Review on Veterinary Important *Haemophilus* Bacteria

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Abstract: *Haemophilus* species are small, gram negative, non-spore forming, pleomorphic and facultative anaerobes which do not grow on MacConkey agar. Therefore, the objective of this paper is overview general characteristics of the genus haemophilus. They are fastidious bacteria which require one or both of the growth factors found in blood. These factors were originally referred to as X and V factors and later identified as haemin and nicotinamide adenine dinucleotide respectively. *Haemophilus paragallinarum, Haemophilus parasuis* and Haemophilus *somnus* are species of veterinary importance. They are commensals or parasites of the mucous membranes of animals, most commonly of the upper respiratory and lower genital tracts. Finally, it can be concluded that since all members of the genus *Haemophilus* have the characteristics of requiring growth factors in common, X and V factor requirement tests are among identification techniques of *haemophilus* species.
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1. Introduction

When the world was suffering from pandemic influenza in 1889 and 1890, bacteriologists were vigorously pursuing the causative agent of the disease. The news of the discovery of the influenza bacillus by Richard Pfeiffer was a sensation, and a preliminary report was published simultaneously in January 1892 in German, English and French medical journals (Smith *et al*., 1933). The investigation of the influenza bacillus was hampered by the difficulty of growing it on laboratory media. When it grew, it did so in minute, pinpoint-size colonies that could easily be overlooked or overgrown by other bacteria present in the sample (Quinn *et al.,* 1994). The genus *Haemophilus* based on the discovered 'influenza bacillus', is currently classified in the family Pasteurellaceae. The taxonomy of this group that now contains seven genera of veterinary importance: *Actinobacillus, Avibacterium, Haemophilus, Histophilus, Mannheimia, Pasteurella and Bibersteinia* (Piechulla *et al*., 1986).

Growth factor requirements have remained of importance for the taxonomy of genus *Haemophilus* which is now recognized as pathogens in man and various animal species. They exist as mucosal parasites, but are capable of being primary are opportunistic pathogens depending on the carrier status and health of the host. At present there is general agreement that the genus *Haemophilus* should be restricted to Gram-negative rods or coccobacilli with a requirement for haemin or certain other porphyrins (X-factor) and NAD (V-factor) or other definable coenzyme- like substances (Zinnemann, 1973).

2. Literature Review

Haemophilus

Genus name derived from Greek meaning "blood loving, due to an in vitro requirement for certain growth factors (Holt, 1961). These factors were originally referred to as X and V factors and later identified as haemin and nicotinamide adenine dinucleotide (NAD) respectively (Howard, 1997). *Haemophilus* contains small, non motile, non-spore forming, oxidase positive, catalase positive, pleomorphic, less than 1µm in width, variable in length, gram negative bacilli that are parasitic on human beings or animals. Cellular structure is typical of gram negative rods. Capsules can be produced by *H. paragallinarum (*Quinn, *et al.,* 1994).

Taxonomy

Genus *Haemophilus* belongs to domain of bacteria, Phylum of *proteobactereia*, Class of gamma proteobacteria Order of *pasteurellales* and family of *pasteurellaceace* (Euzeby,2013). Nucleic acid hybridization studies and the development of a sensitive porphyrin test for factor X requirement have resulted in reclassification of some species in the genus (Corbeil *et al.,* 1995).

Species

There have been a number of taxonomic changes to organisms within the genus *Haemophilus* in recent years (Piechulla *et al*., 1986). *Haemophilus paragallinarum, Haemophilus parasuis* and *Histophilus somni* are species of veterinary importance. *Histophilus somni* is the name now given to those micro-organisms previously knwon as

"*Haemophilus somnus*" *"Haemophilus agni", "Histophilus ovis'*(Quinn, *et al.,* 1994).

