



REPORT OF THE WHO CONSULTATION ON VETERINARY PUBLIC
HEALTH ASPECTS OF PREVENTION AND CONTROL OF
CAMPYLOBACTER INFECTIONS

Moscow, 20-22 February 1984

CONTENTS

	<u>Page</u>
1. Introduction.	2
2. Clinical Aspects.	3
3. Review of recent trends in the epidemiology	4
3.1 Industrialized countries	4
3.2 Developing countries	5
4. Ecology of <u>Campylobacter jejuni</u> including its survival in the environment and in food of animal origin.	7
5. Methods for detection of <u>Campylobacter jejuni</u> in food animals and in food of animal origin.	8
6. Special preventive and control measures	9
6.1 In animal husbandry.	9
6.2 In the meat and dairy processing industry.	10
6.3 In food establishments (restaurants, kitchens, etc.)	11
7. Conclusions and recommendations	12
8. Recommendations for future research	13
Annex I List of participants	15
Annex II Methods for detection of <u>Campylobacter jejuni</u> in food of animal origin.	16

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The Consultation was opened by Dr A. Koulikovskii who welcomed the participants on behalf of Dr H. Mahler, Director General of the World Health Organization. He also expressed his great appreciation to the local organizers of the meeting and to the Ministries of Health and Agriculture which contributed so much to the organization and preparation of this meeting. He described the role of the Organization in the prevention and control of foodborne enteric diseases throughout the world and in particular the collaboration of the Veterinary Public Health Unit with the Diarrhoeal Diseases Control Programmes (CDD) and the Food Safety Programme in this field. Campylobacter jejuni* is a newly recognized important causative agent of enteric infection in humans and he expressed his confidence that such a distinguished international forum of experts could contribute greatly to the proper assessment of the global problem of C. jejuni as well as advise the WHO Member States on the appropriate measures for prevention and control of foodborne diseases caused by this pathogen.

Dr D. Barua informed the participants of the activity of the CDD Programme in relation to campylobacteriosis. In particular, he drew attention to the research component of the Programme which is now supporting 32 projects on campylobacters including 13 studies relating solely to campylobacters and 19 studies on the aetiology and epidemiology of acute diarrhoeas in Africa, Asia and Latin America with a significant campylobacter component.

These activities reflect the widespread interest among research workers, veterinarians and public health workers in collecting more information on the antigenic and other characteristics of campylobacters and on the natural history of the infections caused by these agents in man and animals.

He also described how the WHO Collaborating Centre for Campylobacter jejuni** is helping the national health workers by developing simplified techniques for isolation and identification and by providing them with reference strains, reagents and sera and helping them in characterization of epidemiologically important strains by biotyping and serotyping them when required.

Professor Koromyslov (Director of the All-Union Institute for Experimental Veterinary Medicine) on behalf of the Ministries of Health and Agriculture of the USSR cordially welcomed the participants of the Consultation as well as observers from the different Institutes of the USSR. He briefly introduced the participants to the work of the Institute and underlined the necessity for research on campylobacteriosis which should be done in close collaboration with medical colleagues. Therefore, this first WHO meeting on veterinary public health aspects of this zoonotic disease, which gathered together scientists (veterinarians, physicians, biologists), is extremely important.

Dr R. G. A. Sutton was elected Chairman, Professor G. Koromyslov Vice-Chairman and Dr M. B. Skirrow Rapporteur.

1. Introduction

For over 70 years infections caused by organisms that we now classify as campylobacters (formerly Vibrio fetus) have been known to occur in cattle, sheep, pigs and birds, and veterinarians are familiar with their role as a cause of abortion in sheep and cattle. However, it is only in the past decade, when simplified culture methods became available enabling the organisms to be isolated from animals, man and food, that C. jejuni emerged as an important cause of acute diarrhoeal diseases in humans throughout the world.

* The term Campylobacter jejuni is used in this document to include the closely related species C. coli except when the context renders it clear that each species is indicated separately.

** Director: Professor J. P. Butzler, St Pierre University Hospital, 322, Rue Haute, 1000 Brussels, Belgium (tel. 5380000 ext. 2530)

Unfortunately, food microbiologists and hygienists are still not very familiar with this pathogen though the WHO Scientific Working Group on Epidemiology and Etiology of Enteric Infections drew attention to the role of food in the transmission of C. jejuni and the Joint FAO/WHO Expert Committee on Food Safety included this organism in its list of causative agents of foodborne diseases in 1982.

The public health aspects of this infection were extensively discussed by a WHO Scientific Working Group¹ which noted in particular that "it will not be possible to understand fully the relationship between human and animal Campylobacter infections unless more work is done in this field".

There is no doubt that veterinary public health should and could contribute greatly to the prevention and control of this human infection. However, successful actions in this direction are restricted because the ecology of C. jejuni is still not clear. More information is needed on the survival of C. jejuni in the environment and in different foods of animal origin, rapid methods of detection and on practical preventive measures which might be taken in animal husbandry and in meat and milk processing plants. Therefore the purpose and objectives of the present Consultation were defined as follows:

- to review the problem of campylobacteriosis in different countries;
- to consider the role of animals and foods of animal origin in the epidemiology of this disease and review new data on the ecology of C. jejuni;
- to select the most suitable methods for the isolation of this organism from animals, foods, and environmental samples;
- to consider the most important and practical veterinary public health measures for the prevention and control of this foodborne disease in humans.

