CHAPTER 2.9.3.

CAMPYLOBACTER JEJUNI AND CAMPYLOBACTER COLI

SUMMARY

Definition of the disease: Campylobacter jejuni and C. coli can colonise the intestinal tract of most mammals and birds and are the most frequently isolated Campylobacter species in humans with gastro-enteritis. Transmission from animals to humans is mainly through consumption and handling of animal food products but also direct contact with colonised animals may contribute to human campylobacteriosis. This chapter focuses on C. jejuni and C. coli in primary livestock production with regard to food safety.

Description of the disease: Campylobacter jejuni and C. coli do not cause clinical disease in adult animals except for sporadic cases of abortion in ruminants and very rare cases of hepatitis in ostriches. The faecal contamination of meat (especially poultry meat) during processing is considered to be a major source of human food-borne disease. In humans, extraintestinal infections, including bacteraemia, can occur and some sequelae of infection, such as polyneuropathies, though rare, can be serious.

Identification of the agent: In mammals and birds, detection of intestinal colonisation is based on the isolation of the organism from faeces, rectal swabs and/or caecal contents. Campylobacter jejuni and C. coli are thermophilic, Gram-negative, highly motile bacteria that, for optimal growth, require microaerobic environment and incubation temperatures of 37–42°C. Agar media containing selective antibiotics are required to isolate these bacteria from faecal/intestinal samples. Alternatively, their high motility can be exploited using filtration techniques for isolation. Enrichment techniques to detect intestinal colonisation are not routinely used. Preliminary confirmation of isolates can be made by light microscopy. The organisms in the log growth phase are short and Sshaped in appearance, while coccoid forms predominate in older cultures. Under phase-contrast microscopy the organisms have a characteristic rapid corkscrew-like motility. Phenotypic identification is based on reactions under different growth conditions. Biochemical and molecular tests can be used to confirm various Campylobacter species. Polymerase chain reaction assays also can be used for the direct detection of C. jejuni and C. coli.

Serological tests: serological assays are not routinely in use for the detection of C. jejuni/C. coli colonisation.

Requirements for vaccines and diagnostic biologicals: There are no effective vaccines available for the prevention of enteric Campylobacter infections in birds or mammals.

A. INTRODUCTION

1. Disease

Campylobacter jejuni and *C. coli* are generally considered commensals of livestock, domestic pet animals and birds. Large numbers of *Campylobacter* have been isolated from young livestock, including piglets, lambs and calves, with enteritis, but the organisms are also found in healthy animals. Outbreaks of avian hepatitis have been reported, but the pathogenic role of *Campylobacter* spp. is unclear. One possible exception is ostriches where *Campylobacter*-associated death and enteritis occurs in young birds. Campylobacter are the main cause of human bacterial intestinal disease identified in many industrialised countries (24). Over 80% of cases are caused by *C. jejuni* and about 10% of cases are caused by *C. coli*. In humans, *C. jejuni/coli* infection is associated with acute enteritis and abdominal pain lasting for 7 days or more. Although such infections are

generally self-limiting, complications can arise and may include bacteraemia, Guillain–Barré syndrome, reactive arthritis, and abortion (21). The primarily source of *C. jejuni/coli* infections in human is believed to be the handling and/or consumption of contaminated meat, especially poultry meat. However, contact with pets and livestock, the consumption of contaminated water or raw milk and travelling in high prevalence areas are also considered risks factors in human disease (8). The control of *Campylobacter* in the food chain has now become a major target of agencies responsible for food safety world-wide.

2. Taxonomy

In 1991 a revision of the taxonomy and nomenclature of the genus *Campylobacter* was proposed. According to Bergey's Manual, the genus *Campylobacter* comprises sixteen species and six subspecies. More recently, two additional species have been proposed (25). Members of the genus are typically Gram-negative, non-spore-forming, S-shaped or spiral shaped bacteria (0.2–0.8 µm wide and 0.5–5 µm long), with single polar flagella at one or both ends, conferring a characteristic corkscrew-like motility. These bacteria require microaerobic conditions, but some strains also grow aerobically or anaerobically. They neither ferment nor oxidise carbohydrates. Some species, particularly *C. jejuni, C. coli* and *C. lari*, are thermophilic, growing optimally at 42°C. They can colonise mucosal surfaces, usually the intestinal tract, of most mammalian and avian species tested. The species *C. jejuni* includes two subspecies (*C. jejuni* subsp. *jejuni* and C. *jejuni* subsp. *doylei*) that can be discriminated on the basis of several phenotypic tests (nitrate reduction, selenite reduction, sodium fluoride, and safranine) and growth at 42°C (subsp. *doylei* does not grow at 42°C) (9). Subspecies *jejuni* is much more frequently isolated then subspecies *doylei*.

