding of cellular infiltration in biopsy specimens from patients with *campylobacter* colitis suggest that tissue invasion can occur.

An important determinant of the propensity for an organism to cause extraintestinal spread is its susceptibility to the bacteriocidal activity present in normal serum. In general, *C.jejuni/coli* strains are serum sensitive, although not to the same degree as *shigellae*, *V.cholerae* and most *E. coli* strains. This susceptibility is mediated by specific antibodies and by complement, chiefly through the classical pathway. Plasmids are present in at least some isolates of *C. jejuni/coli*, but these do not necessarily specify virulence properties (Taylor et al., 1981).

It has been reported that *C. jejuni* is frequently encountered in mixed infections with other known bacterial pathogens (Svedhem and Kaijser, 1980; Walder, 1982). This observation prompted many observers to study whether co-infection with other pathogens could influence the interaction between *C. jejuni* and cultured cells. In vitro studies by Bukholm and Kapperud (1987) showed that the ability of *C. jejuni* to localize intracellularly in epithelial cells is significantly enhanced by the presence of other enteric pathogens as coinfectants. However, the mechanism for interaction is still obscure.

Research aimed at elucidating these questions may increase our understanding of the little-known determinants of bacterial synergism.

1.1.7 CLINICAL FEATURES

a) ENTERIC FORMS

Symptoms and signs of *campylobacter* infection are not so distinctive that the physician can differentiate it from symptoms caused by other enteric pathogens (Plotkin et al., 1979). At the mild end of the spectrum, symptoms and signs may last for 24 hours and may be indistinguishable from those seen in a viral gastroenteritis. In contrast, *C.jejuni/coli* may also cause colitis that mimics ulcerative colitis or Crohn's disease (Blaser et al.,1980d; Lambert et al., 1979).

Knowledge of the clinical features of *campylobacter* infection is based largely on studies of patients whose symptoms were sufficiently severe to prompt their physicians to obtain a faecal culture. The dominating clinical symptom of *C. jejuni/coli* infection is enterocolitis (Blaser et al., 1979; Butzler and Skirrow, 1979; Svedhem and Kaijser, 1980).

The onset of illness is fairly sudden with fever and frequent watery stools. Only a minority of the patients, particularly adults, vomit. Bloody stools are less common, but do appear. Severe tenesmus is not uncommon. The majority of cases recover within one week without any treatment except restoration of the loss of fluid. In a minority of cases the diarrhoea is more severe and the patient might need antibiotic treatment.

b) EXTRAIESTINAL MANIFESTATIONS

Extraintestinal manifestations are much less common than enteric infection, but probably significantly under-reported, since the organisms may be difficult to grow and identify.

Most diagnosed extraintestinal infections have occurred in compromised hosts (Guerrant et al., 1978). Clinically the illness manifests as acute gram negative septicemia and the mortality associated with this type of infection is high if untreated.

Other common extraintestinal manifestations are Guillain Barré syndrome (Kuroki et al., 1991), reactive arthritis in patients carrying the HLA-B27 histocompatibility antigen (Bremell et al., 1991), meningitis (Goosens et al., 1986) and haemolytic uraemic syndrome (Haq et al., 1985). Recently attention has been focused on *campylobacter* infections in patients with immunodeficiency disorders including AIDS (Lebar et al., 1985).

c) **LABORATORY FINDINGS**

Many patients with enteric campylobacteriosis have an elevated ESR and CRP and white blood cell counts show leucocytosis of $10-12 \times 10^9/l$, but in some patients, the results may be normal. As might be expected microscopic or chemical examination of stool specimens reveal higher frequencies of blood in stools than visual inspection. Polymorphonuclear leucocytes are seen microscopically in almost all stool samples (Mäki et al., 1979).

d) **DIFFERENTIAL DIAGNOSIS**

In principle, enterocolitis caused by enteric bacteria and viruses, may give rise to the same clinical picture as the enteritis caused by enteric *campylobacter* and this has been observed by many investigators (Blaser et al., 1980d ; Lambert et al., 1979). In patients with frequent bloody stools, inflammatory bowel disease (IBD) and intussusception as non-infectious differential diagnoses should be considered.

1.1.8 **IMMUNITY**

Clinical studies have shown that *C.jejuni/coli* enterocolitis induces a very marked antibody response as measured in serum, with IgM, IgA, and IgG antibodies developing. IgM antibodies disappear in a few weeks, but IgA and IgG antibodies may remain at detectable levels for several months. IgG and IgM antibodies can be quantitated separately by the DIG-ELISA technique introduced by Svedhem et al. (1983). This allows

discrimination between individuals with acute infections and those with antibody remaining after a previous infection.

In developing countries, specific serum IgA levels rise progressively with age, which reflects recurring exposure to *C. jejuni/coli*. It has been found that healthy individuals in developed countries have low or undetectable levels of anti-*campylobacter* antibodies in serum (Svedhem et al., 1983). However, individuals working in slaughter houses, handling *campylobacter* infected animals, have been reported to have higher levels of antibodies to *campylobacter* in serum without any signs of infection (Svedhem et al., 1983). Considering these reports, it is likely that humoral immunity plays a role in the protection against *C. jejuni/coli* infection. Prolonged, severe, and recurrent *C. jejuni/coli* infection has been observed in patients with congenital or acquired hypogammaglobulinemia (Ahnen and Brown, 1982). In addition it has been reported that patients infected with human immunodeficiency virus (HIV) as well as with *C. jejuni/coli* failed to respond to antimicrobial therapy. This failure to respond to antimicrobial therapy correlated with a failure to produce a humoral response to the infection (Perlman et al., 1988).

1.1.9 TREATMENT

Fluid and electrolyte replacement are the cornerstones for treating diarrhoeal illnesses. Patients with *campylobacter* infection who are badly dehydrated should undergo rapid volume expansion using intravenous solutions of electrolyte in water. For those with less severe depletion, oral rehydration using glucose and electrolyte solutions is indicated.

In the beginning, erythromycin was the drug of choice when antimicrobial treatment was indicated (McNulty, 1987), because of the ease of administration, the lack of serious toxicity, and its apparent efficacy. Few strains are as yet resistant to erythromycin

(Svedhem et al., 1981c; Lind and Kaijser, 1991). The recommended dosage for adults is 250 mg taken orally four times a day, or 500 mg twice a day, for 5-7 days; the recommended dosage for children is 30-50 mg/kg/day, in divided doses, for the same period. Other alternatives today are the new fluoroquinolones e.g. norfloxacin and ciprofloxacin. Ciprofloxacin is a broad spectrum antibiotic with activity against several bacteria which cause diarrhoeal illness as well as against *Campylobacter* species. The dosage is 500-750 mg orally twice a day for 5-7 days.

Many *C. jejuni/coli* isolates are not susceptible to ampicillin, penicillin, or trimethoprim-sulphamethoxazole, and these agents should not be used.

1.1.10 CONTROL MEASURES

Control of *C. jejuni/coli* infections will ultimately be based on a better understanding of the reservoirs, epidemiology and pathophysiology of the infection. Since the available evidence shows that farm and household animals constitute a major reservoir for these organisms, interruption of transmission to human beings from these sources should have a high priority. Awareness of the necessity for hand washing after contact with animal products and the importance of proper cooking and storage of foods of animal origin are probably as important for preventing *C. jejuni/coli* infection as they are for preventing *salmonella* infections. Pasteurization of milk, chlorination of water supplies and proper cooking of foods all readily kill *C. jejuni/coli*.

There have been no reports of asymptomatic excretors transmitting *C. jejuni/coli* infection. Infected food handlers or hospital employees who are asymptomatic need not be excluded from work, but the need for hand washing after defecation should be stressed to these persons.

The situation is different for persons infected with *C. jejuni/coli* who have diarrhoea. Because persons with diarrhoea often spread enteric pathogens, symptomatic hospital employees with responsibilities for patient care and symptomatic food handlers should not be permitted to work until the diarrhoeal episode has ended.

1.1.11 **SUMMARY**

C. jejuni/coli has been known since the beginning of the century under other names. Its ability to cause diarrhoea or sometimes other infections has been known for approximately fifteen years. It is one of the most common bacterial causes of enterocolitis all over the world.

The bacteria can be easily isolated, if special culture techniques are used. There is a serotyping system recognized for *C. jejuni/coli*. Antibodies are induced in infected individuals and can be detected and measured, preferably using an ELISA technique.

Some strains of *C. jejuni/coli* produce an enterotoxin which in combination with adherence to mucous membranes and an invasive capacity probably contribute to pathogenicity. Most patients recover from their disease within a week. In prolonged or unusually severe cases antibiotic treatment, usually erythromycin, is used.

C. jejuni/coli is a common normal commensal in the gut of many wild and domestic animals. The majority of the episodes of disease are solitary infections. Undercooked foods are probably the most common sources of infection. Water and unpasteurized milk also cause infection, sometimes in the form of epidemics.

2.0 AIMS OF THE STUDY

- 2.1 To investigate the occurrence of *campylobacter* enteritis in patients with diarrhoea at Tikur Anbassa Hospital, Addis Ababa, Ethiopia.
- 2.2 To study the clinical features of enteric campylobacteriosis in patients with special reference to their age.
- 2.3 To identify other enteric bacterial pathogens such as *shigellae*, *salmonellae*, *Yersinia enterocolitica* and enteropathogenic *E. coli* (in children less than 2 years of age) from all specimens examined in order to ascertain the relative importance of *Campylobacter* species as bacterial agents of diarrhoea.
- 2.4 To study the antibiotic sensitivity of the isolated *campylobacter* strains to certain selected antimicrobial agents.
- 2.5 To differentiate to species level and serotype the isolated *campylobacter* strains from infected Ethiopian patients in order to provide additional epidemiological markers as a basis for future studies.

3.0 MATERIAL AND METHODS

3.1 STUDY AREA

Tikur Anbassa Hospital, Addis Ababa, Ethiopia.

