

# CAMPYLOBACTER

## Learning objectives

To know in detail about,

- Principal host and diseases caused by *Campylobacter species*
- Morphology, cultural and biochemical characters of *Campylobacter species*
- Selective media for Campylobacter
- Pathogenesis and pathogenicity of Campylobacteriosis in domestic animals
- Different diagnostic methods for Campylobacteriosis
- Mating test or virgins heifer test for Campylobacteriosis

## SYSTEMATICS

Domain	Bacteria
Phylum	<i>Proteobacteria</i>
Class	<i>Epsilonproteobacteria</i>
<u>Order</u>	<i>Campylobacterales</i>
Family	<i>Campylobacteraceae</i>
Genus	<i>Campylobacter</i>

- The most **important** animal pathogens are *C. fetus* subsp. *fetus*, *C. fetus* subsp. *venerealis*, *C. jejuni* and possibly *C. mucosalis* and *C. hyointestinalis*.
- The *Campylobacter* species were once placed in the genus *Vibrio* and some of the diseases are still occasionally referred to as 'vibriosis'.

## HABITAT

- The *Campylobacter* species are worldwide in distribution.
- Many of the animal species are commensals on the mucosa of the oral cavity and intestinal tract.
- *C. fetus* subsp. *venerealis* occurs in the prepuce of bulls and in the genital tract of cows in herds where bovine genital campylobacteriosis is, or has been, present.
- There are also some nonpathogenic species that are saprophytes in the environment.

## MORPHOLOGY AND CULTURAL CHARACTERISTICS

### Morphology

- The *Campylobacter species* are thin, curved, Gram-negative, microaerophilic, motile rods.
- The cells are 0.2-0.5 u.m in width and when daughter cells remain joined, S-shaped, seagull-shaped and sometimes long spiral forms may be seen.
- They are motile by means of polar flagella. Small, curved or seagull-shaped rods can be demonstrated by using DCF-stained (dilute carbol fuchsin for 4 minutes) smears prepared from colonies. Wet mounts under phase contrast or darkfield microscopy reveal the characteristic curved forms with darting motility.

## Cultural characteristics

- The organisms grow at 25 °C or 45 °C. They grow best on nutritious basal media supplemented with 5-10 per cent blood under reduced oxygen tension.
- The *Campylobacter fetus* (both subspecies) grows very well on nutritious base (Brucella, Columbia or brain heart infusion agars) supplemented with 5-10 per cent blood.
- The medium can be made selective by the addition of polymyxin B sulphate (2 units/ml), novobiocin (2 µg/ml) and cycloheximide (20 µg/ml).
- Incubate the plates at 37°C for 4-6 days in a microaerophilic atmosphere containing 6 per cent O<sub>2</sub>, 10 per cent CO<sub>2</sub> and 84 per cent N<sub>2</sub>.
- These atmospheric conditions must be adhered to strictly. Both subspecies of *C. fetus* have small (1 mm), round, slightly raised, smooth, translucent colonies- said to have a 'dewdrop' appearance.
- *C. jejuni* grows very well on charcoal-cefoperazone-deoxycholate agar or Blaser's Campy-BAP medium.
- This latter medium is Brucella agar with 5 per cent sheep blood and vancomycin (10 ug/ml), polymyxin B sulphate (2-5 units/ml), trimethoprim lactate (5 ug/ml) cephalothin (15 ug/ml), and amphotericin B (2 ug/ml).
- The plates should be incubated under the atmosphere described above for 2-3 days at 42° C.
- The colonies of *C. jejuni* are usually flat, grey, and larger than those of *C. fetus* and can be spreading and watery on moist plates. *C. mucosalis* / *C. coli* grows on Columbia blood agar with and without 1:60,000 brilliant green.
- Incubate the plates anaerobically at 37°C for 2-5 days. After primary isolation the bacterium will grow well microaerophilically.

- *C. coli* produces a pink-tan pigment and the growth of *C. mucosalis* may appear as a dirty-yellow colour.

## BIOCHEMICAL PROPERTIES, RESISTANCE AND ANTIGENICITY

### Biochemical properties

- *Campylobacter* species are non-fermentative, non-hemolytic, oxidase-positive, produced hydrogen sulphide, reduced nitrate and catalase-variable.
- *C. jejuni* is the only species that hydrolyses sodium hippurate.

### Resistance

- *C. fetus* is killed at a temperature of 60°C for 5 minutes.
- It will survive for 10-20 days in soil, hay and manure, depending upon humidity and temperature. They are readily killed by disinfectants.