Natural habitat

Haemophilus species are commensals or parasites of the mucous membranes of animals, most commonly of the upper respiratory and lower genital tracts (Quinn, *et al.,* 1994). They are susceptible to desiccation and do not survive for long periods away from their hosts (Corney *et al*., 2008). *H. parasuis* inhabits the nasopharynx of normal pigs and H.*somnus* is present in the respiratory tract of healthy cattle, but *H. paragallinarum is* more closely associated with the respiratory tract of sick or recovered birds (Quinn, *et al.,* 1994).

Growth characteristics

They are typically cultured on blood agar platesbut cannot grow on MacConkey agar; grow best on chocolate agar (supplying the X and V factors) (Quinn, *et al.,* 1994). Successful culture of many *Haemophilus* species is enhanced in an atmosphere of 10 percent Co_2. Hence, inoculated Chocolate agar incubated in 5-10% CO2 at 35-37°C for 16-48hr while Blood agar incubated in 5-10% CO2 at 35-37°C for 16-48hr, although some may grow in 24 hours. On blood agar, although X factor is directly available to the organism, growth is restricted by the limited availability of V factor which is only released by breaking up the blood cells. Different species require either or both of these factors. Improved growth can easily be obtained by heating blood media to produce "chocolate agar", which counters the effects of these enzymes releasing extra V factor into the medium and produces plates dark brown in colour. A heated blood medium is therefore often preferred to facilitate identification of other pathogens that may be present in a specimen (Kilian, 1985).

The pattern of X and V dependency, usually determined by a simple paper disc method, is extensively used for identification (Howard, 1997). The test organism requiring X factor alone, grows only in the vicinities of X and X+V factor discs. Those which require V factor alone grow in the vicinities of V and X+V factor discs. If both X and V factors are required, then the organism will grow only in the vicinity of the X+V factor discs. This satellite growth is seen around the disc promoting growth (Murray *et al*., 2003).

Alternatively, *Haemophilus* is sometimes cultured using the "Staph streak" technique. Both Staphylococcus and *Haemophilus* organisms are cultured together on a single blood agar plate. In this case, *Haemophilus* colonies will frequently grow in small "satellite" colonies around the larger *Staphylococcus* colonies because the metabolism of *Staphylococcus* produces the necessary blood factor by-products required for *Haemophilus* growth. All *Haemophilus* species grow more readily in an atmosphere enriched with CO_2 (BID, 2015)

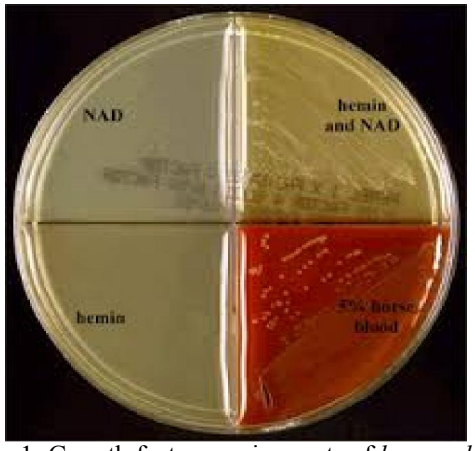

Figure1: Growth factor requirements of *haemophilus*

Virulence factors

The organism has a number of virulence attributes, which include endotoxin production (Outer membrane lipopolysaccharide or lipooligosaccharide), capsule and immunoglobulin-binding proteins (Adhesins). The cell well lipopolysacharide of *Histophilus somni* is termed lipooligosaccharide (has shorter side chains). LOS is a major virulence factor, because of both its toxic lipid A component and the organism can modify the structure of its LOS, under the control of the gene lob (for LOS biosynethesis). A capsule found on the cell wall of some bacteria is used to interfere with phagocytosis (antiphagocytic effect). *Haemophilus* species have generally been thought not to make toxins or other extracellular products that account for their ability to produce infection (Czuprynski *et al*., 2004).

