Table 2.7. Diseases caused by pasteurella organisms in agricultural and domestic animals.

<table>
<thead>
<tr>
<th>Host species</th>
<th>Disease</th>
<th>Organism(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle/buffalo</td>
<td>Haemorrhagic septicaemia (HS)</td>
<td><em>P. multocida</em> serotypes B:2 and E:2</td>
</tr>
<tr>
<td>Cattle</td>
<td>Occasionally, HS-like septicaemic disease</td>
<td><em>P. multocida</em> serotype B:3,4</td>
</tr>
<tr>
<td>Cattle</td>
<td>Bovine pneumonic pasteurellosis</td>
<td><em>P. haemolytica</em> A1, <em>P. multocida</em> A</td>
</tr>
<tr>
<td>Sheep and goats</td>
<td>Pneumonic pasteurellosis, Septicaemic pasteurellosis</td>
<td><em>P. haemolytica</em> A, <em>P. haemolytica</em> T</td>
</tr>
<tr>
<td>Pigs</td>
<td>Sporadic outbreaks of HS, Atrophic rhinitis</td>
<td><em>P. multocida</em> serotype B:2, Toxigenic strains of <em>P. multocida</em> type D, occasionally, type A</td>
</tr>
<tr>
<td>Poultry/turkeys</td>
<td>Fowl cholera</td>
<td><em>P. multocida</em> type A, Common serotypes are A:1, A:3, A:4. Other serotypes of groups A and D are less common (and type F in turkeys)</td>
</tr>
</tbody>
</table>

Note: For *P. multocida* ‘A’ refers to the capsular serogroup whereas for *P. haemolytica* ‘A’ refers to the biotype.

2.4.2 Bovine pasteurellosis

Bovine pasteurellosis is known by a number of synonyms that are descriptive of the condition and are relevant in specific circumstances. The terms shipping fever, transit fever, bovine enzootic pneumonia, and bovine respiratory disease (BRD) complex are all very meaningful terms used to describe the disease. It is believed that in the United States, the losses to the beef and dairy industries from this disease complex are greater than the losses due to all other diseases put together. In the BRD complex, more than one species and serotype of pasteurellae are incriminated as playing a role that is secondary to respiratory viruses and ‘stress’. This is unlike HS, which is a primary pasteurellosis caused by specific serotypes of the species *P. multocida*. The pasteurellae associated with BRD are predominantly *P. haemolytica* type A, and *P. multocida* capsular serogroup A. There is no consistency in the somatic types involved.

The pasteurellae that cause pneumonic pasteurellosis are carried in the upper respiratory tract (URT) of calves. In the case of *P. haemolytica* type A1, the bacterium is not easily detected in the URT of healthy calves, but is shed and can be easily isolated in calves that are stressed in some way or affected with another concurrent infection. The URTs of stressed or otherwise diseased calves can be easily colonised by *P. haemolytica* A1. In the case of *P. multocida*, no such relationship between stress and ability to colonise has been observed. The explosive multiplication of *P. haemolytica* that results from stress leads to two processes. Firstly, there is the invasion of the lungs, resulting in pneumonia. Secondly, there is excessive shedding resulting in dissemination of infection to healthy incontact calves. The exact mechanisms underlying the rapid proliferation are not completely understood.

The most effective preventive method is good management and avoidance of stress. Vaccines are of limited use, since a multiplicity of other causative agents such as viruses are also involved. Vaccines against the pasteurellae involved will help to reduce the severity of the disease, since it is the secondary bacterial phase of the disease that contributes to both its severity.
and fatality. Treatment with appropriate antibiotics helps to reduce the severity of the disease and prevent death. In problem herds where the condition occurs frequently, a knowledge of the antibiotic sensitivity patterns of the strains involved is useful.

2.4.3 Pasteurellosis in sheep and goats

This is probably the most economically important bacterial disease of sheep and goats. The predominant organism that causes pasteurellosis in sheep and goats in temperate climates is *P. haemolytica*. Biotype A causes pneumonia in all ages of sheep, and septicaemia in young lambs. Biotype T, on the other hand, is associated with a distinct septicaemic syndrome in young adult sheep. *P. haemolytica* is carried in the nasopharynx and tonsils of apparently healthy sheep. Lambs acquire infection soon after birth, presumably by contact. The carrier rate is low in normal healthy flocks and there is an assortment of serotypes. In flocks undergoing outbreaks, on the other hand, the carrier rate is high and a few specific serotypes dominate. The carrier status has also been found to display seasonal variations.

