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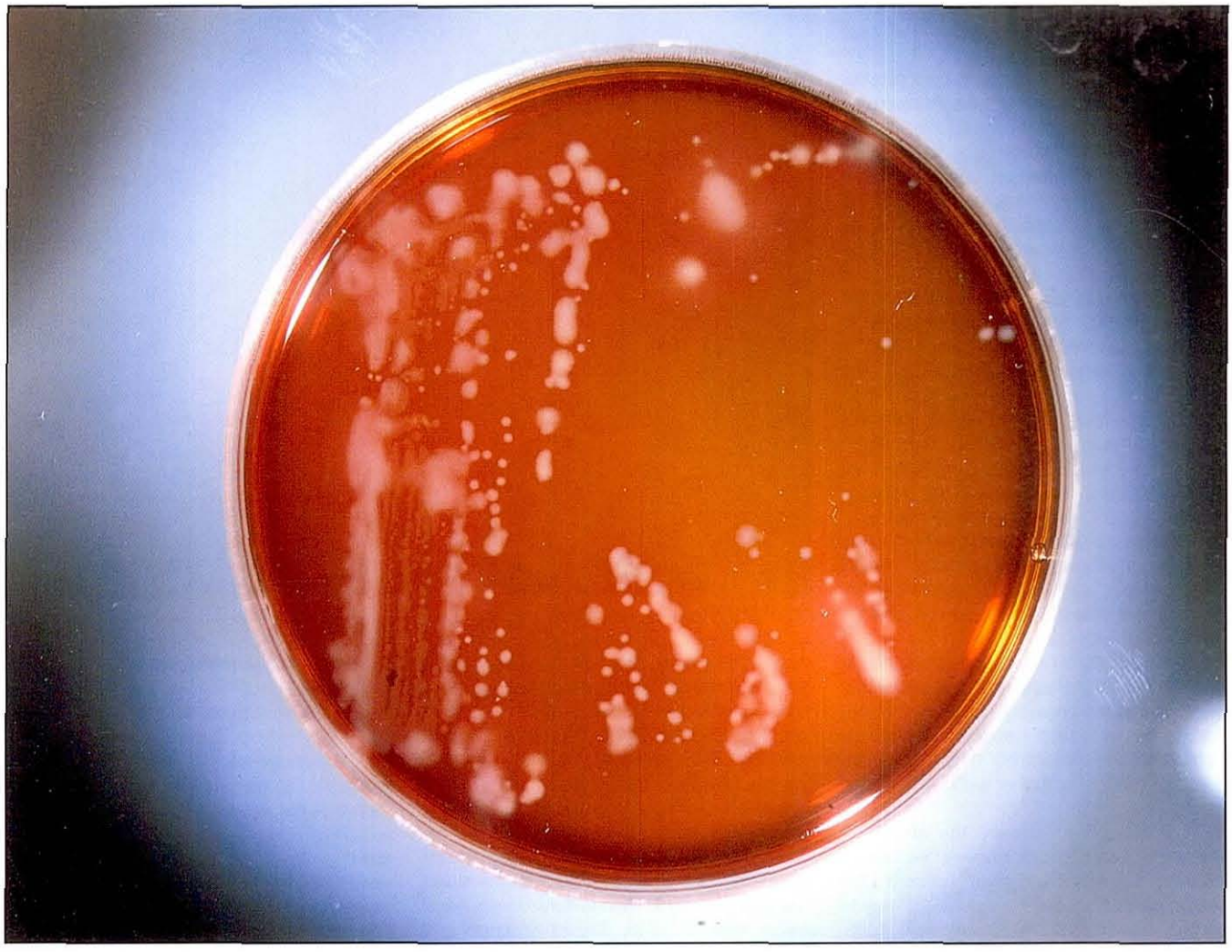

Campylobacter coli cultured on Skirrow Selective Medium.

Hydrophila Group Aeromonads in Environmental Waters

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Aeromonas species can cause infection and so should be controlled in drinking and environmental waters.

Epidemiology of Campylobacter

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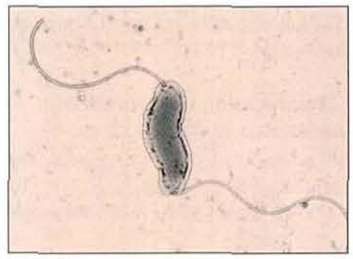

An overview of one of the most important emerging pathogens in human diarrhoeal disease.

Hydrophila Group Aeromonads in Environmental Waters

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Introduction

Most aquatic environments have complex and variable microflora. *Aeromonas* spp. can represent a high percentage of the bacteria that constitute this heterotrophic flora in fresh waters, especially when there are favourable conditions. This organism has a cosmopolitan distribution because of its ability to survive in a wide variety of aquatic systems.

A.hydrophila comprises a portion of the normal microbial flora of fishes as well as other aquatic animals and it can cause infections in a variety of animals including man. For this reason the potential public health significance of large numbers of aeromonads in aquatic environments cannot be ignored. Several reports have demonstrated a circumstantial link between the infections caused by *Aeromonas* spp. and water or foods known to contain these organisms. Moreover, the strains isolated from patients and those isolated from the environment seem to have similar characteristics.[1,2]

Inland lake.

