

## Haemorrhagic Septicaemia: A General Review

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### ملخص البحث

التسمم الدموي مرض بكتيري، سارى فوق الحاد و يسبب معدلات نفوق عالية في الحيوانات الأليفة و البرية. و هو مستوطن في معظم المناطق المدارية و شبه المدارية في أفريقيا و آسيا. يحدث المرض في الحيوانات نتيجة الإصابة بالأمراض المصلية ب2 (B:2) وهـ2 (E:2) لبكتيريا باستوريلا ملتوسيدا. يوجد النمط المصلى ب2 في قارة آسيا بينما تم عزل النمط هـ2 من أفريقيا. من ناحية أخرى يوجد النمطين معاً في بعض الأقطار الأفريقية من بينها السودان. تم تسجيل حالات الإصابة بالمرض في مختلف أجزاء السودان. يتم تشخيص المرض بالطرق التقليدية وكذلك التقنيات الجزيئية الحديثة. كما تتم السيطرة على المرض بواسطة التمنيع بواسطة مستضد بكتيري (bacterin) ميت بإضافة عامل مساعد (adjuvant) مناسب. في السودان ينتج لقاح التسمم الدموي من العزتين ب و هـ. في هذه الورقة استحداث للمعلومات عن هذا المرض في النواحي الوبائية و المرضية و البكتيرية والمناعية مع التركيز على المعلومات الواردة من السودان.

### Summary

**Haemorrhagic Septicaemia(HS) is a contagious, peracute, acute or chronic highly fatal bacterial disease of domestic and wild animals. The disease is endemic in most parts of tropical and subtropical Asia and Africa, and is caused by *Pasteurella multocida* serotypes B:2 and E:2, respectively. Both serotypes have been reported from Sudan and other African countries. The disease is endemic in the Sudan and was reported nearly from all parts of the country. Conventional methods and recently developed molecular techniques are used for the diagnosis. The control of the disease depends mainly upon immunization with killed adjuvant vaccines. In Sudan, *P.multocida* B:2 and E:2 vaccine strains are routinely used for immunization against HS. Research has been directed mainly towards production and development of an improved vaccine.**

**Up-to-date literature on the epidemiological, pathological, bacteriological and immunological aspects is reviewed with emphasis on, and relevance to, Sudan condition.**

### Geographical distribution:

Haemorrhagic Septicaemia is worldwide in distribution and endemic in most tropical and subtropical Africa and Asia (Carter and DeAlwis, 1989; DeAlwis, 1984). In Africa it was reported in most countries (Mustafa *et al.*, 1978; Shigidi and Mustafa, 1979). The

disease as found across Asia from the Middle East region to South Eastern Asian countries.(Anon., 1979; DeAlwis, 1984; DeAlwis *et al.*, 1986).No confirmatory reports exist on the presence of the disease in Europe except Italy, East European countries, one sporadic case in the UK (Johnes and Hussaini, 1982); and a case of HS in Danish fellow deer (Aalbaek *et al.*, 1999). In the United States the disease has been reported as well (Dunbar *et al.*, 2000; Dyer *et al.*, 2001).The disease was reported in Australia, Oceania and Canada (Carter and DeAlwis, 1989). HS, which is sometimes mixed with pneumonic pasteurellosis of cattle, is of great significance in Central and South American countries (Carter and DeAlwis, 1989).

In Sudan the disease was reported from the Blue Nile province, Central Sudan, in 1939, and subsequently reported from Kassala province, Eastern Sudan, in 1939, from Northern Kordofan Province, Western Sudan, in 1943 and from Upper Nile Province, Southern Sudan, in 1947 (Anon., 1933-1959). The disease is endemic, was reported nearly from all parts of the country and has a seasonal prevalence as outbreaks usually occur early after the onset of the rainy season (Mustafa *et al.*, 1978).