2. Clinical aspects

The clinical features of C. jejuni infection vary from a brief insignificant enteritis to an enterocolitis with abdominal pain and profuse diarrhoea. Watery diarrhoea seems to be more commonly associated with this infection in the developing countries. In severe cases, the stools contain fresh blood and pus, which suggests colo-rectal inflammation. Since the disease is usually self-limiting, antibiotics should be reserved for severe cases; it is certain that education and better hygiene have far greater roles to play in reducing these infections than have antibiotics.

The mechanisms by which C. jejuni causes disease are not yet known. The finding of dysenteric stools suggests that mucosal damage due to an invasive process analogous to that seen in shigellosis is important in pathogenesis. Thus Campylobacter colitis has to be considered in the differential diagnosis of acute colitis.

In-vitro studies have shown adhesions to and penetrations of epithelial tissue culture cells by C. jejuni. Penetration has also been observed in the intestinal epithelium of experimentally infected cattle and in the chorio-allantoic membranes of chick embryos. The significance of these findings has yet to be established, particularly in view of the failure of the organism to invade the guinea pig cornea (Séreny test). The frequent occurrence of profuse watery stools in many cases suggests that an enterotoxin may also be involved in pathogenesis. Preliminary studies have shown that C. jejuni also produces substances that are cytotoxic for tissue culture cells. The role of these toxins as virulence factors needs to be further investigated.

¹ Report of a Sub-Group of the Scientific Working Group on Epidemiology and Etiology "Enteric Infections due to Campylobacter, Yersinia, Salmonella, and Shigella", WHO/DDC/EPE.80.4.

With regard to the minimum infective dose of the organisms, a number of factors tend to suggest that it is about 10^5 . These factors include: 1) a study in which it was shown that only 500 organisms (in milk) were needed to initiate illness; 2) outbreaks did result from contaminated food vehicles (milk, poultry and water) despite the fact that the organism does not grow in such vehicles at ambient temperatures and atmosphere. Probably the infectivity of C. jejuni lies somewhere between shigellas (about 10^2) and salmonellas (about 10^8), but it must be emphasized that in addition to possible strain to strain variation, such variables as the type of food or drink containing the organisms, and certain host factors, e.g. the contents of the stomach may greatly influence the size of the infective dose.

3. Review of the recent trends in the epidemiology of Campylobacter infection in industrialized and developing countries.

3.1 Industrialized countries

Several species of Campylobacter are found in animals, but only C. jejuni and C. coli commonly infect man. In the UK, the laboratory isolations of these bacteria reported to the Communicable Disease Surveillance Centre numbered over 17,000 in 1983 as compared to about 14,000 Salmonella isolations. The incidence is also higher in rural than urban areas and in summer than winter months.

Campylobacter enteritis is a zoonosis: animals are the reservoir of infection. In industrialized countries, man is a relatively unimportant secondary reservoir for direct or indirect transmission. Humans are only a temporary source of infection as chronic carrier states have not been described.

A review of the data¹ from the developed countries on the isolation rates of thermophilic campylobacters from water and healthy animals and man indicate that they may be found in up to 53% of river water samples, 43% of cattle, 91% of poultry, 88% of pigs, 49% of dogs, 53% of cats but in only about 1.6% of man.

With regard to animal reservoirs C. jejuni has been found in almost every species of warm blooded animal, including exotic animals in zoos. C. jejuni is especially prevalent in birds; indeed wild birds almost certainly form the main natural reservoir of the organism. High carrier rates have been found in gulls and crows, especially those with access to refuse dumps in urban areas. But significant carriage has also been found among non-scavenging rural birds such as migratory waterfowl, pigeons, geese and rooks.

It appears that C. jejuni may form a normal part of the flora of birds, and counts of $10^4 - 10^7$ cfu/gram of faeces are common. The high optimum growth temperature of C. jejuni probably reflects an adaptation to birds which have high body temperatures.

Domestic animals and poultry are infected to a varying degree and they may carry the organism for long periods without apparent harm. C. jejuni can also cause abortion in sheep like C. fetus subsp. fetus.

Farmers, veterinarians, gamekeepers and kennel maids, in fact anyone engaged in animal husbandry, may acquire infection from direct contact with infected animals, especially animals that have diarrhoea. Killed animals or their products may also be a source of infection to slaughtermen, workers in poultry processing plants, and butchers. Housewives or other foodhandlers may acquire infection from handling raw animal products, especially poultry, in the kitchen.

Infection acquired from live animals in the home is virtually confined to close contact with a sick puppy or occasionally a kitten, though a statistical association of disease with the presence of caged birds in the home has been observed. There are very few reports of human infection resulting from contact with healthy dogs and cats, even if they are found to be excreting campylobacters.

¹ Proc. Int. Workshop on Campylobacter infection, London, 1982

Campylobacters of various sorts, including C. jejuni and C. coli, can be readily isolated from natural water: lakes, rivers, streams, and even inshore sea water. As they are always accompanied by faecal type coliforms, it is presumed that they originate from the intestinal tracts of animals. Thus, it is not surprising that the consumption of untreated or inadequately treated water has given rise to human infection.

Any raw meat from animals bred for consumption may be contaminated with campylobacters, but in practice there is a wide range of contamination depending on the type of animal and the history of the meat since slaughtering. For example, cattle, sheep and pig carcasses may show high rates of contamination in the abattoir immediately after slaughter, but by the time the meat has reached retail outlets contamination rates are low for reasons discussed below.

Almost all the parts of poultry carcasses are frequently contaminated with C. jejuni, whether fresh, chilled or frozen, and counts are often high - about 10^5 Campylobacter per/g of carcass. In one study on poultry end-products¹, 49% of carcasses, 73% of liver, 50% of stomachs and 65% of hearts were found to be contaminated. It is, therefore, not surprising that poultry constitutes the largest potential source of foodborne infection in man. It is interesting to note that preliminary serotyping surveys indicate considerable overlap in the spectrum of serotypes found in poultry and man.