B. DIAGNOSTIC TECHNIQUES

1. Isolation and identification of the agent

Two ISO (International Organization for Standardization) procedures for detection of *Campylobacter* exist, a horizontal method for detection of thermotolerant *Campylobacter* in food and animal feeding stuffs (10) and a procedure for the isolation of *Campylobacter* from water (11). However, neither of these standard methods may be optimal for the isolation of campylobacters from live animals. An appendix to ISO 10272 on this topic is currently being developed.

a) Collection of samples

i) Poultry at the farm

Poultry is frequently colonised with *C. jejuni* (65–95%) less often with *C. coli* and rarely with other *Campylobacter* species (16). Colonisation rates in broiler chickens are age-related. Most flocks are negative until 2 weeks of age. Once *Campylobacter* colonisation occurs in a broiler flock, transmission, via coprophagy, is extremely rapid and up to 100% of birds within a flock can become colonised within 72 hours. Samples from live birds, destined for the food chain, should therefore be taken as close to slaughter as possible (16). The majority of birds shed large numbers of organisms (>10⁶ colony-forming units/g faeces). Campylobacters can be isolated from fresh faeces/caecal droppings or cloacal swabs. For reliable detection of *Campylobacter* by culture, freshly voided faeces (preferably without traces of urine) should be collected. Such samples must be prevented from drying out before culture. When swabs are used, a transport medium such as Amies, Cary Blair or Stuart must be used.

ii) Cattle, sheep and pigs at the farm

Campylobacters are frequent colonisers of the intestine of livestock such as cattle, sheep and pigs (2, 26, 27). Cattle and sheep are found to be colonised mainly with *C. jejuni, C. coli, C. hyointestinalis*, and *C. fetus*, whereas pigs are predominantly colonised by *C. coli*. In young animals, the numbers are higher than in older animals. In older animals, the organisms can be intermittently detected in faeces, probably due to low numbers or due to intermittent shedding. Fresh samples have to be taken (rectal samples if possible) and they should be prevented from drying out. When swabs are used, a transport medium (like Amies, Cary Blair or Stuart) <u>must</u> be used.

iii) At slaughter

In poultry, the caeca are usually used for the detection of *Campylobacter*. They can be cut with sterile scissors from the remaining part of the intestines and submitted intact to the laboratory in a plastic bag or Petri-dish.

Samples from cattle, sheep and pigs are collected from the intestines by aseptically opening the gut wall or by taking rectal swabs.

b) Transportation and treatment of samples

i) Transport

Campylobacters are remarkably sensitive to environmental conditions, including dehydration, atmospheric oxygen, sunlight and elevated temperature. Transport to the laboratory and subsequent processing should therefore be as rapid as possible, preferably the same day, but within at least 2 days. The samples must be protected from light.

No recommendation on the ideal temperature for transportation can be made, but it is clear that freezing or high temperatures can reduce viability. High temperatures (>20°C), low temperatures (<0°C) and fluctuations in temperature must be avoided. When the time between sampling and processing is longer, storage at 4°C (\pm 2°C) is advised.

ii) Transport media

Swabs: When samples are collected in swabs, the use of commercially available transport tubes, containing a medium, such as Amies, is recommended. This medium may be plain agar or charcoal-based. The function of the medium is not for growth of *Campylobacter* spp., but to protect the swab contents from drying and the toxic effects of oxygen.

When only small amounts of faecal/caecal samples can be collected and transport tubes are not available, shipment of the specimen in transport medium is recommended. Several transport media have been described: Cary-Blair, modified Cary-Blair, modified Stuart medium, Campythioglycolate medium, alkaline peptone water and semisolid motility test medium. Good recovery results have been reported using Cary-Blair (13, 20).

iii) Maintenance of samples

On arrival at the laboratory, samples should be processed as soon as possible, preferably on the day of arrival but no longer than 3 days after collecting the samples. To avoid temperature variation, samples should only be refrigerated when they cannot be processed on the same day, otherwise they should be kept at room temperature. When samples are submitted or kept in the laboratory at 4°C, they should be allowed to equilibrate to room temperature before processing to avoid temperature shock.

c) Isolation of *Campylobacter*

For the isolation of *Campylobacter* from faecal/caecal or intestinal samples, no pretreatment is needed; samples can be plated on to selective medium or the filtration method on non-selective agar can be used. In the case of caecal samples, caeca are aseptically opened by cutting the end with a sterile scissors and squeezing out the material to be processed. Enrichment is recommended to enhance the culture sensitivity of potentially environmentally stressed organisms or in the case of low levels of organisms in faeces, for example from cattle, sheep or pigs. However, enrichment from the latter samples is not carried out routinely and in research setting only.