Predisposing factors undoubtedly play a vital role in most outbreaks. Climatic changes and stressful management practices such as transport, dipping and shearing may precipitate outbreaks. Proper management and the use of vaccines help to reduce the prevalence of the disease.

2.4.4 Pasteurellosis in pigs

Apart from occasional outbreaks of septicaemic disease caused by the HS serotype (B:2), two well-defined syndromes occur in pigs. These are atrophic rhinitis and pneumonia.

Atrophic rhinitis is a disease associated with intensive pig breeding in most parts of the world. It was originally described in Germany nearly 160 years ago, but it was only during the last decade that its complex aetiology and pathogenesis were revealed. Economic losses due to this disease are due not only to deaths, but also to reduced weight gains. The disease is characterised by the atrophy of the nasal turbinates resulting in a shortening and sometimes twisting of the snout. It is accompanied by sneezing and epistaxis. Two bacterial organisms have been incriminated in the disease. These are *Bordetella bronchiseptica* and *P. multocida*. The former organism is a normal inhabitant of the URT of pigs. Turbinate atrophy that occurs in atrophic rhinitis is preceded by a rapid proliferation of certain toxigenic strains of *P. multocida* type D. Concurrent infection with *B. bronchiseptica* or the action of certain irritants creates an environment favourable for such proliferation. Vaccines used in the past were bacterins containing both organisms. Modern vaccines are a combination of *B. bronchiseptica* bacterin with pasteurella toxoids prepared from toxigenic strains. Both components play a role in the protective mechanism.

2.4.5 Fowl cholera

This is a disease of considerable economic importance to the poultry industry in the developed countries of the world. In the developing countries, where the poultry industry is rapidly changing from a scenario dominated by village chicken and smallholder operations to one of large-scale commercial undertakings, it has become an emerging disease of economic importance. In 1986, one estimate gave the worldwide losses due to fowl cholera as US$200 million. The disease in wild birds is often referred to as avian cholera or avian pasteurellosis and is considered to be a threat to the survival of some endangered birds.

The earliest historical record of what is now known to be fowl cholera dates back to the year 1600. Much of the earliest work on the role of microorganisms in infectious diseases, the use of immunising agents and the contribution of Louis Pasteur in this regard was related to Pasteur's work on fowl cholera in the 1880s.

Fowl cholera is caused by *P. multocida* and most serotypes involved belong to serogroup A. Serotypes A:1, A:3 and A:4 appear to be the common ones in most countries, although all of the 16 somatic serotypes of capsular group A, and some types of group D, have also been implicated. In turkeys, capsular serogroup F has been incriminated. Fowl cholera is a primary pasteurellosis resulting in septicaemia and death. A chronic form has also been described and chronically affected birds may serve as reservoirs of infection.
The disease is associated with poor sanitation. The most important control measure is to improve sanitation. Chemotherapeutic agents are used for treatment as well as on a prophylactic basis at low dose levels in situations of high prevalence. The latter use, however, is not encouraged. Vaccines are also used. Polyvalent vaccines containing the commonly occurring serotypes as bacterins, with or without adjuvants, are available commercially. Their efficacy is variable.

2.4.6 Other animals

In addition to these diseases of economic importance caused by pasturella organisms in agricultural and domestic animals, occasional, sporadic outbreaks of disease have been reported in a variety of host species. Some of these are summarised in Table 2.8. Carter (1959) recorded pasturellae associated with disease in deer, cats, dogs, horses, mink and monkeys. More recent reports of disease in donkeys and horses (Pavri and Apte 1967), and deer (Jones and Hussaini 1982; Carrigan et al. 1991), are available. Pasteurellosis has also been reported among elephants in Sri Lanka, bison in the United States, camels in Sudan and in a snow leopard in the Himalayas (Carter 1957; De Alwis and Thambithurai 1965; Bain et al. 1982; De Alwis 1982a; Wickremasuriya and Kendaragama 1982; Chaudhuri et al. 1992).

