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ENTERIC INFECTIONS DUE TO CAMPYLOBACTER, YERSINIA, SALMONELLA, AND SHIGELLA

campylobacter infections
Yersinia

Report of a Sub-group of the
Scientific Working Group on Epidemiology and Etiology
(Geneva, 14 -16 November 1979)

Diarrhoeal Diseases

This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization

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1. INTRODUCTION

At the request of its Member States, WHO has launched a global Diarrhoeal Diseases Control (CDD) Programme with the primary objective of decreasing diarrhoeal disease-related mortality and morbidity.[1] In this Programme, the Organization is cooperating with its Member States in the implementation of national CDD programmes in the context of primary health care, and is supporting research to design better tools and methods for prevention and treatment.

Since August 1978, the Programme has convened a number of Scientific Working Groups to define research priorities in the areas of treatment, prevention, and control of diarrhoeal disease. In the area of epidemiology and etiology, Scientific Working Sub-Groups have met to review available knowledge and recommend research priorities in the fields of Escherichia coli Diarrhoea[2], Rotavirus and other Viral Diarrhoeas[3], and Cholera and other Vibrio-Associated Diarrhoeas[4].

The purpose of the present Sub-Group was to consider enteric infections due to Campylobacter, Yersinia, Salmonella, and Shigella. Of these, Campylobacter jejuni and Yersinia enterocolitica are organisms that have only recently been recognized as important causes of enteric infection, and accordingly, the available knowledge on these pathogens was reviewed in extenso. In the better known fields of salmonellosis (including typhoid fever) and shigellosis, on the other hand, the Group limited its review to new and important epidemiological and related information that had implications for their control.[5] The Group further outlined research activities in each of these areas that should receive priority support under the CDD Programme.

2. REVIEW OF RECENT KNOWLEDGE

2.1 Campylobacter jejuni

In the last few years, Campylobacter jejuni (previously called "related vibrios") has emerged as an important cause of acute diarrhoeal disease. Although this organism was suspected of being a cause of acute enteritis in man as early as 1954, it was not until 1972, in Belgium, that it was first shown to be a relatively common cause of diarrhoea. Since then, workers in Australia, Canada, Netherlands, Sweden, United Kingdom, and USA have reported its isolation from 5-14% of diarrhoea cases and less than 1% of asymptomatic persons, and most of the information given below is based on conclusions drawn from these studies in developed countries. There have been only a few studies in which an attempt has been made to isolate C. jejuni from the stools of diarrhoea cases in developing countries. The results of these studies (carried out in Rwanda, South Africa and Zaire) suggest that C. jejuni infection is common and that this organism may be of even greater importance as a cause of diarrhoeal disease in the developing countries than it is in the industrialized countries. However, it is evident that the global magnitude of the problem of C. jejuni enteric infection has still to be determined.

2.1.1 Clinical features

As with other intestinal pathogens, the clinical picture of C. jejuni infection varies from asymptomatic excretion or mild symptoms to severe disease. The incubation period in most

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[1] WHO Wkly epidem. Rec., 1979, 54, No. 16, 121-123

[2] Unpublished document WHO/DDC/EPE/79.1 (1979)

[3] Unpublished document WHO/DDC/EPE/79.2 (1979)

[4] Unpublished document WHO/DDC/EPE/80.3 (1980)

[5] Additional background information on non-typhoid salmonellosis, typhoid fever, and shigellosis is provided in WHO Tech. Rep. Ser., 1976, No. 598 (Microbiological aspects of food hygiene) and Tech. Rep. Ser., 1978, No. 624 (Surveillance for the prevention and control of health hazards due to antibiotic-resistant enterobacteria).

cases averages 3-5 days, but may range from 1.5 to 7 or even 10 days.

In the majority of cases of C. jejuni enteritis, there is a febrile prodromal illness, which usually lasts 12-24 hours but may range from a few hours to few days, and which is characterized by some or all of the following symptoms: malaise, headache, dizziness, backache, myalgia, and rigors. Temperature is commonly raised to 40°C and may be accompanied by delirium. Vomiting occurs in about 25% of cases. Periumbilical abdominal pain occurs early in the course of illness in most cases, and eventually becomes colicky, heralding the onset of diarrhoea. The stools rapidly become liquid, foul-smelling, and often bile-stained. Those severely affected may become prostrate. Dysenteric stools characterized by blood and mucus sometimes appear after a day or two, and most diarrhoea stool samples that have been examined microscopically contain polymorphonuclear leukocytes. In one reported series, 34 of 37 children had frank blood in their stools. Occasionally, patients have even presented with signs of acute colitis and have been mistakenly thought to have acute ulcerative colitis.

In the most severely affected patients, dehydration and electrolyte imbalance have necessitated hospital admission, but most cases have been treated as outpatients. The most frequent reason for hospital admission has probably been abdominal pain, which can be severe enough to mimic an acute abdomen and is usually seen in young adults and teenagers. Occasionally these patients have had peritonitis, but most of those who have undergone emergency laparotomy have had patchy inflammation of the ileum and jejunum with associated mesenteric lymphadenitis. Septicaemia appears to be uncommon; this may reflect inadequate blood culture techniques, or possibly a failure to take blood cultures early enough in the disease.

Sometimes young infants with Campylobacter enteritis pass blood in their stools and have little diarrhoea. This has led in two reported instances to a mistaken diagnosis of intussusception, resulting in an unnecessary laparotomy. A few patients have developed aseptic arthritis following Campylobacter enteritis. Others have had acute cholecystitis apparently caused by C. jejuni, which were isolated from the bile in pure culture.

C.jejuni can be isolated for 2 to 7 weeks following illness from the faeces of patients with enteritis who have not been given chemotherapy. However, in mild cases, the organism is excreted for only a few days. In rare instances, patients have excreted the organism for much longer periods.

There has been no controlled trial of the efficacy of antibiotics in the treatment of C. jejuni enteritis. However, it is the impression of most investigators thatantibiotic therapy is needed for severe cases, and for the present it is felt that chemotherapy should be reserved for such patients. The most common antibiotic resistance pattern of the organism is sensitivity to aminoglycosides, erythromycin, clindamycin, the tetracyclines (particularly minocycline), chloramphenicol, and furazolidone, and resistance to penicillin, cephalosporins, lincomycin, colistin, and trimethoprim; ampillicin and the sulfonamides are intermediate in activity. In one laboratory study, 57 of 62 strains tested produced β-lactamase. In vitro studies have shown that resistance to some antibiotics is plasmid-determined. The antibiotic that has been used most is erythromycin (erythromycin stearate,500 mg twice daily for adults, and erythromycin ethylsuccinate,50 mg/kg/day in children), but a possible disadvantage of this antibiotic is the finding that 2-10% of strains tested (from Northern Europe and North America) are resistant to it.

2.1.2 Laboratory characteristics

The failure to recognize C. jejuni as an important enteropathogen in some earlier studies stemmed from the inability to cultivate and identify the organism. The development of highly selective culture media for the isolation of C. jejuni has greatly simplified the study of this organism. The identification of C. jejuni is now a relatively simple procedure, applicable in most clinical microbiology laboratories.