i) Selective media for isolation

Many media can be used in the recovery of *Campylobacter* spp. Modified charcoal, cefoperazone, desoxycholate agar (mCCDA), is the recommended medium, .although alternative media may be used. A detailed description on *Campylobacter* detection by culture and the variety of existing media is given by Corry *et al.* (6, 7). The selective media can be divided into two main groups: blood-containing media and charcoal-containing media. Blood components and charcoal serve to remove toxic oxygen derivatives. Most media are commercially available. The selectivity of the media is determined by the antibiotics used. Cefalosporins (generally cefoperazone) are used, sometimes in combination with other antibiotics (e.g. vancomycin, trimethoprim). Cycloheximide (actidione) and more recently amphotericin B are used to inhibit yeasts and molds (15). The main difference between the media is the degree of inhibition of contaminating flora. All the selective agents allow the growth of both *C. jejuni* and *C. coli*. There is no medium available that allows growth of *C. jejuni* and inhibits *C. coli* or vice versa. To some extent, other *Campylobacter* species (e.g. *C. lari, C. upsaliensis, C. helveticus, C. fetus* and *C. hyointestinalis*) will grow on most media, especially at the less selective temperature of 37°C.

Examples of selective blood-containing solid media:

- Preston agar
- Skirrow agar
- Butzler agar
- Campy-cefex

Examples of charcoal-based solid media:

- mCCDA (modified charcoal cefoperazone deoxycholate agar), slightly modified version of the originally described CCDA) (4, 5)
- Karmali agar or CSM (charcoal-selective medium) (12)
- CAT agar (cefoperazone, amphotericin and teicoplanin), facilitating growth of *C. upsaliensis* (1).

ii) Passive filtration

Passive filtration, a method developed by Steele and McDermott (22) obviates the need for selective media; thus it is very useful for the isolation of antimicrobial-sensitive *Campylobacter* species. As the method does not use expensive selective media, it may be used in laboratories with fewer resources. For passive filtration, faeces are mixed with PBS (approximately 1/10 dilution) to produce a suspension. Approximately 100 μ l of this suspension are then carefully layered on to a 0.45 or 0.65 μ m filter, which has been previously placed on top of a non-selective blood agar plate. Care must be taken not to allow the inoculum to spill over the edge of the filter. The bacteria are allowed to migrate through the filter for 30–45 minutes at 37°C or room temperature. The filter is then removed, the fluid that has passed through the filter is spread with a sterile glass or plastic spreader, and the plate is incubated microaerobically at 42°C.

iii) Incubation

Atmosphere

Microaerobic atmospheres of 5–10% oxygen, 5–10% carbon dioxide are required for optimal growth (7, 25). Appropriate atmospheric conditions may be produced by a variety of methods. In some laboratories, (repeated) gas jar evacuations followed by atmosphere replacement with bottled gasses are used. Gas generator kits are available from commercial sources. Variable atmosphere incubators are more suitable if large numbers of cultures are undertaken.

• Temperature

Media may be incubated at 37°C or 42°C, but it is common practice to incubate at 42°C to minimise growth of contaminants and to select for optimal growth of *C. jejuni/C. coli*. The fungistatic agents cycloheximide or amphothericin are added in order to prevent growth of yeasts and mould at 37°C (5). In some laboratories, incubation takes place at 41.5°C to harmonise with *Salmonella* and *Escherichia coli* O157 isolation protocols (10).

• Time

Campylobacter jejuni and *C. coli* usually show growth on solid media within 24–48 hours at 42°C. As the additional number of positive samples obtained by prolonged incubation is very low, 48 hours of incubation is recommended for routine diagnosis (5).

d) Confirmation

A pure culture is required for confirmatory tests, but a preliminary confirmation can be obtained by direct microscopic examination of suspect colony material.