3.2 PATIENTS

3.2.1 **Adults**

Adult patients (15 or more years of age) with diarrhoeal disease from the outpatient and inpatient departments of internal medicine were investigated for enteric pathogens.

3.2.2 **Children**

Sick children (less than 15 years of age) with diarrhoeal disease as the major complaint were investigated for enteric pathogens. These children were those who attended the outpatient department of the Ethio-Swedish Paediatrics and Child Health Clinic and referred to the Diarrhoeal Disease Control and Training Centre for oral rehydration. Some of the sick children were admitted to the inpatient department for further investigation and management.

Diarrhoea was defined as three or more loose or watery stools passed in a day (WHO Bull, 1989). Chronic diarrhoea was defined as diarrhoea of fourteen days' duration or more.

3.3 CONTROLS

3.3.1 **Adults**

Patients (15 or more years of age) without symptoms of diarrhoeal disease, who were admitted for other illnesses, to the wards of the Department of Internal Medicine served as controls.

3.3.2 **Children**

Sick children (less than 15 years of age) admitted to the Department of Paediatrics and Child Health Clinic for illnesses other than diarrhoea served as controls.

Patients and controls treated with broad spectrum antibiotics within the preceding one week were excluded. All patients and controls were investigated for enteric pathogens between February 1992 and January 1993 and stool specimens were collected from patients during regular working hours between 9:00 am and 12:00 noon every day except Sundays. Stool samples were collected from controls during regular working hours once a week. One stool sample for examination was collected from each patient and each control.

Histories were taken from each patient and control. The hospital charts for every individual included in the study were reviewed and the patients or their parents were interviewed by physicians. All the relevant data were transferred to the questionnaire prepared for this study (see Appendix).

Assessment of the nutritional status of children less than 5 years of age was done according to an international standard (Editorial, 1970).

The degree of dehydration was graded according to clinical signs as described by Ironside et al. (1970).

Informed consent was obtained from all patients, controls and in the case of children from a parent before collection of the stool samples.

3.4 COLLECTION, HANDLING AND TRANSPORT OF SPECIMENS

All stool specimens were obtained from defecated material and put into double screw-capped containers. They were delivered to the laboratory immediately and processed within one hour.

3.5 CULTURE AND IDENTIFICATION

3.5.1 *Campylobacter* species

All stool specimens were cultured directly on *campylobacter* blood free selective agar (Oxoid Ltd., Basingstoke, Hampshire, England), which is selective for the isolation of *Campylobacter jejuni*, *coli* and *laridis* (Bolton et al., 1984).

The medium was supplemented with cefoperazone (Sigma, Ltd., U.S.A) 32 mg/litre and crystal violet (Kebo, Sweden) 0.1%, 1 ml/litre, to suppress the normal faecal flora.

Cultures were incubated in a microaerophilic atmosphere which was achieved in anaerobic jars (Oxoid) with a palladium catalyst by using activated CO₂ generating kits (Oxoid). These kits generate an atmospheric gas concentration of 10% CO₂ and 5% O₂. Cultures were incubated at 42°C for 48 hours.

The growth of *Campylobacter* species was detected by their characteristic appearance on culture media i.e. the presence of flat greyish colonies resembling droplets of water sprayed on the medium. Preliminary identification was done based on the characteristic gram staining reaction and positive tests for oxidase and catalase.

All the isolated *campylobacter* strains were kept frozen at -70°C as stab cultures in 1% nutrient agar until species differentiation, antibiotic sensitivity testing and serotyping were done.

3.5.2 Differentiation of the isolated *Campylobacter* species

The isolated *campylobacter* strains were defined as *C. jejuni/coli* by the rapid hippurate hydrolysis test as proposed by Lior et al. (1984). A 1% sodium hippurate solution (Sigma, Ltd., U.S.A) was prepared in sterile distilled water, dispensed in 0.4 ml amounts into screw-capped tubes (10x75mm) and kept frozen at -20°C until used. A 3.5% solution of ninhydrin was freshly prepared each time before testing by dissolving 0.350g

of ninhydrin (Sigma, Ltd., U.S.A) in a tube containing 10 ml of a 1:1 mixture of acetone-butanol. A small (1 mm diameter) loopful of a 48 hour culture from a blood agar plate was emulsified well in a tube of thawed 1% sodium hippurate and incubated in a heating block at 37°C. After 2 hours of incubation, 0.2 ml of the ninhydrin reagent was slowly added on the sides of the tubes.

Without mixing the tubes were returned to the heating block for 20 minutes and examined immediately, without shaking, for colour development. A deep purple colour (crystal-violet like) indicating the presence of glycine which resulted from the hydrolysis of hippurate was considered a positive test. Those organisms giving a positive test were considered as *C. jejuni*. A pale purple colour or colourless tubes were considered negative for hippurate hydrolysis. Organisms showing a negative reaction were considered as *C. coli*. All negative samples were retested.

3.5.3 *Shigellae, Salmonellae, Yersinia enterocolitica and EPEC*

Specimens were cultured directly on xylose-lysine-desoxycholate agar (XLD) and desoxycholate agar (DCA) (Oxoid., Ltd) at 37°C for 48 hours for the isolation of *Shigella* species. Inoculation into Rappaport-Vassilidis (RV, Oxoid., Ltd) enrichment broth for 24 hours followed by subculture on XLD agar at 37°C for 24 hours was used for the isolation of *Salmonella* species.

For the isolation of *Y. enterocolitica*, specimens were cultured on desoxycholate agar at room temperature between 25°C and 30°C for 48 hours and for the isolation of *E.coli*, specimens were cultured on MacConkey agar No.2 (Oxoid., Ltd) at 37°C for 24 hours. These bacteria were identified by their characteristic appearance on their respective media and confirmed by the pattern of biochemical reactions using the standard API 20E system (Marcy-l'Etoile, France) and serologically by slide agglutination tests using commercial

polyvalent and group specific antisera (NBL, Stockholm, Sweden).

Logistically it was not possible to include virological and parasitological examinations of the stools in this study.

3.6 SEROGROUPING AND SEROTYPING

3.6.1 *Campylobacter*

The strains of *campylobacter* identified as *C. jejuni* and *C. coli* were serotyped with 16 HL antisera by slide agglutination tests using the method of Lior et al. (1982). The HL antisera used were chosen because in earlier studies in Sweden (Kaijser and Sjögern, 1985) and Canada (Lior et al., 1982) it had been shown that 90% of the strains isolated could be typed with these 16 antisera.

The definition of a non typeable strain used in this study was a strain not typeable with any of the 16 HL antisera used.

3.6.2 *Shigellae*

The isolated *shigella* strains were serogrouped by slide agglutination tests using A₁, A₂, A₃, B, C₁, C₂, C₃ and D antisera.

3.6.3 *Salmonellae*

The isolated *salmonella* strains were serogrouped by slide agglutination tests using poly O and groups A, B, C, D, E antisera. These strains were further tested against poly H antisera. Those strains identified biochemically as *Salmonella typhi* were tested against V_i antisera.

3.6.4 *ENTEROPATHOGENIC E.COLI (EPEC)*

For the screening of *EPEC*, 5-6 lactose positive colonies with typical *E.coli* morphology were picked from the MacConkey agar and subcultured on blood agar (Oxoid., Ltd) supplemented with 5% sheep blood. After growth for 24 hours on blood agar,

organisms were serotyped by slide agglutination tests using *E. coli* polyvalent antisera containing a mixture of pool I, II and III antisera. The individual pools of antisera comprised the following serogroups:

Pool I (O26:B6, O55:B5, O111:B4, O127:B8)

Pool II (O86:B7, O119:B14, O125:B15, O126:B16, O128:B12)

Pool III (O25:11L, O44:74L, O78, O114, O124).

3.7 ANTIMICROBIAL SENSITIVITY TESTING OF *CAMPYLOBACTER* STRAINS

The antimicrobial sensitivity testing of all *campylobacter* strains was done by the standard agar disc diffusion method (Bauer et al., 1966) using commercial antibiotic discs (Oxoid). Each isolate was taken from the freezing medium and subcultured on blood agar (Oxoid blood agar base supplemented with 5% citrated sheep blood) and incubated in a microaerophilic atmosphere at 37°C for 48 hours.

When a pure culture was obtained, a loopful of bacteria was taken from a colony and was transferred to a tube containing 5 ml phosphate buffered saline (PBS) and mixed gently until it formed a homogenous suspension. The turbidity of the suspension was then adjusted to the optical density of a McFarland 0.5 tube (0.14-0.15nm) measured at 500nm absorbance in order to standardize the inoculum size.

A sterile cotton swab was then dipped into the suspension and the excess was removed by gentle rotation of the swab against the surface of the tube. The swab was then used to distribute the bacteria evenly over the entire surface of PDM Antibiotic Sensitivity Medium II (PDM ASM II, Bio disk., Solna, Sweden) containing 5% sheep blood.

The inoculated plates were left at room temperature to dry for 3-5 minutes. With the aid of an automatic dispenser (Oxoid) a set of 10 antibiotic discs (Oxoid) with the following concentrations were then delivered on the surface of the PDM II plate:

ampicillin(AMP), 10µg; cephalothin(KF), 30µg; chloramphenicol(C), 30µg; erythromycin(E), 15µg; gentamicin (CN), 30µg; nalidixic acid (NA), 30µg; norfloxacin(NOR), 10µg; sulphonamide(S3), 300µg; tetracycline(TE), 30µg and trimethoprim sulphamethoxazole(SXT), 25µg. The discs were gently pressed onto the medium with sterile forceps to ensure firm contact.

The plates were then incubated at 42°C for 48 hours in anaerobic jars (Oxoid) using CO₂ generating kits (Oxoid).

A standard reference strain *E. coli* (ATCC 25922), sensitive to all of the antimicrobial drugs being tested was used as a control in this study. Diameters of the zone of inhibition around the discs were measured to the nearest millimetre using a metal calliper, and the isolates were classified as sensitive, intermediately sensitive and resistant according to the standardized table supplied by the manufacturers (Matsen and Barry, 1974).