In countries where drinking water is free from aeromonads, the data available suggest that the presence of aeromonads in faeces is of much greater significance compared with countries where they are present in water but further work is needed to confirm this.

It is difficult to classify the strains of *Aeromonas* spp. to species level because its classification was in a state of confusion until recent genetic studies. However, it is easy to identify the genus. Gram-negative rods, facultative anaerobes, oxidase positive and resistant to O/129 vibriostatic agent are presumed to be *Aeromonas* spp. Classification *a posteriori* to species level is more complex. Two groups of species can be clearly distinguished: a relatively homogenous group of psychrophilic, non-motile *A. salmonicida* and another, more heterogeneous group of motile organisms, *A. hydrophila*. In the last edition (1984) of *Bergey's Manual of Systematic Bacteriology*[3] the *hydrophila* group was composed of three species: *hydrophila*, *caviae* and *sobria* (**Table 1**). Nowadays the number of genospecies has increased to 12.[4]

Classification of aeromonads

Aeromonas phenotyping has been confused for years and it is still not well established. The history of the genus began in 1890 when Zimmerman isolated a Gram- negative, non-sporulated motile bacillus from tap water which he called *Bacillus punctatus*. At that time, Ernst isolated a bacillus from frogs with red leg disease which he called *Bacillus ranicida* and Sanarelli isolated *Bacillus hydrophillus fuscus* from the lymph of a frog with a haemorrhagic septicaemia. During successive years there were many new isolations with different names but with similar characteristics. It was in 1936 that Kluiver and Van Niel created the genus *Aeromonas*. A few years later, in 1943, Stanier grouped and rearranged all the strains of *Aeromonas* under a sole species, *A. hydrophila*.

Studies of the biochemical and genetic properties of different strains demonstrated a wide heterogeneity. In 1984 Popoff[3] described the genus with four species: *A. hydrophila*, *A. caviae*, *A. sobria* and *A.salmonicida*. The genus was included in the Vibrionaceae family under Section 5 'Facultatively Anaerobic Gram-Negative Rods'.

This classification has proved insufficient and studies performed using DNA-DNA hybridisation recognised 12 genospecies of which 11 phenotypes have been named:[4] *A. caviae*, *A. eucrenophila*, *A. hydrophila*, *A. jandei*, *A. media*, *A. schubertii*, *A. sobria*, *A. trota*, *A. veronii* and *A. salmonicida*. Simultaneously, a new family: Aeromonadaceae, independent from Vibrionaceae, has been proposed.[5,6]

Environmental distribution

A. salmonicida and *A.hydrophila* groups occupy two ecological niches that are well differentiated. *A. salmonicida* is a natural

Table 1: Differentiation between *A. hydrophila*, *A. caviae* and *A. sobria*.[15]

	hydrophila	*caviae*	*sobria*
Esculin hydrolysis	+	+	–
Gas from glucose	+	–	+
Voges-Proskauer	+	–	V
Acid from arabinose	+	+	–

Estuary waters.

parasite of fish and other poikilotherms and normally is not found free in the water. On the other hand, though the *A. hydrophila* group belongs to the fish microflora, it is ubiquitous in aqueous environments. The *A. hydrophila* group is present virtually in all types of water, e.g. rivers, lakes and sewage. Hazen[7] demonstrated this by studying its distribution in 147 aqueous locations in the USA. He found that aeromonads were not recovered from 12 but these locations could be considered as extreme environments. There are several more studies from different parts of the world that reach similar conclusions.[2,8,9] Hence, *Aeromonas* spp. are considered to have a cosmopolitan distribution.

These organisms can be isolated in waters within a wide range of physico-chemical limits, *i.e. waters with pH values* between 5.2 and 9.8 and temperatures lower than 10°C or as high as 45°C, 35°C being the thermal optimum.[10] Aeromonad biotypes are isolated from water with very low organic matter content as in oligosaprobiotic lakes and streams and they can also grow in polysaprobiotic waters such as sewage.

There is no agreement in the literature to call *Aeromonas* spp. marine organisms. They can be isolated from coastal waters but there is no published study about isolation from open sea water and they do not grow *in vitro* with 4% NaCl. The presence of aeromonads on the coast is probably due to outflows from waste-water discharges and rivers.

Environmental water is an important site for these organisms. However, this is not the only place where we can find them. *A. hydrophila* can be isolated from faeces of homeothermic animals; for example, in human stools the percentage is between 0.1% and 11.7%. Another important site is raw food.[11] *A.hydrophila* has been found in fish, meat, milk and vegetables. The presence of these organisms in food can be a source of human infection.

Aeromonads can be a suitable index organism for the assessment of the trophic state of water.[12] They have a close relationship to environmental factors such as temperature, nitrogen, phosphates, chlorophyll a and organic matter. Mass production of these organisms can occur in waste water[9] and similar biotopes where an intense degradation of certain high molecular compounds takes place, e.g. proteins, fats, starches, etc. In general, *Aeromonas* growth rate can be increased in natural aquatic habitats by raising the level of nutrients such as phosphates, nitrogen and organic matter. The same effect was shown by Van der Kooij in tap water,[13] where these micro-organisms can grow with the addition of very small quantities of certain substrates (phosphates, glucose). Schubert[9] found that the numbers of aeromonads in natural waters was increased by sewage pollution although he considered aeromonads themselves did not have a faecal origin. The high correlation between the biological oxygen demand (an indirect measure of biodegradable organic matter) and *Aeromonas*[14] (**Figure 1**), indicates that these organisms can obtain nutrients from material of faecal origin. This explains the high numbers of aeromonads in waters with high faecal pollution levels.

