# Laboratory diagnosis of veterinary important agents from genera Brucella and Campylobacter

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# Brucella in animals

- Brucellosis is a zoonotic disease caused by Gram-negative coccobacilli of the genus *Brucella*.
- In livestock, the disease results in significant economic losses due to reproductive impairment caused by abortion, stillbirth or weak calves and neonatal mortality, infertility.
- The genus *Brucella* is currently classified into 12 known species, according to basic differences in pathogenicity and host preference .
- Each one may infect different host species, but each *Brucella* species has a preference for its host species.

- **Epidemiological features of Brucella species.**

| Species | Natural host | Prevalent region | Reported human cases |
|---|---|---|---|
| *B. melitensis* | Sheep, goats | Mediterranean littoral, Arabian Peninsula, Latin America | Several cases |
| *B. abortus* | Cattle | Asian countries, Europe | Several cases |
| *B. suis* | Pigs | Latin America, Southern China, Southeast Asia, Europe | Several cases (biovar 1) |
| *B. canis* | Dogs | Argentina, Brazil, China, Czech Republic, Germany, Japan, Madagascar, Mexico, Papua New Guinea, Peru, Philippines | Rare cases |
| *B. ovis* | Sheep | Argentina, Chile, France, Germany, South Africa, USA, Spain, countries of the former Soviet Union | No human cases |
| *B. neotomae* | Rodents (desert wood rats) | United States | Two human cases |
| *B. microti* | Wild voles | North Europe | No human cases |
| *B. ceti* | Marine mammals | Mainly Northern Hemisphere | One laboratory infection |
| *B. pinnipedialis* | Marine mammals | Mainly Northern Hemisphere | No human cases |
| *B. inopinata* | Unknown | - | Prosthetic breast implant infection (one human case) |
| *B. papionis* | Baboons | - | No human cases |
| *B. vulpis* | Red foxes | Austria | No human cases |

# Laboratory diagnosis

- **Extreme care must be applied when working with *brucellae* because humans are highly susceptible to brucellosis and laboratory infections could happen.**

# Laboratory diagnosis

- **Phenotyping identification, through biochemical profiling, is not conclusive for all *Brucella* species due to the high similarity between them.**
- **There is little antigenic variation among *Brucella* spp., therefore the differentiation of species and strains is based on approximately 25 biological and physiological characteristics (phenotype)**
- **Therefore a combination of the following techniques are required:**
  1. **Classical microbiological identification**
  2. **Serology**
  3. **Molecular techniques.**

# Samples for *Brucella* spp. For isolation, serology, and molecular techniques:

- Fetal membranes
- fetal organs such as the lungs, bronchial, lymph nodes, spleen and liver, as well as fetal gastric contents.
- Milk, vaginal secretions and semen.
- Blood and serum