3.8 REFERENCE STRAINS

Reference strains for *C. jejuni* (NCTC 11351), *C. coli* (LMG 6440), *C. laridis* (NCTC 11352), *Shigella flexneri* (NBL SC 530), *Salmonella typhimurium* (NBL IS-11) and *Yersinia enterocolitica* (NBL 482 III) were used for quality control throughout the study.

3.9 STATISTICAL METHODS

Statistical analyses were done using the Chi-square test with Yates' correction by EPI-INFO Version 5 microcomputer statistical analysis programme to test differences in the isolation frequency of enteric pathogens between cases and controls. Probabilities of $P < 0.05$ were considered statistically significant.

4.0 **RESULTS**

4.1 **PATIENTS AND CONTROLS**

The age and sex distribution of patients and controls are shown in Figure 2. During a 12-month period, 630 patients were examined for enteric pathogens (Figure 2). Of the 630 patients, 232 were adults (15 or more years of age) and 398 were children (less than 15 years of age); 371 (58.9%) were males and 259 (41.1%) were females, resulting in an overall male to female ratio of 1.4:1.

There were 546 (86.7%) outpatients (170 adults and 376 children) and 84 (13.3%) inpatients (62 adults and 22 children). Children aged less than one year predominated among the patients and represented 42.7% of all, whereas the age group 15-34 years represented 24.7%.

Of the 220 controls, 112 were adults and 108 were children; 121 (55%) were males and 99 (45%) were females resulting in an overall male to female ratio of 1.2:1 (Figure 2). All controls were inpatients.

4.2 **ISOLATION RATE OF *CAMPYLOBACTER*, *SHIGELLA* AND *SALMONELLA* SPECIES**

The number and percentage of each pathogen isolated from patients and controls are shown in Tables 2 and 3, and the number of patients investigated for enteric pathogens and the number of isolates during a 12-month period are shown in Figure 3.

A total of 184 enteric pathogens were isolated from 630 patients and 17 from 220 controls (29.1% vs. 7.7%, $p < 0.001$).

Campylobacter species were the most common bacterial pathogens isolated. They were isolated from 88 (10.3%) of the 850 investigated patients and controls.

Of the 88 isolates, 86 were recovered from patients and 2 from controls (13.6% vs. 0.9%,



Age and sex distribution of patients (n=630) and controls (n=220) investigated for enteric pathogens

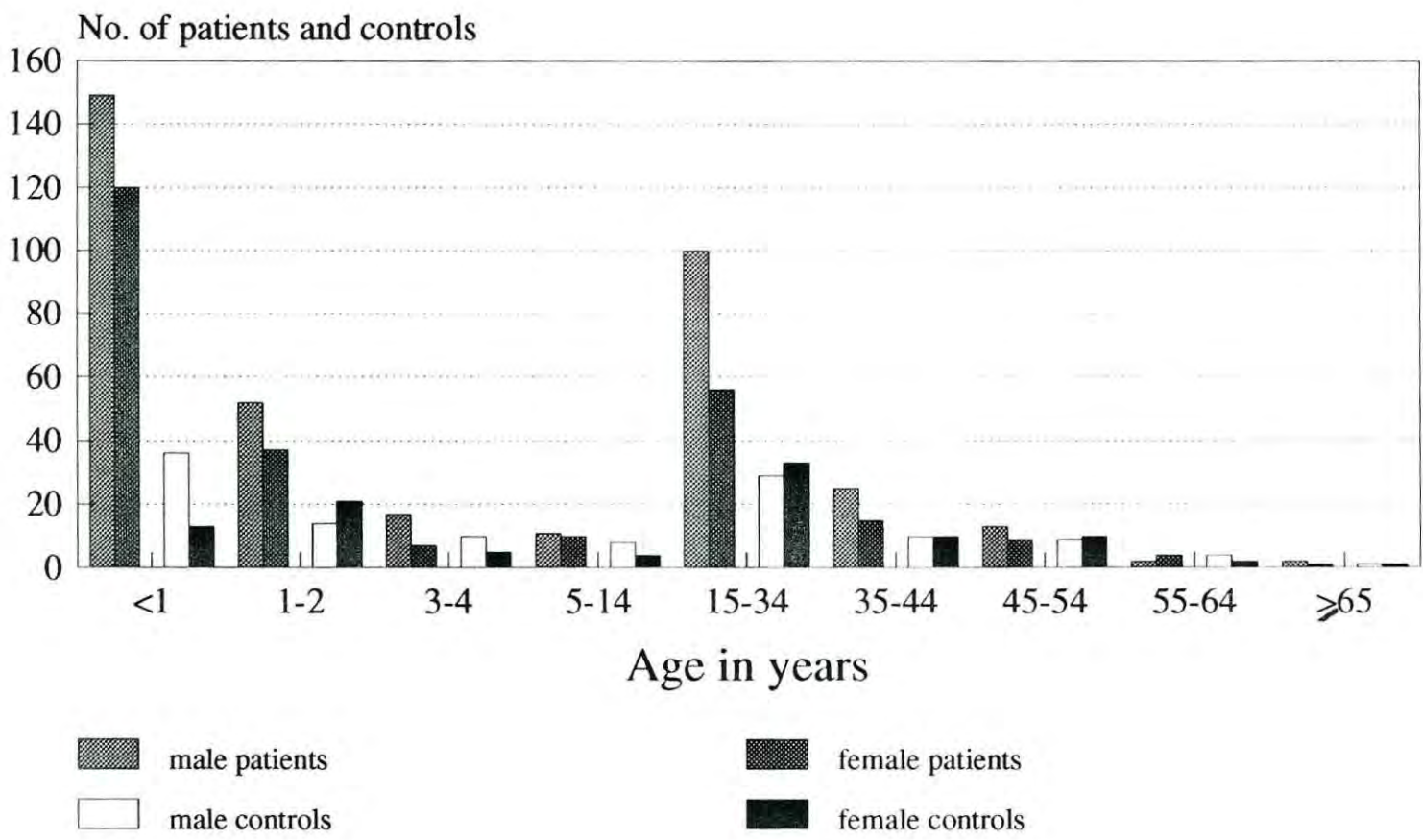


Figure 2

Table 2. Bacterial enteric pathogens isolated from 630 patients with diarrhoea in Tikur Anbassa Hospital during one year (Feb 1992-Jan 1993).

Enteric pathogen	No. of patients					
	Adults (n=232)		Children (n=398)		Total ^e (n=630)	
	No.	%	No.	%	No.	%
<i>Campylobacter</i> spp. ^a	25	10.7	61	15.3	86	13.6
<i>Shigella</i> spp. ^b	45	19.4	29	7.3	74	11.7
<i>Salmonella</i> spp. ^c	8	3.4	16	4.0	24	3.8
<i>Yersinia enterocolitica</i>	-	-	-	-	-	-
Total ^d	78	33.6	106	26.6	184	29.1

Table 3. Bacterial enteric pathogens isolated from 220 control patients without diarrhoea in Tikur Anbassa Hospital during one year (Feb 1992-Jan 1993).

Enteric pathogen	No. of controls					
	Adults (n=112)		Children (n=108)		Total ^e (n=220)	
	No.	%	No.	%	No.	%
<i>Campylobacter</i> spp. ^a	-	-	2	1.8	2	0.9
<i>Shigella</i> spp. ^b	1	0.9	1	0.9	2	0.9
<i>Salmonella</i> spp. ^c	1	0.9	12	11.1	13	5.9
<i>Yersinia enterocolitica</i>	-	-	-	-	-	-
Total ^d	2	1.8	15	13.8	17	7.7

^ap < 0.001, adult patients vs. adult controls, Chi-square test.

^ap < 0.001, children patients vs. children controls, " " .

^bp < 0.001, adult patients vs. adult controls, Chi-square test.

^bp < 0.005, children patients vs. children controls, " " .

^cp = 0.28, adult patients vs. adult controls, Fishers exact test.

^cp < 0.01, children patients vs. children controls, Chi-square".

^dp < 0.001, adult patients vs. adult controls, Chi-square test.

^dp < 0.001, children patients vs. children controls, " " .

^ep < 0.001. patients vs. control, Chi-square test.

Number of patients investigated for
enteric pathogens and number of isolates
during 12 months (Feb 1992 - Jan 1993)