The types of infections recorded are highly varied and range from septicaemias and respiratory infections, which are the most common forms, to wound infections, abscesses, mastitis, peritonitis and encephalitis. The most common form of infection in the human occurs as a result of animal bites.

Table 2.8. Sporadic incidence of *Pasteurella multocida* infections in other species.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Country</th>
<th>Serogroup</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bison</td>
<td>United States</td>
<td>B</td>
</tr>
<tr>
<td>Yak</td>
<td>China</td>
<td>B</td>
</tr>
<tr>
<td>Deer</td>
<td>England, Australia</td>
<td>B</td>
</tr>
<tr>
<td>Elephant</td>
<td>Sri Lanka</td>
<td>B</td>
</tr>
<tr>
<td>Camel</td>
<td>Sudan</td>
<td>–</td>
</tr>
<tr>
<td>Horse</td>
<td>India, Egypt, Sudan</td>
<td>B,D</td>
</tr>
<tr>
<td>Snow leopard</td>
<td>India</td>
<td>F</td>
</tr>
<tr>
<td>Cat</td>
<td>United States, France</td>
<td>–</td>
</tr>
<tr>
<td>Mink</td>
<td>United States</td>
<td>A,D</td>
</tr>
<tr>
<td>Monkey</td>
<td>United States</td>
<td>A</td>
</tr>
</tbody>
</table>
The Disease: Infection, Clinical Signs and Pathology

Overview

The disease
Haemorrhagic septicaemia is a primary pasteurellosis in cattle and buffaloes caused by Pasteurella multocida serotypes B:2 and E:2. More recently, disease syndromes indistinguishable from HS have been found in other host species associated with other B serotypes.

Infection
Clinically affected animals and active carrier animals serve as a source of organisms in outbreaks, and infection occurs by inhalation or ingestion. Although the virulence factors responsible for the pathogenesis are not yet completely understood, recent work has thrown considerable light on this aspect. There are no permanent reservoirs of infection outside susceptible animals but carcasses, freshly infected pasture, bedding etc. may be infective, particularly in moist conditions.

Clinical signs and pathology
Clinical signs, including increased temperature and respiratory distress, appear after a brief incubation period. The disease lasts a few hours to a few days and most animals die with septicaemia in the terminal stages. Recovery from clinical disease is rare and occurs only when animals are treated in the initial stages of the disease.

Subcutaneous oedema and widespread petechial haemorrhages are seen at postmortem examination, as well as with congested lungs and enlarged lymph nodes.

Pathogenesis and bacteriology
The bacteria appear to multiply in the tonsils. During the terminal phase of the disease, pasteurellae can be isolated from blood and rapid multiplication occurs in the carcase. The nature of the bacterium’s virulence is not fully understood but lipopolysaccharides in the outer membrane have been implicated, as they inhibit phagocytosis enabling rapid multiplication. Some animals can become immune carriers.

3.1 The Disease

Haemorrhagic septicaemia (HS) was previously defined as: an acute, fatal septicaemic disease caused in cattle and buffaloes by one of two specific serotypes of the bacterium Pasteurella multocida.

The two serotypes are popularly known as the Asian and African serotypes, but were designated 6:B and 6:E by the Namioka–Carter system and more recently B:2 and E:2 by the Carter–Heddleston system, respectively (see Section 2.2). Occasionally, these same serotypes cause septicaemic disease in other species (e.g. sheep and goats). In such instances, the term ‘septicaemic pasteurellosis’ has been used to describe the condition (see Table 2.7).

More recently, a re-examination of the bacterial strains associated with the classical HS syndrome, using modern serotyping or DNA fingerprinting techniques, has indicated that type B strains of P. multocida, other than serotype B:2, have also been involved in some instances (Rimler and Wilson 1994). In view of the wider spectrum of serotypes of the B and E groups incriminated in the disease, and the involvement (though rarely) of species of animals other than cattle and buffaloes, it may now be appropriate to give the disease a somewhat broader definition. Hence, HS may now be considered as: an acute, fatal, septicaemic disease caused by strains of P. multocida belonging to the serogroups B or E, commonly in cattle and buffaloes and also in pigs and feral ruminants.