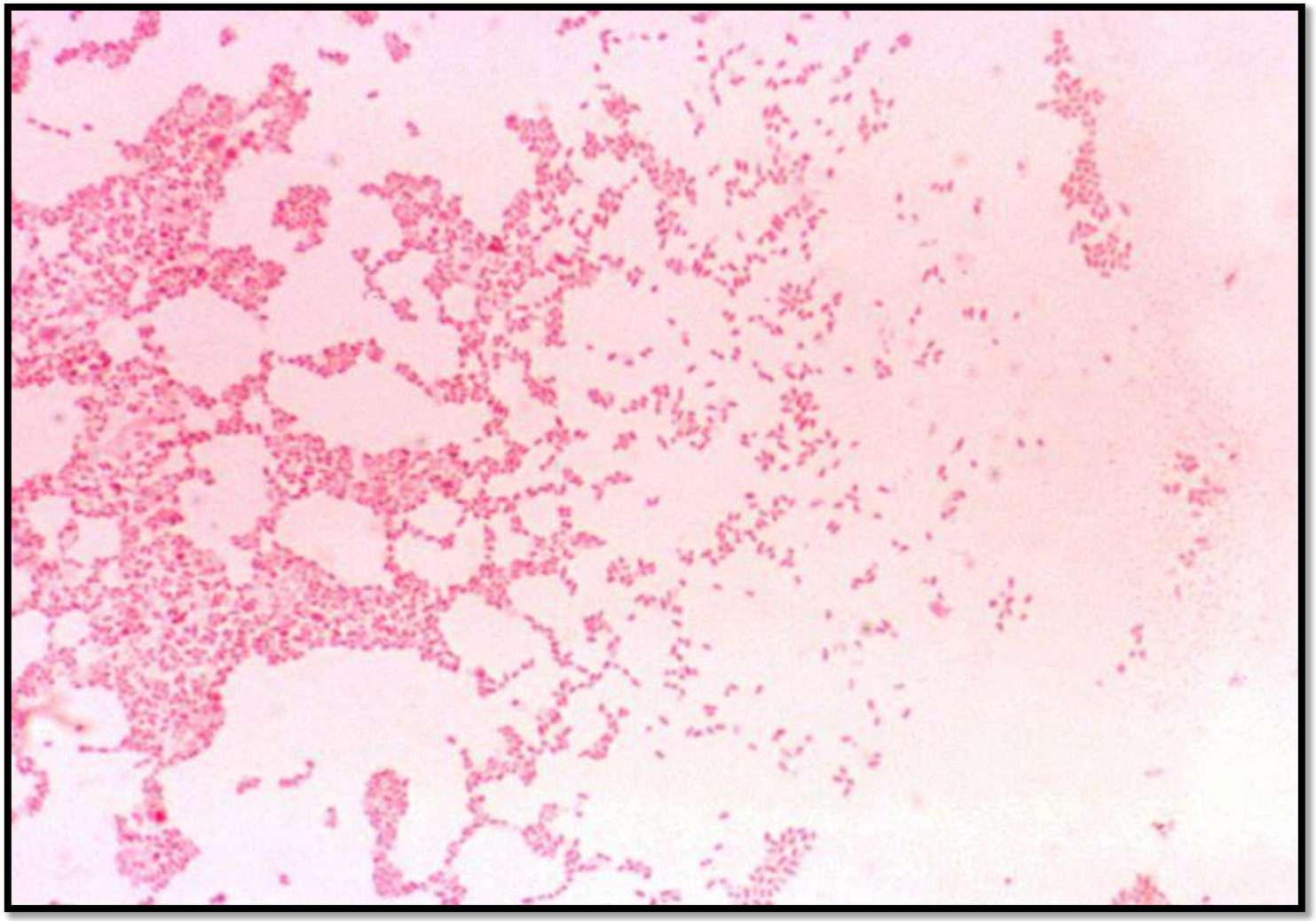
# 1- Classical microbiological identification

General microbial features for *Brucellae* :

1. Aerobic
2. Gram-negative cocci, coccobacilli or short rods of 0.5-0.7 by 0.6-1.5 µm in size
3. Usually individual and less frequently are found in pairs, short chains, or small groups.
4. They are non-motile and do not produce flagella.
5. Multiplication is slow at the optimum temperature of 37°C but the organism can grow under temperatures ranging from 20 oC to 40 oC

5. ***Brucella*** **spp. are fastidious bacteria that need rich culture medium to support adequate growth. The growth occurs on *Brucella* agar, MacConkey Agar, Trypticase Soy agar, Sheep Blood agar and Standard Nutrient agar at 25–42ºC.**
6. **Colonies on media are transparent, convex and have an entire edge. They are usually small (0.5–1.0 mm after 2–3 days of incubation of a fresh inoculum).**
7. ***Brucella*** **strains are catalase positive and superoxide dismutase positive, most of them are also oxidase positive.**
8. **Many strains require supplementary CO2 for growth.**

- ***Brucella melitensis*, gram, negative, coccobacillus,**



- ***Brucella abortus,***
- **Grown on  blood agar**

# Example : Phenotypic features of *Brucella* species and biotypes, and the tests for their identification and differentiation

