CHAPTER 2.9.3.

INFECTION WITH *CAMPYLOBACTER JEJUNI* AND *C. COLI*

SUMMARY

Description of the disease: Campylobacter jejuni (C. jejuni) *and* Campylobacter coli (C. coli) *can colonise the intestinal tract of most mammals and birds and are the most frequently isolated* Campylobacter *species in humans with gastroenteritis. Although poultry is the main reservoir of* Campylobacter, *transmission to humans is only partly through handling and consumption of poultry meat; other transmission routes are also considered to be important. This chapter focuses on* C. jejuni *and* C. coli *in primary livestock production with regard to food safety.*

Campylobacter jejuni *and* C. coli *do not normally 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 an important 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 or caecal contents, or the use of polymerase chain reaction* (*PCR*). 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 examining the morphology and motility using a light microscope. The organisms in the log growth phase are short and S-shaped 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, including PCR and MALDI-TOF* (*matrix assisted laser desorption ionisation–time of flight*) *mass spectrometry can be used to identify* Campylobacter *strains at species level. PCR assays can also 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 colonisation by* C. jejuni and C. coli.

Requirements for vaccines: *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 with enteritis, including piglets, lambs and calves, but the organisms are also found in healthy animals. Outbreaks of avian hepatitis have been reported, but although *C. jejuni* is associated with the disease, it is not the causative agent (Jennings *et al.,* 2011). Recently, a new *Campylobacter* was isolated as the causative agent of spotty liver disease in layers (Crawshaw *et al.,* 2015). *Campylobacter* is the main cause of human bacterial intestinal disease identified in many industrialised countries (Havelaar *et al.,* 2013; Scallan *et al.,* 2011). Over 80% of cases are caused by *C. jejuni*

and about 10% of cases are caused by *C. coli*. In humans, *C. jejuni/C. 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 (WHO, 2013). Attribution studies show that poultry is the main reservoir of *Campylobacter* and responsible for between 50 and 80% of the human infections. In the European Union (EU), an estimated 30% of the human infections are associated with handling and consumption of poultry meat; but a considerable proportion of the poultry-derived strains has a non-poultry meat transmission route, e.g. via environmental contamination (EFSA, 2010b). 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 (Domingues *et al.,* 2012). The control of *Campylobacter* in the food chain has now become a major target of agencies responsible for food safety world-wide.

Laboratory manipulations should be performed with appropriate biosafety and containment procedures as determined by biorisk analysis (see Chapter 1.1.4 *Biosafety and biosecurity: standard for managing biological risk in the veterinary diagnostic laboratory and animal facilities*).

2. Taxonomy

There are currently 34 *Campylobacter* species recognised, but with the improved diagnostic techniques and genomic analysis, this number is expected to increase over time (*cf* List of prokaryotic names with standing in nomenclature: http://www.bacterio.net/index.html). Members of the genus *Campylobacter* 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) (Garrity, 2005). Subspecies *jejuni* is much more frequently isolated then subspecies *doylei*.

B. DIAGNOSTIC TECHNIQUES

Table 1. *Test methods available for the diagnosis of* Campylobacter jejuni *and* C. coli *and their purpose*

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Agent identification[1]						
Isolation	+++	–	+++	+++	+++	n/a
MALDI-TOF	+++	–	+++	+++	+++	n/a
Antigen detection	++	–	++	–	++	n/a
16S rRNA sequencing	++	–	++	++	++	n/a
Real-time PCR	++	–	++	++	++	n/a
Detection of immune response: n/a for *Campylobacter jejuni* and *C. coli*						

Key: +++ = recommended method, validated for the purpose shown; ++ = suitable method but may need further validation;
+ = may be used in some situations, but cost, reliability, or other factors severely limits its application;
– = not appropriate for this purpose; n/a = purpose not applicable.
Maldi-tof = matrix assisted laser desorption ionisation–time of flight; PCR = polymerase chain reaction;

1 A combination of agent identification methods applied on the same clinical sample is recommended.

1. Isolation and identification of the agent

Two ISO (International Organization for Standardization) procedures for detection of *Campylobacter* exist, a horizontal method for detection and enumeration of thermotolerant *Campylobacter* spp. (ISO 10272) in food and animal feeding stuffs with 2 parts: (part 1 detection method and part 2 colony count technique. Both parts of the ISO are under revision and will be published in 2017. The revised standard will include methods for the isolation of *Campylobacter* from live animals, and a procedure for ISO 17995 concerns water quality, with detection and enumeration of thermotolerant *Campylobacter* spp. from water (ISO, 2005 – last reviewed in 2014).

