


BACTERIOIDES AND FUSOBACTERIUM

Learning objectives

To know in detail about,

- *Bacterioides* and *Fusobacterium* species
- Media and cultivation methods for *Bacterioides* and *Fusobacterium* species
- Colonial morphology and microscopic appearance of *Bacterioides* species
- Different types of foot rot in sheep
- Choice of specimens and collection procedures, isolation and identification methods of *Bacterioides* infections in animals

SYSTEMATICS

Domain	Bacteria	Bacteria	Bacteria
Phylum	<i>Proteobacteria</i>	<i>Bacteroidetes</i>	<i>Fusobacteria</i>
Class	<i>Gammaproteobacteria</i>	<i>Bacteroidetes</i>	<i>Fusobacteriales</i>
<u>Order</u> 	<i>Cardiobacteriale</i>	<i>Bacteroidales</i>	<i>Fusobacteriales</i>
Family	<i>Cardiobacteriaceae</i>	<i>Bacteroidaceae</i>	<i>Fusobacteriaceae</i>
Genus	<i>Dichelobacter</i>	<i>Bacterioides</i>	-

- *Bacterioides*, *Fusobacterium*, *Campylobacter mucosalis*, *Campylobacter hyointestinalis* and *Serpulina hyodysenteriae* are non spore forming, Gram-negative anaerobic bacteria.
- They are commonly implicated in necrotic and suppurative conditions, often as mixed infections with facultative anaerobic bacteria.

HABITAT AND MORPHOLOGY

Habitat

- *B. nodosus* are obligate anaerobic bacteria of the digital epidermis of sheep under normal climatic conditions.
- They will not survive on pastures for more than a week.
- Other *Bacterioides* species can be normal inhabitants of the skin, mucous membrane and G.I. tract of domestic animals.
- *Fusobacterium* species occurs as a commensal in the alimentary tract and mucous membranes of a variety of animals.

Morphology

- *Bacterioides nodosus* appear as Gram-negative, fairly large (1.7 x 3-6 μm), slightly curved and non-motile rods.
- Often swollen at one or both ends. They occur singly or occasionally in pairs
- *Fusobacterium necrophorum* is Gram-negative, long and filamentous but does not branch.
- Filaments can be up to 100 μm in length and 0.5-0.7 μm in diameter.
- May have tapered or rounded ends. Irregular staining is characteristic

CULTURAL CHARACTERISTICS

- Anaerobic jars with a catalyst, an anaerobic indicator and an atmosphere free of oxygen (10% Hydrogen, 5% CO₂ and 85% Nitrogen) is essential for the culture of these strict anaerobes.
- Enriched blood agar is highly suitable for these fastidious anaerobes.
- Eugon, Columbia, trypticase soy or brain-heart infusion agar enriched with 0.5 per cent yeast extract, vitamin K (10 µg/ml), haemin (5 µg/ml), kanamycin (100 µg/ml) and vancomycin (7.5 µg/ml) are most commonly used.
- *Bacteroides* spp. (except *B. ureolyticus*) are resistant to kanamycin but the *Fusobacterium* spp. is sensitive to this antibiotic.
- A 'Fastidious Anaerobe agar' is available commercially with various antibiotic supplements, depending on the anaerobe that is being sought.
- Eugon agar base with 0.2 per cent (w/v) yeast extract, 10 per cent defibrinated horse blood and one µg/ml lincomycin is the selective medium for *Bacteroides nodosus*.
- Lemco agar containing pulverized hoof powder can also be used. Members of the *B. fragilis* group will grow on bile aesculin medium with 5 per cent sheep blood.
- Agar media should be pre-reduced in an anaerobic jar for 6-24 hours before use. The plates are incubated at 35-37°C immediately after streaking for 4-8 days.
- Liquid media such as Cooked meat broth with 0.4 per cent glucose or thioglycollate medium is also suitable with the addition of the vitamin K-haemin supplement.

Colonial morphology and microscopic appearance

- The cellular morphology, and sometimes the colonial morphology, can be very variable depending on the strain, medium and cultural conditions.

Bacteroides nodosus

- *B. nodosus* in a Gram-stained smear from enriched blood agar, appears as straight or slightly curved rods with the characteristic terminal knobs on one or both ends of the cells
- Three basic colonial types are described
 - B-type: papillate or beaded (most pathogenic) from ovine foot rot.
 - M-type: mucoid (less pathogenic) from non-invasive infections of sheep and cattle.
 - C-type: circular (non-pathogenic) and resulting from repeated passage in media.