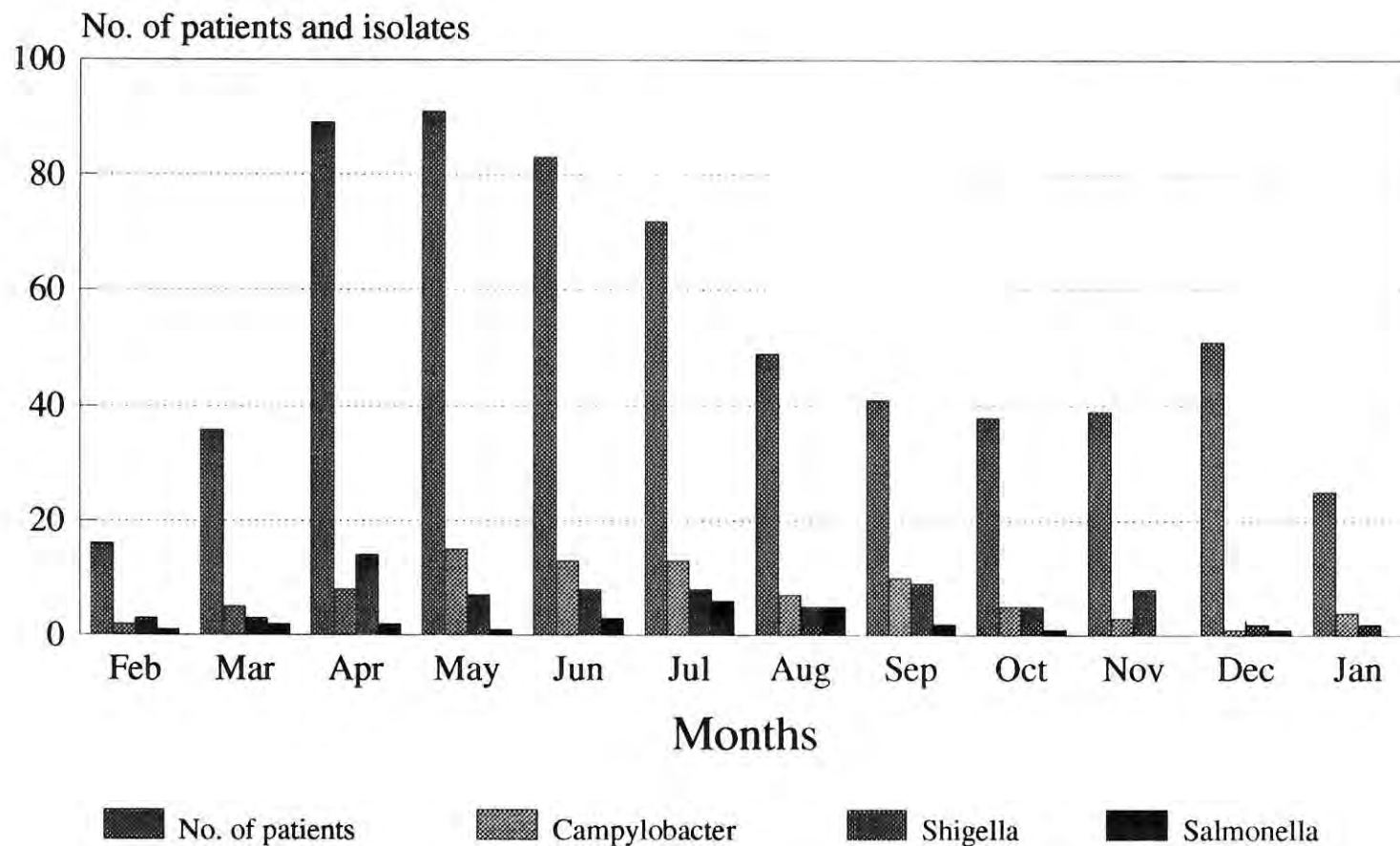


Figure 3

$p < 0.001$) (Tables 2 and 3). Of the 25 *campylobacter* isolates from adults, 25 were from patients and none from controls (10.7% vs. 0%, $p < 0.001$). Out of the 63 *campylobacter* isolates from children, 61 were from patients and 2 were from controls (15.3% vs. 1.8%, $p < 0.001$) (Tables 2 and 3).

Of the 88 *campylobacter* isolates, 68 strains were recovered after freezing and storage. Of these, 56 (82.4%) were identified as *C. jejuni* and 12 (17.6%) as *C. coli*. Of the 56 *C. jejuni* strains, 40 (71.4%) were isolated from children and 16 (28.6%) from adults. Of the 12 *C. coli* strains, 10 (83.3%) were isolated from children and 2 (16.7%) from adults. These results are shown in Table 4.

The relative frequency of other bacterial pathogens isolated from patients and controls during the same period is shown in Tables 2 and 3. *Shigella* spp. were isolated from 76 (8.9%), and *Salmonella* spp. from 37 (4.4%) of the 850 patients and controls investigated. *Y. enterocolitica* was not isolated from any person during this study.

More patients (53.1%) presented for investigation of diarrhoea during the months of April through July but there was no difference in the isolation frequency of the three enteric pathogens studied throughout the year (Figure 3).

More than one enteric pathogen was isolated from 12 (14.0%) of the 86 patients with stool cultures positive for *Campylobacter* species; *campylobacters* and *shigellae* from 9 patients; *campylobacters* and *salmonellae* from 3 patients (Table 5).

Of the 12 patients with double infections, 6 were adults (4 inpatients and 2 outpatients) and 6 were children, all of whom were outpatients.

Table 4

Distribution of *Campylobacter jejuni* and *Campylobacter coli* isolated from patients and controls.

Source	<i>C. jejuni</i>		<i>C. coli</i>	
	(n=56)		(n=12)	
	No.	%	No.	%
Children with diarrhoea	38	67.8	10	83.3
Children without diarrhoea	2	3.6	0	-
Adults with diarrhoea	16	28.6	2	16.7
Adults without diarrhoea	-	-	-	-
Total	56	100.0	12	100.0

Table 5

Number of concomitant isolations of *Shigella* and *Salmonella* species from 86 patients with stool cultures positive for *Campylobacter* species.

Enteropathogen	<u>Patients</u>					
	Adults		Children		Total	
	(n=25)		(n=61)			
	No.	%	No.	%	No.	%
<i>S. dysenteriae</i> (A ₁)	-	-	1	1.6	1	1.2
<i>S. dysenteriae</i> (A ₂)	-	-	1	1.6	1	1.2
<i>S. flexneri</i> (B)	3	12	1	1.6	4	4.6
<i>S. boydii</i> (C ₁)	1	4	1	1.6	2	2.3
<i>S. sonnei</i> (D)	-	-	1	1.6	1	1.2
<i>Salmonella</i> (B)	1	4	1	1.6	2	2.3
<i>Salmonella typhi</i>	1	4	-	-	1	1.2
Total	6	24	6	9.8	12	14.0

4.3 CAMPYLOBACTER INFECTED PATIENTS

4.3.1 Age and sex distribution

The age and sex distribution of patients with *campylobacter* infections are shown in Figure 4. Among the 86 patients infected with *Campylobacter* species, there were 48 males and 38 females (55.8% vs, 44.2%), resulting in an over all male to female ratio of 1.2:1.

The peak isolation rate for *campylobacters* was from patients less than one year of age (32.5%). Of the 86 patients with stool cultures positive for *campylobacter*, 59 (68.6%) of the *campylobacter* isolations were from children less than five years of age and 25 from patients 15 or more years of age (Table 2 and Figure 4).

4.3.2 Clinical features

The clinical features of 19 adults and 55 paediatric patients with *campylobacter* infections are summarized in Table 6. Patients from whom a concomitant isolate of *Shigella* or *Salmonella spp.* was recovered were excluded (Table 5).

a) Adults

In adult patients low grade fever was the most commonly reported symptom (84.2%); followed by watery diarrhoea in 13/19 (68.4%), abdominal pain accompanied by tenesmus or colicky pain in 4/19 (21%), and vomiting in 4/19 (21%). In 6/19 patients (31.5%) bloody, mucoid or mixed diarrhoea was noted. Seven patients (36.8%) had had diarrhoea for more than fourteen days.

b) Children

Watery diarrhoea was the most commonly reported complaint (87.2%) by the children's parents; low grade fever was documented in 42/55 (76.4%) and vomiting was observed in 30/55 (54.6%) of the patients. The duration of diarrhoea for most of these

Age and Sex distribution of 86 patients with stool cultures positive for Campylobacter

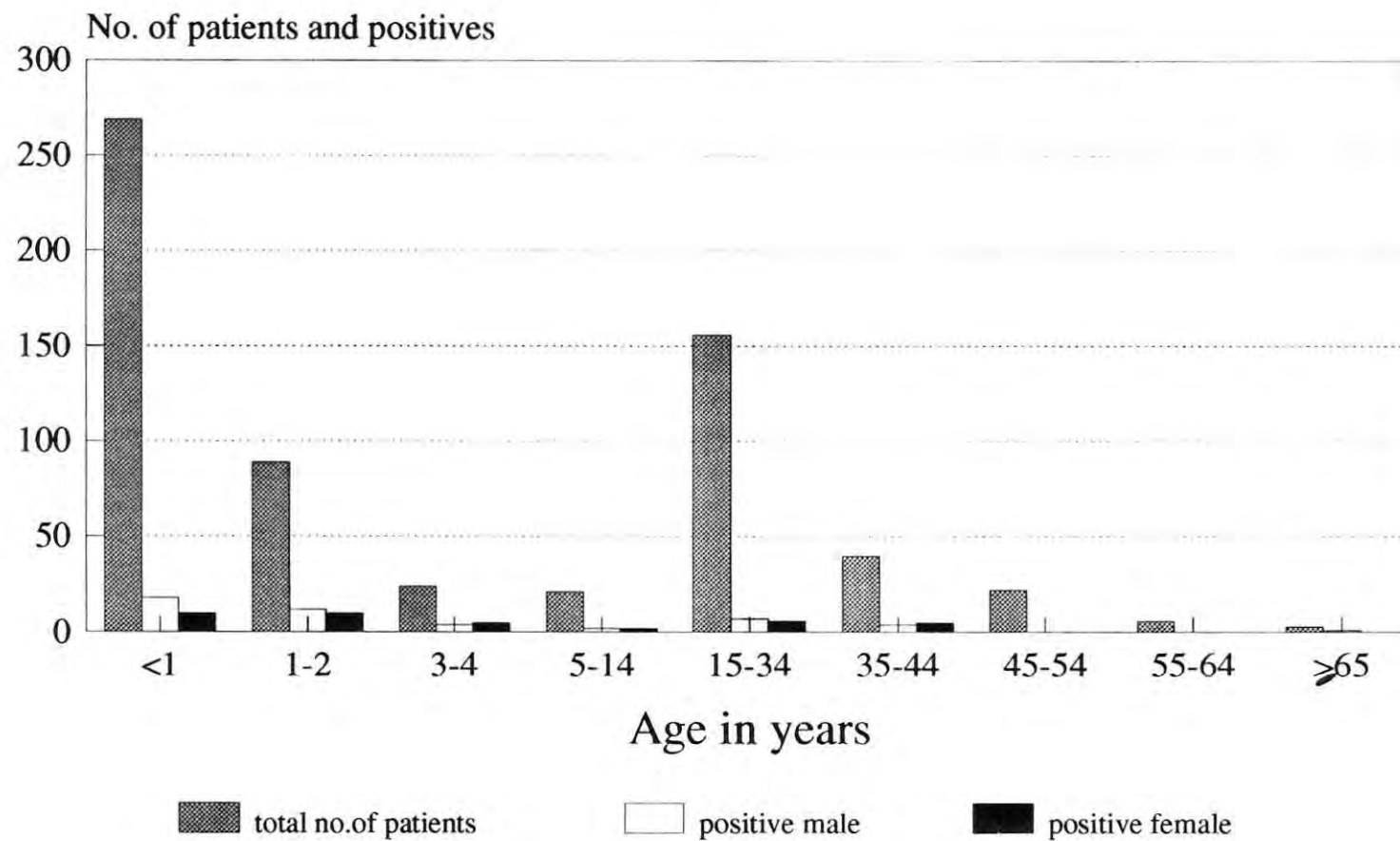


Figure 4

Table 6.