1.1. Collection of specimens

1.1.1. Poultry at the farm

Poultry is frequently colonised with *C. jejuni* (65–95%), less often with *C. coli* and rarely with other *Campylobacter* species (Newell & Wagenaar, 2000). 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 exposure to faecal contamination, is extremely rapid and up to 100% of birds within a flock can become colonised within a few days. Samples from live birds, destined for the food chain, should therefore be taken as close to slaughter as possible (Newell & Wagenaar, 2000). The majority of birds shed large numbers of organisms (>10^6 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 Cary Blair, Amies, or Stuart must be used. Sampling strategy in primary poultry has been reviewed (Vidal *et al.,* 2013) and is normally based on boot-swab samples, faecal/caecal droppings or cloacal swabs.

1.1.2. Cattle, sheep and pigs at the farm

Campylobacters are frequent colonisers of the intestine of livestock such as cattle, sheep and pigs; data have been reviewed by Newell *et al.,* in press. 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 Cary Blair, Amies, or Stuart) must be used.

1.1.3. 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 suitable container.

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

1.2. Transportation and treatment of specimens

1.2.1. Transport

Campylobacters are 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 3 days. The samples must be protected from light, extreme temperatures and desiccation.

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 than 48 hours, storage at 4°C (±2°C) is advised.

1.2.2. Transport media

Swabs: When samples are collected on boot-swabs or rectal swabs, the use of commercially available transport tubes, containing a medium, such as Cary Blair or 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 (Luechtefeld *et al*., 1981; Sjogren *et al*., 1987).

1.2.3. 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.

1.3. Isolation of *Campylobacter*

For the isolation of *Campylobacter* from faecal/caecal or intestinal samples, no pre-treatment is needed; samples can be plated on 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 of faecal samples is usually subject to overgrowth by competing bacteria and is not carried out routinely.

1.3.1. Selective media for isolation

Many media can be used in the recovery of *Campylobacter* spp. Modified charcoal, cefoperazone, desoxycholate agar (mCCDA), is the most commonly 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.* (Corry *et al*., 1995; 2003). The selective media can be divided into two main groups: blood-based media and charcoal-based 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 (Martin *et al*., 2002). 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:

i) Preston agar
ii) Skirrow agar
iii) Butzler agar
iv) Campy-cefex

Examples of charcoal-based solid media:

i) mCCDA (modified charcoal cefoperazone deoxycholate agar), slightly modified version of the originally described CCDA) (Bolton *et al*., 1984; 1988)
ii) Karmali agar or CSM (charcoal-selective medium) (Karmali *et al*., 1986)
iii) CAT agar (cefoperazone, amphotericin and teicoplanin), facilitating growth of *C. upsaliensis* (Aspinall *et al*., 1993).

1.3.2. Passive filtration

Passive filtration, a method developed by Steele & McDermott (1984) 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 µl of this suspension are then carefully layered on to a 0.45 or 0.65 µm 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 and the filter is then removed. The plate is incubated microaerobically at 42°C.

1.3.3. Incubation

i) Atmosphere

Microaerobic atmospheres of 5–10% oxygen, 5–10% carbon dioxide are required for optimal growth (Corry *et al*., 2003; Vandamme, 2000). 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.

ii) 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* and *C. coli*. The fungistatic agents cycloheximide or amphothericin are added in order to prevent growth of yeasts and mould at 37°C (Bolton *et al*., 1988). In some laboratories, incubation takes place at 41.5°C to harmonise with *Salmonella* and *Escherichia coli* O157 isolation protocols (ISO, 2006).

iii) 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 (Bolton *et al*., 1988).

1.4. Confirmation

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

1.4.1. 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.

1.4.2. 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.

1.4.3. 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.

1.4.4. Aerobic growth at 25°C

Inoculate the pure culture on to a non-selective blood agar plate and incubate at 25°C in an aerobic atmosphere for 48 hours.

1.4.5. Latex agglutination tests

Latex agglutination tests for confirmation of pure cultures of *C. jejuni* and *C. coli* (often also including *C. lari*) are commercially available.