Raw or inadequately heat treated milk has been repeatedly incriminated as a source of Campylobacter infection in countries such as the UK, USA and Canada. The dairy cows are apparently the source of the organisms which could get into the milk by faecal contamination at the time of milking or presumably by excretion in the milk from a cow with Campylobacter mastitis. Although Campylobacter mastitis has been produced, to date it has not been found as a naturally occurring entity. Anyhow, outbreaks have been caused by raw milk produced in model dairies where hygiene methods were adequate. Thus, good hygiene itself will not prevent milkborne infection; some form of heat treatment of milk is essential.

Flies caught in a chicken farm and piggery were found to have a high Campylobacter carrier rate compared with flies caught in domestic houses. Transmission of infection by flies is a possibility, particularly where food is prepared near animals, but the number of infections caused in this way is likely to be small.

3.2 Developing countries

Information on Campylobacter enteritis from developing countries is sketchy and it is more difficult to draw a clear picture of the epidemiology of the disease there.

In recent years Campylobacter enteritis has been reported from many countries: Africa (Algeria, Egypt, The Gambia, Nigeria, Rwanda, South Africa), Asia (Bangladesh, China, India, Indonesia, Saudi Arabia) and Latin America (Brazil, Chile, Costa Rica, Peru).

Several of these studies have shown a uniformly high detection rate of C. jejuni from the faeces of healthy persons, which is in striking contrast to observations in developed countries where asymptomatic infection is rare. Another enigmatic feature of the disease in these countries is that mixed infections with other bacterial, viral or protozoal pathogens are much more common with C. jejuni than with other pathogens - mixed infections have been observed in about 20% of cases in China and 59% of cases in Bangladesh.

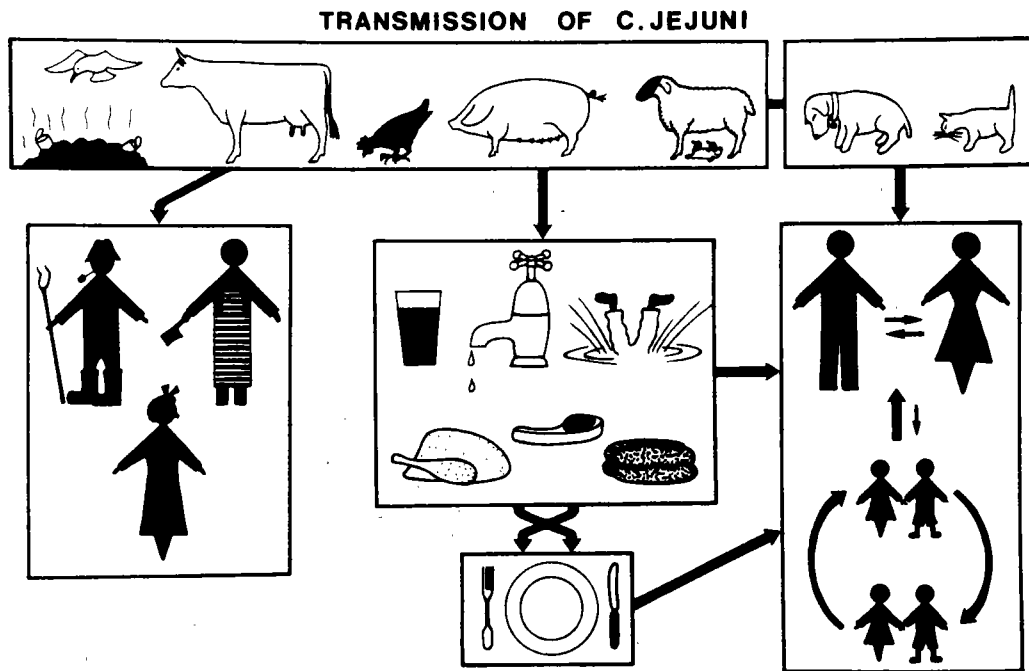
¹ Oosterom et al (1983). In: Food Prot. 46(4), 339-344.

The investigation of Campylobacter enteritis in South Africa, Bangladesh, The Gambia, Indonesia, China and India has highlighted the prevalence of this infection among children. As age increases, fewer infections are associated with symptoms until in adults the disease is rarely seen. This suggests the progressive acquisition of immunity, but little is known about the degree of exposure necessary to bring this about or whether the maintenance of immunity depends on continuing exposure. No sex-specific prevalence has been documented.

Reports on reservoirs of C. jejuni in developing countries are few. In a survey conducted in South Africa and India, fowl faeces showed extremely high isolation rates of C. jejuni and a large number of dogs were found to harbour this organism. It was suggested that the high incidence of this disease in Soweto could be due to the substantial consumption of fowls, bovine tripe and intestines by the local inhabitants.

In developed countries the disease is primarily acquired by consumption of food of animal origin derived from infected animals or by cross-contamination during processing. The transmission in developing countries appears to be predominantly through improper handling of food and water contaminated by faecal material from sick persons and animals or by direct or indirect contact with them or their faeces. Thus, environmental contamination appears to be an important determinant influencing the spread of infection in developing countries, particularly among the poorer sectors where domestic cattle and fowl are frequently housed in close contact with humans.

The diagram below illustrates some of the ways that C. jejuni commonly circulates among animals, birds and man.