- **Not for memorization**

| Species | Biotype | $CO_2$ | $H_2S$ | Biochemical tests | | | | | Growth on dyes ** | | | Agglutination in serat | | | Lysis by phage (RTD)Tb# | Motility | Acriflavin test |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Urease | Oxidase | Catalase | Citrate | Nitrate | Thionin *** a | Thionin *** b | Basic fuchsin | Anti -A | Anti -M | Anti -R | Tb 10 000 x RTD | | |
| *B. melitensis* | 1 | - | - | + | + | + | - | + | - | + | + | - | + | - | - | - | - |
| | 2 | - | - | + | + | + | - | + | - | + | + | + | - | - | - | - | - |
| | 3 | - | - | + | + | + | - | + | - | + | + | + | + | - | - | - | - |
| *B. abortus* | 1 | (+) | + | + | + | + | - | + | - | - | + | + | - | - | - | - | - |
| | 2 | (+) | + | + | + | + | - | + | - | - | - | + | - | - | - | - | - |
| | 3 | (+) | + | + | + | + | - | + | + | + | + | + | - | - | + | - | - |
| | 4 | (+) | + | + | + | + | - | + | - | - | (+) | - | + | - | + | - | - |
| | 5 | - | - | + | + | + | - | + | - | + | + | - | + | - | + | - | - |
| | 6 | - | -or+ | + | + | + | - | + | - | + | + | + | - | - | - | - | - |
| | 7 | - | -or+ | + | + | + | - | + | - | + | + | + | + | - | - | - | - |
| | 8 | + | - | + | + | + | - | + | - | + | + | - | + | - | - | - | - |
| | 9 | -or+ | + | + | + | + | - | + | - | + | + | - | + | - | - | - | - |
| *B. suis* | 1 | - | ++ | + | + | + | - | + | + | + | (-) | + | - | - | - | - | - |
| | 2 | - | - | + | + | - | - | + | - | + | - | + | - | - | - | - | - |
| | 3 | - | - | + | + | - | - | + | + | + | + | + | - | - | - | - | - |
| | 4 | - | - | + | + | + | - | + | + | + | (-) | + | + | - | + | - | - |
| | 5 | - | - | + | + | + | - | + | + | + | - | - | + | - | + | - | - |
| *B. neotomae* | | - | + | + | - | - | - | + | - | - | - | + | - | - | (+) | - | - |
| *B. ovis* | | + | - | - | - | - | - | - | + | + | (-) | - | - | + | - | - | + |
| *B. canis* | | - | - | + | + | + | - | + | + | + | (-) | - | - | + | - | - | + |
| *B. ceti* | | - | - | + | + | - | - | + | + | + | + | + | +or- | - | - | - | - |
| *B. pinnipedialis* | | - | - | + | + | + | - | + | + | + | + | + | +or- | - | - | - | - |
| *B. microti* | | - | - | + | + | + | - | + | + | + | + | - | + | - | + | - | - |
| *B. inopinata* | | - | + | + | + | + | - | + | + | + | + | - | - | + | + | - | - |

+ positive; - negative; (+) usually positive; (-) usually negative; ** Species differentiation is obtained on Trypticase-soy agar or tryptose agar with the concentrations of dyes: (a)1:25 000, (b)1:50 000; ***B. *abortus*. 1, 2 and 4 do not grow in 1:100 000 thionine; † Monospecific antiserum: A-*abortus*; M-*melitensis*; R-*rough*; d-doubtful; # Tbilisi; RTD-routine test dilution

* Adapted from Alton et al. 1975; Foster et al., 2007; Jacques **et al.** 2007; Poester et al., 2010.

# 2-Serological tests

- Serological tests are crucial for laboratorial diagnosis of brucellosis
- Inactivated whole bacteria or purified fractions (i.e. lipopolysaccharide or membrane proteins) are used as antigens for detecting antibodies generated by the host during the infection.