Frequency of symptoms and signs in 19 adults and 55 children with campylobacter infections.

	<u>Adults</u>		<u>Children</u>		Total	
	(n= 19)		(n= 55)			
	No.	%	No.	%	No.	%

1) <u>Diarrhoea</u>						
a) nature						
watery	13	68.4	48	87.2	61	82.4
bloody	2	10.5	3	5.5	5	6.8
mucoid	2	10.5	2	3.6	4	5.4
mixed	2	10.5	2	3.6	4	5.4
b) Duration						
< 3 days	1	5.3	14	25.4	15	20.3
≥ 3-7 "	6	31.6	34	61.8	40	54.1
8-14 "	2	10.5	3	5.5	5	5.4
> 14 days	7	36.8	3	5.5	10	13.5
data missing	3	15.8	1	1.8	4	5.4
2) <u>Vomiting</u>						
present	4	21.0	30	54.6	34	45.9
absent	15	79.0	24	43.6	39	52.7
data missing	-	-	1	1.8	1	1.4
3) <u>Abdominal pain</u>						
present	4	21.0	Difficult to			
absent	15	79.0	assess			
data missing	-	-				
4) <u>Temperature°C</u>						
≥ 39	2	10.5	4	7.3	6	8.1
37-38	16	84.2	42	76.4	58	78.4
< 37	1	5.3	7	12.7	8	10.8
data missing	-	-	2	3.6	2	2.7

children (61.8%) was between 3-7 days. Abdominal pain is difficult to evaluate in small children and therefore not reported in Table 6.

In 3/55 patients (5.5%) bloody diarrhoea was noted; mucoid or mixed diarrhoea was observed in 4/55 (7.2%) of the patients. Dehydration ranging from mild to severe was observed in 14 (25.4%) of the 55 children with *campylobacter* infections (Table 7). Of the 55 children 37 (67.2%) had signs of malnutrition and most of them (47.2%) were underweight.

c) Patients with a double infection

Patients with a double infection did not differ clinically from those with a single infection. In 3/6 adult patients bloody diarrhoea was the most commonly reported complaint (50.0%); followed by low grade fever in 2/6 (33.7%) and vomiting in 1/6 (16.7%). In 5/6 (83.3%) of the paediatric patients watery diarrhoea, low grade fever, vomiting or a combination of the three were noted.

Table 7

Nutritional status and degree of dehydration in 55 children (< 5 years) with *Campylobacter* infections.

	<u>Children</u>	
	(n=55)	
	No.	(%)
<u>Nutritional status</u>		
normal	15	27.3
underweight	26	47.2
marasmus	9	16.4
marasmic kwashiorkor	2	3.6
kwashiorkor	-	-
data missing	3	5.5
total	55	100.0
<u>Degree of dehydration</u>		
none	40	72.7
mild	1	1.8
some	11	20.0
moderate	1	1.8
severe	1	1.8
data missing	1	1.8
total	55	100.0

4.4 SEROGROUPS OF *CAMPYLOBACTER*, *SHIGELLA* AND *SALMONELLA*

SPECIES

4.4.1 *Campylobacter* species

Serotyping of *campylobacter* isolates was done using the Lior system. Of the 68 *campylobacter* isolates available for screening, 59/68 (86.8%) of the strains were typeable, whereas the remaining 9 (13.2%) isolates were untypeable (Table 8).

Of the 56 *C. jejuni* and 12 *C. coli* isolates, 50 (89.3%) of the *C. jejuni* and 9 (75%) of the *C. coli* isolates were typeable.

A total of 11 serotypes were represented among the *C. jejuni* and 3 among the *C. coli* isolates (Table 8). Lior serotypes 1, 2, 4, 5, 6 and 7 were most common among the *C.jejuni*, while Lior serotypes 1 and 2 were dominant among the *C. coli* isolates. Lior serotypes 1, 2, 4, 5, 6 and 7 accounted for 63.2% of all isolates. Of the 56 *C. jejuni*, 10 (17.9%) and of the 12 *C. coli* strains, 2 (16.7%) of the strains were positive for more than one of the Lior serotypes as shown in Table 8. Serotypes 1 and 2 were common for both *C. jejuni* and *C. coli*, whereas the remaining serotypes were found mainly among the *C.jejuni* isolates.

4.4.2 *Shigella* species

Of the 76 isolates of *Shigella* spp. 74 were found in patients and 2 in controls (11.7% vs. 0.9%, $p<0.001$) (Tables 2 and 3). Of the 74 *shigella* isolates from patients, 45 (60.8%) were from adults and 29 (39.2%) from children. Of the 2 *shigella* isolates from the controls, 1 was a *S. dysenteriae* from an adult and the other one was a *S. flexneri* from a child. Out of the 46 *shigella* isolates from adults, 45 were from patients and 1 from controls (19.4% vs. 0.9% , $p<0.001$). Of the 30 *shigella* isolates from children, 29 were from patients and 1 from controls (7.3% vs. 0.9%, $p<0.005$).

Table 8.

Frequency of Lior serotypes of *Campylobacter jejuni* and *Campylobacter coli* isolated from patients and controls.

HL (Lior serotypes)	<i>C. jejuni</i> (n=56)		<i>C. coli</i> (n=12)	
	No.	%	No.	%
1	11	19.6	3	25.0
2 ^a	10	17.8	4	33.3
4 ^b	4	7.1	-	-
5	2	3.6	-	-
6	5	9.0	-	-
7	4	7.1	-	-
8	-	-	-	-
9	1	1.8	-	-
11	-	-	-	-
12	-	-	-	-
13	1	1.8	-	-
17	-	-	-	-
20	1	1.8	-	-
21	-	-	-	-
35	-	-	-	-
36	1	1.8	-	-
1 & 2	4	7.1	1	8.3
1 & 6	1	1.8	-	-
2 & 5	2	3.6	-	-
2 & 6	-	-	1	8.3
2 & 36	1	1.8	-	-
6 & 21	1	1.8	-	-
4, 5, 6, 9 & 36	1	1.8	-	-
NT ^c	6	10.7	3	25.0
Total	56	100.0	12	100.0

^a1 *C. jejuni* serotype 2
& were from controls.

^b1 *C. jejuni* serotype 4

^cNT= non typeable strains.

Table 9

Serogroups of 74 strains of *Shigella* species isolated from 630 patients and 2 strains from 220 controls.

Serogroups	No of isolates	Isolation frequency (%)
<i>A₁(S. dysenteriae)</i>	14*	18.4
<i>A₂(S. dysenteriae)</i>	3	4.0
<i>A₃(S. dysenteriae)</i>	-	-
<i>B(S. flexneri)</i>	41*	54.0
<i>C₁(S. boydii)</i>	2	2.6
<i>C₂(S. boydii)</i>	3	4.0
<i>C₃(S. boydii)</i>	1	1.3
<i>D (S. sonnei)</i>	12	15.7
Total	76	100.0

* 1 *S. dysenteriae* and 1 *S. flexneri* were from controls.

Serogrouping of the 76 *Shigella* isolates showed that *S. flexneri* was the most commonly isolated species (54.0%), followed by *S. dysenteriae* (22.4%), *S. sonnei* (15.7%) and *S. boydii* (7.9%) as shown in Table 9.

4.4.3 *Salmonella* species

Of the 37 isolates of *Salmonella* species, 24 were isolated from patients and 13 from controls (3.8% vs. 5.9%, $P=0.26$) (Tables 2 and 3). Out of the 24 *Salmonella* isolates from patients, 8 (33.3%) were from adults and 16 (66.7%) from children. The corresponding figures for the *Salmonella* isolates from the controls were 1 and 12, respectively. Out of the 9 *Salmonella* isolates from adults, 8 were from patients and 1 from controls (3.4% vs. 0.9%, $P=0.28$). Of the 28 *Salmonella* isolates from children, 16 were from patients and 12 from controls (4.0% vs. 11.1%, $p<0.01$) (Tables 2 and 3).

Among the 37 *salmonella* strains, the most commonly isolated serogroup was group B, 30 (81.1%), followed by *Salmonella typhi*, 4 (10.8%) and group C, 3 (8.1%). All of the four *Salmonella typhi* isolates were recovered from adult outpatients and these patients had diarrhoea. In 2/4 (50.0%) watery diarrhoea was noted; bloody or mixed diarrhoea was observed in 2/4 (25% each). Mixed infections with *campylobacters* were noted in 1/4 (25%) of the patients (Table 5).

4.5 EPEC (Enteropathogenic E.coli)

A total of 421 patients and controls less than 2 years of age were screened for *EPEC* using polyvalent and pooled antisera. Of the 421 children screened for *EPEC*, 345 were patients and 76 were controls.

Of the 345 paediatric patients, 72 (20.8%) and 13 (17.1%) of the 76 controls had *EPEC* in their stools ($p=0.56$) (Table 10). Of the 345 sick children screened for *EPEC*, 21 (6.1%) harboured *E. coli* colonies which reacted with more than one pool of antisera. Of the 76

Table 10

Isolation rate of screened *EPEC* in patients and controls less than 2 years of age with pooled antisera.