Aeromonads have also been recovered from drinking water. They have been isolated from both unchlorinated and chlorinated supplies. However, their numbers are *reduced by chlorination and other disinfec*tion methods. The ability of *Aeromonas* spp. to grow in tap water with the addition of very small quantities of organic and inorganic substrates suggests that its presence in drinking water supplies is the result of contamination by nutrients.

There is little information available about species distribution in aquatic environments. Most of the environmental studies are on the *hydrophila* group as a whole. It is well known however, that there is more diversity in fresh, unpolluted waters, where *A. hydrophila* is the dominant species, than in sewage, where *A. caviae* predominates.[15,16]

It is not difficult to isolate aeromonads. They grow well on any complex medium such as Nutrient Agar or Trypticase Soy Agar as well as on selective media for faecal coliforms, such as McConkey agar. This ability can produce false-positive reactions which may lead to over-estimation of the latter. Specific selective media have been proposed for the isolation of motile aeromonads from other micro-organisms in mixed populations from stools, food and environment.[17] The most common selective media for aeromonads

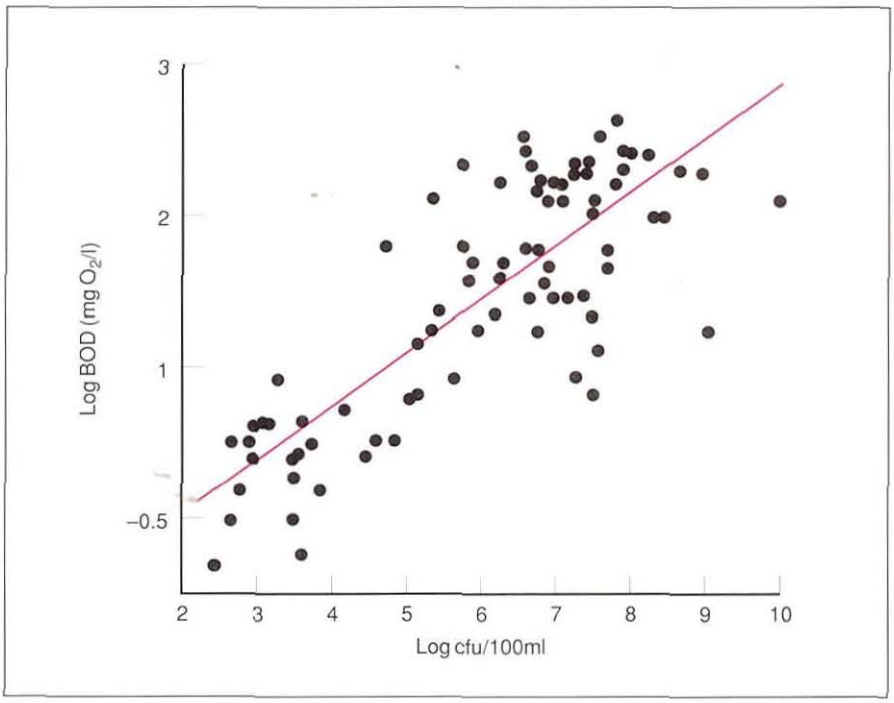

Figure 1: Regression line showing the relationship between the sample data of *A. hydrophila* group (log CFU/100ml) and the biological oxygen demand in faecal polluted waters (correlation coefficient r = 0.82, n = 86).[14] BOD is an indicator of the organic matter content.

use carbohydrates as the main carbon source and penicillin or ampicillin as inhibitors of the background flora.

Pathogenesis in animals and man

Interest in the aquatic presence of *Aeromonas* spp. has been largely related to pathogenesis in aquatic animals.[8] *A. hydrophila* belongs to the microbial flora of fish, frogs, reptiles and turtles. However, in certain conditions, it can cause disease in its hosts. It can cause several different types of infection. In fish, the most common is haemorrhagic septicaemia and in frogs, red leg disease. These infections can cause a high rate of death in fish hatcheries and in natural environments, so their control is of economic interest. Epizootic outbreaks have been related to environmental changes. For example, high temperature increases the stress of fish, causing greater susceptibility to infection. High temperature also raises the numbers of *A. hydrophila* and thus increases the exposure of host populations to the pathogen.[10]

Water is the principal source of *Aeromonas* infections in man. The origins of gastric disease and wound infection,the two principal infections caused by aeromonads, are linked circumstantially to water.[2] However, aeromonad pathogenesis in man is not universally accepted.[4] Several factors have helped to maintain this uncertainty. The infections it causes are associated with a variety of clinical manifestations and are sometimes underdiagnosed due to lack of recognition of their significance. Moreover, for many years aeromonads have been misclassified. *A. hydrophila* is largely recognised as an opportunistic pathogen, generally associated with other organisms, causing mild infections which can be fatal in immunocompromised patients. Although recognised as an enteric pathogen, a definitive direct aetiological role remains undocumented in animal models or studies on human volunteers. The origin of human gastric infection with *Aeromonas* spp. is considered to be drinking untreated water. Cellulitis or wound infections involving skin, muscle or bone form the second large group of isolations. The infection occurs as a result of exposure to water or soil, most frequently during warm weather. The most serious sequela that *Aeromonas* spp. cause is sepsis, usually occurring in immunologically compromised hosts and those with malignancies. In these cases the origin of infection is often the intestinal tract.