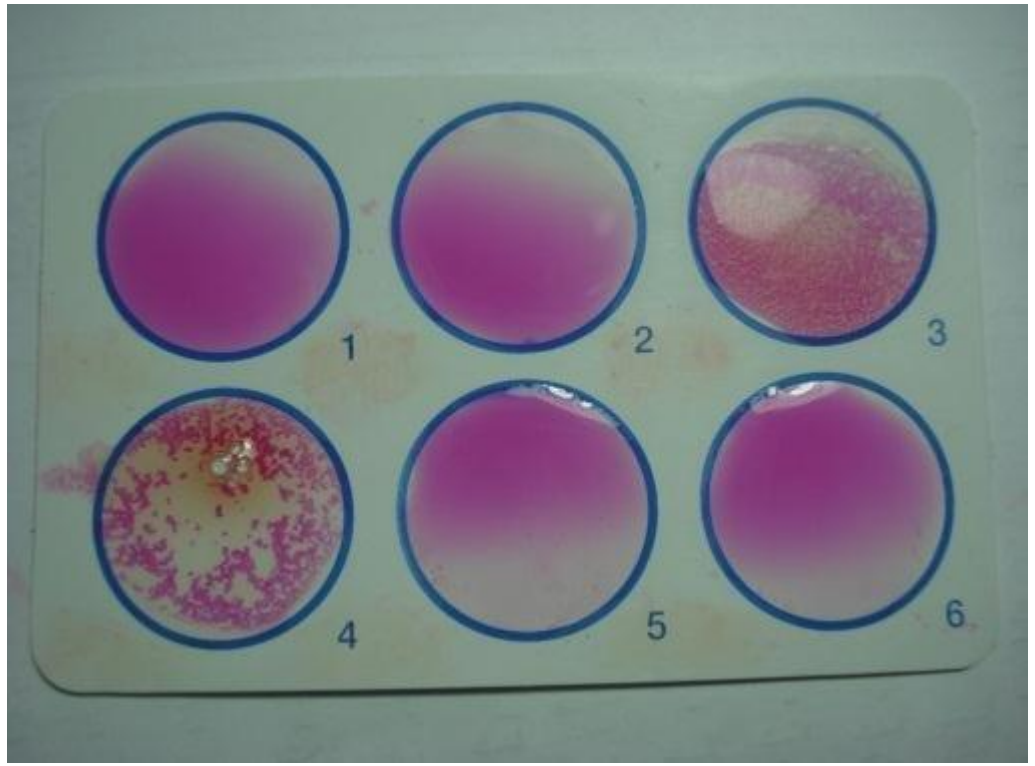
- Problem = cross reaction

**Several serological methods are available. They can be classified as :**

- **Screening tests (e.g. buffered antigen plate agglutination - BPAT),**
- **Monitoring or epidemiological surveillance tests (e.g. milk ring test),**
- **Complementary or confirmatory tests (e.g. 2-mercaptoethanol, complement fixation, ELISAs, and fluorescence polarization assay).**

- **Example: Rose Bengal test is a traditional methods that has been extensively used for diagnosis of brucellosis in cattle, pigs, and goats**

- **Rose Bengal Plate Test; Positive test showing agglutination no.3 and 4.**



# 3-Molecular diagnosis

- Molecular techniques are important tools for diagnosis and epidemiologic studies.
- They provide information for identification of species and biotypes of *Brucella* spp.
- Molecular detection of *Brucella* sp. can be done directly on clinical samples without previous isolation of the organism.
- These techniques can be used to complement results obtained from phenotypic tests.
- Most of the molecular diagnostic methods for brucellosis have sensitivity ranging from 50% to 100% and specificity between 60% and 98%. This is because the DNA extraction protocol, type of clinical sample, and detection limits of each protocol, are factors that can influence the efficiency of the technique.

- Polymerase Chain Reaction (PCR): amplification of specific genomic sequences of the genus or virulence genes to determine pathogenicity.
- Real time PCR - qPCR is much more sensitive and can be used even on low number of bacteria in the samples.