Explanation to the diagram: At the top are common animal reservoirs and sources of C. jejuni and C. coli (the gull is feeding on a rubbish tip and the sheep has just given birth to a C. jejuni infected stillborn lamb). On the left is portrayed direct transmission to occupational groups (farmers, butchers, poultry processors) and on the right, direct transmission among persons in contact. The centre panel shows indirect transmission through milk, water and meat, with cross-contamination to other foods below.

4. Ecology of Campylobacter jejuni: survival in the environment and food of animal origin

As a general rule the common food poisoning organisms either multiply in food to numbers sufficient to cause infection (e.g., Salmonellae, Vibro parahaemolyticus, Clostridium perfringens) or produce a toxin in the food associated with the disease (e.g., Staphylococcus aureus or Clostridium botulinum). Multiplication due to storage conditions favourable for growth, is therefore an important element in the epidemiology of these diseases. However, this is not necessarily the case with campylobacteriosis - a factor which sets it apart from other foodborne enteric infections.

That C. jejuni requires specific conditions for growth, has been well documented. In the laboratory the organism has been shown to require strict microaerophilic conditions - an atmosphere containing 5% oxygen, usually with about 8-10% CO₂ and 85% N₂, is most favourable for growth. Moreover, the range of temperatures at which the organism grows is narrower than other enteric pathogens. A number of studies have shown that the organism grows poorly, if at all, below 30°C or above 47°C. The optimum temperature is 42° - 45°C. Not only does the organism fail to grow at normal ambient temperatures, it dies off rapidly when stored at 15 - 25°C. It favours a roughly neutral pH (6.5 - 7.5) although it has been shown to grow at as high as 8.0 and as low as 4.8. As with other organisms, variations between strains occur in relation to all of these growth conditions. It is not surprising, therefore, that in most instances growth in foods is usually poor.

Nevertheless, some growth can occur in red meat with its relatively high pH of about 6.4. It has also been observed that in the early stages of growth on high pH meat, C. jejuni was not inhibited by other organisms, although when spoilage due to other organisms reached an advanced stage, C. jejuni died off progressively over 2-3 days.

C. jejuni has also been observed to grow in minced meat and sliced chicken. Therefore, although the opportunities for growth of C. jejuni are limited, it should not be assumed that conditions that might enable some growth do not occur as multiplication may occur in slowly cooling poultry, or barbeque chickens held in "warming ovens".

In cows' milk at a temperature of 4°C, the organisms can survive for up to 3 weeks but at a temperature of 25°C they die in 3 days. They die within 1 minute when heated at 60°C in skimmed milk. Therefore, the process of pasteurization of milk is effective in killing this pathogen. In milk products, such as soft cheeses, C. jejuni died off very rapidly.

A sharp reduction in the number of campylobacters takes place during cooling and freezing of meat depending upon the way in which these processes are performed. However, once frozen, campylobacters are preserved for several months at -20°C. Low temperatures extend the survival of the organisms in meat of cattle and pigs, e.g. in minced pork the bacteria survive 72 hours of storage. Thus, Campylobacter infections may occur after the consumption of such ground meat if not cooked properly.

Campylobacters are sensitive to drying and may die overnight on surfaces and equipment in meat processing plants, slaughterhouses and other food enterprises. In soil and dung they can survive up to 30 days. In autoclaved stream water C. jejuni survived at a temperature of 4°C for 33 days, but for much shorter periods as the temperature increased.

Although C. jejuni does not grow well at normal oxygen concentrations, it is not readily killed in contact with air. Again, although C. jejuni does not grow in the presence of 2% sodium chloride, some strains have been shown to survive for 24 hours in solutions containing 30% sodium chloride. However, it is still not clear whether C. jejuni can survive for extended periods in salt concentrations commonly used in food processing. Sodium nitrite also has a destructive effect on C. jejuni, especially in combination with freezing at -20°C. The addition of glucono-delta-lacton (0.4%) to

minced meat also causes a reduction in the number of organisms. These observations indicate that disinfectants and the regimens for their use which are normally applied in food processing plants and animal husbandry are highly effective for the control of C. jejuni and could destroy organisms in 5 minutes.

5. Methods for detection of C. jejuni in food animals and food of animal origin.

It is recognized that the procedures for isolating C. jejuni from animal derived foods described below may not be appropriate for many laboratories in the developing countries and that considerable research is needed to further develop more simplified technologies for the isolation of C. jejuni from foods and other environmental specimens.

The procedures for detecting C. jejuni in foods and food animals were considered under four headings: (i) specimen handling and storage conditions; (ii) enrichment procedures; (iii) selective plating media, and (iv) microaerobic conditions.

Specimen handling and storage

Because of the known susceptibility to the environment at ambient temperatures, the procedures for sampling, transporting, and preserving specimens are extremely important. Hence, improper handling of specimens before testing often negates the value of a sensitive isolation procedure.

C. jejuni is best stored at 4°C in oxygen-free medium containing 0.01% sodium bisulfite. Under these conditions, bacteria will survive 10 times longer than when held in a bisulfite-free medium exposed to air at 25°C.

Milk samples, which are to be transported and assayed at a later date, would best be held refrigerated (4°C) after the addition of 0.01% sodium bisulfite and either 0.15% sodium thioglycollate or in an atmosphere of 100% N₂.

Ideally meat and solid food specimens should be refrigerated and stored in an atmosphere of 100% N₂. However, a more practical approach is to store meat samples at 4°C with an equal amount of modified Cary-Blair medium (0.16% agar) which results in little change in C. jejuni viability during 14 days of storage.