- i) Identification on solid medium: On Skirrow or other blood-containing agars, characteristic *Campylobacter* colonies are slightly pink, round, convex, smooth and shiny, with a regular edge. On charcoal-based media such as mCCDA, the characteristic colonies are greyish, flat and moistened, with a tendency to spread, and may have a metal sheen.
- ii) *Microscopic examination of morphology and motility:* material from a suspect colony is suspended in saline and evaluated, preferably by a phase-contrast microscope, for characteristic, spiral or curved slender rods with a corkscrew-like motility. Older cultures show less motile coccoïd forms.
- iii) Detection of oxidase: take material from a suspect colony and place it on to a filter paper moistened with oxidase reagent. The appearance of a violet or deep blue colour within 10 seconds is a positive reaction. If a commercially available oxidase test kit is used, follow the manufacturer's instructions.
- iv) *Microaerobic growth at 25°C:* Inoculate the pure culture on to a non-selective blood agar plate and incubate at 25°C in a microaerobic atmosphere for 48 hours.
- v) Aerobic growth at 41.5°C: Inoculate the pure culture on to a non-selective blood agar plate and incubate at 41.5°C in an aerobic atmosphere for 48 hours.
- vi) Latex agglutination tests for confirmation of pure cultures of *C. jejuni/C. coli* (often also including *C. lari*) are commercially available.

e) Identification of Campylobacter to the species level

Among the *Campylobacter* spp. growing at 42°C, the most frequently encountered species from samples of animal origin are *C. jejuni* and *C. coli*. However, low frequencies of other species have been described. Generally, *C. jejuni* can be differentiated from other *Campylobacter* species on the basis of the hydrolysis of hippurate as this is the only hippurate-positive species isolated from veterinary or food samples. The presence of hippurate-negative *C. jejuni* strains has been reported (23). Table 2 gives some basic classical phenotypic characteristics of the most important thermophilic *Campylobacter* species (10). Sensitivity to nalidixic acid used to be one of the most commonly tested characteristics, but nowadays may give difficulties in interpretation, both due to an increase in nalidixic acid-resistant strains of *C. jejuni* and *C. coli* and to the isolation of nalidixic acid-sensitive genogroups of *C. lari*. More extensive speciation schemes have been described in the literature (18, 25). Speciation results should be confirmed using defined positive and negative controls.

The confirmatory tests for the presence of thermophilic campylobacters and the interpretation (10) are given in Table 1. Confirm results of confirmation tests using positive and negative controls.

Confirmatory test	Result for thermophilic Campylobacter	
Morphology	Small curved bacilli	
Motility	Characteristic (highly motile and cork-screw like)	
Oxidase	+	
Aerobic growth at 41.5°C	-	
Microaerobic growth at 25°C	-	

Table 1. Confirmatory tests for thermophilic Campylobacter

Table 2. Basic phenotypic characteristics of selected thermophilic Campylobacter species

Characteristics	C. jejuni	C. coli	C. lari
Hydrolysis of hippurate	+	-	-
Hydrolysis of indoxyl acetate	+	+	-

Key: + = positive; - = negative; S = sensitive; R = resistant

- Detection of hippurate hydrolysis: Suspend a loopful of growth from a suspect colony in 400 µl of a 1% sodium hippurate solution (care should be taken not to incorporate agar). Incubate at 37°C for 2 hours, then slowly add 200 µl 3.5% ninhydrin solution to the side of the tube to form an overlay. Re-incubate at 37°C for 10 minutes, and read the reaction. Positive reaction: dark purple/blue. Negative reaction: clear or grey. If commercially available hippurate hydrolysis test disks are used, follow the manufacturer's instructions.
- ii) Detection of indoxyl acetate hydrolysis: Place a suspect colony on an indoxyl acetate disk and add a drop of sterile distilled water. If indoxyl acetate is hydrolysed a colour change to dark blue occurs within 5–10 minutes. No colour change indicates hydrolysis has not taken place. If commercially available indoxyl acetate hydrolysis test disks are used, follow the manufacturer's instructions.

Biochemical speciation may be supplemented or even replaced with molecular methods. A variety of DNA probes and polymerase chain reaction (PCR)-based identification assays has been described for *Campylobacter* species (18, 25). On *et al.* (19) evaluated the specificity of 11 PCR-based identification assays for *C. jejuni* and *C. coli.*

f) Molecular detection of *Campylobacter*

PCR-based methods for the detection of *Campylobacter* in animal faecal samples and enriched meat samples have been previously described in the literature (17). One of these assays is in use in Denmark for routine screening of cloacal swabs from broilers at the slaughterhouse (3, 14).

g) Antigen-capture-based tests

Enzyme immunoassays are available for the detection of *Campylobacter* in human stool samples only.

2. Serological tests

There are no serological assays in routine use for the detection of colonisation of *C. jejuni/C. coli* in livestock.

C. REQUIREMENTS FOR VACCINES AND DIAGNOSTIC BIOLOGICALS

There are no vaccines specifically developed for *C. jejuni* or *C. coli* in animals or birds.

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NB: There is an OIE Reference Laboratory for Campylobacteriosis (see Table in Part 3 of this *Terrestrial Manual* or consult the OIE Web site for the most up-to-date list: www.oie.int).