Not all species are equally pathogenic. However, the combination of poor classification and the confused taxonomy makes it difficult to determine which species are the most virulent and which require the most rigorous control measures in aquatic systems.

In the literature, pathogenesis is not generally related to a species of aeromonad and only the group hydrophila is usually considered. In most studies where the species are cited, the aerogenic species such as *hydrophila* or *sobria* are usually related to pathogenesis. Anaerogenic species such as *A. caviae* are not so closely related. The interest in *Aeromonas* pathogenicity initiated studies on their virulence factors. These bacteria can produce several toxins, e.g. haemolysins, cytotoxins and enterotoxins. Moreover, aeromonads produce extracellular factors that, associated with these toxic factors, can increase their cellular virulence: e.g. proteases, lecitinases, nucleases, amylases, haemaglutinins and adhesins. The precise function of these toxic factors in pathology has yet to be determined but most of the strains of *A. hydrophila* and *A. sobria* have virulence factors, whereas very few *A. caviae* have them.

Conclusion

The presence of the *A. hydrophila* group should be controlled especially in drinking and recreational waters, as well as aquaculture where there is a risk of infection to humans and animals. The exclusive use of faecal bacterial indicators can underestimate the problem, especially in eutrophic waters. Moreover, although a few studies have already been performed[16] with the aim of assessing the risk to public health and understanding their role in ecology, environmental studies are required using the new species classification[4] together with the main virulence factors.

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Epidemiology of Campylobacter Infections

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Introduction

The story of campylobacter may have started more than 100 years ago in Germany when the renowned bacteriologist Theodore Escherich, in 1886, observed in the diarrhoeal stools of children and kittens, a non-culturable, spiral-shaped micro-organism, closely resembling the campylobacter of today, which he named *Vibrio felinus*. In the period following the description by Escherich other similar organisms were described as *Vibrio fetus* and *Vibrio jejuni* and were considered mainly animal pathogens. In 1963 these organisms were assigned to a new genus, *Campylobacter*. Campylobacter, by their ability to grow in a micro-aerophilic environment, have eluded detection by the usual microbiological techniques until Butzler and colleagues in Belgium in 1973 reported the isolation by a filtration technique of 'related vibrios' from the stools of diarrhoeal patients.[1]

Almost 20 years ago, in 1977, Skirrow, in England, using a simpler isolation technique based on antibiotics contained in blood agar media, corroborated Butzler's findings that these organisms, *Campylobacter jejuni*, were indeed a common cause of human diarrhoea.[2] By the middle of the 1980s, the genus *Campylobacter* contained species with many diverse characteristics which led to the restructuring of the genus. In 1991 Vandamme[3] proposed that the family of Campylobacteraceae should include three genera:

1. **Campylobacter**
   — *19 species and subspecies*
2. **Arcobacter**
   — 4 species
3. **Helicobacter**
   — 16 species

Genus *Campylobacter*

Campylobacter are Gram-negative, slender, spirally curved bacterial rods which require a micro-aerobic atmosphere for growth. Most campylobacter associated with human enteritis grow at 42°C and 37°C. Today, campylobacter are recognised as the major aetiologic agents in human diarrhoeal disease around the world.

Nineteen species and subspecies presently identified in the genus *Campylobacter* have been isolated from humans and/or animals (**Table 1**). Amongst these, *C. jejuni* ssp *jejuni (C. jejuni)* and *C. coli* are most commonly isolated from diarrhoeal disease in humans and animals while, to a lesser extent, other species, such as *C. lari* and *C. upsaliensis*, have been isolated from sporadic and outbreak cases from humans and/or dogs and cats. *C. fetus* ssp *fetus*, known for many years as an important animal pathogen associated with abortions in sheep and cattle, has also been associated with disease in humans.

Table 1: Campylobacter species — human and non-human sources.

C. coli	*C. mucosalis*
C. concisus	*C. rectus*
C. curvus	*C. sputorum biovar bubulus*
C. fetus ssp. *fetus*	*C. sputorum biovar fecalis*
C. fetus ssp. *venerealis*	*C. sputorum biovar sputorum*
C. hyointestinalis	*C. upsaliensis*
C. jejuni ssp. *jejuni*	*'C. helveticus'*
C. jejuni ssp. *doylei*	*'C. showae'*
C. lari	*'C. hyoilei'*
	'C. gracilis'

In the United States, Canada and the UK, isolation rates for campylobacter from diarrhoeal disease are estimated to be about 50–60/100,000,[4,5] whilst in some developing countries the rates may be 30–50 times higher.[4] In Canada, the number of reported isolates increased from about 2,000 in 1983 to about 13,000 in 1995 (provisional data) and since 1989, surpassed salmonella as the most common agent of diarrhoeal disease (**Figure 1**). In the UK, in 1995 there were about 43,000 campylobacter and about 29,700 salmonella isolations reported to the PHLS (provisional data) (**Figure 2**).