**The causal agent:**

HS is caused by the serotypes B:2 and E:2 of *P. multocida* (Carter, 1955; Carter, 1961; Dawkins *et al.*,1990),commensally present in the upper respiratory and digestive tracts of healthy animals as well as a frequent secondary invader, opportunist pathogen or a primary cause of the disease (Quinn *et al.*, 1999).

*P. multocida* is a primary causative agent of fowl cholera and HS in cattle and buffalo, or a secondary invader in pneumonia in cattle, swine, sheep, goat, and other species as well as it is frequently involved in bovine shipping fever and enzootic pneumonia of pigs (Thomson *et al.*, 1975). Also the organism was isolated from a wide range of sporadic infections in many species, and is one of the causes of severe mastitis of sheep and cattle. Dogs, cats and other animals, frequently carrying the organism commensally in their mouths (Smith, 1955), may infect man and animals through biting (Bailie *et al.*, 1978; Woolfery *et al.*, 1985).In rabbit, *P. multocida* causes rhinitis (snuffles), pneumonia, otitis media, septicaemia, metritis and death (Jarvinen *et al.*, 1998). In man, *P. multocida* infection is usually associated with close contact with pets such as dogs and cats; it is

frequently associated with localized wound infections, cellulitis, meningitis, septic arthritis (Layton, 1999), endocarditis (Vasques *et al.*, 1998) and peritonitis subsequent to peritoneal dialysis (Musio and Tiu, 1998).

**Classification:**

The serotyping of *P. multocida* depends on the identification of its capsular and somatic antigens by three serological methods. The capsular serotypes are detected by an indirect haemagglutination (IHA) test (Carter, 1955) whereas somatic serotyping is done by agglutination and agar gel immunodiffusion (AGID) tests (Heddleston *et al.*, 1967; Namioka and Murtata, 1961). The Asian and the African strains belong to capsular types B and E, respectively. By the somatic serotyping, strains of both capsular serotypes B and E, belong to type 6 (agglutination test) and type 2 (AGID).

Serotype B:2 is the causative agent of the disease in Asia while E:2 was isolated from the disease only in Africa (Carter, 1984). Both serotypes have been reported from Sudan, Egypt and other African countries (Shigidi and Mustafa, 1979; Dizva *et al.*, 2000; Martrenchar and Njanpop, 1994).

**Characterization:**

*P. multocida* is a non-motile, Gram-negative bacterium, usually forming small coccobacilli or short rods. Isolates obtained from infected tissues show a bipolar staining affinity, while those from healthy animals are often pleomorphic. A degree of pleomorphism will also be noted particularly in old cultures with longer rods of varying length (OIE, 2000). The serotypes vary in their fermentation of sugars but conform to their inability to grow on MacConkey medium (Carter, 1967; Carter and Chengappa, 1981). Muroid, smooth and rough colony variations were reported (Carter and Bain, 1960). Growth on blood agar is usually in the form of small non-haemolytic gray colonies. On the contrary, haemolysis under anaerobic conditions was also reported (Adler *et al.*, 1999; Hunt *et al.*, 2000).

The recent application of molecular methods such as the polymerase chain reaction (PCR), restriction endonuclease analysis, ribotyping, pulsed-field gel electrophoresis, gene cloning characterization and recombinant protein expression, mutagenesis,

plasmid and bacteriophage analysis and genomic mapping, have greatly increased the understanding of *P. multocida* and has provided

researchers with a number of molecular tools to study its pathogenesis and epidemiology at the molecular level (Hunt *et al.*, 2000). Characterization of *P. multocida* could be done by serotyping, sodium dodecyl sulphate-polyacrylamide gel electrophoresis of whole cell proteins (WCPs) and outer-membrane proteins (OMPs), and PCR fingerprinting. Combination of conventional and molecular typing methods enhances the differentiation of *P. multocida* isolates (Dabo *et al.*, 1999). DNA fingerprinting of *P. multocida* is a precise characterization method and, undoubtedly, in conjunction with serological typing can further classify *P. multocida* strains for epidemiological studies (Wilson *et al.*, 1992).