1.5. 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, including *Helicobacter* 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 (Steinhauserova *et al*., 2001). Table 2 gives some basic classical phenotypic characteristics of the most important thermophilic *Campylobacter* species (ISO, 2006). More extensive speciation schemes have been described in the literature (On, 1996; Vandamme, 2000). Speciation results should be confirmed using defined positive and negative controls.

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; *not all strains.

The confirmatory tests for the presence of thermophilic campylobacters and the interpretation (ISO, 2006) are given in Table 3. Confirm results of confirmation tests using positive and negative controls.

Table 3. *Confirmatory tests for thermophilic* Campylobacter

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

1.5.1. 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.

1.5.2. 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 replaced with molecular methods or MALDI-TOF mass spectrometry. MALDI-TOF can be used to identify *Campylobacter* isolates rapidly and efficiently at the genus and species level (Bessede *et al.*, 2011). A variety of DNA probes and polymerase chain reaction (PCR)-based identification assays has been described for the identification of *Campylobacter* species (On, 1996; Vandamme, 2000). On & Jordan (2003) evaluated the specificity of 11 PCR-based assays for *C. jejuni* and *C. coli* identification. A fast method to differentiate *C. jejuni* and *C. coli* strains is a duplex real-time PCR, targeting gene *mapA* for *C. jejuni* identification and gene *CeuE* for *C. coli* identification (Best *et al*., 2003). Another real-time PCR method commonly used to identify and differentiate between *C. jejuni, coli* and *lari* is described by Mayr (2010). A gel-based method that is commonly used differentiates between *C. jejuni, C. coli, C. lari and C. upsaliensis* (Wang *et al.,* 2002).

Campylobacter isolates can also be molecular identified at species level with 16S rRNA sequencing (Gorkiewicz *et al.,* 2003).

1.6. Molecular detection of *Campylobacter*

PCR-based methods for the detection of *Campylobacter* in animal faecal samples and enriched meat samples have been extensively described in the literature (Bang *et al*., 2001; Lund *et al*., 2003; Olsen *et al*., 1995). Many molecular tests are available to identify *Campylobacter* species, but there is not a specific recommended one. *Campylobacter* isolates can be identified at species level with 16S rRNA sequencing (Gorkiewicz *et al.,* 2003). Inclusion of positive and negative reference strains and process controls to detect inhibition of the PCR reaction by the sample matrix are required for all molecular *Campylobacter* detection methods.

1.7. Antigen-capture-based tests

Enzyme immunoassays are available for the detection of *Campylobacter* in human and animal stool samples. Some are of the lateral flow format. The sensitivity and specificity should be critically evaluated through an in-house validation.

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.

REFERENCES

ASPINALL S.T., WAREING D.R.A., HAYWARD P.G. & HUTCHINSON D.N. (1993). Selective medium for thermophilic campylobacters including *Campylobacter upsaliensis. J. Clin. Pathol*., **46**, 829–831.

BANG D.D., PEDERSEN K. & MADSEN M. (2001). Development of a PCR assay suitable for *Campylobacter* spp. mass screening programs in broiler production. *J. Rapid Methods Autom. Microbiol*., **9**, 97–113.

BESSEDE E., SOLECKI O., SIFRE E., LABADI L. & MEGRAUD F. (2011). Identification of *Campylobacter* species and related organisms by matrix assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry. *Clin. Microbiol. Infect*., **17**, 1735–1739.

BEST E.L., POWEL E.J., SWIFT C., KATHLEEN A.G. & FROST J.A. (2003). Applicability of a rapid duplex real-time PCR assay for speciation of *Campylobacter jejuni* and *Campylobacter coli* directly from culture plates. *FEMS Microbiol.*, **229**, 237–241.

BOLTON F.J., HUTCHINSON D.N. & COATES D. (1984). Blood-free selective medium for isolation of *Campylobacter jejuni* from faeces. *J. Clin. Microbiol*., **19**, 169–171.

BOLTON F.J., HUTCHINSON D.N. & PARKER G. (1988). Reassessment of selective agars and filtration techniques for isolation of *Campylobacter* species from faeces. *Eur. J. Clin. Microbiol. Infect. Dis*., **7**, 155–160.

CORRY J.E.L., ATABAY H.I., FORSYTHE S.J. & MANSFIELD L.P. (2003). Culture media for the isolation of campylobacters, helicobacter and arcobacters. *In:* Handbook of Culture Media for Food Microbiology, Second Edition, Corry J.E.L., Curtis G.D.W. & Baird R.M. eds. Elsevier, Amsterdam, The Netherlands, 271–315.

