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RESEARCH ARTICLE

DIAGNOSTIC TECHNIQUES FOR CAMPYLOBACTER IN LIVESTOCK

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ABSTRACT

Campylobacter colonize are main cause for instestinal disease in human. They are largely present in livestock which are consumed by humans as food. This paper presents various diagnostic techniques for the detection and confirmation for members of campylobacter colonizes in livestock. With help of these techniques, we can confirm the presence of Campylobacter jejuni and C. coli and also identify the various other campylobacter in sample.

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INTRODUCTION

Members of the genus Campylobacter colonize the gastrointestinal tract of a broad range of animals. Campylobacter jejuni and C. coli are generally considered commensals of livestock, domestic pet animals and birds, but notably are associated with disease in humans. The main cause of human bacterial intestinal disease coli such as food-borne diarrhea identified in many industrialized countries are C. jejuni and Campylobacter [17]. 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 [15]. Focusing on C. jejuni and C. coli, the objective of this review is to summarize the current knowledge of pathogenesis of Campylobacter in crucial livestock production with regard to food safety [1].

Definition and description of disease

Campylobacter jejuni and C. coli can take possession of the intestinal tract of most mammals and birds. These are the most often isolated Campylobacter species in humans with gastro-enteritis. Transmission of this pathogenesis from animals to humans is mainly through consumption and handling of animal food products as well as direct contact with colonised animals [1].

Except for sporadic cases of abortion in ruminants and ostriches with hepatitis, which is very rare case, these members of genus Campylobacter do not cause clinical disease in adult animals. The major source of human food-borne disease is faecal contamination of meat (especially poultry meat) during processing. In humans, extraintestinal infections, including bacteraemia, can occur and some squeal 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 Gram-negative, thermophilic, highly motile bacteria that for optimal growth require incubation temperatures of 37–42°C and microaerobic environment. To isolate these bacteria from faecal/intestinal samples Agar media containing selective antibiotics are required [1].

On the other hand, their high motility can be exploited using filtration techniques for isolation whereas Enrichment techniques are not routinely used. Preliminary confirmation of isolates can be made by use of light microscopy. In the log growth phase the appearance of organisms are short and S-

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shaped, while in older cultures they are predominated by coccoid forms. The organisms have a characteristic of rapid corkscrew-like motility under phase-contrast microscopy.
Phenotypic identification is based on reactions under different growth conditions. Molecular and biochemical tests can be used to verify diverse Campylobacter species whereas Polymerase chain reaction assays can be used for the direct detection [1].

Diagnostic Techniques

Isolation and identification of the agent

Two ISO (International Organization for Standardization) procedures for detection of Campylobacter exists, first-horizontal method for detection of thermotolerant Campylobacter in food and animal feeding stuffs [8] and second-a procedure for the isolation of Campylobacter from water [9] but neither of these standard methods possibly will be most favorable for the isolation of campylobacters from live animals. An appendix to ISO 10272 on this topic is currently being developed.

Collection of Samples

Livestock and Poultry at farm

Campylobacters are frequent colonisers of the intestine of livestock such as cattle, sheep and pigs [3, 19, 20]. 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.

Poultry is frequently colonised with C. jejuni (65–95%) less often with C. coli and rarely with other Campylobacter species [13]. 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 [13]. 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 rectal samples without traces of urine) should be collected and they should be prevented from drying out before culture. When swabs are used, a transport medium such as Cary Blair, Amies or Stuart must be used.

At Slaughter

Samples from cattle, sheep and pigs are taken from the intestines by aseptically opening the gut wall or by taking rectal swabs while in poultry, the caeca are frequently 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.

Transportation and treatment of samples

Transport

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

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

Transport media

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 charcoalbased. 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 such as Cary-Blair, modified Cary-Blair, semisolid motility test medium, modified Stuart medium, alkaline peptone water and Campythioglycolate medium. Good recovery results have been reported using Cary-Blair [11, 14].

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, before processing to avoid temperature shock, they should be allowed to equilibrate to room temperature.

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. In the case of low levels of organisms in faeces, enrichment is recommended to enhance the culture sensitivity of potentially environmentally stressed organisms [1].

Selective media for isolation

There are various medias available for to recover Campylobacter spp. The recommended medium is Modified charcoal, cefoperazone, desoxycholate agar (mCCDA). A detailed description on Campylobacter detection by culture and the variety of existing media is presented by Corry et al. [6, 7]. The selective media can be divided into two main groups: blood-containing media and charcoal-containing media which are used to eliminate toxic oxygen derivatives. Preston agar, Skirrow agar, Butzler agar and Camply-cerex are examples of selective blood-containing solid media and mCCDA (modified charcoal cefoperazone deoxycholate agar), slightly modified version of the originally described CCDA) [4, 5], Karmali agar or CSM (charcoal-selective medium) [10], CAT agar (cefoperazone, amphotericin and teicoplanin), facilitating growth of C. upsaliensis [1] are example of charcoal based solid media.