As with foods, recovery of C. jejuni from animal faecal specimens is greatest when samples are held at 4°C. It is advisable to refrigerate faecal specimens or rectal swabs immediately after they are obtained when, even without a transport medium, they are satisfactory for up to 3 days. If they are to be held for longer than 3 days, suspending faecal specimens and rectal swabs in the modified Cary-Blair medium and storing at refrigeration temperature is recommended.

Enrichment procedures

Enrichment methods are essential for the isolation of C. jejuni from food and environmental samples. They are also desirable for faecal samples from animals and man without diarrhoea. Several enrichment procedures for isolating C. jejuni from food of animal origin are available and a comprehensive study is needed to compare the efficacy of these existing procedures. However, at the present time, the direct enrichment procedure, given in Annex II, has been shown to be effective for recovering C. jejuni from chicken skin, raw milk, ground meat, and from other foods of animal origin when present in small numbers.

Selective plating media

Many satisfactory media are in use (e.g. Skirrow's, Butzler's, Blaser-Wang's, Preston agar). Recently developed media are: (i) blood-free selective medium developed in Preston - in developing countries where blood is sometimes difficult to obtain, the new

Preston blood-free medium offers an advantage¹; (ii) Butzler medium Virion², which is especially suitable for use in candle jar conditions.

Microaerobic conditions

Special attention was given to the microaerobic growth requirements of C. jejuni. An evacuation-replacement procedure in which the atmosphere in an anaerobic jar is replaced with a gas mixture of 5% O₂:10% CO₂:85% N₂ was considered optimal for this purpose. However, in countries where this is not practical, or is too expensive, the use of the traditional candle jar will give satisfactory results. Similarly, although the optimum incubation temperature is 42°C, satisfactory results can be obtained using Butzler's Virion medium incubated at 37°C.

6. Special preventive and control measures

6.1 In animal husbandry

Prevention of any zoonotic disease is difficult, particularly when the causative agent is as widely distributed among animals and the environment as C. jejuni. Although few studies have been done to prove or disprove that immunity is acquired as a result of repeated infection, the wide range of serotypes found to date suggests that a vaccine would be neither technically effective nor economically feasible in controlling the disease. Attention has, therefore, been focussed on breaking the chain of transmission as a means of control.

The epidemiology of Campylobacter infection is, in general, similar to that of Salmonella. Therefore, the approaches and difficulties encountered in the control of campylobacteriosis in animal husbandry are similar to those experienced with salmonellosis.³ It is also obvious that procedures for the control of Campylobacter infection should be included in the general control programme for other enteric pathogens of a zoonotic nature.

Control measures are facilitated by the fact that C. jejuni can rarely multiply outside the animal's body. However, hygienic measures in animal husbandry, such as permanent cleaning and disinfection of stables, cannot alone prevent a possible dissemination of bacteria regularly excreted with faeces. It could, however, decrease the risk of infection of animals through a contaminated environment.

The ideal form of control, which is difficult to implement, is the gradual establishment of Campylobacter-free farms. This depends on the definition and control of the sources of infection on a farm, which poses practical and economic difficulties. Control will cost money, but the benefits resulting from healthier stock may ultimately prove profitable. Cost/benefit studies should be carried out. In this connexion, the fact that some chicken flocks remain free from Campylobacter infection suggests that properly housed poultry could be protected without too much extra cost. The establishment of Campylobacter-free farms has to be a long-term sanitation programme. Together with the control of latent Salmonella infections this will be one of the main tasks of the veterinary public health service in the future.

¹Bolton et al (1984), Journal Clinical Microbiology, 19, 169-171.

²Goossens et al (1983), Eur. J. Clin. Microbiol., 2, 389-394.

³WHO Guidelines on Prevention and Control of Salmonellosis, Document VPH/83.42, Geneva, 1983

6.2 In the meat and dairy processing industry

The need for enforcement of strict hygienic rules during slaughter of animals and further processing of meat, to prevent cross-contamination was emphasized. Proper hygienic handling of meat as prescribed in various meat hygiene regulations and in international guidelines, e.g. those of the FAO/WHO Codex Alimentarius Commission, is considered highly important, especially in view of the current voluminous international trade in meat and meat products.

In the case of pigs, contamination of their carcasses during the slaughtering process originate not only from the animals' intestines but also from surfaces, equipment and utensils in the slaughter hall. Contamination could be significantly reduced during cooling of the carcasses, especially with forced ventilation, which usually takes 16-24 hours (Table 1). This procedure is a normal part of pig processing in many countries and should be considered as an important tool for prevention of the spread of the microorganism.

Table 1. Number of Campylobacter jejuni on the skin of pig carcasses during cooling (per cm² of skin, Log₁₀)¹

Time (°C) (hours)	Cooling		Resulting temperature
	With ventilation (rel. humidity 60-70%)	Without ventilation (rel. humidity 95%)	
0	3.51	3.52	30.5
1	3.11	3.57	19.5
3	2.40	3.11	12.5
6	2.36	3.15	9.5
24	1.00	3.18	4.0

The same approach is also valid for cattle carcasses. The relatively low contamination of these carcasses compared with poultry carcasses may be explained by the fact that they are also air chilled in the same manner as pig carcasses in accordance with technological requirements. This procedure significantly decreases the surface contamination of the carcasses.

It was observed that cross-contamination of poultry carcasses takes place especially during defeathering and evisceration of the birds. The policy of cooling poultry by means of a spin chiller may also cause massive cross-contamination. After scalding at 58°C, C. jejuni contamination can be significantly reduced, but not eliminated. Scalding at 52°C appears to have little decontaminating effect.