There are at present two types of selective agar in common use: Butzler's medium and Skirrow's medium. Both are based on the incorporation of multiple antibiotics into blood agar medium, which results in the suppression of normal enteric flora and allows C. jejuni to grow. The contents of the media are described below:

Butzler's medium - Thioglycollate agar with 10% sheep blood and bacitracin 25 I.U./ml, novobiocin 5 µg/ml, cycloheximide 50 µg/ml, colistin 10 units/ml, and cefazolin 15 µg/ml.[1]

Skirrow's medium - Oxoid BA Base No. 2 with 5-7% lysed horse blood and vancomycin 10 µg/ml, polymyxin B sulphate 2.5 I.U./ml, and trimethoprim lactate 5 µg/ml.[1]

Butzler's medium is preferable to Skirrow's medium for use in developing countries because it is more selective, it can be used at 37°C, and it does not require horse blood (often difficult to obtain).

Either fresh stool or rectal swabs can be used for culture. If swabs must be transported and stored, Cary-Blair semi-solid transport medium should be used, which will maintain the viability of C. jejuni for up to 72 hours. Swabs or liquid stool should be applied directly to 1/4 of a selective agar plate and streaked for separation with a loop. If solid stool is being cultured, it should first be emulsified with saline before it is applied to the agar. With both Butzler's and Skirrow's media, plates must be incubated under conditions of reduced oxygen tension, preferably with added carbon dioxide. One easy way to achieve this is by using an anaerobic jar without a catalyst, along with a carbon dioxide and hydrogen GAS-PAK. It is expected that in the near future techniques will be available that will permit the cultivation of C. jejuni in a candle jar or an ordinary incubator. For optimum selectivity, the plates should be incubated at 42-43°C for 18-24 hours, but they can be incubated at 37°C, preferably for 48 hours.

Campylobacter colonies are typically flat, glossy, and effuse, and have a tendency to spread along the tracks left by the inoculating wire. When well spaced they resemble droplets of fluid that have been splattered on the agar. Some strains, however, particularly those commonly found in pigs, form more discrete, domed colonies. Any suspicious colonies should be smeared and stained with a strong stain such as crystal violet or carbol fuchsin, as campylobacters do not take up stain readily. Campylobacter can be seen as slender, Gram-negative, spiral or S-shaped organisma with tapering ends. Occasionally, the spiral morphology is not obvious, and in some cases spindle-shaped bacilli predominate. A highly characteristic feature of Campylobacter is that they degenerate into coccoid forms after a few days of culture, especially when grown on solid media. Generally, these coccoid forms have lost their motility and fail to subculture.

In the experience of investigators familiar with the organism, its morphology is usually sufficiently characteristic to allow its isolation and identification from faecal cultures. However, when attempting to isolate the organism from blood, or from faeces using a 37°C rather than 43°C incubator, confirmatory tests should be done,when possible, to differentiate the organism from C. fetus. Temperature tolerance tests are the most reliable for this purpose, but the triphenyltetrazolium chloride (TTC) test is useful (Table 1).

TABLE 1

Tests for distinguishing Campylobacter jejuni from Campylobacter fetus

Growth	Campylobacter fetus	Campylobacter jejuni
At 43°C	-	+
At 37°C	+	+
At 25°C	+	-
On TTC[a] agar	-	+

a = one litre thioglycollate broth plus 15 g agar plus TTC = Triphenyltetrazolium Chloride (400 µg/ml).

[1] Combined antibiotic supplements for each of the media are commercially available from Oxoid Limited.

Problems have been noted with long-term storage of C. jejuni, including lyophilization. The organism has been best maintained by culture in thioglycollate semi-solid medium stored in liquid nitrogen at -70°C. It has also been maintained by immersion in glycerol stored at -70°C.

There have been a number of attempts to develop a C. jejuni antigenic typing scheme using live, formalized, and heated bacterial suspensions. A direct haemagglutination test is now in use and seems to be satisfactory.

One group has reported that a rapid diagnosis of C. jejuni enteritis can be made by direct phase contrast microscopic examination of stool, the organism being readily recognizable by its characteristic morphology and motility; this observation requires confirmation.

The disease can also be confirmed serologically. The great majority of patients develop an antibody response which appears in the first few days of illness, quickly reaches a maximum titre, and then declines during the ensuing few months. The antibody response has been measured by a number of assays, including an agglutination test, complement fixation test, serum bactericidal assay, and indirect immunofluorescence test. However, the specificity of the antibody response requires further study.

2.1.3 Epidemiology

There is some evidence that C. jejuni enteritis is a zoonosis with a world-wide distribution, and there are probably a number of ways by which man can become infected. However, it will not be possible to understand fully the relationship between human and animal infections until suitable methods are available for differentiating strains (i.e., serotyping, phage-typing, etc).

Campylobacters are found in the intestines of many animal species, particularly birds, in which they seem to be normal commensals. Poultry probably constitutes the largest potential reservoir of C. jejuni infection. The carriage rate in poultry flocks is high (contamination of fresh and frozen chicken carcasses has been reported from the United Kingdom) and it is thought that consumption of contaminated poultry is one of the most common means of transmission. Some human infections have been traced to contact with live birds on farms and the handling of dressed carcasses in processing plants, butchers' shops, and kitchens. Studies of the survival of C. jejuni in different foods have not been reported.

C. jejuni has also commonly been recovered from coproculture of cows, and cow's milk has recently been shown to be an important source of infection. In 1979, outbreaks occurred in the United Kingdom in which unpasteurized milk was the incriminated vehicle. It is thought that the organism is introduced into the milk by faecal contamination from bovines.

Dogs can suffer from Campylobacter enteritis and may constitute a source of infection. Several bacteriologically proved cases have been reported in children who had been in close contact with young dogs or puppies with diarrhoea.

C. jejuni, like C. fetus, can cause abortion in sheep. A single instance of human infection from contact with sheep has been reported in a farmer.

Polluted water is thought to have caused a major outbreak in a town in Vermont, USA, in the summer of 1978. There have also been other circumstantial links between infection and the ingestion of untreated water from streams or rivers.

Person-to-person transmission has been observed in nurseries among infants and young children. Five outbreaks have been reported in infant day-care centres in Belgium. Pregnant women infected at or near term have been shown to infect their new-born babies.

For reasons that are unknown, in Western Europe and North America the incidence of C.jejuni enteritis is highest in the warmer months.

There have been no community-based studies describing the age/sex-related incidence of the disease in any setting.

2.1.4 Pathogenesis

C. jejuni enteritis has been reproduced in rhesus monkeys by inoculation of pure cultures, and a human volunteer suffered a typical attack of *Campylobacter* enteritis a few days after swallowing a live culture of *C. jejuni* recently isolated from a patient with the disease.

Experiments in Belgium have suggested that *C. jejuni* produce a predominantly invasive type of infection. Pathological and microbiological observations in children, including observations made from specimens obtained at autopsy and sigmoidoscopy, suggest that *C. jejuni* invades the mucosa of both the small (particularly the ileum) and the large intestine. Preliminary evidence indicates that a few strains can produce a heat-stable enterotoxin.

2.2 Yersinia enterocolitica

Yersinia enterocolitica is another newly recognized cause of enteric infection and has been found in many parts of the world. As in the case of *C. jejuni*, most of the information available to date has been derived from observations in Northern Europe and North America. The global magnitude of the problem of *Y. enterocolitica* infection remains to be determined.