Table 1 ConfirmatoryTests for Thermophilic Campylobacter

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

Table 2 Basic phenotypic characteristics of selected Thermophilic Campylobacter Species

Characteristics	C. jejuni	C. coli	C. lari
Hydrolysis of hippurate	Positive	Negative	Negative
Hydrolysis of indoxyl acetate	Positive	Positive	Negative

Most media are commercially available but the selectivity of the media is determined by the antibiotics used. Cycloheximide (actidione) and more recently amphotericin B are used to inhibit yeasts and molds. Cefalosporins (generally cefoperazone) are used, sometimes in combination with other antibiotics (e.g. vancomycin, trimethoprim) [12].

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 existing which can allow growth of C. jejuni and inhibits C. coli or vice versa. To some extent, other Campylobacter species like C. lari, C. upsaliensis, C. helveticus, C. fetus and C. hyointestinalis will grow on most media, mainly at the less selective temperature of 37°C.

Passive filtration

Passive filtration is very useful method for the isolation of antimicrobial-sensitive Campylobacter species since it obviates the need for selective media. Because of the no requirement of expensive selective media, it may be used in laboratories having limited resources.

In this process, 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 while taking care of not allowing 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 after which the filter is removed. The fluid that has passed through the filter is spread with help of sterile glass or plastic spreader. The plate is than incubated microaerobically at 42°C.

Incubation

Atmosphere

Appropriate atmospheric conditions may be produced by a variety of methods. For optimal growth microaerobic atmospheres of 5–10% oxygen and 5–10% carbon dioxide are required [7, 18]. In some laboratories, gas jar evacuations followed by atmosphere replacement with bottled gasses are used. 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 minimize growth of contaminants and to select for optimal growth of C. jejuni/C. coli, they are incubated at 42°C. In some laboratories, incubation takes place at 41.5°C to harmonise with Salmonella and Escherichia coli O157 isolation protocols [8]. The fungistatic agents cycloheximide or amphothericin are added in order to prevent growth of yeasts and mould at 37°C [5].

Time

C. jejuni and C. coli have usually shown growth on solid media within 1 or 2 days time at 42°C. As the number of negative samples obtained from prolonged incubation is very high in compare to positive one 48 hours of incubation is recommended for routine diagnosis [5].

Confirmation

For confirmatory tests, a pure culture is required, but a preliminary confirmation can be obtained by direct microscopic examination.

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.
2. 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.
3. 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.

4. Latex agglutination tests for confirmation of pure cultures of C. jejuni/C. coli (often also including C. lari) are commercially available.
5. 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.

Identification of Campylobacter to the species level

C. jejuni and C. coli are the most frequent encountered species from animal samples growing at 42°C. 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 [16]. Some basic classical phenotypic characteristics of the most important thermophilic Campylobacter species are given in Table 2 [8]. 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. Speciation results should be confirmed using defined positive and negative controls.

The confirmatory tests for the presence of thermophilic campylobacters and the interpretation [8] are given in Table 1. Confirm results of confirmation tests using positive and negative controls.

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, and 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.

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. Biochemical speciation may be supplemented or even replaced with molecular methods. While using commercially available hippurate hydrolysis test disks the manufacturer's instructions must be followed.

Antigen-capture-based tests

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

Serological tests

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

Requirements for vaccines and diagnostic biological

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

References

1. OIE Terrestrial Manual (2008), 1185-1191.
2. 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.
3. Atabay H.I. & Corry J.E.L. (1998). The isolation and prevalence of campylobacters from dairy cattle using a variety of methods. J. Appl. Microbiol., 84, 733–740.
4. 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.
5. 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.
6. 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.
7. 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.
8. 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.
9. 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.
10. 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.
11. 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.

12. 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.
13. 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.
14. 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.
15. SKIRROW M.B. & BLASER M.J. (2000). Clinical aspects of Campylobacter infection. In: Campylobacter, Second Edition, Nachamkin I. & M.J. Blaser, eds. ASM Press, Washington DC, USA, 69–88.
16. 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.
17. Tauxe R.V. (1992). Epidemiology of Campylobacter jejuni infections in the United States and other industrialized nations. In: Campylobacter jejuni: current state and future trends, Nachamkin I., Blaser M.J. & Tompkins L.S., eds. ASM Press, Washington DC, USA, 9–19.
18. 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.
19. Weijtens M. (1996). Campylobacter in pigs (dissertation). Utrecht University, The Netherlands.
20. Wesley I.V., Wells S.J., Harmon K.M., Green a., Schroeder-Tucker l., Glover M. & Siddique i. (2000). Fecal shedding of ampylobacter and Arcobacter spp. in dairy cattle. Appl. Environ. Microbiol., 66, 1994–2000.

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