#### **Pathogenicity of *P. multocida***

The pathogenicity of *P. multocida* is attributed to many factors, notably the capsule and toxins.

#### **Capsule:**

The capsule has been implicated in the virulence of *P. multocida*. Boyce and Adler (2000) have identified and determined the nucleotide sequence of the *P. multocida* B2 capsule biosynthetic locus. Wild-type, mutant and complemented strains were tested for virulence by intraperitoneal challenge infection of mice; the presence of the capsule was shown to be a crucial virulence determinant. Following intraperitoneal challenge of mice, acapsular bacteria were removed efficiently from blood, spleen and liver while wild-type bacteria multiplied rapidly. The former bacteria were readily taken up by the murine peritoneal macrophage, but the latter ones were significantly resistant to phagocytosis. Both wild-type and acapsular bacteria were resistant to the complement in bovine and murine sera (Boyce and Adler, 2000).

#### **Toxins:**

The production of endotoxins in embryonic lung cells and vero cells has been successfully implemented for the study of the toxigenic effect of *P. multocida* (Pennig and Storm, 1984; Rutter and Luther, 1984). Variability of the toxigenic effect in experimental animal species was confirmed. Soluble and heat stable toxin of both avian and bovine strains were reported to be more lethal to rabbits than to mice (Adlam and Rutter, 1989).

Purified toxins of *P. multocida* type 1 were later used experimentally in cattle and buffaloes through intravenous injection and were found to initiate malaise, fever and blood tinged diarrhoea (Bain, 1963). In general, the role of toxin produced by some strains of *P. multocida* in naturally occurring cases was reported in swine, goats and calves (Rutter, 1985; Musa *et al.*, 1972). Drucker (1977) and Nakai *et al.* (1984) reported a dermonecrotic effect of toxins in guinea pigs and rabbits. Another toxigenic effect of *P. multocida* is cytopathic effect attributable to the toxic reduction in DNA synthesis especially in rapidly dividing cells (Chanter *et al.*, 1986). The nature of toxigenic *P. multocida* is ascribed to a cell membrane structural component, lipopolysaccharide (LPS), which is the endotoxin fraction (Heddleston *et al.*, 1967; Rebers and Rimler, 1984), besides a protein fraction (Rimler and Brogden, 1986). The chemical properties of *P. multocida* LPS-protein complex are similar to those of many Gram-negative bacteria endotoxins in addition to its immunogenic nature (Rimler and Brogden, 1986).

#### Economical importance

HS is rooted in Africa and Asia with regular sporadic or massive seasonal outbreaks resulting in tremendous losses. There is no definite statistical data to estimate its actual economic impact. As a killing disease, HS represents one of the most serious diseases of livestock; it causes great losses in buffaloes and cattle, which are vitally important for the rural economy in many countries (Bain, 1963; Bain *et al.*, 1982).

#### Epidemiology

There are many integrated factors that affect the incidence of HS; these include clinical, immunological and epizootiological factors (Carter and DeAlwis, 1989).

#### **Host:**

Cattle and water buffaloes are the most susceptible animals (Francis *et al.*, 1980; Myint *et al.*, 1987). Buffaloes are more susceptible than cattle (DeAlwise, 1981). Bain (1963) confirmed experimentally the high susceptibility of rabbits and mice to *P. multocida* infection, while guinea pigs, pigeons, pigs and horses have moderate degree of susceptibility. The susceptibility is variable in sheep and goat, while fowl (Chandrasekaran *et al.*, 1985) and dogs are not susceptible to HS. Swines are also susceptible (Lungtenberg *et al.*,

1986). Highly fatal septicaemic pasteurellosis was reported in camels (Momin *et al.*, 1987), elephants (DeAlwis, 1982), bisons (Carter, 1982) and fellow deer (Johnes and Hussaini, 1982; Eriksen *et al.*, 1999).

