

GENUS: HISTOPHILUS, HAEMOPHILUS AND AVIBACTERIUM



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BACILLUS

- Domain: Bacteria
- Phylum: Proteobacteria
- Class: Gammaproteobacteria
- Order: Pasteurellales
- Family: Pasteurellaceae
- Genus: Haemophilus

Winslow et al. 1917



INTRODUCTION

- There have been a number of taxonomic changes to organisms within the genus *Haemophilus* in recent years.
- The species *Histophilus somni* now includes the bovine commensal and opportunistic pathogen '*Haemophilus somnus*' and the ovine organisms *Histophilus ovis* and *Haemophilus agni*.
- *Haemophilus paragallinarum* has been re classified as *Avibacterium paragallinarum*.
- These species are small (less than $1\mu\text{m} \times 1$ to $3\mu\text{m}$), 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.**

INTRODUCTION

- They are fastidious bacteria; *H. parasuis* and *Avibacterium paragallinarum* require growth factor V (nicotinamide adenine dinucleotide, NAD).
- Optimal growth for all these species occurs in an atmosphere of 5 to 10% CO₂ on chocolate agar which supplies both X (haemin) and V factors although, in the case of *H. somni*, X and V factors are not absolute requirements for growth.
- Most isolates of these organisms form small, transparent, dewdrop - like colonies after incubation for 48 hours.
- Colonies of *H. somni* have a yellowish hue and some isolates are haemolytic on sheep blood agar.



USUAL HABITAT

- These three 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.



Table 29.1 Disease conditions caused by *Histophilus somni*, *Haemophilus parasuis* and *Avibacterium paragallinarum*.

Organism	Hosts	Disease conditions
<i>H. somni</i>	Cattle	Septicaemia, thrombotic meningoencephalitis, bronchopneumonia (in association with other pathogens), sporadic reproductive tract infections
<i>H. somni</i> (ovine strains)	Sheep	Epididymitis in young rams; vulvitis, mastitis and reduced reproductive performance in ewes; septicaemia, arthritis, meningitis and pneumonia in lambs
<i>H. parasuis</i>	Pigs	Glasser's disease, secondary invader in respiratory disease
<i>A. paragallinarum</i>	Chickens Pheasants, turkeys, guinea fowl	Infectious coryza Respiratory disease

Table 29.2 *Haemophilus* and *Avibacterium* species which occur as commensals in domestic animals.

Species	Host	Comments
<i>Avibacterium avium</i>	Chickens	Commensal
<i>A. gallinarum</i>	Birds	Possible low-grade infections of upper respiratory tract
<i>Haemophilus felis</i>	Cats	Commensal of nasopharynx; occasionally involved in respiratory disease
<i>H. haemoglobinophilus</i>	Dogs	Commensal of the lower genital tract
<i>H. paracuniculus</i>	Rabbits	Isolated from intestines

- Differentiation of *Histophilus somni*, *Haemophilus parasuis* and *Avibacterium paragallinarum*
- Isolation techniques:
- Both X and V factors are required in media for isolation of some *Haemophilus* and *Avibacterium* species.
- Although *Histophilus somni* does not have an absolute requirement for these factors, its growth is enhanced by their presence.
- The X factor is heat stable and is present in red blood cells.
- The heat - labile V factor, which is also present in red blood cells, is susceptible to NADases in plasma.
- There are two common methods for ensuring the availability of both X and V factors in culture media:

- Chocolate agar, which supplies both factors, is prepared by heating molten blood agar in a water bath at 80 °C for about 10 minutes.
- The chocolate - brown colour of the medium is due to lysis of the red cells.
- The heat - stable X factor, released from the lysed cells, is unaffected by this procedure.
- The V factor, which is also released from the lysed cells, tolerates a temperature of 80 ° C for a short period whereas the plasma NADases which degrade V factor are destroyed.
- *Staphylococcus aureus* growing on blood agar releases V factor into the medium.
- Colonies of *Haemophilus* species which require V factor grow close to the *S. aureus* colony, a phenomenon referred to as satellitism.

Tests for X and V factor requirements:

- The disc method for determining X and V factor requirements is illustrated and explained in figure. This test is particularly suitable for determining V factor requirement.