# Campylobacter

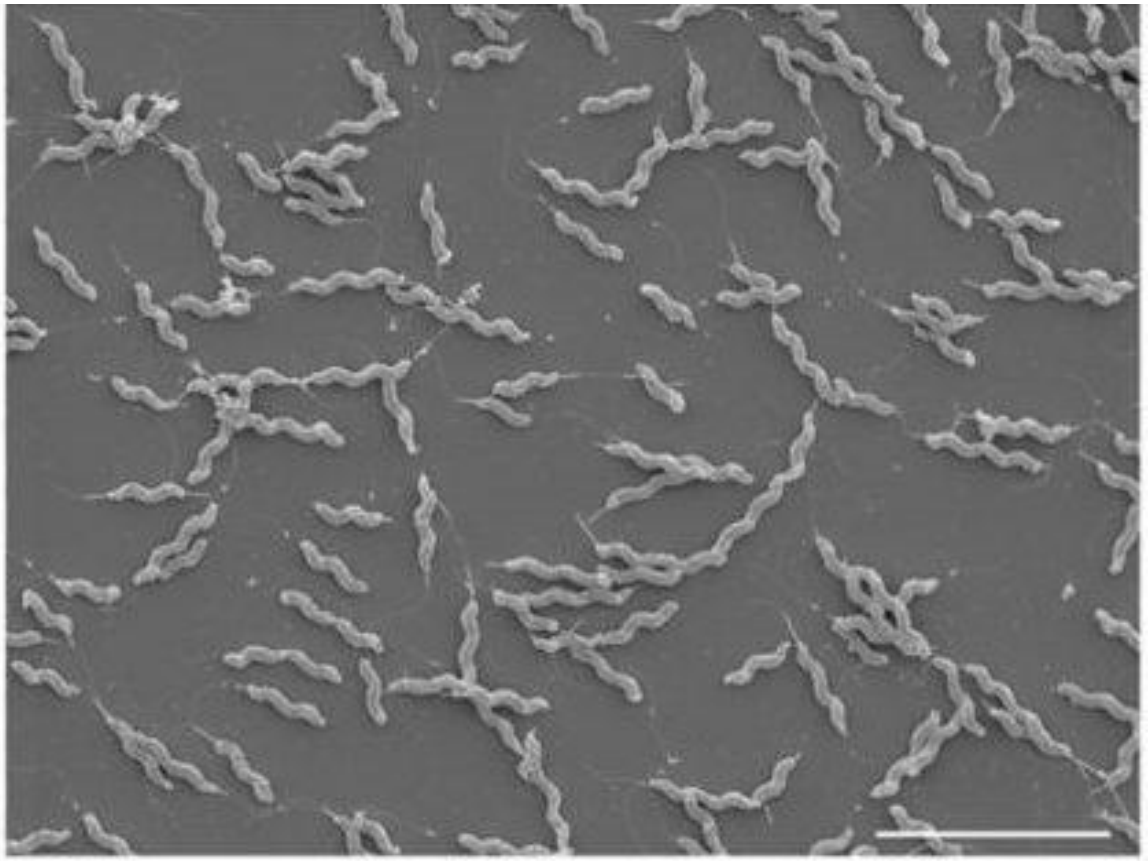
- Campylobacter spp. belong to the family *Campylobacteriaceae*.
- *Campylobacter* constitute a diverse group of organisms, some of which are well-known causes of clinical illness in animals and humans, whereas many other members of the genus appear to be commensals in the intestinal tract.
- They are Gram-negative rods.
- *Campylobacter* cells are S/seagull-shaped, spirally curved rods 0.2–0.8 µm wide and 0.5–6.0 µm long, but cells may transform to spherical or coccoid forms in response to stress conditions.
- non-spore-forming
- Most are motile, exhibiting a characteristic corkscrew-like or darting motility motion. Motility is conferred by flagella, usually in the form of a single flagellum at one or both ends of the cell, although non motile species or species with multiple flagella have been described
- Gram-negative bacteria and

- Optimal growth occurs at **37–42**◦C for thermophilic members (*Campylobacter jejuni* and *Campylobacter coli* ), whereas *C. fetus* grows well at **25**◦C and 37◦C and usually does not grow at **42**◦C .
- *Campylobacter* spp. are typically unable to ferment or oxidize carbohydrates, and thus energy is derived from the degradation of amino acids or tricarboxylic acid cycle intermediates.
- They are slow-growing, fastidious organisms and require a microaerobic atmosphere (containing **5%** O2, 10% CO2, and **85%** N2) for optimal growth.
- In general, *Campylobacter* is sensitive to oxygen, desiccation, osmotic stress, low pH, and high temperatures.

- ***Campylobacter*** **species (primarily *C. jejuni* and *C. coli* ) are essentially commensals in birds and frequently colonize the intestine in high numbers .**