**Carrier status:**

The healthy carrier animals harbour the pathogen in their nasopharynx and tonsils (DeAlwis, 1990), and disseminate the disease by direct contact or through contamination of soil, water and pasture and hence transmission of infection is through inhalation or ingestion. Carriers have a great role in creating HS outbreaks (Gupta, 1982; Wijewantha and Karunaratne, 1986).

**Natural acquired immunity:**

This phenomenon was observed in endemic areas (Dhanda, 1959). It may be due to the natural exposure of animals to arrested infection or avirulent organism; affected animals show no clinical signs of the disease due to the level of the immunity.

**Predisposing factors:**

Parasitic, viral and bacterial infections, exhaustion and over- working specially in buffaloes, transport, successive change in nutrition, management and temperature with changes of seasons and mode of husbandry, were reported to have an influential role in the occurrence of the disease (Annosa and Isoun, 1975; Bain, 1963; Carter, 1967; Gajapathi and D'Souza, 1968; Olsen and Needham, 1952; Perreau, 1961; Rhoades *et al.*, 1967).

**Other factors:**

In endemic areas, which are characterized by high frequency of the disease, the mortality rate among old animals (aged more than two years) is lower than that among animals of all ages in non-endemic areas (Baba, 1984; DeAlwis, 1981). Animal species is another factor, e.g. buffaloes are more susceptible than cattle (DeAlwis, 1981). Vaccination and herd size were also reported to affect the mortality rate. As for an environmental factor, there is an intimate relation between the wet season and HS outbreaks (Sheikh *et al.*, 1996).

**Clinical symptoms and pathological lesions:**

HS is characterized by rapid onset, high fever, salivation, conjunctivitis, lacrimation, cessation of rumination and dullness

followed by sudden death in peracute conditions. In acute cases, symptoms include dyspnea, painful groans, respiratory distress and oedematous swelling in the head-throat-brisket region and fore-limbs followed by recumbency at late stages (Bastianello and Jonker, 1981). The onset of the clinical signs occurs at 30 hrs after experimental inoculation and in two to three days following natural infection (Carter and DeAlwis, 1989). Post-mortem lesions following experimental inoculation were congested lungs with light petechial haemorrhages on the heart (DeAlwis *et al.*, 1975). In acute cases, wide-spread haemorrhages over the pleural surfaces and the parenchymal organs, oedematous and red-gray lobular inflamed lungs, oedematous lymph nodes, pericarditis with serous haemorrhage on the left ventricle and blood stained exudates were observed (Bastianello and Jonker, 1981, Horadagoda *et al.*, 1989). In a case of HS in fellow deer, the major clinical symptoms and peracute or acute pathological changes included extensive swelling of the head and neck, septic pneumonia, petechial and ecchymotic haemorrhages on serous membranes and severely haemorrhagic adrenal gland and abomasum. Rhinitis and necrotic pharyngeal mucosa were common. Histologically, the most advanced lesions were in the nasal mucosa and pharynx. The swelling of the head and the neck had arisen from a diffuse cellulitis in the subcutaneous and intermuscular tissues. The earliest lesions in the lung included presence of large numbers of bacteria in the pulmonary capillaries, but various degrees of fibrinous exudation in the alveoli and infiltration with neutrophils were usually observed (Eriksen *et al.*, 1999).