It is also obvious that during poultry or animal slaughtering processes, Campylobacter cross-contamination can be significantly decreased by cleaning and disinfection. Adequate chlorination of waste water is an additional means of preventing further dissemination of the bacteria. Apart from hygienic measures, the decontamination of end-products might be considered; at the moment, treatment with lactic acid or irradiation of meat offer the most promise.

¹J. Oosterom et al. (1983). J. Food Prot. 46(8), 702-706

Less complicated are the conditions in milk processing plants because C. jejuni cannot survive pasteurization or other accepted forms of heat treatment e.g. ultraheat treatment (UHT). These methods are therefore recommended as the most important means for control of milkborne campylobacteriosis as well as other milkborne bacterial diseases. However, the fact that proper heat treatment is an effective and safe method of terminal decontamination should not be taken as licence to neglect normal standards of hygiene and disinfection in dairy practice. As long as it is still legal in some countries to sell raw milk it is all the more necessary to insist on these standards for such dairies.

6.3 Preventive and control measures in food establishments

The butchers' shops, kitchens for mass catering (canteens, aviation catering, etc.), hotels and restaurants can play a very important role in the prevention and control of C. jejuni infection in humans. In this connexion, reference was made to the comprehensive WHO guidelines which deal with hygiene in food establishments.^{1, 2, 3} The principles and recommendations contained in these publications would go a long way towards preventing foodborne campylobacteriosis. Simple and safe hygienic rules presented in the Recommended International Code of Practice¹, should always be followed in food establishments. In particular, they include hygienic requirements for food establishments (design and facilities), maintenance of hygiene in the establishments, personal hygiene and health requirements for food handlers, and hygienic processing requirements, including the process of cooking and storing ready-to-eat foods.

Since food of animal origin is a frequent source of Campylobacter infection, the above-mentioned principles and requirements are especially important for those establishments which handle raw meat and raw milk.

Although it is difficult to reach the housewives, they need to be made aware of the magnitude of the problem of Campylobacter infections and of the importance of hygienic food handling in order to combat foodborne diseases in general. Specific educational programmes appropriate for different situations should be developed at a local level for this purpose. Of particular importance in this connexion is that sufficient attention be devoted to time/temperature control during the cooking and cooling procedures, as well as the need to prevent the final product from becoming recontaminated with microorganisms from raw foods.

It is essential that good hygienic practice at home becomes a habit. Any food, even though free of campylobacters, may cause other types of foodborne diseases if handled unhygienically. The general public needs to be made aware of the importance of food hygiene and this should be included in the general health education of school children. Opinion formers such as parents, teachers, religious and social leaders and health workers should especially endeavour to teach hygiene by example as well as by precept. The basic principles of food hygiene which are presented in the above-mentioned documents will also be valid and useful for this educational purpose, in particular those which deal with exclusion of domestic animals from living quarters and specially from food producing areas, frequent washing of hands, personal cleanliness etc.

¹ General Principles of Food Hygiene, CAC/vol. A, Ed. 1, 1979.

² WHO Guide to Hygiene and Sanitation to Aviation, 1977, Geneva.

³ Mass Catering, WHO Regional Publications, European Series No. 15, 1983.

7. Conclusions and recommendations

1. C. jejuni enteritis is primarily a zoonotic disease which has a great public health significance for developed and developing countries. In developed countries, the disease is almost exclusively transmitted by foods of animal origin, but in developing countries the limited amount of available evidence indicates that it may be mainly transmitted through faecal contamination of food and water or by close contact with a sick man or animal.
2. C. jejuni unlike other so-called food poisoning organisms, grows poorly in food, even when stored at elevated (35-45°C) temperatures. At ambient temperatures and atmosphere the organism dies off rapidly although it will survive for longer periods in refrigerated (days) and frozen (months) food.
3. For optimum results the isolation of C. jejuni in the laboratory requires elevated temperature (42°C), a microaerobic atmosphere (5%O₂:10%CO₂: 85%N₂) and highly selective media. However, for those laboratories without access to a 42°C incubator or special gas-generating facilities, satisfactory results can be obtained using the traditional candle jar incubated at 37°C, though this procedure may be 5-10% less sensitive.
4. Because only a small number of C. jejuni may be present in foods the procedure for isolation from food requires an enrichment technique.
5. C. jejuni almost always comes from the faeces. Therefore, in many situations, the probability of its presence may be assessed by examination of materials for the presence of Enterobacteriaceae. The isolation and enumeration of Enterobacteriaceae are less laborious and need less sophisticated equipment than for C. jejuni. This principle is already accepted for Salmonella studies.
6. Preventive measures in animal husbandry should be directed at the raising and fattening of Campylobacter-free slaughter animals. For this purpose, it is necessary to have available Campylobacter-free parent animals, Campylobacter-free animal feeds and sties that can be isolated hygienically from the environment. Strict hygienic practices will also be required for the transport of animals.
7. Recycling of animal waste products, in particular raw frozen animal waste feeds given to pet animals, may constitute a health hazard for, e.g. dogs and subsequently man. This needs further investigation.
8. During slaughter of animals and processing of meat it is obligatory to follow strict rules of hygiene to prevent cross-contamination. More attention should be paid to development of new, more hygienic slaughter techniques and equipment.
9. Decontamination of pig, beef and poultry carcasses is an important tool in prevention of campylobacteriosis. For this purpose, lactic acid and irradiation could be used as well as employing cooling of the carcasses with intensive aeration.
10. Adequate education of housewives, kitchen staff in restaurants, hospitals, old people's homes, etc., for prevention of campylobacteriosis should be encouraged. For developing countries such educational efforts will have a wider perspective. These programmes should include among others general measures such as proper disposal of excreta of animals and man of all ages, safe water supply, need for keeping out domestic animals from kitchen, dining and living areas, personal cleanliness and frequent hand-washing. Special attention should be drawn to the importance of proper heating and/or cooling of foods and to the prevention of cross-contamination to those ready for consumption to ensure food safety.