- The colonies, generally, are greyish-white and 0.5-3.0 mm, diameter, in 3-7 days.

Bacteroides melaninogenicus

- Circular, entire, convex and shiny colonies, 0.5- 2.0 mm in diameter.
- Colonies become darker after 5-14 days, being black in the centre with a grey-brown periphery.
- Haematin (black or brown) pigment is seen best on media containing blood.
- A few strains are haemolytic on rabbit blood agar. The colonies fluoresce under ultra-violet light.

Bacteroides asaccharolyticus

- Colonies are 0.5-1.0 mm in diameter, round, convex, opaque and light grey after 48 hours' incubation.
- In 6-14 days the colonies may become black. Some strains are haemolytic on rabbit blood agar

Bacteroides fragilis

- Colonies are circular, entire, low convex, translucent to semi-opaque.
- They tend to have concentric rings of growth. Less than 1 % of strains are haemolytic.

Fusobacterium necrophorum

- *F. necrophorum* produces grey to yellowish shiny colonies on blood agar, that are about 2-3 mm in diameter after 48 hours' incubation.
- Haemolysis is variable.
- A Gram-stained smear from the colonies shows long Gram-negative filaments that are less characteristic than those from direct microscopic examination of specimens.
- Lipase, but not lecithinase, activity is exhibited by many strains of *F. necrophorum* on egg yolk agar.

BIOCHEMICAL CHARACTERISTICS AND RESISTANCE

Biochemical characters

- All pathogenic *Bacteroides species* are catalase negative except *B. fragilis*. *B. nodosus* does not ferment carbohydrates.
- *F. necrophorum* is indole and H₂S positive. Gelatin is not liquefied.
- They ferment glucose and maltose with production of acid and gas but not lactose.

Resistance

- Both the species are highly susceptible to atmospheric oxygen.
- They are readily killed at 50 –60° C for 15 minutes.

TOXINS AND PATHOGENESIS

Toxins

- *B. nodosus* causing foot rot produces keratinolytic enzymes in greater amounts.

- *F. necrophorum* produces an exotoxin (leukotoxin) and necrotizing endotoxin.
- The endotoxin when inoculated intradermally into rabbit causes necrosis.
- The exotoxin when inoculated in skin of rabbits causes mild erythema but when administered intravenously causes maciation or death of rabbits in a few hours.

Pathogenesis

- The infections are often endogenous, arising from normal flora at the site or by wounds contaminated by nearby flora.
- For these strict anaerobes multiply at a focus in animal tissue if the redox potential of the area is lowered.
- This can occur through trauma and necrosis, ischaemia, parasitic invasion or concomitant multiplication of facultative anaerobes.
- *B. nodosus* produces keratinolytic enzymes and *F. necrophorum* produces leukotoxin – which protects *Corynebacterium pyogenes* from phagocytosis. *C. pyogenes* produces a diffusible factor that stimulates the proliferation of *Fusobacterium* in tissue.
- The conditions caused by these non-sporing anaerobes include soft tissue abscesses and cellulitis, post-operative wound infections, periodontal abscesses, aspiration pneumonia, lung and liver abscesses, peritonitis, pleuritis, myometritis, osteomyelitis, mastitis and foot rot. The excessive odour is due to production of volatile fatty acids.
- Diseases caused by *Bacteroides* and *Fusobacterium* species

Species	Host(s)	Disease
<i>Dichelobacter (Bacteroides) nodosus</i>	Sheep Cattle, Goats and Pigs	Contagious (virulent) foot rot Contagious interdigital dermatitis
<i>B. melaninogenicus</i>	Cattle	Foot rot
<i>B. asaccharolyticus</i>	Dogs, cats, horses, cattle	Osteomyelitis
<i>B. fragilis</i>	Calves, lambs, foals, piglets	Diarrhoeal disease
<i>Fusobacterium necrophorum</i> (associated with <i>Corynebacterium</i> <i>pyogenes</i>)	Sheep Cattle Horses Pigs Chickens	Foot abscess , Ovine interdigital dermatitis (Scald) Calf diphtheria Thrush - infections of front of hoof. Ulcerative stomatitis ("sore mouth" or "Bull nose" - via injury from fitting nose rings)