Transmission and sources of infection

Campylobacter enteritis is a zoonosis. In the industrialised world, most human campylobacter infections result from contact with poultry, cattle, raw milk, surface water and pets.[6] The faecal-oral route may play a role in the epidemiology of campylobacter enteritis. Farm workers have been reported to have contracted the disease as a result of handling cattle and chickens. Occupational exposure to monkeys has resulted in human infections, and cage birds may play a role in household outbreaks. The consumption of undercooked chicken has been shown to be the most common cause of sporadic infections and outbreaks. Poultry may be an important means of transmission of infection to man due to cross-contamination of kitchen utensils, cutting boards, hands and raw vegetables. In addition to poultry, red meats have also been a source of infections. Campylobacter have been isolated from about 5% of retail red meats in the USA and from 2–5% of ground beef and beef flank in Canada. In a recent two-year study in a defined geographical area in the UK,

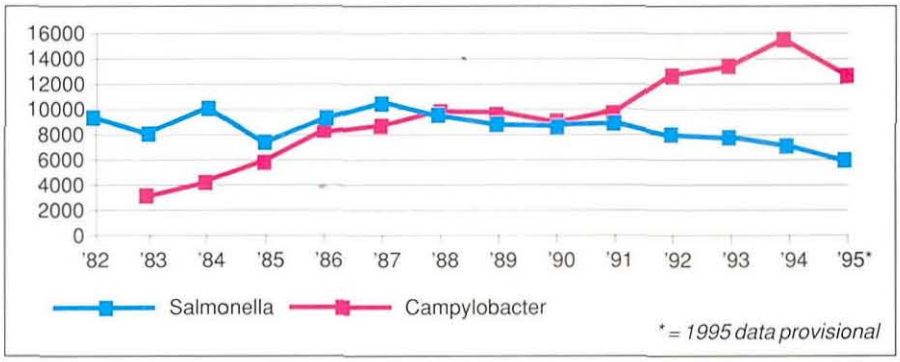

Figure 1: Campylobacter and salmonella — Canada 1982–1995.

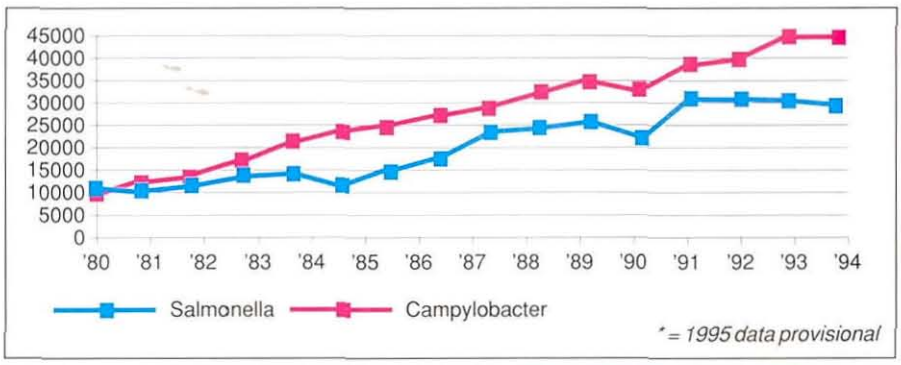

Figure 2: Campylobacter and salmonella — England and Wales 1980–1995*, PHLS - CDSC.

campylobacter were found in 47% of offal, 23% of beef, 18% of pork samples and 15% of lamb samples.

In the past, campylobacter have been associated with very large milk and waterborne outbreaks in several countries.[6] Consumption of raw milk has been shown to be a major risk factor in several large outbreaks in the UK,[7] especially among school children who drank raw milk. Pasteurisation eliminates campylobacter from milk, however post-pasteurisation contamination may lead to milk-borne outbreaks of campylobacteriosis, and recently, pasteurised milk bottles have been found to be contaminated by jackdaws and magpies. Waterborne outbreaks have been reported in several countries and evidence suggests that non-chlorinated water, possibly contaminated with sewage, bird and animal faecal material, was associated with most outbreaks. Although no campylobacter were isolated from the water, untreated municipal water supplies were considered the source of large community outbreaks in the USA and Sweden. In contrast, campylobacter have been isolated from sea water and from 50% of fresh water samples collected from rivers, lakes and estuaries in England, but only in the presence of faecal *Escherichia coli*.

Transmission of campylobacter enteritis by infected puppies has been documented and it has been estimated that about 5% of human cases in England originate from dogs. Human infections have also been associated with sick kittens and healthy cats.

Despite a very low infective dose of less than 2 bacteria/ml, person-to-person transmission is uncommon although vertical transmission from symptomatic or asymptomatic mothers to their neonates has occurred.

Age and sex

The peak incidence in children occurs in the first 5 years of life. In Southern India, and in South Africa isolation rates of between 30–40% have been reported in this age group. In developed countries, although young adults are most affected, infection can occur at all ages. Campylobacter infections tend to occur more frequently in males under the age of 14 years, with a peak between one and four years. In females, the peak incidence is in the first year of life. Among those over 15 years, both sexes appear equally affected with the exception of females over 65 years.