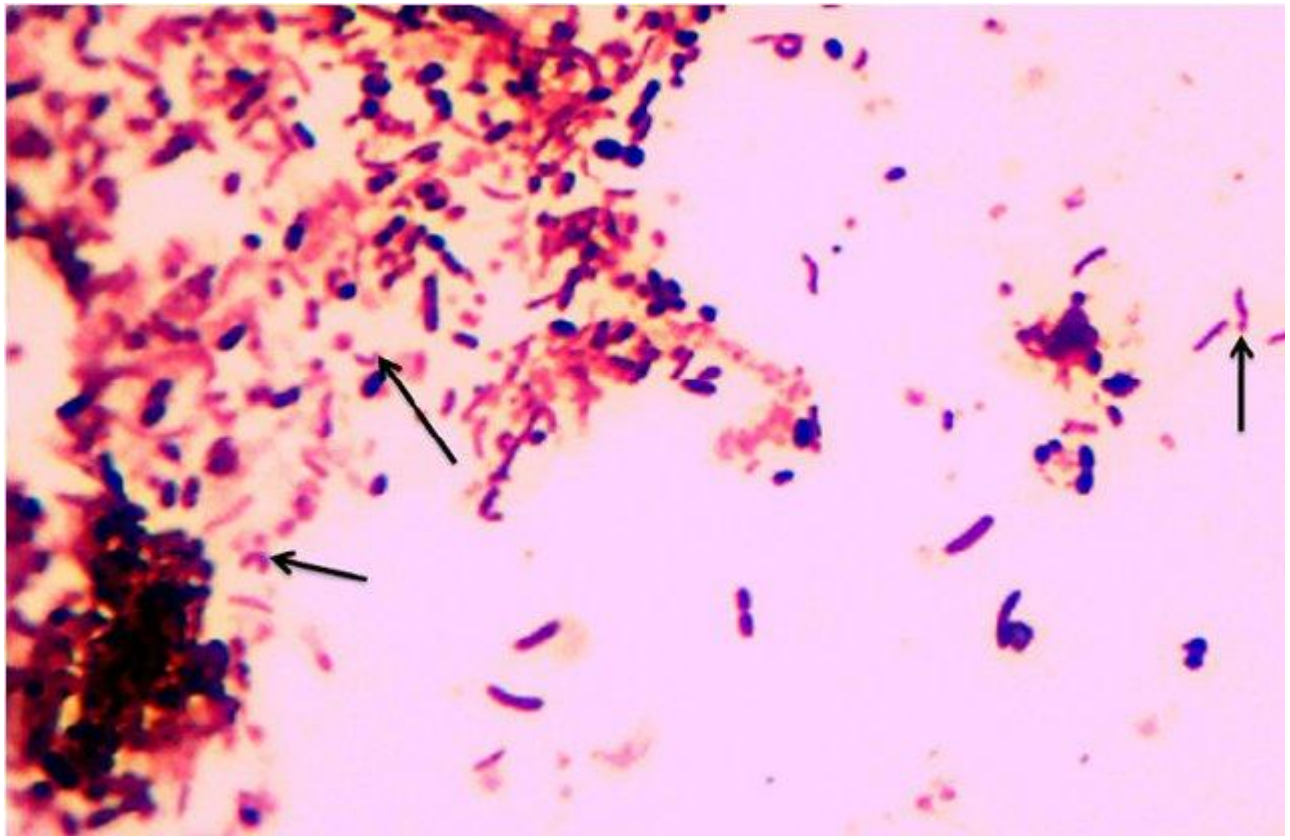
- ***Campylobacter*** **contamination of poultry meat is a significant concern for food safety.**

***Scanning electron micrograph of the spiral shape and flagella of Campylobacter jejuni***



https://www.nature.com/articles/srep38303#f1

**The C, S or gull wing shape of Campylobacter species (arrows) stained by 1% Carbol fuchsin.**



https://www.researchgate.net/publication/271214444_Evaluation_of_detection_methods_for_Campylobacter_infections_among_under-fives_in_Mwanza_City_Tanzania/figures?lo=1