2.2.1 Clinical features

The clinical features of *Y. enterocolitica* infection appear to vary with age. In infants and young children, the predominating symptom is acute watery diarrhoea of 3 to 14 days' duration; blood is observed in the stools in about 5% of cases. In children older than 5 years and in young adults, right lower quadrant abdominal pain is the most common symptom and is often accompanied by fever, moderate leukocytosis, and elevated erythrocyte sedimentation rate. This clinical picture can be so similar to that of acute appendicitis that it is often not possible to distinguish between the two conditions. In studies in Scandinavia, 5% of cases diagnosed as appendicitis were shown to be *Y. enterocolitica* enteritis. *Y. enterocolitica*, however, is rarely a cause of purulent appendicitis or peritonitis. This type of *Y. enterocolitica* infection must also be differentiated from early Crohn's disease. The prognosis in such cases is good.

In adults, erythema nodosum may follow infection with *Y. enterocolitica*; onset is usually 1-2 weeks after enteritis, although 40% of cases give no history of gastrointestinal symptoms. Eighty percent of these erythema nodosum cases are in women, and the condition is most frequent in persons over the age of 40 years. These cases seldom show enlargement of hilar glands or elevated antistreptolysin titres. Prognosis is good and relapses seldom occur.

One serious and not uncommon complication of *Y. enterocolitica* infection in adults is reactive arthritis. About half of these cases have monoarthritis localized in one knee, foot or hand; in the remainder two or more joints are involved. The symptoms may have an acute onset occurring about one week after the onset of enteritis, although 30-40% of the cases have no history of gastrointestinal symptoms. The duration of the symptoms is more than one month in two-thirds of these cases. Most cases have an elevated erythrocyte sedimentation rate. The condition has an equal sex distribution and 65% of cases have been found to belong to the histocompatibility group HLA-B27. Occasionally, suppurative polyarthritis may be observed, which can be severe and very long-lasting, sometimes resulting in severe disability.

About 100 cases of septicaemia in which *Y. enterocolitica* was isolated from the blood have been described, mostly in Europe. Many of these cases had underlying illnesses and no history of enteritis. Rare cases of myocarditis, subacute hepatitis, hepatic abscesses, conjunctivitis, ophthalmitis, meningitis, urethritis, and acute glomerulonephritis have also been described as being complications of *Y. enterocolitica* infection. It has been shown that patients with hyperthyroidism (Graves' disease) may have elevated antibodies to serotype O3 of *Y. enterocolitica* [1] antigen and thyroid tissue cells.

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[1] The term serotype is used in this report to describe the O-antigen characteristics of *Y.enterocolitica*, as has been the common practice in the literature. A more accurate term might be serogroup.

Y. enterocolitica is universally resistant to penicillins and their derivatives, cephalothin, oleandomycin, and novobiocin. Some strains of serotype O8 are an exception, being sensitive to ampicillin. Resistance to the penicillins is dependent on the ability of the strains to produce β-lactamase, which has been shown in a few strains to be plasmid-mediated. This plasmid has been transferred in vitro from one Y. enterocolitica strain to another, and to a strain of Escherichia coli. Most strains are sensitive to streptomycin, tetracycline, chloramphenicol, nitrofurantoin, sulfonamides, trimethoprim-sulfamethoxazole, gentamicin and nalidixic acid.

The effectiveness of antibiotic therapy in cases of Y.enterocolitica enteritis has not been investigated in a controlled trial. Uncomplicated cases appear not to need antibiotic therapy, but most investigators feel it should be given to severe cases and to those with complications. Empirical observations suggest that good results are obtained with the use of tetracycline or trimethoprim-sulfamethoxazole, and that ampicillin may be effective in cases caused by strains of serotype O8. Treatment seems to eradicate carriage of the organism, which may continue for 2-3 months in untreated persons; however, further confirmatory studies are needed.

2.2.2 Laboratory characteristics

Y. enterocolitica grows as a lactose-negative organism on peptone agar, blood agar, and media used for the detection of Salmonella and Shigella, such as SS agar, Desoxycholate agar, and MacConkey agar; its growth is, however, slow. It does not grow well on Endo agar. Colonies of Y. enterocolitica are very small after 24 hours, becoming larger after 48 hours, especially when the plates are incubated at 22-25°C. Development of flagellae (and thus motility) and a positive Voges-Proskauer reaction are seen only after incubation of the media at 22-25°C.

The isolation of Y. enterocolitica from stools may be done simultaneously with the isolation of other lactose-negative enterobacteria. Ideally, this is done by streaking an extra plate, which is incubated at 22-25°C for 48 hours, or by taking a plate that has been incubated for 18-24 hours at 37°C for the isolation of Salmonella and Shigella and placing it at 22-25°C for an additional 24 hours. In laboratories that do not have incubators set at 22-25°C and where the room temperature is above 25°C, the plates can be incubated at 37°C for 48 hours but require careful observation to detect Y. enterocolitica, which appear as tiny colonies resembling enterococci.

It has been shown that to detect low numbers of organisms in stool (e.g., in carriers), stool samples need to be enriched in phosphate buffer solution or peptone broth at 4°C for 3-7 days; this increases the growth of Y. enterocolitica and depresses the growth of E. coli and other bacteria. There is no advantage to this enrichment procedure when trying to isolate Y. enterocolitica from the stools of acute diarrhoea cases. Other selective methods are the use of Leifson selenite broth supplemented by 0.007% malachite green, or a medium containing carbenicillin.

Suspect Y. enterocolitica colonies should be confirmed biochemically; they are usually urease-positive and oxidase- and phenylalanine-negative. Workers in Belgium and Sweden have divided Y. enterocolitica into 5 different biotypes (Table 2).[1] The human pathogenic strains appear to belong to biotypes 2, 3 and 4. Biotype 5 has most commonly been observed in animal epizootics, while biotype 1 includes mostly non-human pathogenic strains, which, because of their biological characteristics, can be referred to Yersinia species although their true species have yet to be decided. Some workers have proposed additional biotypes: one for sucrose-negative strains, one for rhamnose-positive strains, and one for rhamnose-positive and melibiose-positive strains.

In common with the Enterobacteriaceae, Y. enterocolitica has lipopolysaccharide antigens, and an O-antigen serotyping scheme has been devised.[1] At the present time, there is

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[1] Winbald, S. (1979) In: Methods in Microbiology, Vol. 12, New York, Academic Press, pp. 37-50

TABLE 2

Relationship between Biotypes and O-Serotypes and Source of *Y. enterocolitica* [1]

Biotype	Biochemical reactions										Biotype (modified after Niléhn and Wauters) [2]	O-serotypes *	Source
	Indole	Aesculin	Salicin	Voges-Proskauer 25°C	Rhamnose	Sucrose	d-xylose	Trehalose	Melibiose	Lecithinase			
Y. enterocolitica													
d-xylose negative	−	−	−	+	−	+	[−]	+	−	−	4	3	Man, dogs and cats
Trehalose negative	−	−	−	−	−	+	[−]	[−]	−	−	5	2(1)	Hares and goats
d-xylose positive	−	−	−	+	−	+	[+]	+	−	−	3	1,5b(3,4a)	Chinchillas; Man
Sucrose negative	−	−	−	+	−	[−]	+	+	−	−	3	11,12,NT	Animals; Man
Weakly indole positive	(+)	−	−	+	−	+	+	+	−	+	3	9	Man
Indole positive	[+]	−	−	+	−	+	+	+	−	[+]	2	8,(18,20)	Man
Yersinia species													
Indole, Aesculin, Salicin positive	[+]	[+]	[+]	+	−	+	+	+	−	+	1	2a,4,5a,6,7, 7/13,10,13,14 15,16,19,NT	Water, food, animals, milk
Melibiose positive	+	+	+	+	+	+	+	+	[+]	+	1	17	Man

* The less prevalent serotypes are shown in brackets.