### Antigenicity

- *C. fetus* subsp. *venerealis* has "o" and "W" cell wall antigens. "o" antigen is stable, whereas "W" antigen is altered once specific (sIgA) antibodies are produced.
- Now "W" antigen is called Surface Array protein. As sIgA immunologic memory is poor, the bacterium can shift between antigens.
- *C. fetus* subsp. *fetus* has "o" A-2 and B. But the protein antigens are important 📌

## PATHOGENESIS

- Little is known of the pathogenic mechanisms of most *Campylobacter* species. *C. jejuni* produces an adhesin, a cytotoxin and a heat-labile toxin similar to that of *Escherichia coli*.
- **Transmission** 📌 of many of the *Campylobacter* species, including *C. fetus* subsp. *fetus*, is by the faecal-oral route. *C. fetus* subsp. *venerealis* is transmitted by coitus and infection of the female genital tract may lead to metritis with resulting death and resorption of the embryo (infertility), or occasionally to abortion.
- *C. fetus* subsp. *venerealis* - is an obligate parasite of bovine genitalia.
- Encounter is usually through coitus or artificial insemination - in bulls the infection is usually unapparent and involves the epithelium of the penis and the fornix of the prepuce.
- The organism localizes in the anterior vagina and cervix during the ovulatory phase but does not invade the uterus and oviducts until progesterone release - and then causes endometritis and salpingitis for several weeks to a few months during which the animal is infertile - animals usually regain fertility within 5 months.
- *C. fetus* subsp. *fetus* - Ovine abortions, however organism enters via ingestion, (venereal transmission does not occur) causes bacteraemia and finally localizes in the placenta causing placentitis and abortion near the end of gestation.
- Infection of ewes in the first half of the gestation period does not result in abortion.
- Occasionally causes abortion during latter half of gestation in cattle - organism enters through ingestion.
- *C. sputorum* subsp. *mucosalis* - found in mouth of piglets for about 2 weeks following weaning - if conditions favour (stress, overcrowding, underlying gastroenteritis), the organisms can invade intestinal mucosa and multiply intracellularly.

- The result is excessive proliferation of functionally (absorptive capacity) immature epithelial cells which carry the organism.
- Affected pigs do not thrive; lesions are in the lower ileum and cecum (proliferating epithelia) with loss of villi and development of polypoid (polyp-like) masses.
- Animals usually recover in 6 weeks. The diseases caused by *Campylobacter* species are

Species	Host	Disease
<i>C. fetus</i> subsp. <i>venerealis</i>	<ul style="list-style-type: none"> <li>• Cattle</li> </ul>	<ul style="list-style-type: none"> <li>• Epizootic bovine infertility (Bovine genital campylobacteriosis) , early embryonic death and occasional abortion</li> </ul>
<i>C. fetus</i> subsp. <i>fetus</i> Syn: <i>C. fetus intestinalis</i>	<ul style="list-style-type: none"> <li>• Sheep</li> <li>• Cattle</li> </ul>	<ul style="list-style-type: none"> <li>• Abortion (Ovine genital campylobacteriosis)</li> <li>• Occasional abortion</li> </ul>
<i>C. jejuni</i>	<ul style="list-style-type: none"> <li>• Cattle</li> <li>• Sheep</li> <li>• Poultry</li> <li>• Dogs and cats</li> </ul>	<ul style="list-style-type: none"> <li>• Winter dysentery &amp; Mastitis</li> <li>• Abortion</li> <li>• Avian vibronic hepatitis</li> <li>• Enteritis with diarrhoea</li> </ul>
<i>C. mucosalis</i>	<ul style="list-style-type: none"> <li>• Pigs</li> </ul>	Swine proliferative enteritis (Porcine intestinal adenomatosis complex)
<i>C. hyointestinalis</i> ( <i>Serpulina hyointestinalis</i> )	<ul style="list-style-type: none"> <li>• Pigs</li> </ul>	Swine proliferative enteritis (Porcine intestinal adenomatosis complex)
<i>C. coli</i>	<ul style="list-style-type: none"> <li>• Pigs, Man</li> </ul>	<ul style="list-style-type: none"> <li>• Mild diarrhoea in pigs and enteritis in man.</li> <li>• Swine dysentery (along with <i>Serpulina hyodysenteriae</i>)</li> </ul>

## PATHOGENECITY

### Symptoms

#### Cattle

- Affected cattle usually show signs of acute endometritis within 2 weeks after exposure.
- Failure to implant or early abortions, Abortions at 5 -7 months, retained placenta and increase number of services per conception resulting in repeat breeders are most commonly seen.

#### Sheep

- Pregnant ewes that become infected may lose weight and appear unthrifty. Diarrhea may be present. They usually abort late in their pregnancy.
- They may also deliver stillborn or weak lambs. In unvaccinated flocks, the abortion rate may reach 70% of the ewes.
- The aborted fetus is autolyzed (already showing signs of decomposition).
- This is in contrast to a fresh fetus in Chlamydial/Enzootic Abortion of Ewes (EAE), which is another common cause of ovine abortion.
- The placenta is often hemorrhagic (bloody), necrotic (decomposition), and edematous (swollen, leathery).
- Blood tinged fetal fluids. Characteristic necrotic spots in the fetal liver.
- Following the abortion, the ewe may develop an infection of the uterus (metritis) and require additional medical attention.
- Mortality in the ewes may exceed 5%. Surviving ewes may become carriers.