Pathogenesis

Young or previously unexposed animals are most susceptible to *Haemophilus* infections with stress factors contributing to the development of signs of disease (Quinn, *et al.,* 1994). Environmental and other stress factors such as transportation, weaning and viral infections may contribute to the development of infections by this group of organisms (Czuprynski *et al*., 2004). The capsule and cytotoxic factor of *H. paragallinarum* are thought to be virulence factors and endotoxin may play a role in the disease process. *H. somnus* is resistant to the lethal effects of phagocytes and serum, can adhere to epithelium and is toxic to endothelial cells to which it also adheres (Quinn, *et al.,* 1994).

Vasculitis is a major feature of the lesions observed in *H. somni* infections and LOS is involved in induction of apoptosis in endothelial cells and in leukocytes (Czuprynski *et al*., 2004). In addition, *H. somni* produces an exopolysaccharide and filamentous

haemagglutinin proteins, both of which may be involved in biofilm formation (Sandal and Inzana, 2010). The virulence attributes of *H. parasuis* are poorly characterized, and definitive proof of virulence requires reproduction of disease in animals. It is known that *H. parasuis* produces capsular polysaccharide, lipooligosaccharide and outer membrane proteins but detailed information on their exact pathogenic role is not available (Czuprynski, 2009).

Clinical infections

Haemophilus species which are pathogenic for animals tend to be host specific and cause a variety of clinical syndromes (Lees *et al*., 1990). *Haemophilus paragallinarum*, the cause of infectious coryza in chickens, is one of the most important type species of veterinary importance. *H. parasuis,* the cause of a septicemic disease called Glasser's disease or polyserositis, and seconday respiratory disease of swine), and *Haemophilus somnus* the cause of septicemic, respiratory, and genital tract disease in cattle and sheep) (Blood *et al*., 1983). Thrombotic meningoencephalitis is a common consequence of septicaemia, due to *H. somnus*. It is encountered sporadically in young cattle recently introduced to feedlots (Quinn, *et al.,* 1994).

Table 1: Diseases of Veterinary Important *Haemophilus* Species.

Species	Host (s)	**Disease or significance**
H. somnus	Cattle	• Infectious thromboembolic meningoencephalitis (TEME): • Respiratory disease: pneumonia and pleurisy, endometritis and abortion.
	Sheep	epididymitis and orchitis in rams. pneumonia, mastitis, polyarthritis, meningitis and septicaemia
H. parasuis	Pigs	• Glasser's disease: polyserositis and meningitis in young pigs • Arthritis and pneumonia in older pigs, *(Mycoplasma hyopneumoniae)*
H. paragallinarum	**Poultry**	Infectious coryza and edema of the face, reduction in egg production

Source: (Quinn, *et al.,* 1994).

3. Laboratory diagnosis

Specimen

Haemophilus species are fragile and the specimens should be protected from drying and cultured as soon as possible within 24 hours after collection. Refrigeration and transport media do not appear to be beneficial and deep freezing, below -60°C, is the only definite method for the preservation of these bacteria. The type of specimen required will depend on the disease or lesions present (Quinn, *et al.,* 1994).

Direct microscopy

Demonstration of these small Gram-negative rods in tissues is often difficult and specific fluorescent anti-body staining is a sensitive and specific method (Quinn, *et al.,* 1994).

Isolation

X and V factors must be supplied for all the *Haemophilus* species except *H. somnus* which is X and V factor independent (Piechulla *et al.,* 1986). The X factor (haemin) is heat-stable and present in adequate amounts in 5 percent blood agar. V factor is present mainly intracellularly in red cells and is susceptible to NADases present in most bloods. In chocolate agar the V factor is released from the red cells, the NADases are destroyed, and the heat-stable X factor is still present. Also, *Staphylococcus aureus* grown as a streak across a blood agar plate will provide the V factor. V-factor requiring haemophili will grow as satellite colonies near the streak. Commercially available media with supplements are available for *Haemophilus* species but chocolate agar is the most satisfactory medium for the haemophili isolated from animals (Quinn, *et al.,* 1994).