NT Not typeable.

[1] Adapted from Winblad, S. (1979) In: Methods in Microbiology, Vol. 12 New York, Academic Press, p. 46

[2] Niléhn, B. (1969) Acta path. microbiol. scand. Suppl., 206; Wauters, G. (1970) Contribution à l'étude de Yersinia enterocolitica, Vander, Louvain.

agreement among workers in this field on the O-serotypes 1 to 20, and additional work is under way to identify other O-serotypes. Some cross-reactivity has been found between O-serotypes. H-antigens have also been identified in the different O-serotypes. In practice, however, only O-serotyping has been used for the identification of strains, as H-serotyping sera are not widely available. To date, the strains causing disease in man have belonged to serotypes O3, O8 and O9 (see Section 2.2.3) almost exclusively.

Phage-typing of Y. enterocolitica is carried out at the WHO Collaborating Centre for Yersinia at the Pasteur Institute, Paris. On the whole, there has been agreement between phage-typing and serotyping. It is of interest, however, that strains of serotype O3 from cases in Canada have been phage-type VIII, while strains of serotype O3 from cases in Europe have been phage-type IX and IXb.

The diagnosis of Y. enterocolitica enteritis is best made by isolation of the organisms from stool. The organisms should also be looked for (with enrichment) in cases of appendicitis, erythema nosodum, and reactive arthritis. To confirm a diagnosis, a serological test has been used to detect agglutinins against the antigen of the infecting strains (Widal test). These titres generally are detectable 8-10 days after the onset of illness and remain elevated 8-18 months after infection. Some cross-reactivity has been observed, particularly between serotype O9 and Brucella, Salmonella O30 and Vibrio cholerae antigens. There have been no systematic studies of titres in patients with salmonellosis or other diarrhoeal diseases.

A complement fixation test has also been used to measure antibodies against Y.enterocolitica, but with less satisfactory results. An ELISA test has recently been described that measures antibodies against serotype O3 lipopolysaccharide.

2.2.3 Epidemiology

The incidence of Y. enterocolitica enteritis has been studied in a few areas only. In one large study in Sweden in 1978, the organism was isolated from 154 (2%) of 7304 cases of acute enteritis studied. Similar results (ranging from 1 to 3%) have been reported from Belgium, Canada, and the Federal Republic of Germany. There have been no large studies in the developing countries and no community-based studies in any location.

Outbreaks of Y. enterocolitica enteritis have occurred in Finland, Japan and USA, but their source could not be identified. One outbreak in the USA was traced to contaminated chocolate-milk. There have been no studies of the survival of Y. enterocolitica in foods. Outbreaks have occurred in hospitals, in which person-to-person transmission were the most likely mode of spread.

Whether Y. enterocolitica is a true zoonosis is not clear. Studies in Belgium and Denmark have shown that 3-5% of pigs are intestinal carriers of serotype O3. Throat and tongue cultures have been positive in up to 53% of these animals. It has been observed that dogs and cats are often infected, and there have been reports of simultaneous infection of children, dogs, and cats in the same household; however, it is not clear whether the dogs or cats can transmit the disease to man.

In Europe, Y. enterocolitica infection occurs most frequently during the colder months, although a few cases are observed during the spring and summer.

As mentioned above (see Section 2.2.1), acute non-complicated enteritis is usually seen in children and the frequency of such cases decreases with age. This distribution of cases suggests that immunity to enteritis develops with age. In contrast, cases with complications (e.g., erythema nodosum, reactive arthritis) are mostly observed in older persons and are rare in children, suggesting that these complications are associated with reinfection and are a consequence of a secondary immune response.

A relationship has been observed between the O-serotypes associated with disease in man and geographical areas. Infection with serotype O3 is common in Belgium, Federal Republic of Germany, Hungary, Netherlands, Scandinavian countries, and Spain, and cases with this serotype have been reported from Canada, Israel, Japan, Rwanda, South Africa and Zaire. Infection with serotype O9

has also been found in Belgium, Federal Republic of Germany, Finland, Hungary, Netherlands, and Sweden, but is much less common than serotype O3. In contrast, infection with serotype O8 is most common in Canada and USA.

Studies of the biotypes and serotypes of isolated strains suggest that there is a relationship between the bio-serotype and the source of the isolate (Table 2). As mentioned, human pathogenic strains belong to serotypes O3, O8 and O9 and biotypes 2, 3, and 4 (see Section 2.2.2). The most common of the serotypes (serotype O3) is usually d-xylose-negative. Strains from hares and goats are trehalose-negative and belong to serotype O2. A few strains of serotype O3 isolated from man and strains isolated from chinchilla epizootics are d-xylose-positive. Indole positivity is usually associated with the ability to produce lethicinase, as seen in the human pathogenic serotype O8 strains in North America, which are also negative for aesculin and salicin fermentation. Indole, aesculin, and salicin positivity are a common character of all the non-pathogenic strains (O-serotypes 4, 5a, 6, 7, 7/3, 10, 13, 14, 15, 16, 17, 19) isolated from water, food, and animals. It is not clear whether serotypes O17 and O5b are pathogenic for man. In one study in USA, serotype O17 was isolated from non-intestinal sources in 9 persons.

2.2.4 Pathogenesis

Some strains of serotypes O3, O8 and O9 have been shown to be invasive when tested in HeLa and porcine kidney cells, and by the Serény test. In one study this invasive property was shown to be plasmid-mediated.

A small molecular weight, methanol soluble, heat-stable enterotoxin has been shown to be produced by strains of serotypes O3 and O8, and, in one study, by two non-typeable strains. The enterotoxin was detected when these strains were incubated at 25°C (but not at 37°C) and the supernatants or filtrates were tested in the infant mouse and rabbit ileal loop assays; it has been shown to be a potent activator of guanylate cyclase. There is some suggestion that toxin production may also be plasmid-mediated. A few strains have been shown to be both invasive and enterotoxin producing.

Histological studies show that early in *Y. enterocolitica* enteritis the small intestinal lymph nodes and the Peyer's patches are involved and microabscesses can be seen. However, large abscesses have not been observed. In patients operated upon for "appendicitis" the usual findings are mesenteric lymphadenitis and/or terminal ileitis.

Animal models for the disease have been developed in mice, guinea pigs, rats and rabbits.

2.3 Non-typhoid salmonellosis

The genus *Salmonella* now comprises about 2000 serotypes, which can infect a wide range of warm and cold-blooded animals. These infections may be asymptomatic, but when disease occurs in humans two broad patterns are recognized. One pattern is associated with generalized infection of the reticulo-endothelial system, bacteraemia, and prolonged pyrexia, i.e., "enteric fever", and is typical of infections caused by *S. typhi* and *S. paratyphi* A and B. Other serotypes, in particular *S. sendai*, *S. cholerae-suis*, and *S. dublin*, also cause septicaemia but in addition are often associated with metastatic abscesses. The other, and more common, clinical manifestation is enteritis accompanied by fever, which is caused by a wide variety of serotypes.