CORRY J.E.L., POST D.E., COLIN P. & LAISNEY M.J. (1995). Culture media for the isolation of campylobacters. *Int. J. Food Microbiol*., **26**, 43–76.

CRAWSHAW T.R., CHANTER J.I., YOUNG S.C., CAWTHRAW S., WHATMORE A.M., KOYLASS M.S., VIDAL A.B., SALGUERO F.J. & IRVINE R.M. (2015). Isolation of a novel thermophilic *Campylobacter* from cases of spotty liver disease in laying hens and experimental reproduction of infection and microscopic pathology. *Vet. Microbiol.,* **179**, 315–321.

DOMINGUES A.R., PIRES S.M., HALASA T. & HALD T. (2012). Source attribution of human campylobacteriosis using a meta-analysis of case-control studies of sporadic infections. *Epidemiol. Infect*., **140**, 970–981.

EUROPEAN FOOD SAFETY AUTHORITY (EFSA) (2010b). Scientific Opinion on Quantification of the risk posed by broiler meat to human campylobacteriosis in the EU. EFSA Panel on Biological Hazards (BIOHAZ). *EFSA J*., **8**, 1437. [89 pp.].

GARRITY G.M. (Editor-in-Chief) (2005). Bergey's Manual of Systematic Bacteriology, Second Edition. Springer-Verlag, New York, USA.

GORKIEWICZ G., FEIERL G., SCHOBER C., DIEBER F., KÖFER J., ZECHNER R. & ZECHNER E.L. (2003). Species-specific identification of Campylobacters by partial 16S rRNA gene sequencing. *J. Clin. Microbiol*., **41**, 2537–2546.

HAVELAAR A.H., IVARSSON S., LÖFDAHL M. & NAUTA M.J. (2013). Estimating the true incidence of campylobacteriosis and salmonellosis in the European Union, 2009. *Epidemiol. Infect.,* **141**, 293–302.

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION (ISO) (2005). ISO 17995:2005. Water quality – Detection and enumeration of thermophilic *Campylobacter species.* International Organisation for Standardisation (ISO), ISO Central Secretariat, 1 rue de Varembé, Case Postale 56, CH - 1211, Geneva 20, Switzerland.

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION (ISO) (2006). ISO 10272-1:2006 AND ISO/TS 10272-2:2006. Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Campylobacter* spp. Part 1: Detection method; Part 2: Colony count technique. International Organisation for Standardisation (ISO), ISO Central Secretariat, 1 rue de Varembé, Case Postale 56, CH - 1211, Geneva 20, Switzerland.

JENNINGS J.L., SAIT L.C., PERRETT C.A., FOSTER C., WILLIAMS L.K., HUMPHREY T.J. & COGAN T.A. (2011). *Campylobacter jejuni* is associated with, but not sufficient to cause vibrionic hepatitis in chickens. *Vet. Microbiol.,* **149**, 193–199.

KARMALI M.A., SIMOR A.E., ROSCOE M., FLEMING P.C., SMITH S.S. & LANE J. (1986). Evalutaion of a blood-free, charcoal-based, selective medium for the isolation of *Campylobacter* organisms from feces. *J. Clin. Microbiol.*, **23**, 456–459.

LUECHTEFELD N.W., WANG W.L., BLASER M.J. & RELLER L.B. (1981). Evaluation of transport and storage techniques for isolation of *Campylobacter fetus* subsp. *jejuni* from turkey cecal specimens. *J. Clin. Microbiol.*, **13**, 438–443.

LUND M., WEDDERKOPP A., WAINO M., NORDENTOFT S., BANG D.D., PEDERSEN K., & MADSEN M. (2003). Evaluation of PCR for detection of *Campylobacter* in a national broiler surveillance programme in Denmark. *J. Appl. Microbiol.*, **94**, 929–935.

MARTIN K.W., MATTICK K.L., HARRISON M. & HUMPHREY T.J. (2002). Evaluation of selective media for *Campylobacter* isolation when cycloheximide is replaced with amphotericin B. *Lett. Appl. Microbiol*., **34**, 124–129.