Identification

A presumptive identification of the fastidious *Haemophilus* species is based on the host species, clinical signs and lesions, colony morphology, gram stain, X and V factor requirements (V factor, Disc method for X and V factors and Porphyrin tests), oxidase and catalase reactions and whether or not CO2 enhances growth (Quinn, *et al.,* 1994). Identification is confirmed by commercial biochemical tests and serotyping with type-specific antisera (UKSMI, 2001).

Colony morphology: on blood agar, although X factor is directly available to the organism, growth is restricted by the limited availability of V factor which is only released by breaking up the blood cells (Holt, 1961). Small dewdrop-like colonies may appear after 24-48 hours' incubation and none are consistently haemolytic. A few strains of *H.* somnus may show clearing around the colonies especially on Columbia-base sheep blood agar. *H.somnus* colonies may appear yellowish in a loopful of growth in a confluent lawn (Quinn, *et al.,* 1994). Small, round, convex colonies, which may be iridescent and develop after 24hr incubation on chocolate agar (Kilian, 1985).

Figure2: Colonies of haemophilus on chocolate agar

Microscopic examination: gram stain *Haemophilus* species are small coccobacilli or longer rod-shaped Gram negative cells, variable in length with marked pleomorphism and sometimes forming filaments. (Quinn, *et al.,* 1994).

Biochemical reactions: H.parasuis grows on blood agar in the zone around Staphylococcus aureus, does not cause haemolysis, is urease-negative, oxidase-negative, catalase-positive, reduces nitrates, does not produce indole, and causes fermentation of glucose, galactose, mannose, and fructose, saccharose and maltose (Kielstein *et al*., 2001).

Capsular polysaccharide, outer membrane protein and lipooligosaccharide are the major surface antigens (Quinn, *et al.,* 1994). Surveys have shown that antibody to *H. somnus* is widespread in cattle populations. So far there is no test that is used for the diagnosis of clinical cases. In poultry, antibody to *H. paragallinarum* is demonstrable after 1-2 weeks of infection and can be detected for over a year. Serological procedures are used to identify potential carrier birds; these include slide and tube agglutination tests, agar gel precipitation, Latex agglutination and haemagglutination (Chen *et al*., 1996).

Table2: Comparative features of pathogenic Haemophilus species of veterinary importance.

	Growth factor	**Catalase production**	**Oxidase Production**	**Utilization of**		
				Sucrose	Lactose	Mannitol
'H. somnus'	factor v+x	−	+	−	−	+
H. parasuis	factor v	+	−	+	±	−
H. paragallinarum	factor v	−	−	+	−	+

Source: (Quinn, *et al.,* 1994).

4. Conclusion And Recommendations

All members of the genus *Haemophilus* have in common the characteristic of requiring for growth certain accessory factors normally present in blood. They do not grow on MacConkey agar. The genus has been subdivided according to growth-factor requirements. Thus, some species require both X- and V-factors, some require V-factor only, and others require X-factor only.

Optimal growth occurs in an atmosphere of 5-10% CO_2 on blood and chocolate agar (X and V factors) Small, dew-drop like colonies is formed by most *Haemophilus* species after 48 hrs of incubation. V-factor requiring haemophili will also grow as satellite.

Colonies when they streak near *Staphylococcus aureus* on blood agar plate.

They are commensals of the mucous membranes of animals, most commonly of the upper respiratory and lower genital tracts. They have been described as playing significant roles in a variety of serious infectious diseases. *Haemophilus paragallinarum*, the cause of infectious coryza in chickens, *H. parasuis*, causes Glasser's disease in pigs and *Haemophilus somnus* causes septicemic, respiratory, and genital tract disease in cattle and sheep.

Therefore based on the above conclusions the following recommends are forward:

- In addition to providing both growth factors, a chocolate agar often preferred to facilitate identification of hemolytic pathogens that may be present in a specimen
- Different *Haemophilus* species can cause economically important diseases. so, attentions should be given and apply available control techniques.

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