Pooled antisera	<u>Patients^a</u> (n= 345)		<u>Controls^a</u> (n= 76)	
	No.	%	No.	%
I	18	5.2	2	2.6
II	13	3.7	2	2.6
III	20	5.8	4	5.3
I & II	2	0.6	-	-
I & III	2	0.6	1	1.3
I II & III	17	4.9	4	5.3
Total	72	20.8	13	17.1

^ap= 0.56, patients vs. controls, Chi-square, Yate's correction.

controls, screened for *EPEC*, 5 (6.6%) harboured *E. coli* colonies which reacted with more than one pool of antisera. More than one enteric pathogen was isolated from 14 (19.4%) of the 72 children and 2 (15.4%) of the 13 controls with stool cultures positive for *EPEC*. Both *EPEC* and *campylobacters* were found in 10 patients, *EPEC* and *shigellae* in 2 patients, *EPEC* and *salmonella* in 1 patient; *EPEC*, *campylobacter* and *shigella* in 1 patient. Both *EPEC* and *salmonellae* were isolated from 2 of the controls.

4.6 ANTIMICROBIAL SENSITIVITY TESTING

Of the 88 *Campylobacter* isolates, 3 could not be recovered after freezing and storage and only 85 strains were available for antimicrobial sensitivity testing.

The sensitivity patterns of these 85 strains isolated from patients and controls against 10 chosen antimicrobial agents are presented in Table 11.

All strains were sensitive to chloramphenicol, erythromycin, gentamicin, nalidixic acid, norfloxacin, sulphonamide and tetracycline and all were resistant to cephalothin.

Resistance was found against ampicillin in 60% of the strains and against trimethoprim-sulphamethoxazole in 58.8%. No intermediate sensitivity was found in any strain tested.

There were no differences in the sensitivity pattern between *C. jejuni* and *C. coli* (data not shown).

Table 11

Sensitivity of 85 *campylobacter* strains against 10 antimicrobial agents.

Antimicrobial agents	sensitive		intermediate		resistant	
	No	(%)	No	(%)	No	(%)
Ampicillin	34	40.0	-	-	51	60.0
Cephalothin	-	-	-	-	85	100.0
Chloramphenicol	85	100.0	-	-	-	-
Erythromycin	85	100.0	-	-	-	-
Gentamicin	85	100.0	-	-	-	-
Nalidixic acid	85	100.0	-	-	-	-
Norfloxacin	85	100.0	-	-	-	-
Sulphonamide	85	100.0	-	-	-	-
Tetracycline	85	100.0	-	-	-	-
Trimethoprim-sulphamethoxazole	35	41.2	-	-	50	58.8

5.0 DISCUSSION

Diarrhoea is one of the most common diseases throughout the world. Numerous bacteria, viruses and intestinal parasites have been identified as causative agents of diarrhoea. In many instances, no etiological agent is identified, even when the most sophisticated microbiologic procedures are used. However, it has been recognized recently that *Campylobacter* species are among the commonest causes of bacterial diarrhoea throughout the world. As *campylobacter* enteritis is a relatively "new" human disease, it is necessary to have a clear understanding of the role played by *Campylobacter* species as causative agents of diarrhoea in developing countries, such as Ethiopia, where diarrhoea is a common cause of malnutrition and death, especially in children less than five years of age. The present study was undertaken in order to investigate the importance of *Campylobacter* species as causative agents of diarrhoea in Ethiopian patients. It was necessary to isolate other known enteric bacterial pathogens such as *shigellae*, *salmonellae* and *Y. enterocolitica* in order to ascertain the relative importance of *Campylobacter* species as bacterial agents of diarrhoea. It was not possible logistically to include virological and parasitological examinations of the stool specimens in this study.

All patients and controls in the present study, were from the outpatient clinics and inpatient wards of the Department of Internal Medicine and from the Ethio-Swedish Paediatric Clinic of Tikur Anbassa Hospital in Addis Ababa. Tikur Anbassa Hospital is a referral hospital located in the centre of the city. Most of the patients come from Addis Ababa but some may be referred from different parts of the country for further investigation and management.

The frequency of isolation of *campylobacters*, *shigellae* and *salmonellae* from 630 patients with and 220 controls without diarrhoeal illnesses are presented in Tables 2 and 3.

Campylobacter spp. were isolated from 86 (13.6%), *Shigella* spp. from 74 (11.7%) and *Salmonella* spp. from 24 (3.8%) of the 630 patients investigated. In addition, *Campylobacter* spp. were isolated from 2 (0.9%), *Shigella* spp. from 2 (0.9%) and *Salmonella* spp. from 13 (5.9%) of the 220 controls investigated.

These results indicate that there was a statistical difference in the isolation rates of *Campylobacter* spp. from patients and controls (13.6% vs. 0.9%, $p < 0.001$), adult patients and adult controls (10.7% vs. 0%, $p < 0.001$) and paediatric patients and paediatric controls (15.3% vs. 1.8%, $p < 0.001$) (Tables 2 and 3).

The frequency of *Campylobacter* spp. isolation in this study (13.6%) is similar to that reported in other studies performed in England (13.9%) (Bruce et al., 1977) and in Sweden (10.9%) (Svedhem and Kaijser, 1980). However there are reports from different parts of the world with lower frequencies of isolation e.g. 5.1% in England (Skirrow, 1977), 2.27% in Italy (Varoli et al., 1989), 4.5% in Saudi Arabia (Zaman, 1992) and 1.2% in Singapore (Lim and Tay, 1992). Of the 86 *campylobacters* isolated from patients, 61 (70.9%) were from children, whereas the remaining 25 (29.1%) isolates were from adults (Table 2). In fact, *Campylobacter* species were the most commonly isolated bacterial pathogen in children (15.3%).

The frequency of *campylobacter* isolates from paediatric patients in our study (15.3%) was almost the same as in studies performed in Gambia (Billingham, 1981) (13.6%), in Thailand (13%) (Taylor et al., 1991), earlier in Ethiopia (13%) (Thorén et al., 1982) and in south Africa (16%) (Bokkenheuser et al., 1979). Higher isolation rates were reported from Bangladesh (35%) (Blaser et al., 1980a) and Egypt (25.9%) (Pazzaglia et al., 1991).

A possible explanation for the high isolation rate of *campylobacter* from patients in our study as compared with reports from some of the different countries mentioned earlier may be related to socioeconomic factors. It is well known that, in developing countries, isolation of *C. jejuni/coli* is common from patients with diarrhoea and from those who are healthy (Bokkenheuser et al., 1979; Blaser et al., 1980a; Billingham, 1981), which is in contrast with observations in developed countries in which asymptomatic infection is rare (Skirrow, 1977; Walder, 1982).

A possible explanation for the relatively low isolation rate of *campylobacters* from controls in Ethiopia, as compared with the results of some of the other studies from developing countries (Bokkenheuser et al., 1979; Blaser et al., 1980a), may be that, since most of our patients were from an urban population they may not have been persistently exposed to the sources of the infection.

Mixed infections of *Campylobacter* species with other enteric pathogens are common and have been reported by many investigators (Bokkenheuser et al., 1979; Svedhem and Kaijser, 1980; Lim and Tay, 1992). In our study nearly 14.0% of the 86 patients infected with *Campylobacter* had another enteric pathogen recovered from their stools (Table 5). Nine patients had mixed infections with *Campylobacter* species and *Shigella* species, whereas three patients harboured *Campylobacter* species along with *Salmonella* species.

The isolation of *C. jejuni* and *C. coli*, respectively, show geographical variations. Studies in Great Britain have revealed a percentage of 97% *C. jejuni* isolation versus 3% *C. coli* isolation (Skirrow and Benjamin, 1980b). In some other countries, for example the Central African Republic (Martin et al., 1988) and Poland (Dzieranowska and Rozynek, 1987) about 40% of the *campylobacter* isolates from patients with diarrhoea were *C. coli*.

Of the *campylobacters* that were identified at the species level in this study, *C. jejuni* accounted for 82.4% and *C. coli* accounted for 17.6% as shown in Table 4. These results are similar to those of studies done in Canada (Lior et al., 1984) and in Singapore (Lim and Tay, 1992).

Work done in Europe and North America (Lauwers et al., 1978; Blaser et al., 1979) has shown that *campylobacter* infections are most common during the summer. In developing countries the isolation frequency of *C. jejuni/coli* from patients with diarrhoea has been reported to be much the same in the rainy season as in the dry season (Demol and Bosmans, 1978; Bokkenheuser et al., 1979; Blaser et al., 1980a). In this study, more patients (53.1%) presented for investigation of diarrhoea during the months of April through July. There were no significant differences in the isolation frequency of *campylobacters* and the other two enteric pathogens studied during this period as compared to other months of the year as shown in Figure 3.

In 1977, the article of Skirrow started a world-wide search for *Campylobacter* species (Skirrow, 1977). It became evident that *C. jejuni* and *C. coli* were common causes of bacterial diarrhoea in all parts of the world and different serotyping systems have been developed to provide additional significant markers in the study of the epidemiologic features of these organisms (Lior et al., 1982; Penner et al., 1983). The serotype distribution of *C. jejuni* and *C. coli* strains from different parts of the world has been investigated. These investigations have shown that a limited number of serotypes dominate, and that these are the most commonly found serotypes both in outbreaks and in sporadic cases of enteric campylobacteriosis (Kaijser and Sjögren, 1985; Melby et al., 1986). In the course of this study, 68 isolates were serotyped using the methods of Lior et al. (1982), with 16 HL antisera. Eighty to 90% typeability can be achieved with these 16

antisera and for routine purposes this is considered sufficient (Kaijser and Sjögren, 1985). Of the 68 isolates available for serotyping, 59 (86.8%) of the strains were typeable (Table 8). A total of 11 serotypes were represented among the *C. jejuni* and 3 serotypes among the *C. coli* isolates. Lior serotypes 1, 2, 4, 5, 6 and 7 were the most common among the *C. jejuni*, while serotypes 1 and 2 were dominant among the *C. coli* isolates. Lior serogroups 1, 2, 4, 5, 6, and 7 accounted for 63.2% of all isolates. These results also show that the most common HL antigens among *campylobacter* strains isolated from Ethiopia during this study were similar to those reported from Sweden (Kaijser and Sjögren, 1985) and Canada (Lior et al., 1982).