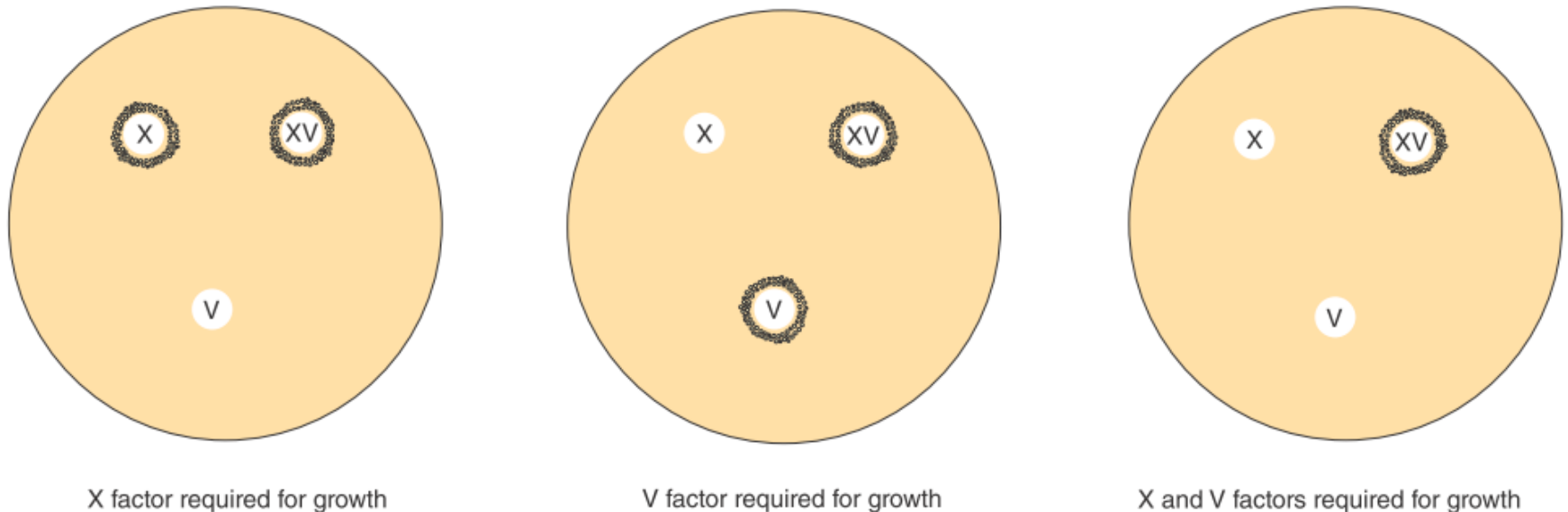


Figure 29.1 Disc method for determining the requirement for X and V growth factors. Isolates of *Haemophilus* species are spread over nutrient agar, and discs containing X, V, and X and V factors are placed on the inoculated media. After incubation in 10% CO₂ at 37°C for 3 days, colonies of *Haemophilus* species grow around the discs supplying the growth factor required by the particular isolate.

- The porphyrin test is a more accurate method for determining the growth requirement for X factor.
- The isolate is grown at 37°C for 4 hours in broth containing a porphyrin precursor.
- When the culture is exposed to UV light in the dark, porphyrin production is detected by a red fluorescence indicating that the isolate does not have a requirement for the X factor.

Table 29.3 Comparative features of *Histophilus somni*, *Haemophilus parasuis* and *Avibacterium paragallinarum*.

Organism	Growth factor required	Catalase production	Oxidase production	Carbohydrate utilization		
				Sucrose	Lactose	Mannitol
<i>Histophilus somni</i>	None	–	+	–	–	+
<i>Haemophilus parasuis</i>	Factor V	+	–	+	±	–
<i>Avibacterium paragallinarum</i>	Factor V	–	–	+	–	+

Pathogenesis and pathogenicity

Histophilus somni has a number of virulence attributes, which include:

- Endotoxin production
- Phase variation of its lipo-oligosaccharide
- Induction of apoptosis in endothelial cells
- production of transferrin and
- immunoglobulin - binding proteins.
- The lipo-oligosaccharide (LOS) of *H. somni* is a major virulence factor, both because of its toxic lipid A component and because the organism can modify the structure of its LOS, resulting in phase variation and evasion of the host immune response.



- The virulence attributes of *H. parasuis* are poorly characterized, and definitive proof of virulence requires reproduction of disease in animals.



GLASSER'S DISEASE

- Glasser's disease, caused by *Haemophilus parasuis*, manifests as polyserositis and leptomeningitis usually affecting pigs from weaning up to 12 weeks of age. Some cases present as polyarthritis.
- *Haemophilus parasuis* is part of the normal flora of the upper respiratory tract of pigs.
- Piglets acquire the organism from sows shortly after birth either by direct contact or through aerosols.
- However, Glasser's disease may occur sporadically in 2 to 4week - old piglets subjected to stressful environmental conditions



- The incubation period is 1 to 5 days.
- Clinical signs usually develop in conventionally reared pigs 2 to 7 days following exposure to stress factors such as weaning or transportation.
- Anorexia, pyrexia, lame-ness, recumbency and convulsions are features of the disease.
- Cyanosis and thickening of the pinnae are often encountered.
- Pigs may die suddenly without showing signs of illness.



Infectious coryza of chickens

- Infectious coryza, caused by *A. 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.



- 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 post-mortem in the infraorbital sinuses.
- Tracheitis, bronchitis and airsacculitis may be present also.



DIAGNOSIS

- Facial swelling is a characteristic finding.
- Isolation and identification of *A. Paragallinarum* from the infraorbital sinuses of several affected birds is confirmatory.
- Immunoperoxidase staining can be used to demonstrate *A. 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 *A. paragallinarum* in a flock.



FURTHER READINGS

- Clinical Veterinary Microbiology 2nd Edition 2013 By Bryan Markey
- Veterinary Microbiology and Microbial Disease