2.3.1 Magnitude of the problem

The WHO *Salmonella* Surveillance Programme receives laboratory-confirmed data from about 30 countries, but the representativeness of the information varies considerably. Regular surveillance information is available from only a small number of countries. However, information from occasional surveys and from studies such as those carried out by the WHO Diarrhoeal Diseases Advisory Team in seven developing countries in 1960-65[1] has confirmed that salmonellosis has a

[1] van Zijl, W.J. (1966) *Bull. Wld Hlth Org.*, 35, 249-261

worldwide distribution and has given an indication of the magnitude of the problem.

Much of the currently available information has come from North America and the United Kingdom, where surveillance reports are published regularly. It has been estimated that there are about 2 million Salmonella infections in the USA each year, of which up to 500 000 require hospitalization. In England and Wales, a similar extrapolation of laboratory-confirmed data would give an estimated 200 000 human infections yearly. In these, and no doubt also in other developed countries, while salmonellosis is not a significant cause of mortality, it is an important cause of diarrhoea and, as such, is of considerable economic importance because of the associated costs of medical care and lost working time. In these countries, the surveillance data suggest that the highest incidence of salmonellosis occurs in the first year of life, and more particularly in the early months. Whether this is a true age-specific attack rate or merely reflects a greater tendency to investigate diarrhoea in very young children is not known.

In the developing countries, the lack of surveillance data makes it particularly difficult to evaluate the importance of salmonellosis in the family unit or community, although a few extensive common-source food or water-borne outbreaks and hospital outbreaks have been described. In some, a high mortality rate, possibly related to co-existent malnutrition, has been documented.

2.3.2 Epidemiology

Salmonellosis most commonly results from the ingestion of contaminated food. In industrialized countries, water-borne outbreaks have occurred as a result of sewage contamination of water supplies; while uncommon, such outbreaks are usually dramatic and easily recognizable. In the developing countries, where water is often obtained from local sources that are not purified or protected, water-borne salmonellosis is probably more common.

The relative frequency of salmonellosis as a cause of foodborne disease varies from country to country and depends on such factors as dietary habits, hygienic standards in food production and service establishments, and animal husbandry practices such as intensive-rearing systems. In the USA, about 40% of reported food poisoning cases are due to Salmonella, whilst in England and Wales the comparable figure is about 80%. In these countries, salmonellosis is a zoonosis and the overall epidemiological pattern is related to the predominant source of animal protein in the diet. Thus, in the USA, food of bovine origin is the main source of Salmonella infection, while in England and Wales poultry accounts for about 50% of outbreaks and beef for only about 2%. In many other developed countries, poultry and pigs are the commonest sources of Salmonella infection.

In some developed countries where intensive-rearing methods have been introduced over the last 20 years, up to 30% of intensively-reared poultry are reported to be infected with Salmonella. In countries where infected poultry and pork are responsible for most cases of salmonellosis, imported Salmonella-contaminated animal feeds have been found to be an important source of infection and have been responsible for introducing a succession of different serotypes. The recycling of waste material from processing plants has helped to maintain the situation. Suitable heat treatment of animal feeds has been found to be a useful control measure.

In developing areas where animal protein does not constitute a major part of the diet, it is unlikely that salmonellosis is or will be such an important cause of foodborne disease. In these situations, intensive-rearing of food animals will be uncommon and its associated problems insignificant. However, hygiene standards in food production and catering are likely to be lower and thus Salmonella carriers probably are a more important source of food infection than they are in developed countries.

Nosocomial outbreaks of Salmonella enteritis have been reported from many developed and developing countries in which cross-infection has been documented. Most outbreaks have occurred in maternity or paediatric units, or on wards for the aged or the chronically sick. These outbreaks are often difficult to control and may be associated with high mortality. Outbreaks have occurred even in modern units with sophisticated facilities. Common-source foodborne infections may occur in hospital units, but their explosive epidemiological pattern usually makes them readily distinguishable from cross-infection outbreaks.

2.3.3 Laboratory facilities for surveillance

To obtain meaningful Salmonella surveillance data, laboratory-derived information is essential. Salmonella strains require serotyping and some particularly prevalent serotypes such as S. typhimurium, S. enteritidis and S. panama need the more precise "finger-printing" provided by phage-typing.

In many developing countries, a stool culture is not very often performed on diarrhoea cases, and even when it is, the primary isolation media used are not infrequently selected to facilitate the isolation of V. cholerae and, as such, are not conducive to the isolation of Salmonella. Most countries have at least one laboratory with the technical expertise to perform basic serotyping, but often these laboratories lack the diagnostic antisera to identify even the most common serotypes. Phage-typing facilities are even less readily available, and only a few countries are able to phage-type S. typhimurium, which is the most prevalent serotype worldwide. A further complication is the existence of two distinct schemes for phage-typing S. typhimurium (one developed by the WHO Collaborating Centre in London, England, the other by the National Institute of Public Health in Bilthoven, Netherlands).

2.3.4 Antibiotic resistance

In many countries, a high proportion of Salmonella strains show multiple antibiotic resistance, which is often plasmid-mediated. This problem has been increasing over the last 20 years. The most important contributing factor has been the excessive use of antibiotics, both as growth promoters in animal feeds and for prophylaxis and treatment in human and veterinary medicine.[1]

Some countries in Western Europe have recently prohibited the inclusion of antibiotics used in the treatment of humans in animal feeds; this has led to a documented reduction in the incidence of resistant Salmonella strains isolated from animals, food, and man. However, in some of these countries, the value of this prohibitive legislation is being offset by the continued illicit use of antibiotics by farmers. It is recognized that in food-producing animals, especially in bovines and to a lesser degree in porcines, salmonellosis is a serious disease often accompanied by high mortality and consequent economic loss, so that the use of antibiotics to treat sick animals is justified. It is rather their use for prophylaxis that has been seriously questioned. As an example of the problem, in recent years in the United Kingdom, overuse of antibiotics in animal husbandry has been an important factor in the emergence of multiresistant clones of S. typhimurium, which have become established in epidemic proportions in bovines throughout the country, causing serious disease with high mortality in these animals.

In man, it has been established that antibiotic therapy is not beneficial in uncomplicated enteritis due to Salmonella; such treatment does not accelerate clinical recovery and prolongs the period of excretion in convalescence. For this reason, clinicians must be discouraged from the unnecessary use of antibiotics in the treatment of Salmonella enteritis. In developing countries in particular, the high incidence of multiresitant strains is believed to be due to the general over-use and abuse of antibiotics in human medical care.

2.3.5 Outbreaks and epidemics due to multiple drug-resistant strains

In the last decade there have been a number of outbreaks and epidemics of salmonellosis with common clinico-epidemiological features caused by strains of different serotypes that possess multiple, plasmid-mediated drug resistance. These epidemics have occurred in neonatal or paediatric units, and in many instances have shown a high incidence of septicaemia or meningitis with an associated high case fatality. The strains appeared to spread nosocomially with an unusual degree of communicability. Examples of serotypes causing such outbreaks have been S. isangi (Zaire), S. stanleyville (Senegal), S. typhimurium (Kenya), and S. oranienburg (Brazil).