Seasonal variation

In temperate countries of Europe and North America, infections with campylobacter are more prevalent in the warmer months, usually June to September. In countries with constant mean temperatures throughout the year, isolations are more frequent in the wet season than in the dry season. In England, increased isolations occur in the beginning of the second quarter and last until September. In Sweden, more isolations are made in July and August, with another increase noted in January, probably due to travellers from warmer climates.[6,8]

The seasonal distribution of campylobacter in Canada follows the pattern seen in temperate countries, with a peak in late June lasting through August and September. Slight increases are recorded following holidays such as Thanksgiving, Christmas etc., probably as a result of increased consumption of poultry and turkey (**Figure 3**).

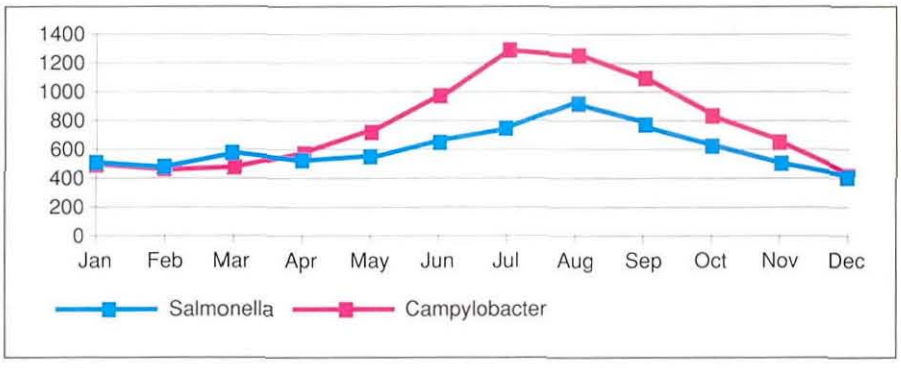

Figure 3: Campylobacter and salmonella — Canada 1994 — distribution by month.

Travellers' diarrhoea

Campylobacter infections present a frequent risk to travellers, and should be suspected along with other enteric pathogens which cause travellers' diarrhoea. Increased campylobacter isolations occurring in wintertime in England, Sweden, Switzerland, and more recently in Japan and Finland, revealed that 3–60% of patients affected acquired their infection while travelling abroad.[4]

Carriers

Follow-up cultures of untreated children with acute campylobacter enteritis showed that 80% of patients ceased to excrete the organisms and 100% were stool negative within 6 weeks. In other cases, untreated patients have presented with intermittent diarrhoea over a period of many months. In developing countries, continuous exposure to campylobacter may result in long-term carriage, especially in children.[4] Relapses and persistent infections may occur.

Clinical aspects

Campylobacter infections may occur in two forms:

1. a disseminated form, and
2. a localised form, mostly enteritis, affecting all ages and sexes.

C. jejuni have been isolated from cases of bacteraemia, appendicitis and more recently have been associated with the Guillain-Barré syndrome (acute inflammatory polyneuropathy) — one of the most serious sequelae of infection with campylobacter.[9]

Like *C. jejuni*, *C. fetus*, a species known for many years as an important animal pathogen, has been implicated in human disease, such as: diarrhoea, meningitis, peritonitis, salpingitis, septic abortion as well as bacteraemia in older patients and immunocompromised hosts.

In contrast to the infrequent occurrence of systemic campylobacteriosis, campylobacter enteritis has acquired prominence as a human disease and has been reported from all parts of the world. In developing countries, campylobacter have been isolated from 2–35% of diarrhoeic cases presenting mostly as watery diarrhoea. In industrialised countries, 4–15% of diarrhoeal cases, mostly in young adults, have been associated with these organisms; in contrast to developed countries, 60% of cases presented with bloody diarrhoea. Simultaneous recoveries from stool and blood in acute diarrhoeal disease have been reported.

Prevention

Control of campylobacter infections requires a better understanding of the reservoirs of these organisms and the epidemiology of the disease. General hygienic measures will prevent the spread of infection. Handwashing after contact with animals or animal products, proper cooking and storage of foods, pasteurisation of milk and chlorination of water supplies, are all important in the prevention of infections.

Acceptance of irradiation of food products will certainly contribute to the reduction of foodborne pathogens and diarrhoeal disease in man.

Campylobacter in animals

Campylobacter species such as *C. jejuni* and *C. coli* are found in the normal intestinal flora of a wide range of domestic and wild animals and birds. For many years these organisms have been the object of investigations due to their frequent colonisation of poultry and turkey flocks. Studies of retail broiler chickens have shown contamination rates of 30–100%.[10]

C. jejuni is commonly present in the bovine intestinal tract, and has also been implicated as a possible cause of diarrhoea in calves. *C. fetus* ssp *venerealis* causes infertility and early embryonic death in cattle and may cause abortion in some infected cows, but has not been associated with human disease. *C. fetus* ssp *fetus* and *C. jejuni* cause abortion in sheep, and may lead to heavy economic losses. *C. coli* causes diarrhoea in piglets, while *C. jejuni* may cause diarrhoea in older pigs. *C. jejuni* has been isolated in up to 50% of both healthy and diarrhoeic puppies and dogs.

Campylobacter upsaliensis

C. upsaliensis is a new emerging pathogen, isolated from dogs and cats, and increasingly, from human cases of enteritis and bacteraemia in both healthy and immunocompromised persons.[11]

Epidemiological markers

The dramatic increase in the isolation of campylobacter from human diarrhoeal disease and the high rate of carriage of these organisms in animals and especially poultry, necessitated the identification and characterisation of markers to support epidemiological investigations. A host of methodologies have been developed over the years which include Phenotyping Methods: species identification, serotyping, biotyping, phagetyping and molecular Genotyping techniques, such as: pulsed-field gel electrophoresis (PFGE), random amplified polymorphic DNA (RAPD),

ribotyping and others.