11. Close cooperation should be promoted between the veterinary and the medical professions for the successful elaboration and implementation of the national programmes on prevention and control of campylobacteriosis, as well as other human enteric infection of zoonotic origin.

8. Recommendations for future research

Epidemiology and immunity

1. There is need for more information on the epidemiology of C. jejuni infections in developing countries. Such research should include:
 - longitudinal studies (including serotyping) in which children are studied from birth to 5 years of age;
 - case control aetiology studies using the WHO protocol for multicentre studies which must include the entire spectrum of bacterial, viral and protozoan enteropathogens so as to evaluate the role of "mixed" infections;
 - studies designed to determine the relative importance of the environment (including household animals), of food and water and of intrafamily spread in the epidemiology of the disease.
2. More work needs to be carried out in both developed and developing countries on the seasonal incidence of Campylobacter infection to determine whether seasonal peaks are temperature-related or due to other factors, e.g. humidity, changes in food consumption patterns, etc.
3. More information is needed on the development of immunity to Campylobacter infections in man and animals. Evidence suggests that immunity does occur in human beings continually exposed to the organism such as poultry processors and persons living in developing countries where the organism is frequently found in the environment.
4. There is still a need for more data on the minimum infective dose of C. jejuni, including a study of variations that might occur from strain to strain and with different food vehicles and indeed whether all strains can cause disease.
5. There is a need for the development of an animal model that can be used to study the pathogenesis of the disease, the nature and role of protective immunity and to determine whether strains differ in their virulence.
6. The development of resistance to multiple antibiotics has been observed in Salmonella strains particularly from antibiotic-fed animals. Studies are needed to determine whether similar resistance develops in C. jejuni strains.
7. More research is needed in developing countries on the incidence of C. jejuni in raw foods and in prepared foods immediately before consumption.
8. There is a need for further research on the multiplication of C. jejuni in foods, and especially poultry, under specific conditions of pH, temperature and atmosphere.

Laboratory methods

1. Although methods are available for the isolation of C. jejuni from food and the environment, they are often complicated and expensive. More simple procedures are needed that can be used in developing countries and in field conditions.
2. More research is needed on simple inexpensive means of transporting and storing specimens and cultures of C. jejuni.
3. There is need for the further development and standardization of serotyping, biotyping, and phage typing schemes for C. jejuni.
4. There is need for the identification of virulence factors of C. jejuni, including the development of suitable laboratory methods that can be used to detect such "markers".

Prevention and control

1. It is necessary to develop appropriate approaches and methodologies for educating housewives, school children and other community members in developed and especially in developing countries to make them aware of the problem due to campylobacteriosis as well as on what could be done by them to reduce the risks. Such messages should be broad and must include all similarly transmitted enteric infections.
2. More data are needed on ways of controlling and reducing the number of organisms present at different stages of the food chain (beginning from live animals and ending with ready-to-eat foods). Proper assessment of these methods as well as of existing decontamination procedures (e.g. irradiation, lactic acid, air cooling) should be investigated.
3. Microbiological veterinary research on the role of animals as important reservoirs of human campylobacteriosis should be promoted, as well as the methods on prevention and control of the disease in animals.

ANNEX I

ANNEX I

LIST OF PARTICIPANTS

- Professor J. P. Butzler, Director, WHO Collaborating Centre for Campylobacter jejuni,
St Pierre University Hospital, 322, Rue Haute, 1000 Brussels, Belgium
- Professor M. P. Doyle, Department of Food Microbiology and Toxicology, University of
Wisconsin, 1925 Willow Drive, Madison WI 53706, USA
- Professor D. Grossklaus, President, World Association of Veterinary Food Hygienists,
Löhleinstrasse 23, 1000 Berlin (West)
- Professor G. Koromyslov, Director, All-Union Institute of Experimental Veterinary Medicine,
Kuzminki, VIEV, 109472 Moscow USSR (Vice Chairman)
- Dr Daya Nzuempb, Department of Agriculture, Laboratoire Vétérinaire, BP 8842, Kinshasa,
Zaire*
- Dr J. Oosterom, Head of the Section Bacterial Zoonoses, National Institute of Public
Health and Environmental Hygiene, Antonie van Leeuwenhoeklaan 9, Postbus 1,
3720 BA Bilthoven, The Netherlands
- Dr S. C. Pal, National Institute of Cholera and Enteric Diseases, WHO Collaborating Centre
for Research and Training in Diarrhoeal Diseases, P.33 C.I.T. scheme X M, Beliaghata,
Calcutta 700 010, India
- Professor V. Pokrovsky, Central Research Institute of Epidemiology of the USSR, Ministry
of Public Health, Novogireevskaya 3a, Moscow, USSR
- Dr M. B. Skirrow, Department of Pathology (Microbiology), Worcester Royal Infirmary,
Castle Street Branch, Worcester WR1 3AS, UK (RAPPORTEUR)
- Dr R. G. A. Sutton, Macquarie Pathology Services, 17 Moore Street, Leichhardt, New South
Wales, 2040 Australia (CHAIRMAN)

