

VMC- 602 (BACTERIOLOGY II)
UNIT III

**ANAEROBIC NON-SPORE FORMING GRAM NEGATIVE BACILLI
(LECTURE-2)**

Dr. Savita Kumari
Assistant Professor-cum-Jr. Scientist
Department of Veterinary Microbiology
Bihar Veterinary College, BASU, Patna

Dichelobacter nodosus

- Formerly known as *Bacteroides nodosus*, *Fusiformis nodosus*
- Primary etiologic agent of foot rot in sheep and goats

Structure

- Gram- negative, Straight or slightly curved rod measuring 1–1.7 to 3–10 μm
- In smears from lesions, rods exhibit terminal swellings- appearance of “dumbbell”
- Feature less discernible when clinical isolates sub-cultured in broth



Image source

(<https://alchetron.com/cdn/dichelobacter-nodosus>)

■ Colony characteristics:

- 0.5–3.0 mm in diameter
- Smooth, convex, and translucent or semi-opaque in appearance
- Colonies often etch into surface of culture medium immediately beneath them, producing a sunken appearance
- Three basic colony types, namely papillate or beaded (B)-type, mucoid (M)-type, and circular (C)-type

- Papillate or beaded (B)-type colonies: Fresh isolates from advanced foot rot
- Muroid (M)-type colonies: Isolates from non-invasive infections of the interdigital skin of sheep and cattle, less pathogenic
- Circular (C)-type colonies: Predominated in repeatedly passaged liquid subcultures, avirulent
- Non- flagellated, possesses long filamentous appendages (type IV fimbriae)
- Type IV fimbriae confer flagella-independent motility, called twitching motility
- Fimbrial proteins highly immunogenic, basis of serotyping of *D. nodosus*

Biochemical characters

- Catalase -ve
- Oxidase -ve
- Indole -ve
- Urease -ve
- H₂S +ve
- Does not ferment carbohydrates

Virulence factors

- Have a wide range of virulence and are categorized as virulent, benign, and intermediate
- Virulence factors (e.g., proteases and twitching motility)
- Certain genes, such as *intA* (formerly *vap*) and *vrl* (virulence-related locus), associated with virulence, do not encode virulence factors, may play a regulatory role in gene expressions

Fimbriae

- Adherence to epithelial cells of interdigital epidermis, close contact between *D. nodosus* and host cells
- Translocation of organism into a more anaerobic microenvironment by twitching motility
- Required for bacterial growth and extracellular protease production, ultimately leading to lesion formation
- Twitching motility and production of extracellular proteases- for virulence

Cell Wall

- Similar to LPS of other gram-negative bacteria

Outer Membrane Proteins

- Promotes adherence to host cell surface
- Interfere with host's immune response
- Highly antigenic and phase variant
- Phase variance allows *D. nodosus* to undergo antigenic shifts to evade the immune system during the course of infection

Serine Proteases

- Secrete three closely related serine proteases
- Postulated to be responsible for the tissue damage in foot rot infections
- All proteases have a similar structure
- Highly virulent strains produce two acidic proteases, AprV2 and AprV5, and one basic protease, BprV
- AprV2 - essential for virulence
- Strains producing benign lesions
 - Produce similar proteases designated as AprB2, AprB5, and BprB
 - Show minor differences in amino acid sequences compared to the proteases of the virulent strains
- The protease genes are located on pathogenicity islands

Growth Characteristics

- Requires carbon dioxide
- Rich medium, preferably containing protein for growth
- Colonies may be visible on plates after 2 days, at least 4–5 days of incubation required to see smooth colonies of about 1–2 mm in diameter

Resistance

- Highly aerotolerant, can survive on plates exposed to air for up to 10 days
- Survives in environment for 2–3 days
- Killed by disinfectants and many antibiotics
- Can survive in and transmitted via soil
- Can also persist for months as a subclinical infection in the interdigital skin, or in small cryptic lesions within the hoof

Variability

- Colony morphological variation and virulence- related to abundance of fimbriae
- Virulence also varies with proteolytic activities of strains
- Benign strains - thermolabile, extracellular protease and a low degree of motility
- Intermediate and virulent strains - thermostable protease
- Ten major serogroups (A–I and M), based on differences in antigenic constitution of fimbrial adhesins
- Two major classes- Based on structural variation within fimbrial protein FimA and genetic organization of fimA gene
 - ❖ Class I -Isolates in serogroups A–C, E–G, I, and M, have fimB gene
 - ❖ Class II- Isolates in serogroups D and H, contain three genes adjacent to fimA, fimC, fimD, and fimZ

