

Haemophilus

By

Assist. Prf. Dr. Ali Aldeewan

Haemophilus is a genus of Gram-negative, pleomorphic, coccobacilli bacteria belonging to the family **Pasteurellaceae**. While Haemophilus bacteria are typically small coccobacilli, they are categorized as pleomorphic bacteria because of the wide range of shapes they occasionally assume



Key points

- Small, motile Gram-negative rods
- Fastidious, requirement for the X and V factors in chocolate agar
- Optimal growth in 5-10% CO₂,
- Facultative anaerobes
- Commensals on mucous membranes of many animal species
- Important pathogens include '*Haemophilus somnus*' (cattle) *H. parasuis* (pigs) and *H. paragallinarum* (poultry)

Haemophilus species are small (less than 1 µm x 1 to 3 µm), Gram-negative rods, which often appear coccobacillary and may occasionally form short filaments. These motile organisms, which are facultative anaerobes with variable reactions in catalase and oxidase tests, do not grow on MacConkey agar. They are fastidious bacteria requiring one or both of the growth factors X (haemin) and V (nicotinamide adenine dinucleotide, NAD). Optimal growth occurs in an atmosphere of 5-10% CO₂ on chocolate agar which supplies both X and V factors. Small, transparent, dewdrop-like colonies are formed by most *Haemophilus* species after incubation for 48 hours. Colonies of '*H. somnus*' have a yellowish hue and some isolates are haemolytic on sheep blood agar. The main pathogens in the genus are '*H. somnus*' in cattle and sheep, *H. parasuis* in pigs and *H. paragallinarum* which is responsible for infectious coryza of chickens

Usual habitat

***Haemophilus* species are commensals on the mucous membranes of the upper respiratory tract. They are susceptible to desiccation and do not survive for long periods away from their hosts.**

Differentiation of Haemophilus species

Haemophilus species are differentiated by requirements for X and V growth factors, by growth enhancement in an atmosphere of CO₂, by catalase and oxidase reactions and by carbohydrate utilization

-Isolation techniques

Both X and V factors are required in media for isolation requirements

- The porphyrin test is a more accurate method for determining the growth requirement for X factor.

- Biochemical reactions:

-Some biochemical tests can be carried out using conventional media. For testing **carbohydrate utilization**, a phenol red broth containing 1% of the sugar under test, filter-sterilized V and X factors and 1% serum is used.

-Commercially-available biochemical kits are used for testing isolates in a wider range of tests.

Pathogenesis and pathogenicity

Young or previously unexposed animals are particularly susceptible to infections by *Haemophilus* species. Specific-pathogen-free pigs, which do not harbour *H. parasuis* as a commensal, often develop signs of disease on primary exposure to the pathogen.

Environmental stress factors appear to contribute to the development of *Haemophilus* infections. Although virulence factors have not been fully identified, endotoxin is thought to play a role in the pathogenesis of infections. '*Haemophilus somnus*' can adhere firmly to several host cell types, including endothelial and vaginal epithelial cells. The organism is reported to cause degeneration of macrophages and it suppresses neutrophil function.

Degeneration of vascular endothelial cells and transmural neutrophil infiltration are prominent findings in thrombotic meningoencephalitis. Certain outer membrane proteins which confer virulence allow widespread dissemination of the bacteria in the host. Immunity to '*H. somnus*' appears to be predominantly **humoral in nature**. However, phase variation in the lipopolysaccharide antigen types may influence survival and persistence in host animals.

Diagnostic procedures

Specimens for laboratory examination depend on the clinical condition and type of lesions. *Haemophilus* species are fragile and neither refrigeration nor transport media maintain viability. Ideally, clinical specimens should be frozen in dry ice and delivered to a laboratory within 24 hours of collection. Either chocolate agar or blood agar inoculated with a streak of *S. aureus*, incubated under 5-10% CO₂ at 37°C for 2 to 3 days in a moist atmosphere, is used for isolation.

Identification criteria for isolates :

- Small, dew drop-like colonies after 1 to 2 days
- Enhancement of growth by CO₂,
- Requirement for X and V growth factors
- Biochemical profile

Although serological tests have been developed for epidemiological purposes, these tests are of little diagnostic value because *Haemophilus* species are widely distributed in animal populations.

Infectious coryza of chickens

Infectious coryza, caused by *H. paragallinarum*, affects the upper respiratory tract and paranasal sinuses of chickens. Its economic importance relates to loss of condition in broilers and reduced egg production in laying birds. Chronically ill and, occasionally, clinically normal carrier birds act as reservoirs of infection. Transmission occurs by direct contact, by aerosols or from contaminated drinking water. Chickens become susceptible at about 4 weeks after hatching and susceptibility increases with age.

Clinical signs

The mild form of disease manifests as depression, serous nasal discharge and slight facial swelling. In severe disease, swelling of one or both infraorbital sinuses is marked and oedema of the surrounding tissues may extend to the wattles. In laying birds, egg production may be severely affected. A copious, tenacious exudate may be evident at postmortem in the infraorbital sinuses and tracheitis, bronchitis and airsacculitis may be present.

Diagnosis

- Facial swelling is a characteristic finding.
- Isolation and identification of *H. paragallinarum* from the infraorbital sinuses of several affected birds is confirmatory.
- Immunoperoxidase staining can be used to demonstrate *H. paragallinarum* in the tissues of the nasal passages and sinuses
- Serological tests such as agglutination tests, ELISA or agar gel immunodiffusion tests are used to demonstrate antibodies about 2 to 3 weeks after infection and to confirm the presence of *H. paragallinarum* in a flock.

Treatment and control

- Medication of water and feed with oxytetracycline or erythromycin should be initiated early in an outbreak of disease.**
- An all-in/all-out management policy should be implemented and replacement birds should be obtained from coryza-free stock. Good management of poultry units minimizes the risk of infection.**
- Bacterins may be of value in units where the disease recurs. Vaccines should be administered about 3 weeks before outbreaks of coryza are anticipated.**