### Swine

- Proliferative enteritis in swine with adenomatous changes due to excessive proliferation of immature epithelial cells. 6 to 20 week old pigs are affected mostly.

## DIAGNOSIS

### Based on Direct microscopy

- Both subspecies of *Campylobacter fetus* can be demonstrated in foetal abomasal contents using the DCF stain.
- Fluorescent antibody staining of smears from foetal abomasal contents, cervical mucus and preputial washings is most reliable, especially when small numbers of the bacterium are present.
- *C. jejuni* can be seen in wet mounts of faeces by phase contrast or darkfield microscopy.
- The typical darting motility of corkscrew-like organism is suggestive of *Campylobacter species*.
- Characteristic slender, curved rods can be demonstrated in DCF-stained smears, or by phase contrast of ovine foetal abomasal contents and in bile from chickens with hepatitis.
- *C. mucosalis* can be visualised using the modified Ziehl-Neelsen (MZN) stain on heat-fixed smears from mucosal scrapings.
- Large numbers of pink-staining, slender, curved rods, located intracellularly are seen. Inoculate the above said specimens on to any one of the selective agar and identification of the organism is based on colonial morphology as described earlier.

### Based on Isolation and identification

- *Campylobacter fetus* (both subspecies): cervical mucus and preputial washings can be passed through a 0.65 µm membrane filter to reduce contamination.
- Foetal abomasal contents and filtrates are useful for isolation and identification.
- *C. jejuni*: rectal swabs or faeces are the most suitable specimens.
- *C. mucosalis*: Homogenates of mucosal scrapings from the affected intestine are useful.

### Based on Biochemical and other tests

- Susceptibility or resistance to nalidixic acid or cephalothin, hydrogen sulphide production, nitrate reduction growth at 25° C or 45° C and the catalase reaction, are

some of the criteria on which a definitive identification of the *Campylobacter species* is made.

### Hippurate test

- *C. jejuni* is the only species that hydrolyses sodium hippurate.
- For this test a large loopful of a 24-48 hour culture of *C. jejuni* is emulsified in 0.4 ml of a 1 per cent aqueous solution of sodium hippurate and incubated at 37°C for 2 hours.
- Then 0.2 ml of a ninhydrin solution is added to the tubes at 37°C.
- A positive reaction is given by a deep purple colour developing after 10 minutes.

### By Serology

- The cervical mucus agglutination test for *C. fetus* subsp. *venerealis* is accurate if carried out 2-7 months post-infection.
- A vaginal mucus agglutination test has been found useful but the serum agglutination test is unreliable.
- As *C. jejuni* is present in the intestines of many normal animals, its isolation from faeces may not necessarily be significant.
- A four-fold increase in an agglutinating antibody titre to the bacterium would suggest involvement of the organism in the diarrhoea.
- Fluorescent antibody-stained smears can be used to identify *C. fetus*, but the technique does not distinguish between the subtypes.

### By mating test or vigin heifer test

- To detect infected bull, virgin heifers are inseminated with semen and preputial washings from suspected animals and examined the heifer's vaginal mucous during the following 3-4 weeks.
- This test is effective but expensive and time consuming.

### DNA probes are available for diagnosis

## CONTROL, PREVENTION AND PUBLIC HEALTH SIGNIFICANCE

- This disease is very contagious and spreads rapidly among the remaining animals unless very strict hygiene is practiced.
- The fetus, placenta, birth fluids, vaginal discharge, and feces from the affected animals are all sources of infection.
- If the water or feeding areas become contaminated with these materials, the abortion rate can be very high.
- Isolate the aborting animals immediately, along with proper disposal of the aborted fetus/placenta, and disinfection procedures.
- Prevent the disease from spreading by limiting access to the aborted materials by wild birds and wild or domestic mammals, which can carry the bacteria to other lots or ranches. Cleanliness is absolutely essential.
- Many *Campylobacter species* produce beta-lactamase, thus accounting for their resistance to penicillin and ampicillin.
- Killed bacterins are effective both prophylactically and therapeutically. Heifers and cows should be vaccinated 1 to 4 months before breeding.
- Segregate affected bulls and test new additions. If infected, suspend breeding for 90 days and use antibiotics and artificial insemination.

- In Sheep, vaccinate all incoming and unvaccinated ewes thirty days prior to breeding season and again sixty to ninety days later.
- Follow up with a booster every year at the onset of breeding season.
- While some immunity is obtained following an outbreak, immunity against one strain of *Campylobacter* is not cross-protective against the other strain.
- This false sense of security combined with the presence of carrier animals can result in further abortion storms.

### **Public health significance**



- *Campylobacter* has potential public health significance. *C. jejuni* - a very important zoonotic pathogen - Milk, minced meat, dogs, cats, and poultry are important reservoirs of infection for humans.
- It is widely distributed in the intestine of domestic and wild animals but is rarely isolated from the feces of normal humans.
- In human even 500 c.f.u./ml in milk are infective, the signs are fever, abdominal pain, nausea, vomiting, and bloody stools. Some may become persistently infected.