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[1] Wld Hlth Org. techn. Rep. Ser., 1978, No. 624 (Surveillance for the prevention and control of health hazards due to antibiotic-resistant enterobacteria)

Epidemics caused by multiresistant strains of S. wien and S. typhimurium phage type 208 have shown an additional epidemiological feature in that they involved numerous hospitals over wide geographical areas. An epidemic due to S. wien commenced in Algeria in 1969, and spread to France, Italy, Yugoslavia, Iraq, and eventually India in 1976. In each country the multiresistant strain became one of the most prevalent causes of human salmonellosis and no food-chain of infection could be identified. The multiresistant strain of S. typhimurium type 208 spread widely in the Middle East between 1969 and 1976 and sporadic isolates occurred in India and the United Kingdom. Detailed investigation of the plasmid content in the epidemic S. wien and S. typhimurium type 208 strains showed that they belonged to single clones; moreover, both clones carried the same plasmid ($F_{I}me$), although other plasmids were also present. More recently (1978), preliminary evidence has suggested that a similar situation is occurring with a multiresistant clone of S. typhimurium in South-East Asia, where outbreaks have occurred in many areas of India and the Philippines.

In these situations, the use of antibiotics was probably an important contributor to the emergence and persistence of the clones, and it is theoretically possible that in some cases the drug resistance plasmids also carried genes coding for enhanced virulence or communicability. In such instances, antibiotic use might select for strains that not only possess drug resistance but also have an increased pathogenicity. These situations reinforce the need to curb all unnecessary use of antibiotics.

2.3.6 Pathogenesis

The pathogenesis of Salmonella enteritis has recently received much study in animal models. In rats, Salmonella produce an ileocecitis, while in primates diffuse colitis is seen in addition to ileitis. In monkeys with experimentally induced diarrhoea due to S. typhimurium, morphological changes have been seen in the colonic and ileal mucosa but not in the jejunal mucosa; those with severe diarrhoea, however, exhibited fluid and electrolyte transport abnormalities in the jejunum, ileum, and colon. When strains of S. typhimurium were studied in rabbit ileal loops invasiveness was found to be a prerequisite for the induction of fluid secretion; however, not all invasive strains induced fluid secretion. One laboratory has reported that S. typhimurium can produce a cholera-like enterotoxin that causes elongation of Chinese hamster ovary cells and exhibits vascular permeability factor activity in the rabbit skin, both of which can be neutralized by cholera antitoxin. Other workers have described a heat-stable enterotoxin produced by Salmonella. If these reports are corroborated, it will mean that Salmonella possess both invasive and enterotoxic properties.

2.4 Typhoid fever

Typhoid fever has remained an important public health problem in many developing areas where it is endemic. Cases observed nowadays in industrialized countries are often a result of infection acquired during travel to endemic areas.

2.4.1 Epidemiology

It is now believed that infection with Salmonella typhi is more common in endemic areas than is reported; this is because about 80% of infections are mild or subclinical. The typical epidemiological pattern in these situations shows typhoid fever to be predominantly a disease of school-age children and young adults. This infers that most transmission of S. typhi occurs outside the home. However, some transmission does occur within the home via chronic carriers, as exemplified by the occurrence of cases in young children. Since the clinical pattern of disease in infants and toddlers can often be atypical, a high degree of suspicion must be maintained and a blood culture examined for S. typhi in cases of pyrexia of unknown origin.

It has often been noted that typhoid fever is common in certain geographical regions and rare in others within the same country; the reasons for this are not clear.

Man is both the only known reservoir and natural host of S. typhi. The size of the inoculum ingested is an important determinant of whether clinical or sub-clinical disease occurs. Studies in volunteers have shown that when an inoculum of 10^7 - 10^9 organisms is ingested, the attack rate for clinical typhoid fever approaches 100%, while a dose of 10^5 organisms causes clinical illness in 25-50% of healthy adults. The critical inoculum in actual life is probably considerably lower.

There is epidemiological evidence to suggest that repeated ingestions of S. typhi by persons living in endemic areas usually result in sub-clinical infection followed by immunity. For instance, in endemic areas, clinically apparent typhoid fever is much less common in older adults, and serological surveys in healthy adults have demonstrated a high prevalence of IgG-specific S. typhi H (d) antibodies.

One of the most notable events in typhoid fever epidemiology in recent years was the occurrence of large epidemics in Mexico and South-East Asia in the early 1970s due to strains of S. typhi that exhibited plasmid-mediated resistance to a range of antibiotics, including chloramphenicol. It was found in these epidemics that cases could be successfully treated with oral trimethoprim-sulfamethoxazole, parenteral ampicillin, or oral amoxicillin. Towards the end of the Mexican epidemic, a few strains that were ampicillin-resistant appeared. The explanation for the sudden appearance, epidemic spread, and equally sudden disappearance of this epidemic strain is not clear. Sporadic isolations of multiresistant S. typhi continue to occur.

2.4.2 Carriers

Chronic biliary carriers of S. typhi represent the most important reservoir of infection; they are a major factor determining the endemic level of the disease and an important source of sporadic outbreaks. Chronic carriers shed S. typhi continuously into the intestine via the bile. However, stool cultures may be only intermittently positive because survival of the bacilli and their recovery in coproculture depend on the stool pattern and the inhibitory effects of normal stool flora. The carrier state may follow clinical or sub-clinical infection and the propensity to become a carrier is related to the presence of gall-bladder disease. Therefore, the chances of becoming a carrier increase with age at the time of infection and are greater in females than in males.

In circumstances where chronic carriers are eliminated from the population by natural death more quickly than new carriers are added, the endemicity of the disease decreases. When a certain threshold level of carrier prevalence is reached, the incidence of typhoid fever has a tendency to diminish relatively rapidly.

Simple, reliable serological screening procedures for presumptive detection of carriers in large populations are not available. However, a non-surgical method for culturing S. typhi from chronic biliary carriers has recently been described. It involves the swallowing of a gelatin string capsule to which a small, weighted rubber bag is attached, which, within 4 hours, passes into the small intestine. After 4 hours, the string is withdrawn and liquid is expressed from the bile-stained portion of the string and cultured.

The most successful treatment of carriers is cholecystectomy with concomitant ampicillin therapy. Cure rates of approximately 80% can be expected with this regimen. Persons who continue to shed S. typhi after cholecystectomy probably have chronic infection of the intrahepatic biliary system. Since cholecystectomy cannot be used as a control measure, more practical, non-surgical, and less costly regimens to eradicate the carrier state are needed. Preliminary evidence suggests that 2-4 week courses of intravenous ampicillin or oral (high-dose) amoxicillin may achieve eradication of the carrier state in about 70% of cases. Failure of therapy may be related to low antibiotic levels in the blood; probenecid therapy may enhance the efficacy in such cases. Both ampicillin and amoxicillin are concentrated in the bile.

2.4.3 Control

In general, control measures for typhoid fever include: identification of chronic carriers, their elimination from food-handling, and their treatment; identification of the vehicles of transmission followed by appropriate specific intervention; general improvement of water supplies, sanitation, food preparation techniques, and personal hygiene; and alteration of host susceptibility by immunization. Selection of the most cost-effective control measure(s) is a practical problem for public health administrators. A mathematical model has recently been proposed that may be of assistance in deciding which control measures should be given priority in different situations.[1]

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1 Cvjetanović, B., Grab, B. & Uemura, K. (1978) Dynamics of acute bacterial diseases - Epidemiological models and their application in public health, Bull.Wld Hlth Org., 1978, 56, Suppl. No. 1.