Of the 76 isolates of *Shigella* spp. 74 were isolated from patients and 2 from controls (Tables 2 and 3). There was a statistical difference in the isolation rates of *Shigella* spp. from patients and controls (11.7% vs. 0.9%, $P < 0.001$), adult patients and adult controls (19.4% vs. 0.9%, $p < 0.001$) and paediatric patients and paediatric controls (7.3% vs. 0.9%, $p < 0.005$) (Tables 2 and 3). Of the 74 *Shigella* spp. isolated from patients, 45 (60.8%) were from adults, whereas the remaining 29 (39.2%) were from children (Table 2). *Shigella* spp. were the most commonly isolated bacterial pathogens in adult patients (19.4%) followed by *Campylobacter* (10.7%). The isolation rate among the adult patients (19.4%) in this study is rather high in comparison with an earlier study performed in Ethiopia (9%) (Ashenafi, 1983). In children *shigellae* were the second most commonly isolated pathogens (7.3%). In fact, in studies from developing countries *shigellae* generally belong among the three or four most commonly isolated enteric pathogens in children with diarrhoea (Black et al., 1980; Taylor et al., 1991). The rate of isolation among the paediatric patients in this study is similar to the rates reported in some of the studies quoted above.

Shigellosis is further, primarily a paediatric disease and in developing countries it becomes a problem by the second half of the first year of life with a peak incidence in the 2-4 year age group (Black et al., 1980). The changes from breast feeding to solid food and also water are probably responsible for the high number of diarrhoeal episodes in this age group. The disease does not confine itself to one type of climate, and defective hygiene rather than a specific climate, influence the attack rate.

Serogrouping of the 76 shigella isolates, showed that *S. flexneri* was the most commonly isolated species (54.0%), followed by *S. dysenteriae* (22.4%), *S. sonnei* (15.7%) and *S. boydii* (7.9%) as shown in Table 9. These findings are in accordance with previous Ethiopian studies except that in those studies *S. boydii* was the third most commonly isolated species (Gedebou and Tassew, 1982; Ashenafi, 1983).

Of the 37 isolates of *Salmonella* spp., 24 were found in patients and 13 in controls (Tables 2 and 3). Thus, in this study there was no statistical difference in the isolation rates of *salmonellae* between patients and controls. (3.8% vs. 5.9%, $p=0.26$). Furthermore, there was no statistical difference between adult patients and adult controls (3.4% vs. 0.9%, $p=0.28$), but a statistical difference between paediatric patients and paediatric controls was noted (4.0% vs. 11.1%, $p<0.01$) (Tables 2 and 3). A possible explanation for the high percentage of *salmonellae* in paediatric controls in this study is that the controls were healthy carriers. Since all controls in this study were inpatients who had stayed in a hospital for a number of days, it is also possible that during this period they may have acquired the bacteria from the hospital environment.

Among the *salmonella* strains, the most commonly isolated serogroup was group B, 30 (81.1%), followed by *Salmonella typhi*, 4 (10.8%) and group C, 3 (8.1%) This finding is different from a previous study done in Ethiopia by Ashenafi (1983), in which group C

was the most frequent isolate. It is not unusual for one serogroup to replace another in the community from time to time.

Of the 345 paediatric patients, 72 (20.8%) and 13 (17.1%) of the 76 paediatric controls had *EPEC* in their stools (Table 10). No statistical difference in the isolation rates of *EPEC* between patients and controls was found in this study ($p=0.56$).

In an earlier study from Ethiopia Thorén et al. (1982) isolated *EPEC* in nearly the same frequency (19%) as in this study. The corresponding figure for the controls was somewhat lower (8%). The high percentage of *EPEC* in the controls in this study may indicate that the children either were non symptomatic carriers or they had acquired the bacteria during their stay in the paediatric ward.

In this study screening for *EPEC* was done only by using slide agglutination tests and a positive test was not confirmed with individual specific antisera by tube titration of a boiled suspension of organisms which would indicate the exact serotype. The relevance of *EPEC* serogrouping in endemic cases of diarrhoea in developing countries is debatable (Gangarosa and Merson, 1977). Furthermore, our findings that the isolation rates among patients and controls were almost the same makes the interpretation of the finding of *EPEC* in the faecal samples difficult.

Attempts to isolate *Y. enterocolitica* were not successful during this study. Several attempts to isolate *Y. enterocolitica* have been made in Ethiopia. In the study reported by Stintzing et al. (1977) one strain of *Y. enterocolitica* type 03 was isolated from a child with diarrhoeal illness. Although all specimens were examined for this pathogen in another study, no strains were isolated from either patients or controls (Thorén et al., 1982).

A similar finding was also reported by Ashenafi (1983) in a study of adult Ethiopian patients with diarrhoea.

The method used to isolate *Y. enterocolitica* in this study was more selective in comparison to the methods used in the earlier studies reported from Ethiopia. In spite of this fact, no *Y. enterocolitica* was isolated from any person during this study. Based on the findings in other studies as well as in our own study we conclude that yersiniosis is uncommon in Ethiopia. This is not surprising since it is well-known that most sporadic infections caused by *Y. enterocolitica* occur in countries with a cold climate (Swaminathan et al., 1982).

As mentioned in the epidemiological review earlier, enteric campylobacteriosis is endemic in developing countries. Symptomatic infections are more frequent in children less than five years of age than in adults (Demol and Bosmans, 1978; Bokkenheuser, 1979; Blaser et al., 1980a; Billingham, 1981). Our studies are in agreement with those quoted above. In this study, 68.6% of the *Campylobacter* isolations were from children less than five years of age (Figure 4). Rates of isolation of *Campylobacter* spp. between males and females (55.8% vs. 44.2%), resulted in an over all male to female ratio of 1.2:1. These figures indicate that there was no significant difference in the isolation rates of *Campylobacter* spp. between males and females. These results are in agreement with those of studies done in Sweden (Walder, 1982) and in Saudi Arabia (Zaman, 1992) and our study makes it much clearer in this situation.

During this study, an attempt was made to study the clinical features of enteric campylobacteriosis in patients (Table 6). Patients from whom a concomitant isolate of *Shigella* or *Salmonella* spp. was recovered were excluded (Table 5).

In this study, there were minor differences in the frequency and duration of certain symptoms and signs between adults and children.

In adult patients low grade fever was the most commonly reported complaint (84.2%), while in children low grade fever was documented in 42/55 (76.4%). In children watery diarrhoea was the most commonly reported complaint (87.2%) by the children's parents, while in adults watery diarrhoea was observed in 13/19 (68.4%). In a significant number of adults (47.3%) the diarrhoea lasted longer than a week, while in a majority of the children (61.8%) it lasted between 3-7 days. A possible explanation for the differences in the duration of diarrhoea between adults and paediatric patients may be that many of the adult patient waited for a number of days, or else visited a number of clinics in the city before coming to the hospital. In contrast diarrhoea is common in children and causes a significant number of deaths, and therefore children with diarrhoea are more likely to be taken to hospital sooner than adults.

In 2/19 adult patients (10.5%) bloody diarrhoea was noted. This finding is more or less similar to those of studies done elsewhere (Editorial, 1978; Walder, 1982). Bloody diarrhoea was noted only in 5.5% of the infected children. This finding is different from a study reported by Karamali and Fleming (1979) from Canada; in that study 92% of the children with *campylobacter* enteritis had bloody diarrhoea. It is well known that *Campylobacter* spp. possess more than one type of virulence factor which may be involved in the pathogenesis of campylobacteriosis. A possible explanation for the low percentage of bloody diarrhoea in the children in our study as compared with the study mentioned above is that, the virulence factors of their strains may be different from those of the strains isolated in our study.

In children vomiting was observed in 30/55 (54.6%), while in adults only 21% of the 19 patients complained of vomiting. Abdominal pains accompanied by tenesmus or colicky pain were observed in 4/19 (21%) of the adult patients. In small children it is difficult to

evaluate abdominal pain and therefore no figures are given.

Dehydration ranging from mild to severe was observed in 25.4% of the 55 children with *Campylobacter* infections (Table 7). Of the 55 children, 67.2% had signs of malnutrition and most of them (47.2%) were underweight. These findings may indicate that the episodes of diarrhoea bringing the children to hospital may have been one of many with successive impairment of their nutritional status. Although some differences exist, the reported frequency of symptoms and signs of this infection in this study are well in accordance with what has been found in other investigations (Demol and Bosmans, 1978; Blaser et al., 1979; Svedhem and Kaijser, 1980; Walder, 1982).

In this study, we were not able to identify any characteristic symptoms and signs, specific for *Campylobacter* infections, that would enable physicians to differentiate enteric campylobacteriosis from diarrhoeas caused by other enteric pathogens. These findings are similar to those reported by Plotkin et al. (1979).

The question of the mode of transmission as well as the source of *Campylobacter* infections in Ethiopia are not yet known. The route of transmission is probably oral, but the sources may differ. There are studies and case reports implicating poultry (Svedhem and Kaijser, 1981a; Lindblom et al., 1986), domestic animals (Svedhem and Kaijser, 1981a; Elgebe, 1983), pets (Svedhem and Kaijser, 1981a), milk (Robinson and Jones, 1981) and drinking water (Vogt et al., 1982) as sources or reservoirs of the infection. Person-to-person transmission is said to be uncommon with *Campylobacter* infections (Vesikari et al., 1981), but reports of nosocomial spread and intrafamilial infections in neonates suggest that it does occur (Kapperud et al., 1992).