Secretariat

- Dr D. Barua, Consultant, Diarrhoeal Diseases Control Programme, World Health Organization,
Geneva, Switzerland
- Dr A. Koulikovskii, Food Hygienist, Veterinary Public Health, Division of Communicable
Diseases, World Health Organization, Geneva, Switzerland
- Professor N. Skovgaard, The Royal Veterinary and Agricultural University, 13 Bülowsvej,
DK-1870 Copenhagen V. Representing the World Health Organization, Regional Office for
Europe, Copenhagen, Denmark

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* Unable to attend

ANNEX II

METHODS FOR DETECTION OF CAMPYLOBACTER JEJUNI
IN FOOD OF ANIMAL ORIGIN

A. Procedure for isolating C. jejuni from animal-derived foods

1. Specimen handling

a. Food specimens should be refrigerated (not frozen) immediately after sampling and, if possible, held in an atmosphere of 100% nitrogen.

b. Raw milk samples should, in addition to being held refrigerated and in an atmosphere of 100% nitrogen, be supplemented with 0.01% sodium bisulfite. If a means of maintaining samples in a nitrogen atmosphere is not available, 0.15% sodium thioglycollate should also be added to the milk.

2. Enrichment procedure. The following procedure is recommended:¹

a. Enrichment broth is composed of brucella broth, 7% lysed horse blood, 0.3% sodium succinate, 0.01% cysteine hydrochloride, vancomycin (15 ug/ml), trimethoprim (5 ug/ml), polymyxin B (20 IU/ml), and cycloheximide (50 ug/ml)

b. Food specimen (25 g) is added to 100 ml of enrichment broth in a 250-ml stoppered sidearm Erlenmeyer flask. If the specimen is solid, it is macerated in the broth by stomaching for 15 sec before being transferred to the flask.

c. The flask is evacuated three times to 500 mm Hg (below atmospheric pressure) and replaced with an atmosphere of 5% O₂:10% CO₂:85% N₂.

d. The flask is shaken (100 gyrations/min) at 42°C for 16 to 18 h.

3. Isolation procedure

a. After incubation, 0.1 ml of enriched broth and 0.1 ml each of two serial dilutions (1:10 1:100) of enrichment broth are plated separately onto Blaser-Wang's (Campy-BAP) selective agar.

b. Plates are incubated at 42°C in a microaerobic atmosphere for 48 h. The microaerobic atmosphere may be obtained by the evacuation/replacement method as above or by using commercially available gas generating systems (such as BBL Campy Pak II or Oxoid BR56 envelopes). The candle jar technique is not suitable for culturing C. jejuni from food or environmental samples.

4. Identification

a. Characteristic Campylobacter colonies on Blaser-Wang's selective agar plates are grey (some tan or slightly pink), nonhemolytic, small, and mucoid.

b. A wet-mount slide of a typical colony should be prepared and observed by phase-contrast microscopy. Cells having vibroid morphology (comma, "S", gull, or spiral shapes) and corkscrew, darting movements can be presumptively identified as campylobacters.

c. The following tests will further narrow the identity of isolates to C. jejuni, C. coli, or C. laridis.

¹ Doyle, M. P. & Roman, D. J. (1982), Appl. Environ. Microbiol. 44, 1154-1158

- (1) Growth at 42°C but not at 25°C.
- (2) Catalase and oxidase-positive.
- (3) Production of H₂S detected by a lead acetate strip over a medium containing 0.02% cysteine-HCl (TSI agar not suitable).
- (4) Growth in 1% glycine.
- (5) No growth in 3.5% NaCl.
- (6) No production of acid from glucose.
- (7) Reduction of nitrate to nitrite.
- (8) Resistance to cephalothin (30 ug disc).

B. Procedure for isolating C. jejuni from faecal specimens of food animals

1. Specimen handling

- a. Faecal specimens should be refrigerated (not frozen) immediately after sampling. Samples should be obtained within 1 h after defaecation; if not possible, specimens may be suspended in cold (4°C) alkaline peptone water to be assayed within 3 days. If specimens are to be held for longer, they should be suspended in cold Cary-Blair medium with 0.16% agar.
- b. Rectal swabs may be suspended in cold alkaline peptone water (3 ml) or Cary-Blair medium with 0.16% agar and should be refrigerated until assayed. Samples should preferably be assayed the same day they are collected.

2. Enrichment procedure

Results of several studies indicate that the enrichment procedure of faecal specimens substantially increases isolation rates of C. jejuni. Until studies are done to compare and fully evaluate the efficacy of enrichment procedures for food animal faecal specimens, no one enrichment procedure can be recommended. Of the enrichment methods that have been published, the procedures of Bolton et al.¹ and Doyle and Roman² appear to have the greatest potential. Because of the small sample size (cotton-tipped swab soaked in a faecal suspension) used in the Bolton et al. procedure, the procedure of Doyle and Roman (which uses a 25% sample) is likely to be more sensitive.

3. Direct plating

Although direct plating is not nearly as sensitive for isolating C. jejuni as an enrichment procedure, direct plating is satisfactory for faeces containing large numbers of them.

- a. Faecal suspension (0.1 ml) is streak plated onto modified Blaser-Wang's supplemented with 50 ug of cycloheximide/ml to suppress mould growth) and/or Preston selective agars. Skirrow's medium and Eutzler's medium may also be used.
- b. Plates are incubated at 42°C in a microaerobic atmosphere for 48 h (as described above in Section A.3.b.).
- c. Characteristic Campylobacter colonies on Blaser-Wang's media are identified as above (Section A.4.).

¹ Bolton et al (1983). J. Clin. Path., 36, 78

² Doyle, M. P. & Roman, D. J. (1982). Appl. Environ. Microbiol., 43, 1343