The subject of typhoid vaccine has been extensively reviewed.[1] Since that report, more information has become available from the first field trial under way in Egypt of an oral vaccine prepared from an attenuated strain of S. typhi (Ty 21a)[2]. This vaccine had previously been intensively studied in healthy adult volunteers in the USA where it was found to cause no adverse reactions in 155 persons who received doses as high as 5×10^{10} organisms, and there was no evidence of genetic instability in over 950 isolates recovered from coproculture. In the Egyptian trial, approximately 15 000 children aged 6-7 years received 3 doses of vaccine (10^9 organisms per dose, given with $NaHCO_3$) on alternate days of one week. There were no significant adverse reactions among the vaccinees. The preliminary results show that, during almost 2 years of surveillance, the incidence of typhoid has remained high in the children who received placebo, but no cases have occurred among the vaccinated children.

The polysaccharide Vi antigen of S. typhi has been highly purified and shown to be antigenic and non-reactogenic in a small group of adult volunteers. This antigen is now available for evaluation in appropriate field trials.

2.5 Shigellosis

The genus Shigella is subdivided into 4 subgenera or subgroups according to their biochemical reactions: Sh. dysenteriae, Sh. flexneri, Sh. boydii, and Sh. sonnei. The first three subgroups may be further subdivided by serotyping, but for Sh. sonnei colicin-typing, and less commonly phage-typing, is used. There are 10 serotypes of Sh. dysenteriae, 8 of Sh. flexneri, and 15 of Sh. boydii. There are also a small number of sub-judice serotypes of Sh.dysenteriae and Sh. boydii. Sh. sonnei has been differentiated into 15 colicin types.

2.5.1 Epidemiology

Shigella produce bacillary dysentery which typically presents as fever and watery diarrhoea, the latter often changing on the first or second day of illness to frequent, small volume stools containing blood and mucus. Although it has frequently been reported that Sh. dysenteriae 1 (Shiga's bacillus) produces the most severe disease and Sh. sonnei the mildest, in fact the disease caused by any of the subgroups has a wide spectrum. The typical case is of short duration (about 4 days), but exceptionally the symptoms may last for up to 2 weeks. Host factors seem to play an important role in determining the severity and duration of the disease. In contrast to salmonellosis, extra-intestinal complications are rare and Shigella are rarely recovered from blood culture. A long-term carrier state is exceptional but does exist.

Shigellosis has a global distribution, with the highest incidence in countries where hygiene is poor. As the general level of environmental and personal hygiene rises in a country, the proportion of cases due to Sh. sonnei increases and that of cases due to Sh. flexneri falls. Thus, in more developed areas, Sh. sonnei is most common, Sh. flexneri next most common, and Sh. boydii and Sh. dysenteriae infections are rare, while in many developing areas infection with the latter two subgroups is more common and Sh. flexneri infection is more frequent than Sh. sonnei infection. This subgroup distribution pattern is exemplified by the frequency of the subgroups in travellers returning to the United Kingdom between 1972 and 1978; during this period, about 80% of Sh. dysenteriae, 70% of Sh. boydii, and 50% of Sh. flexneri infections occurred in persons who had recently returned from developing countries, while Sh. sonnei was most frequent in the indigenous population.

Man is both the reservoir and natural host of Shigella. Infection is by the faecal-oral route and the most common mode of spread is by person-to-person transmission owing to the low infectious dose of Shigella (10^1 - 10^2 organisms). In developing countries, food and water-borne transmission are also common, and in areas with inadequate excreta disposal facilities, flies may be an important vector. In these countries, shigellosis is very common during the

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[1] Intestinal immunity and vaccine development: a WHO memorandum. Bull. Wld Hlth Org., 1979, 57, 719-734 (or unpublished document WHO/DDC/78.2).

[2] Germanier, R. (1977) Present status of immunization against typhoid fever. Bol. Ofic. sanit. panamer., 82, 300-311 - in Spanish (or unpublished document WHO/ENT/75.13 - in English and French).

weaning period and is thought to be a major contributor to childhood mortality. In the developed countries, food and water-borne outbreaks are unusual. The disease is often endemic in institutions such as infant schools and day-care nurseries, and on geriatric and other chronic care wards. These sometimes constitute foci from which the community at large may be infected, and vice versa. In developed countries, in contrast to salmonellosis, infants less than 6 months of age, and especially neonates, are rarely infected with Shigella.

2.5.2 Epidemics due to Shiga's bacillus

Since the 1920s, infection with Shiga's bacillus (Sh. dysenteriae 1) has been uncommon in Europe and North America. No major epidemics had been noted anywhere in the world until, in 1969 and 1970, an epidemic occurred in Central America and Mexico, in which there was a high attack rate and mortality, especially in children; over 13 000 deaths were reported. An important feature of the outbreak was the delay in recognizing the etiological agent; in the early stages, many cases were regarded as acute amoebiasis. As a result of the Central American epidemic, infections were imported into the USA, where 140 cases were reported from 1970 to 1972, compared with only 10 cases between 1965 and 1968. A severe epidemic of Shiga dysentery also occurred in Bangladesh in 1972 and more recently in Sri Lanka, beginning in 1976. In the Central American and Bangladesh outbreaks, some cases had atypical clinical features, notably bacteraemia with intra-vascular haemolysis. In all 3 outbreaks strains with plasmid-mediated multiple drug resistance were involved. Why and how the epidemics declined in these areas remains obscure.

2.5.3 Antibiotic resistance

In many countries a high incidence of antibiotic resistance has been observed in Shigella. Globally, the most common pattern is resistance to sulfonamides (Su), frequently combined with resistance to streptomycin (S) and determined by a single plasmid; it has been suggested that the same SSu plasmid has spread throughout Shigella as a global epidemic of a plasmid. Multiple plasmid-mediated resistance to 4 or more antibiotics, involving in particular tetracycline, ampicillin, and chloramphenicol, is now not uncommon. Multiresistance has been shown to be prevalent in many developing countries, where it is probably related to the unrestricted sale and use of antibiotics in man. It has also been seen in developed countries, especially among infections due to Sh. sonnei, although recent information from the United Kingdom suggests that, at least in one country, the incidence of drug resistance in Sh. sonnei may be decreasing.

Although most cases of shigellosis are mild and require only supportive therapy, effective antimicrobial therapy can be life-saving in severe cases, such as those in the Central American Shiga outbreak. Because of the high incidence of antibiotic resistance, the sensitivity pattern of the strain should be determined before initiating antibiotic treatment. A reduction in the frequency of use of antibiotics is essential to reduce the prevalence of multiple resistance, which restricts the choice of antibiotic for use when needed in severe cases.

2.5.4 Shigella vaccines[1]

Various types of parenteral vaccines have been tested and none has been shown to be efficacious. Oral live vaccines, however, using streptomycin-dependent strains in a polyvalent preparation, have given highly significant protection against clinical disease, although infection still spread in the immunized community; however, the protection conferred by this type of vaccine was serotype-specific, required 3-4 doses given with preparations to neutralize gastric acidity, and lasted for only 6-12 months. Single booster injections prolonged the protection for a further year. Although this kind of vaccine could be used in closed communities such as institutions, it cannot at present be considered practical for large-scale use in the control of shigellosis in the general population.