		Necrotic enteritis Avian diphtheria (Secondary to fowl pox)
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PATHOGENICITY

Symptoms

- Infections in animals generally occur when animals are kept in filthy, manure-laden surroundings.
- Among the *Fusobacterium* only *necrophorum* regularly causes disease in animals.
- It is frequently a secondary invader (e.g., liver abscesses in cattle often found with *Corynebacterium pyogenes* and is characterized by a necrotic process, commonly causing diseases collectively referred to as necrobacillooses and present as necrosis, abscess formation, and putrid odour (most common fermentation product is butyric acid).

Symptoms will vary according to the sites of the lesions.

Cattle

- In foot rot – acute painful swelling of a hoof which leads to lameness.
- Necrosis in digits spreads to tendons, ligaments and joints.
- Calf diphtheria is more common in 3 to 18 months old.
- Excessive salivation, purulent discharge from nose, coughing, temperature and loss of appetite and is found in necrotic foci in the mouth, larynx and trachea.

Sheep

- Foot rot (interdigital dermatitis, infective bulbar necrosis and heel abscess), mouth lesions and abortions (rare).

Swine

- Principal cause of bull nose or Ulcerative stomatitis via injury from fitting nose rings.

Cats

- Opportunistic. Highly suppurative. Involves nasal passages, oral cavity and bone.
- Secondary invaders to tissue damage.
- Dental tartar leads to gingivitis and periodontal disease.

DIAGNOSIS

Choice of specimens

- As the non-sporing anaerobes constitute a major portion of the normal flora, the specimens must be collected with care to avoid contamination from the normal anaerobic flora, situated mainly on mucous membranes and in the intestinal tract. The following samples are suitable for culture of the non-spore-forming anaerobes.
 - Pus from abscesses

- Discharges from wounds (surgical and traumatic)
- Direct pleural aspirates
- Peritoneal aspirates
- Joint fluids
- Urine if taken by suprapubic puncture
- Tissue specimens (biopsy, necropsy and post-operative)

Collection of specimens

- Specimens for the isolation of these strict anaerobes should be placed immediately in an oxygen-free container, especially small pieces of tissue or material taken on swabs.
- Larger pieces of tissue (over 2 cm³) usually maintain an anaerobic microenvironment deep in the tissue and can be placed in an air-tight jar for transportation.
- Fluid specimens can be collected in a sterile syringe, the air expelled and the needle bent over or plugged. However, if the specimen cannot be processed within an hour, a fluid specimen should be placed in an oxygen-free tube or vial.
- All specimens for anaerobic culture should be processed within a few hours of collection.
- It is best to keep the specimens at ambient temperature rather than in the refrigerator, as oxygen absorption is greater at lower temperatures.
 - Direct examination
 - Gram-stained smears of the specimens are useful as a screening process, although many of these anaerobes are not morphologically distinctive.
 - Dilute carbol fuchsin (4-8 minutes) stained smears are more useful for *Bacteroides* and *Fusobacterium species* as they tend to stain faintly with the Gram-stain.
 - *Fusobacterium necrophorum* in clinical specimens is long and filamentous (about 1µm in diameter) and characteristically stains in an irregular manner.
 - *Bacteroides nodosus* is a large rod characterised by the presence of terminal enlargements at one or both ends (barbell or club shaped).
 - FAT is reported as being specific and sensitive
 - By Isolation and identification in anerobic media
 - By animal inoculation
 - Mice, rats and guinea pigs inoculated with cultures of *B. nodosus* will produce abscess and sepsis.
 - Inoculate rabbit subcutaneously with material suspected for *F. necrophorum* - produces lesions throughout the body.
- Differential diagnosis should be made with strawberry foot rot caused by *Dermatophilus species*, FMD and pyogenic infections associated with *Corynebacterium pyogenes*.

TREATMENT AND CONTROL

- In *B. nodosus* infection the affected hooves should be trimmed and treated with 10% formalin or chloramphenicol or tetracycline.
- Parental treatment with penicillin and streptomycin may be of value.
- In *Fusobacterium* infections, 5-10% CuSO₄ is recommended for the treatment of foot lesions.
- For early treatment sulphonamides, tetracycline and erythromycin are useful.