### **Diagnosis:**

As a routine diagnosis, smears made from samples of blood collected from the jugular vein or heart and from bone marrow of the suspect are useful, but not conclusive for the diagnosis of infection. Isolation of the pathogen from the blood is possible just prior to death (Carter and DeAlwis, 1989). Aseptically removed bone marrow is ideal for pure culture isolation on suitable media. A conclusive diagnostic procedure that comprises mouse inoculation with the infected blood or bone marrow, death of these animals and isolation of a bacterium with morphological features and biochemical reactions identical to *P. multocida*, is used (DeAlwis, 1989). Many

serological methods are used for the diagnosis of *P. multocida*, such as rapid slide agglutination, indirect haemagglutination, agar gel immunodiffusion,

counter immuno-electrophoresis and serum agglutination tests (OIE, 2000). An ELISA, as a diagnostic technique for identification of *P. multocida*, was developed and is recommended by OIE (2000). It is also a reliable diagnostic tool for screening *P. multocida* infection (Zaoutis et al., 1991) and detection of maternal antibodies against *P. multocida* in newborn calves (El-Eragi et al., 2001).

The feasibility of using PCR for accurate, rapid detection of toxigenic *P. multocida* from swabs was investigated (Lichtensteiger et al., 1996). In addition to accuracy, as required for a rapid direct specimen assay, toxigenic *P. multocida* was recovered efficiently from inoculated swabs. This shows that PCR can detect toxigenic *P. multocida* directly from clinical specimens.

**Treatment:**

Treatment of HS can be successful if only antibiotics are given at the initial stages of the disease. Various combination of sulphur drugs and antibiotics were considered to be more effective (Sheikh et al., 1996). However, considerable resistance of some strains of *P. multocida* to certain antibiotics was reported (Dyer et al, 2001; Singer et al., 1998).

The therapeutic administration of immune serum was found to be impractical as well as ineffective (Carter, 1967).

**Control:**

Hygienic planning, basic management measures including rearing and feeding, as well as immuno- and chemo-prophylaxis will help in the effective control of many diseases. Restriction of animal movement especially the non-vaccinated animals, on to endemic areas should be strictly implemented. Strict quarantine measures in case of animal transport from the endemic to non-endemic areas should be adopted. The proper disposal of carcasses and adoption of improved husbandry practices are recommended in order to reduce the incidence and dissemination of the disease (DeAlwis, 1984; Seifert, 1978). A more effective control of HS can be achieved by a better understanding of its epidemiology, the production of a better vaccine, and adoption of a country-wide vaccination programme (DeAlwis, 1984).

The only practical approach to the control of HS seems to be through the application of prophylactic measures. Combination of regular vaccination and the use of antimicrobials in the early stages of infection especially in sporadic cases proved to be essential for the disease elimination in endemic areas. Immuno-prophylaxis through vaccination is widely practised and in reality different types of inactivated vaccines with or without adjuvants, have been used in the tropics (Muna *et al.*, 1995; Verma and Jaiswal, 1998). Boostering through repeated natural infection is believed to convey long duration immunity (Bain, 1963).

HS vaccines are simple formalin-killed bacterins, or dense bacterins with adjuvants. The latter enhance the level and prolongs the duration of immunity. The cultures for the production of vaccines should contain capsulated seed cells. There are three types of vaccines used against HS, viz bacterins, aluminium hydroxide precipitated vaccine (APV) and oil adjuvant vaccine (OAV). To provide sufficient immunity with bacterins, repeated vaccination is required. Administration of dense bacterins can run the risk of causing shock reactions, which are less frequent with the APV and almost non-existent with the OAV. These vaccines are standardized as to their bacterial density on the basis of turbidity tests. Potency tests are most conveniently carried out in mice. The recommended age for primary vaccination is 4-6 months. For routine prophylactic vaccination, a vaccination programme comprising a single dose of OAV at 4-6 months of age, a booster one at 3-6 months later and annual revaccination thereafter, is recommended. In the face of an outbreak in vaccinated animals, one dose of APV, followed by one dose of OAV is recommended (OIE, 2000). In the Sudan annual vaccination against HS is practised, using APV which is produced at the CVRL, Soba, Khartoum. Mass cultivation of *P. multocida* strains B:2 and E:2 using the IBT-Gottingen bioreactor is carried out to produce a bivalent vaccine (Elbashir, 1993).

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