Isolation and identification

In the majority of cases, a diagnosis of 'campylobacter' relies on the isolation of the organisms. A variety of excellent selective media are available commercially — among them non-blood containing agar such as the charcoal media. In recent years, return to older methods such as the filtration technique on media without antibiotics, has allowed the isolation of human pathogenic species which did not grow on antibiotic-containing agars. The use of enrichment broths has been justified only when dealing with food and water samples, or material undergoing prolonged transport.

For 'presumptive identification', simple tests such as a Gram stain, a wetmount for specific motility and an oxidase test are sufficient. the hippurate hydrolysis test remains a reliable and necessary test for the differentiation of campylobacter. For 'presumptive confirmation' of the culture, two slide latex tests are commercially available — Campyslide (BBL) for identification at the genus level and Meritec-Campy (Meridian Diagnostics) which will identify *C. jejuni*, *C. coli* and *C. upsaliensis*. The use of antibiotics such as nalidixic acid is no longer reliable for the identification of campylobacter, given the increased resistance to quinolones displayed by some campylobacter.

Detection

In spite of advances in isolation techniques, epidemiological investigations of outbreaks have often failed to isolate the organisms from suspected samples, especially water. The survival strategy of campylobacter under adverse environmental conditions, such as exposure to air and water resulted in the formation of coccoid forms which were characterised as 'non-viable' on culture. Similar viable, non-culturable forms have been known to occur in *Vibrio cholerae, S. enteritidis* and enteropathogenic *E. coli*.

In recent studies of aquatic environments *using acridine orange stains or electron* microscopy, coccoid, non-culturable campylobacter on laboratory media have been observed. The recovery of these non-culturable forms has been partially achieved following passage in animals (such as suckling mice). These forms have been shown to colonise chickens but not other animals. The development of new molecular techniques such as the polymerase chain reaction (PCR) has allowed detection of non-culturable forms by the amplification of species-specific DNA sequences. PCR, using specific primers for the flagellin gene has been successfully applied to the detection of *C. jejuni* and *C. coli* in stools, chicken and water samples.

Table 2: Biotyping scheme for *C. jejuni* ssp. *jejuni*, *C. coli* and *C. lari*.

	C. jejuni spp. *jejuni*				*C. coli*		*C. lari*	
Biotype	I	II	III	IV	I	II	I	II
Test								
Hippurate hydrolysis	+	+	+	+	−	−	−	−
Rapid H_2S test	−	−	+	+	−	−	+	+
DNA hydrolysis	−	+	−	+	−	+	−	+

Species identification

Correct identification at the species level is probably the first epidemiological marker that can be of some use in preliminary tracing of an infection. Most laboratories report the isolation of campylobacter as *Campylobacter* ssp. or *C. jejuni/coli*, and do not make any attempt to identify the species. The identification of *C. jejuni, C. coli* or *C. lari* is relatively simple and easy and should be performed in every laboratory involved in the isolation of campylobacter.

The speciation of campylobacter can be partially accomplished by using indoxyl acetate hydrolysis disks, a rapid, simple inexpensive differential test.

The biotyping scheme devised by Lior allows the identification of *C. jejuni, C. coli* and *C. lari*[12] (**Table 2**).

Other emerging pathogenic species, such as *C. upsaliensis*, should be differentiated from other catalase-negative or weak-positive organisms.

Phenotypic methods

Serotyping

Different approaches have been developed for the serotyping of campylobacter. Lior *et al.* in 1982[13] described a simple, rapid serotyping technique using slide agglutination of live bacteria and specific absorbed antisera raised against heat-labile antigens. This serotyping scheme has been applied to 3 major species, *C. jejuni, C. coli* and *C. lari* in many countries with a high degree of typability and specificity. At present the serotyping scheme recognises 130 serogroups (**Table 3**). A passive haemagglutination technique using sheep red cells coated with heat-extracted antigens (heat-stable antigens) and unabsorbed antisera was designed for the serotyping of *C. jejuni*.[14,15]

For *C. upsaliensis*, Lior and Woodward developed a serotyping scheme which recognises seven serogroups among human and animal isolates.[16]

Biotyping

Although serotyping provides extremely useful markers, it is limited when strains belong to 'common' serogroups. In these cases, additional typing is required. As campylobacter are

Table 3: Campylobacter serotyping scheme — 130 serogroups.

C. jejuni spp. *jejuni*	74 serogroups 55 human sources 11 non-human sources 8 not stated
C. coli	46 serogroups 24 human sources 14 non-human sources 8 not stated
C. lari	10 serogroups 6 human sources 4 non-human sources

quite inert biochemically, some characteristics such as production of unusual enzymes have been used for the differentiation of isolates.