Reservoir

- Significant reservoir- infected foot of sheep or goats
- Cattle and swine strains- of low virulence

Transmission

- By direct or indirect contact
- Brief environmental survival time requires prompt colonization of new hosts

Pathogenesis

- Fimbriae-mediated attachment to host cells
- Proteolytic activity
- Synergy with *F. necrophorum* to which *D. nodosus* supplies growth factors

Disease

Foot rot

- Characterized by an exudative inflammation
- Followed by necrosis of epidermal tissues of hoof
- Three different forms of the disease : virulent, intermediate, and benign
- Benign strains- benign foot rot of sheep and interdigital dermatitis in cattle and goats
- Intermediate and virulent strains - intermediate and virulent foot rot in sheep, varies in severity according to degree of motility of strain
- Virulence:
 - Population type of *D. nodosus*
 - various factors affecting infections, environmental factors

Virulent form:

- Characterized by destruction of the horn
- Erosion of skin–horn junction that penetrates underlying tissues causing delamination
- Highly contagious
- Significant impact on productivity

Intermediate form:

- Covers a range of disease syndrome

Benign form:

- Milder form
- Inflammation of interdigital skin with dermatitis
- Also called foot-scald
- No destruction of underlying tissues and delamination

Sequence of events:

- The interdigital epidermis is softened and easily damaged because of persistent soaking
- *F. necrophorum*, a soil organism, infects the damaged skin and produces superficial inflammation, hyperkeratosis, parakeratosis, and necrosis
- *D. nodosus* colonizes (with the aid of fimbriae) and proliferates in lesion initiated by *F. necrophorum*, producing interdigital swelling
- Invasion of epidermal structures begins with the help of secreted proteases
- Advances to the epidermal matrix of the hoof, eventually separating it from the underlying dermal tissues (“underrunning”)
- Secondary invaders help maintain or aggravate the process
- Extreme lameness, becomes immobilizing if two or more feet involved

Epidemiology

- Specific to sheep and goats,
- Also reported in foot lesions of other animals, including cattle, horses, pigs, deer, etc.
- The disease occurs on all continents
- Most serious in regions with a mild climate and abundant rainfall (>500 mm)
- Dissemination ceases at ambient mean temperatures of less than 50 °F (10 °C)
- Disease not in arid regions and improves during dry periods in endemic areas
- All ages of animals beyond nursing stages are susceptible
- Genetic differences in susceptibility
- Fine wool breeds are most severely affected
- The agent eliminated from contaminated pastures within 2 weeks

Laboratory Diagnosis

- Usually based on obvious clinical signs
- Direct smears from the foot lesion may reveal stout rods with terminal swellings

Culture-based diagnosis:

- Routinely not done (fastidious, slow growth)
- Special selective media required
- Powdered ovine hoof added to promote enhanced growth
- Elastase activity & Gelatin gel test for detection of protease activity
- ELISA, using monoclonal antibodies against *D. nodosus* proteases and other antigens
- Molecular techniques: based on PCR amplification or nucleic acid probes

Immunologic Aspects

- Natural infection produces no immunity
- Even recently recovered sheep - susceptible to reinfection

Treatment

- Removal and exposure of diseased tissue by hoof trimming (foot paring)
- Followed by topical application of disinfectants or antibiotics
- Repeated treatment with 5–10% formalin, 5% copper sulfate, 10– 20% zinc sulfate, or 5% tetracycline tincture
- Formalin, copper sulfate, and zinc sulfate are used in footbaths
- Three 1 h 20% zinc sulfate soaks at weekly intervals - effective without foot paring
- Systemic treatment with large doses of penicillin and streptomycin

Vaccines:

- Extensively used in sheep-producing countries, particularly Australia and New Zealand
- Integral part of the eradication programs
- Also has some therapeutic benefit in accelerating healing
- Based on fimbrial proteins as protective antigen
- Commercially available vaccines contain 8–10 strains of the common serogroups

Control

- Combination of repeated examination, vaccination, treatment of active cases, and segregation of active cases from the healthy flock
- Avoid adding infected animals to the flock
- Contaminated lots should not be restocked for 2 weeks