Diarrhoea caused by *Campylobacter* is often a mild and self limited disease, but some of the more serious cases require antibiotic treatment, e.g. patients with severe,

relapsing or long lasting enterocolitis or severe extraintestinal manifestations such as septicemia and meningitis.

In this study, the sensitivity pattern of 85 *Campylobacter* strains isolated from patients and controls against ten chosen antimicrobial agents were studied (Table 11).

The disc diffusion method was used in this study, because it is simple and inexpensive to perform and the results obtained with it are comparable to those obtained using the agar dilution method (D'Amato et al., 1985).

All of the strains tested were sensitive to chloramphenicol, erythromycin, gentamicin, nalidixic acid, norfloxacin, sulphonamide and tetracycline and all were resistant to cephalothin. These results are in agreement with those studies done by other investigators (Walder, 1979, 1982; Vanhoof et al. 1978, 1980; Svedhem et al., 1981c; Lind and Kaijser, 1991). There were no differences in the sensitivity patterns between *C. jejuni* and *C. coli*. The resistance found against ampicillin (60%) in this study is higher than the figures reported by Mikhail et al. (1989) from Egypt (40%) and by Lim and Tay (1992) from Singapore (42%), but lower than the figure (100%) in a study from Sweden (Walder, 1982). The frequency of resistance to trimethoprim-sulphamethoxazole (58.8%) in this study was lower than the frequencies reported by Mikhail et al. (1989) from Egypt (100%) and by Lim and Tay (1982) from Singapore (99%).

The high percentage of strains resistant to ampicillin and trimethoprim-sulphamethoxazole in this study may be the result of the easy availability of these drugs. Everywhere in Ethiopia, in hospitals and private pharmacies and in the markets people have easy access to ampicillin and trimethoprim-sulphamethoxazole without prescription. This means that the selective pressure of these commonly used antibiotics on the bacteria circulating in the community has resulted in a high frequency of resistance among our isolates.

No *campylobacter* strains resistant to erythromycin were isolated during this study. In the past this has been true in other countries as well and most authorities have advocated erythromycin as the drug of choice for severe or persistent campylobacteriosis, particularly in view of its low toxicity. However, strains resistant to erythromycin do exist. The frequency of isolation of such resistant strains varies from place to place, e.g. 0% in Indonesia (Ringertz et al. 1981), 2-17% in Sweden (Walder, 1979, 1982; Svedhem et al., 1981c), 1% in Canada (Karamali et al., 1981), 2%-8% in Belgium (Vanhoof et al., 1980, 1978) and 51% in Singapore (Lim and Tay, 1992).

No strains of *campylobacter* resistant to tetracycline were isolated during this study. Resistance to tetracyclines varies throughout the world, e.g. 0-25% in Sweden (Walder, 1979; Svedhem and Kaijser 1981c), 20% in Egypt (Mikhail et al., 1989) and 79% in Singapore (Lim and Tay, 1992). Resistance to tetracyclines has been shown to be transmitted by plasmids, and the occurrence and transfer of these plasmids among strains is probably the reason for the differences reported from the various places.

Other drugs currently used for the treatment of *Campylobacter* are the new fluoroquinolones e.g. norfloxacin and ciprofloxacin. All our isolates were sensitive to norfloxacin. Originally it was thought that the risk for development of resistance was small. However, recent reports have indicated that *C. jejuni/coli* may become resistant to quinolones both in vitro, and in vivo during therapy (Endtz et al., 1991; Wretling et al., 1992).

Since person-person transmission of *campylobacter* is rare and the treatment of *campylobacter* diarrhoea doesn't often require the use of norfloxacin, it is unlikely that the use of norfloxacin in the treatment of human infections has contributed significantly to the emergence of the quinolone resistant mutants of *campylobacter* strains. It seems more

probable that the veterinary use of quinolones for treatment and prophylaxis of *E. coli* and *mycoplasma* infections in the poultry industry have contributed significantly to the development of resistance in the *campylobacters* isolated from both man and poultry (Endtz et al., 1991). If extensive veterinary use of quinolones continues, there is a considerable risk that the spread of resistant organisms will seriously limit the future usefulness of quinolones in the treatment of acute diarrhoea. Therefore, quinolones should be used with caution. Quinolone resistance is probably associated with a single mutation in the DNA gyrase gene (Gootz and Martin, 1991). No strains resistant to chloramphenicol or gentamicin were isolated during this study. However, strains resistant to chloramphenicol do exist (Walder, 1979).

SUMMARY AND CONCLUSIONS

This study has shown that *Campylobacter* spp. were the most common cause of bacterial diarrhoea in Tikur Anbassa Hospital, Addis Ababa, Ethiopia. Both children and adults are affected, but diarrhoea caused by *Campylobacter* spp. was more common in children. On the whole *Campylobacter* infections were more or less equally common in both sexes. Of the *campylobacters* differentiated at species level in this study *C. jejuni* accounted for 82.4% and *C. coli* for 17.6%. Lior serotypes 1, 2, 4, 5, 6 and 7 were most common among the *C. jejuni*, and serotypes 1 and 2 among *C. coli* isolates.

The clinical features of *campylobacter* infected patients were studied and watery diarrhoea was the dominating symptom (82%). Bloody, mucoid or mixed diarrhoeas were noted at lower frequencies (<7%). Diarrhoeal diseases in adults and children are generally self limited. Therefore, chemotherapy is not routinely advisable and treatment, when necessary, should be based on fluid and electrolyte replacement.

When antibiotic treatment is indicated (e.g. in patients with severe, relapsing or long lasting enterocolitis or severe extraintestinal manifestations such as septicemia and meningitis) the potential availability, simplicity of use, safety and low cost of the antimicrobial agent have to be taken into account.

Based on this and other studies the new fluoroquinolones (e.g. norfloxacin and ciprofloxacin) currently represent the drugs of choice in developed countries when treatment is indicated. These drugs also have broad spectrum antibiotic activity against several other bacteria which cause diarrhoeal illnesses such as *shigellae*, *salmonellae* and *E. coli* as well as against *campylobacters*. However, in Ethiopia these drugs are expensive and not easily available. Other alternative drugs for the treatment of *Campylobacter* diarrhoea are erythromycin and the tetracyclines, both of which are fairly cheap and easily available. Tetracycline should not be given to children less than 8 years of age; for these children the best alternative is erythromycin. Ampicillin and trimethoprim-sulphamethoxazole should not be used in Ethiopia as the drugs of choice for the treatment of campylobacteriosis unless culture and sensitivity tests have been done prior to treatment.

In conclusion, based on this study the following recommendations are made.

1 This study has dealt with campylobacteriosis in hospital-based patients in a major city; community-based studies need to be conducted to determine whether these data reflect the situation of the general population.

2 The distribution of campylobacteriosis between the urban and rural populations should be investigated.

3 Epidemiological investigations should be conducted in order to find the sources of human infections caused by *campylobacter* and the modes of transmission in the Ethiopian context.

4 Bacteriological laboratories in hospitals and the National Research Institute of Health (NRIH) should take appropriate measures to isolate *Campylobacter* species as a probable cause of diarrhoea. Such laboratories should be supplied with appropriate facilities, and health personnel should be trained to isolate these organisms. The modified blood free selective agar used in this study is easy to prepare and highly selective; it is recommended for routine use.

The antimicrobial sensitivity patterns of *Campylobacter* species should be ascertained in the different regions of Ethiopia and health personnel should be informed.

5 It is known that *campylobacters* possess more than one virulence factor involved in their pathogenetic mechanisms. The question of which of the virulence factors that is important in the strains isolated during this study remains to be investigated.

6 Diarrhoea caused by *Campylobacter* spp. is often a mild and self limited disease, but a few of the more serious cases may require antibiotic treatment. If antibiotic treatment is deemed necessary, then preferably culture should be obtained beforehand and the sensitivity data taken into consideration.

7 Physicians who diagnose and treat patients with diarrhoea should seriously consider *Campylobacter* species as an important etiologic agent of diarrhoea.

8 The microbiological investigations of stools from patients with diarrhoea yielded a total isolation rate of *Campylobacter*, *Shigella* and *Salmonella* spp. of 29.1%. Still 70.9% of the diarrhoeal episodes were etiologically unexplained. It is known that other bacteria such as enterotoxigenic *E. coli* (ETEC), *V. cholerae* and *Aeromonas hydrophilia*; parasites (*E.histolytica* and *Giardia lamblia*) and viruses (*rotaviruses*) would account for a significant number of diarrhoeas. Further studies should be undertaken in order to ascertain the frequency of the other etiologic agents of diarrhoea, their seasonal incidence and their relative importance in the different age groups of Ethiopian patients.

Appendix

Questionnaire for investigation of enteric pathogens from stool specimens of patients and controls.

I.

1. Serial No. _____	2. Date of collection _____
3. Card No. _____	4. Time of collection _____
5. Patients name _____	6. Age _____
7. Sex _____	8. Address _____
9. Ward/Dept _____	10. Antibiotic therapy _____

If it is given please specify _____

II.

a) Chief complaints or history of present illnesses (Please specify the duration and the frequency of diarrhoea and other illnesses) _____

b) Pertinent physical findings including vital signs & degree of dehydration _____

c) Clinical diagnosis _____

d) Type of treatment given _____

III. To be filled in the lab only

Nature of specimens

_____ Normal	_____ Bloody	_____ Watery
_____ Mucoid	_____ Mixed	

IV. Laboratory report

a) Type of enteric pathogen/s isolated _____

b) Antibiotic susceptibility reading (See next page)

Antibiotic susceptibility reading

Zone of inhibition (in mm)		Ampicilin	Chloramphenicol	Cephalothin	Gentamicin	Erythromycin	Nalidixic acid	Norfloxacin	Tetracycline	Sulphonamide	Trimethoprim- Sulfa methoxazole	S = Sensitive I = Intermediate R = Resistant

V. Comments _____

Date of report _____

Signature _____

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DECLARATION

I, the undersigned, declare that this thesis is my work and that all sources of material used for the thesis have been duly acknowledged.

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