Skirrow and Benjamin,[17] in 1980, proposed the differentiation of campylobacter based on hippurate hydrolysis, production of H_2S on FBP medium and resistance to naladixic acid, dividing *C. jejuni* into two biogroups. *C. coli* and *C. lari* were not subtyped. Lior in 1984,[12] proposed a biotyping scheme which was an extension of Skirrow and Benjamin's, based on hippurate hydrolysis, production of H_2S in FBP medium and DNA hydrolysis in an improved medium. This scheme differentiates *C. jejuni* into four biotypes, *C. coli* and *C. lari* into two biotypes each (**Table 2**). Bolton and colleagues in the UK proposed a typing scheme for campylobacter based on resistance to antibiotics, dyes and chemical substrates. Its application is questionable given that some of these attributes may reside on plasmids of unknown stability.[18]

Phagetyping

Khakhria and Lior in 1992[19] extended the phagetyping scheme for *C. jejuni* of Grajewski,[20] and proposed an improved typing scheme by the addition of new phages. The phagetyping scheme recognises at present more than 50 phagetypes and has been applied to isolates from human and non-human sources from 17 countries. Excellent epidemiological results have been obtained by combining serotyping, biotyping and phagetyping.[21]

Salama and colleagues in the UK proposed a phagegrouping scheme which is less discriminatory than phagetyping.[22]

Plasmid profile analysis

Plasmid analysis of many strains has been restricted by the presence of plasmid DNA in only about 30–50% of *C. jejuni* and *C. coli* isolates. However, the instability of the plasmids may diminish their potential value in epidemiological investigations.[18]

Genotypic methods

Pulsed-Field Gel Electrophoresis (PFGE)

The development of PFGE allows the analysis of chromosomal DNA fragments generated by digestion by restriction enzymes. Yan *et al.* in 1991[23] reported the investigation of *C. jejuni* and *C. coli* strains and found that PFGE analysis can be an alternative method in epidemiological investigations for the differentiation of these organisms.

Random Amplified Polymorphic DNA (RAPD)

Mazurier *et al.*[24] described a protocol for the differentiation of campylobacter by RAPD fingerprinting by PCR. In 1993, Giesendorf *et al.*[25] reported the development of species-specific DNA probes by PCR fingerprinting of *C. jejuni, C. coli* and *C. lari*. Lior and Wang[26] (1993) investigated the differentiation of various campylobacter species by RAPD and obtained excellent correlation among strains isolated from several outbreaks. This technique has been greatly simplified and current protocols use DNA templates from heated whole cells instead of the time consuming extraction of DNA.

Restriction Fragment Length Polymorphism (RFLP)

Chromosomal restriction endonuclease analysis has helped differentiate campylobacter which display identical plasmid profiles. This technique has been used with success in the investigation of three outbreaks.[27]

Other important taxonomic and epidemiological information has been obtained with techniques such as ribotyping, multilocus enzyme electrophoresis (MEE) and whole cell protein profiles, but such techniques are carried out by specialised laboratories.

Genus Arcobacter

The genus *Arcobacter* includes aerotolerant campylobacter-like organisms that have been associated with bovine and porcine abortions.[28] Kiehlbauch *et al.*[29] reported the isolation of *C. butzleri*, from cases of diarrhoeal disease in humans and animals. These recently recognised human pathogens differ from other campylobacter by their ability to grow in the presence of air at an optimum temperature of about 30°C. Vandamme *et al.*[30] proposed the inclusion of these organisms in genus *Arcobacter* as *A. butzleri*. In addition to diarrhoeic stools, isolates have been obtained from blood and peritoneal fluid. Isolations have also been made from poultry, cattle, swine, ovine, equine, primates, sewage and water in several countries. Contaminated water, undercooked poultry and contact with infected animals may contribute to human infections. In 1993, Festy *et al.*[31] in France, reported the isolation of *A. butzleri* from 65% of poultry carcasses, 100% of river water samples and 100% of sewage samples investigated. Others in Germany and Italy have reported the isolation of *A. butzleri* from water.

Lior has developed a serotyping and biotyping scheme to assist in epidemiological investigations of *A. butzleri*.[32] The serotyping scheme, based on slide agglutination of live bacteria, currently recognises 73 serogroups and the biotyping scheme, 16 biotypes.[33] In addition, Lior and Wang have used PFGE and RAPD to investigate *A. buztleri* isolated from outbreak and sporadic cases.[34]

At the present time, genus *Arcobacter* recognises four species: *A. cryaerophilus, A. butzleri, A. nitrofigilis* and *A. skirrowii.*

Genus *Helicobacter*

Spiral bacteria were described in the late 19th century in human and animal gastric mucosa. In the early 1980s, Marshall and Warren in Australia observed spiral organisms in gastric biopsies and were the first to culture these organisms using campylobacter media.[35]

In the last ten years, *Helicobacter pylori* has been recognised as the principal cause of antral gastritis and duodenal ulcers in children and adults. One of the most important findings has been the possible association of *H. pylori* with gastric carcinoma and gastric lymphoma. Epidemiologically, it would appear that the organism is spread person-to-person, especially among close family contacts and there is some evidence that in some environments, cats may harbour these organisms.

Currently, the genus *Helicobacter* comprises 16 species. In addition to *H. pylori*, at least three other *Helicobacter* species have been associated with human disease. Among these, *H. cinaedi* and *H. fennelliae* have been isolated from proctitis, blood and diarrhoea from homosexual men. Twelve species have been associated mostly with animals such as cats, dogs, mice, hamsters, primates, rodents, cheetahs and birds.

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