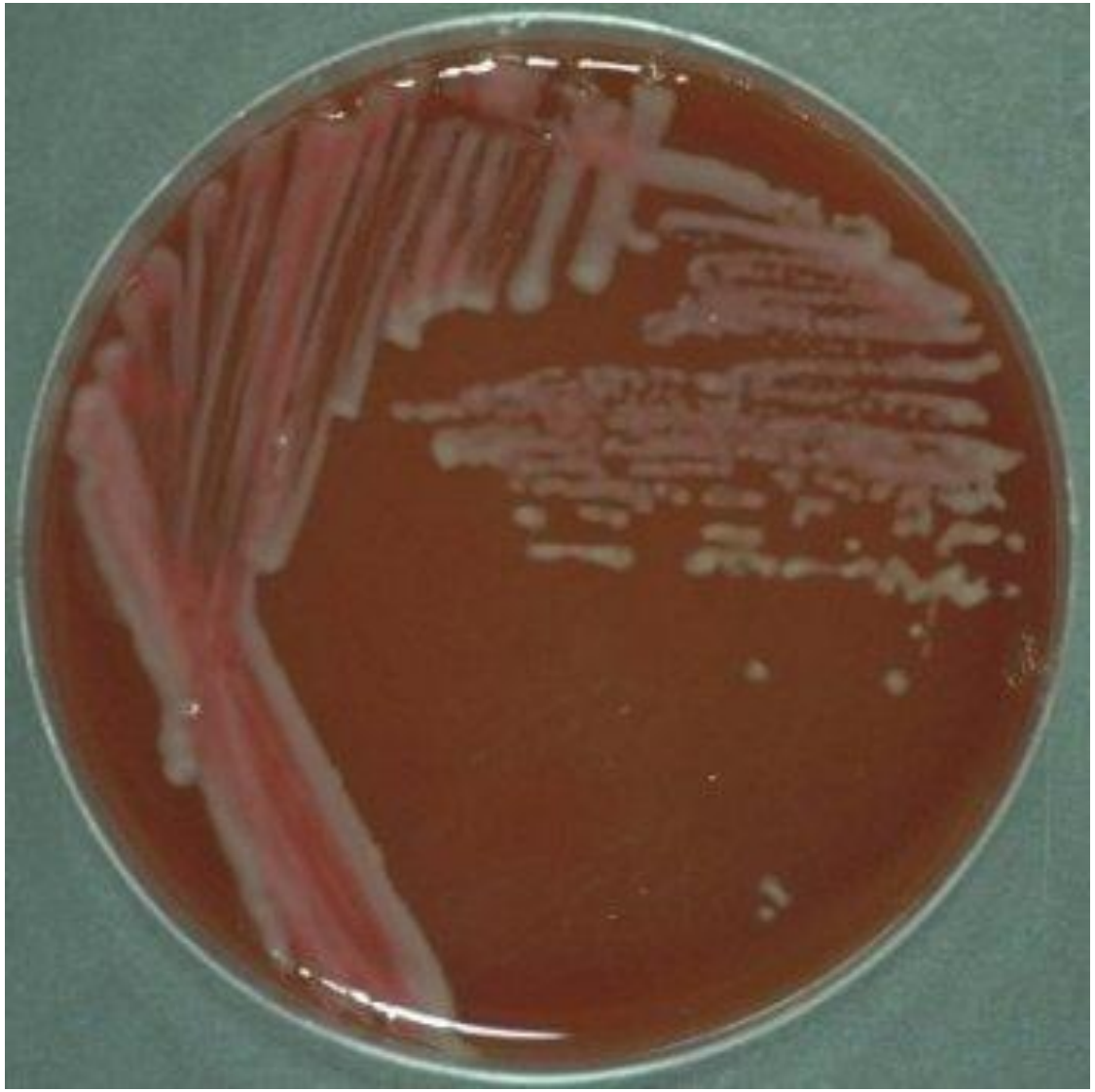
# Diagnosis

- Culture in conjunction with histopathological findings are used for definitive diagnosis of *Campylobacter* in ruminant abortions.
- The organism could be isolated from the placenta and fetal stomach contents, or from fetal lung, and occasionally from fetal liver.
- To isolate *Campylobacter*, a variety of selective culture media can be used such as chrome agar.
- Dark field examination of wet mounts of fetal stomach contents or placenta for bacteria of typical morphology and characteristic of *Campylobacter such as* darting movement of corkscrew-like organisms (especially with *C. fetus* subsp. *fetus*) represents a rapid test to establish a presumptive diagnosis
- Direct staining with dilute carbol fuchsin or fluorescent antibody staining of smears of fetal abomasal contents can also be used to demonstrate *Campylobacter* .

- Following the culture, colonies resembling *Campylobacter* need to be confirmed for definitive diagnosis, which could be done using a variety of assays, including phenotypic tests, polymerase chain reaction (PCR),

- In sheep, a standard PCR is used in detection of both *C. fetus* subsp. *fetus* and *C. jejuni* DNA directly from aborted fetal tissues and placentas

- **Colonies of Campylobacter on Blood Agar plate**



# CHROM agar™ Campylobacter: Typical Appearance of microorganisms *Campylobacter jejuni, coli, lari* → red