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[1] For a more complete review of Shigella vaccines, see: Intestinal immunity and vaccine development: a WHO memorandum, Bull. Wld Hlth Org., 1979, 57, 719-734 (or unpublished document WHO/DDC/78.2).

2.5.5 Pathogenesis

The association between the invasive capacity of Shigella and virulence is indisputable. It has been known for many years that Sh. dysenteriae 1 produces an exotoxin, which has been called neurotoxin, cytotoxin, or enterotoxin depending on the assay used for its detection. Much of the confusion has stemmed from the assumption that evidence of cytotoxin production could be equated with enterotoxin production. Biochemical purification of Shigella "toxin" has shown it to be a mixture of several proteins, and at least two exhibit cytotoxicity for HeLa cells; the more potent cytotoxin, however, lacked enterotoxic activity. In Japan, workers have described the biochemical separation of cytotoxin from the cytotonic toxin. The cytotonic toxin changed the morphology of Chinese hamster ovary cells and exhibited vascular permeability activity in the rabbit skin test.

3. RESEARCH NEEDS

The Group felt that the following research activities should receive priority under the WHO Diarrhoeal Diseases Control Programme because of their relevance for the control of these diseases.

3.1 Campylobacter jejuni

- There is an urgent need to develop a typing scheme for C. jejuni so as to be able to define the epidemiology of C. jejuni enteritis and the immune response to this infection. Serotyping and phage-typing probably offer the best opportunities. To support these studies, there is a need to identify the appropriate antigens of the organism, which should be measured in diagnostic and serological tests.

- An easier and inexpensive technique for the isolation of C. jejuni from stool, blood, and food is required for use in minimally equipped laboratories that have only a 37°C incubator and little or no capability for anaerobic bacteriology.

- As soon as appropriate isolation and typing techniques are available, field studies should be carried out, especially in developing countries, to investigate more thoroughly the epidemiology (age/sex incidence, modes of transmission, seasonality, etc.) of C. jejuni enteritis. These studies should determine the importance of transmission between animals and man.

- Information is needed on the clinical features of C. jejuni enteritis and on the natural history of the disease in various geographical areas and especially in developing countries.

- Controlled trials should be carried out to define the efficacy of antibiotics (e.g., erythromycin, tetracyclines) in the treatment of C. jejuni enteritis.

- The pathogenesis of the disease should be further studied, and a suitable animal model developed.

- The resistance of C. jejuni to antibiotics should be systematically monitored since plasmid-mediated resistance has been identified in some strains. Antibiotic resistance should be correlated with any typing scheme that is developed.

3.2 Yersinia enterocolitica

- More information is needed on the serotypes of Y. enterocolitica strains isolated from human and environmental(food, water, etc.) sources in different parts of the world. In particular, there is a need to evaluate the apparent association of only serotypes 03, 08, and 09 with human disease, as has been observed to date primarily in North America and Europe.

- There is a need to develop standardized techniques and reagents to measure serological responses to Y. enterocolitica infection.

- Further work should be carried out to determine whether an easier and reliable isolation technique for Y. enterocolitica can be developed for use in minimally equipped laboratories that have only a 37°C incubator.

- With improved microbiological and immunological tools, studies should be done to determine the epidemiology (age/sex incidence, modes of transmission, seasonality, etc.) of Y. enterocolitica infections, especially in developing countries from which there is little information at present. These studies should determine the importance of transmission between animals and man.

- The clinical characteristics of Y. enterocolitica infection should be studied in developing countries. There is also a need to determine whether there are differences between the strains that cause enteritis and those that are associated with other clinical conditions (e.g., erythema nodosum).

- Controlled clinical trials are needed to determine the efficacy of antibiotics in the treatment of Y. enterocolitica enteritis and in preventing the development of sequelae (e.g., erythema nosodum). These should be done in children and adults.

- Studies are needed of the pathogenicity of Y. enterocolitica, especially of the relative importance of invasiveness and enterotoxin production.

3.3 Non-typhoid salmonellosis and shigellosis

- Wherever possible, countries should develop national surveillance programmes for salmonellosis and shigellosis, with close cooperation between public health and veterinary services in the case of salmonellosis. Global surveillance is important and the WHO Salmonella Surveillance Programme might be extended to Shigella and cover more countries. To ensure the validity of the data, good diagnostic antisera should be made available. This might involve training to make countries self-sufficient in antisera production. These surveillance programmes should continuously monitor the resistance of strains to antibiotics. Studies might be undertaken, perhaps first in developed countries, to assess the relative costs and benefits of organized national surveillance activities.

- The pathogenic mechanisms in salmonellosis and shigellosis should be investigated further in an attempt to identify virulence factors and their genetic determinants. Such information might facilitate the development of pharmaceuticals for treatment.

- It seems unlikely that vaccines will be a useful intervention measure for Salmonella enteritis. On the other hand, shigellosis vaccines may have a limited use, for example, in the control of institutional outbreaks, and therefore their development should be supported. Vaccine development might make use of information gained from the recognition of virulence factors.

- The two phage-typing schemes at present in use for S. typhimurium (see section 2.3.3) should be compared and arrangements made for the use of a common scheme.

3.4 Typhoid fever

- Intensive studies are needed to reveal the modes and patterns of transmission of S. typhi in geographically diverse populations where the disease is endemic.

- Sero-epidemiological and bacteriological studies of typhoid fever should be carried out in lesser-developed areas where the incidence of diarrhoeal diseases is high but notification data and clinical anecdotes suggest that S. typhi infection is rare. These studies should attempt to determine whether S. typhi infection is indeed rare in such areas, or whether infection is so common early in life that immunity is acquired in early childhood, thereby leaving few older children and adults susceptible to 'typical' clinical typhoid fever.

- Simple, non-invasive methods should be developed to improve the diagnosis of typhoid fever, particularly in rural areas of developing countries and especially in persons who have received antibiotic therapy prior to attending a health care facility.

- Simple tests need to be devised for screening large populations for presumptive chronic carriers of S. typhi, and non-surgical, practicable, inexpensive, and efficacious therapy should be developed for the treatment of chronic biliary carriers.

- There is a need to search for less reactogenic and more effective vaccines and to develop potency tests for their evaluation. Further field trials should be carried out with promising vaccines such as oral attenuated Ty21a to determine: the efficacy of the vaccine when given as an enteric-coated capsule; the minimum number of doses and minimum number of viable organisms/dose that can successfully immunize; and whether and to what extent the mass use of the vaccine in schoolchildren over the course of several years in an endemic area can break the chain of transmission and the cycle of endemicity by creating an immune cohort free of carriers. Methods to improve the viability of this strain upon lyophilization should be developed.

- The relative roles in protection against typhoid fever of cell-mediated immunity, secretory mucosal humoral immunity, and circulating humoral immunity should be studied.

- The role of predisposing host factors in typhoid fever, such as hypochlorhydria, blood group, and HLA allotypes, should be investigated.

- Studies are needed to assess the economic impact and cost-effectiveness of various typhoid control strategies in developing countries.

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