MAYR A.M., LICK S., BAUER J., THARIGEN D., BUSCH U. & HUBER I (2010). Rapid detection and differentiation of *Campylobacter jejuni*, *Campylobacter coli* and *Campylobacter lari* in food, using multiplex real-time PCR*. J. Food Prot.,* **73**, 241–250.

NEWELL D.G., MUGHINI-GRAS L., KALUPAHANA R.S. & WAGENAAR J.A. (2017). *Campylobacter* epidemiology – sources and routes of transmission for human infection. *Campylobacter:* Features, Detection, and Prevention of Foodborne Disease. Elsevier, Amsterdam, Netherlands.

NEWELL D.G. & WAGENAAR J.A. (2000). Poultry infections and their control at the farm level. *In: Campylobacter,* Second Edition, Nachamkin I. & M.J. Blaser, eds. ASM Press, Washington DC, USA, 497–509.

OLSEN J.E., ABO S., HILL W., NOTERMANS S., WERNARS K., GRANUM P.E., POPVIC T., RASMUSSEN H.N. & OLSVIK O. (1995). Probes and polymerase chain reaction for the detection of food-borne bacterial pathogens. *Int. J. Food Microbiol.* **28**, 1–78.

ON S.L.W. (1996). Identification methods for Campylobacters, Helicobacters, and Related organisms. *Clin. Microbiol. Rev*., **9**, 405–422.

ON S.L.W. & JORDAN P.J. (2003). Evaluation of 11 PCR assays for species-level identification of *Campylobacter jejuni* and *Campylobacter coli. J. Clin. Microbiol*., **41**, 330–336.

SCALLAN E., HOEKSTRA R.M., ANGULO F.J., TAUXE R.V., WIDDOWSON M.A., ROY S.L., JONES J.L., GRIFFIN P.M. (2011). Foodborne illness acquired in the United States – major pathogens. *Emerg. Infect. Dis.,* **17**, 7–15.

SJOGREN E., LINDBLOM G.B. & KAIJSER B. (1987). Comparison of different procedures, transport media, and enrichment media for isolation of *Campylobacter* species from healthy laying hens and humans with diarrhea. *J. Clin. Microbiol.,* **25**, 1966–1968.

STEELE T.W. & MCDERMOTT S.N. (1984). The use of membrane filters applied directly to the surface of agar plates for the isolation of *Campylobacter jejuni* from feces. *Pathology*, **16**, 263–265.

STEINHAUSEROVA I., CESKOVA J., FOJTIKOVA K. & OBROVSKA I. (2001). Identification of thermophilic *Campylobacter* spp. by phenotypic and molecular methods. *J. Appl. Microbiol.*, **90**, 470–475.

VANDAMME P. (2000). Taxonomy of the family Campylobacteraceae. *In: Campylobacter*, Second Edition, Nachamkin I. & M.J. Blaser, eds. ASM Press, Washington DC, USA, 3–26.

VIDAL A.B., RODGERS J., ARNOLD M. & CLIFTON-HADLEY F. (2013). Comparison of different sampling strategies and laboratory methods for the detection of *C. jejuni* and *C. coli* from broiler flocks at primary production. *Zoonoses Public Health*, **60**, 412–425.

WANG G., CLARK C.G., TAYLOR T.M., PUCKNELL C., BARTON C., PRICE L., WOODWARD D.L. & RODGERS F.G. (2002). Colony multiplex PCR assay for identification and differentiation of *Campylobacter jejuni, C. coli, C. lari, C. upsaliensis*, and *C. fetus* subsp. *fetus. J. Clin. Microbiol*., **40**, 4744–4747.

WORLD HEALTH ORGANIZATION (WHO) (2013). The global view of campylobacteriosis: report of an expert consultation, Utrecht, Netherlands, 9–11 July 2012, WHO, Food and Agriculture Organization of the United Nations, World Organisation for Animal Health, eds. WHO, Geneva, Switzerland.
http://www.who.int/iris/handle/10665/80751

*
* *

NB: There is an OIE Reference Laboratory for Campylobacteriosis
(see Table in Part 4 of this *Terrestrial Manual* or consult the OIE Web site for the most up-to-date list:
http://www.oie.int/en/our-scientific-expertise/reference-laboratories/list-of-laboratories/).
Please contact the OIE Reference Laboratories for any further information on
diagnostic tests and reagents for campylobacteriosis

NB: FIRST ADOPTED IN 2004. MOST RECENT UPDATES ADOPTED IN 2017