Unlike other pasteurelloses, where the pasteurella organism plays a secondary and opportunistic role, HS is a primary pasteurellosis. It is reproducible experimentally using pure cultures of the causative organism alone, and is preventable by vaccines incorporating the specific serotypes. Thus, HS is a specific form of pasteurellosis, occurring mainly in cattle and buffaloes. This is similar to the situation of typhoid in humans, and pullorum in poultry, which are both caused by a specific strain of salmonella in a specific host species.
It must be stressed, however, that only the classical serotypes B:2 and E:2 are capable of producing disease consistently and predictably upon experimental subcutaneous transmission. The other serotypes occasionally encountered are B:1, B:3,4 and B:4 (see Section 2.2). In the author's experience, B:3,4 fails to produce disease consistently, even upon experimental transmission. No information is available on types B:1 and B:4 in this regard but these serotypes have been associated with occasional sporadic outbreaks of disease, indistinguishable from the classical HS syndrome, mainly in wild animals and, in a few instances, in domestic cattle.

Buffaloes are generally more susceptible than cattle, and young animals are more prone to the disease than adults (De Alwis et al. 1976; De Alwis and Vipulasiri 1980; De Alwis 1981; FAO 1979, 1991). (See Chapter 4 for further details on host and age susceptibility.)

3.2 Source of Infection

In general, *P. multocida* does not survive long enough outside the animal to become a significant source of infection, although survival may be longer in moist conditions.

Some experiments have shown that the organism can survive in sterilised soil for 2–3 weeks (Bain et al. 1982). It was shown in Malaysia, however, that when sterilised earth and mud from rice fields were artificially infected, bacteria could not be recovered after a few hours exposure to sunlight (FAO 1959). When deposited in mud where buffaloes wallow, bacteria could not be recovered after 24 hours (Bain et al. 1982).

Carcasses dumped into rivers and waterways and carried downstream are often incriminated as a likely method of spread of the disease and it is believed that pasteurellae can survive in animal tissues, and perhaps in decomposing carcases, for a few days. Freshly infected pasture, bedding etc. may also be infective. However, no permanent reservoirs of infection have been established outside the animal.

Outbreaks of HS begin when clinically affected or, more likely, carrier animals are introduced into the herd. Once an outbreak has occurred, decomposing carcases not promptly buried or burnt serve as a source of infection. In experimental transmission, it has been found that large numbers of organisms — in the region of $10^7$ to $10^{12}$ colony forming units (CFU) — are required to set up an infection by the natural routes (De Alwis et al. 1990).

How an active carrier animal transmits such a large dose to an incontact animal is not completely understood. It can be speculated that organisms directly shed from a carrier may be more virulent than cultures grown in vitro and used in experimental transmission studies. Alternatively, unknown circumstances may alter the susceptibility of animals so that a smaller infecting dose can cause disease. When isolates from clinical cases and from latent carrier animals were compared, however, no difference in virulence was demonstrated as judged by the median lethal doses (LD50s) for mice (Wijewardana et al. 1986a).

3.3 Routes of Infection

It is believed that the natural routes of infection are by inhalation and/or ingestion, and successful transmission has been made experimentally using intranasal aerosol sprays or oral drenching. However, the dose required to produce clinical disease has not been consistent, and the results obtained with a given dose are not always predictable. Subcutaneous inoculation of bacterial cultures grown in vitro, with doses ranging from $10^4$ to $10^7$ CFU, has produced more consistent results. In experimental transmission, the route of infection is loosely related to the course of the disease, the clinical syndrome and the extent of pathological lesions. Intranasal infection by aerosols and oral drenching results in a longer course of disease and more profound lesions; subcutaneous inoculation results in rapid onset of disease, a shorter course and less marked pathological lesions.

3.4 Clinical Signs

Figure 3.1 shows a buffalo calf with clinical symptoms of HS. The disease occurs mainly in regions where husbandry practices are primitive and the animals are reared under free-range conditions. In such circumstances, the animals are not under constant observation and the only reported sign may be sudden
death. Indeed, first-hand descriptions of the clinical syndrome under natural conditions are scarce. Generally, the observed signs are temperature elevation, loss of appetite, nasal discharge, salivation and laboured breathing, with swellings in the submandibular region spreading to the brisket area and even down to the forelegs.

![Image of a buffalo calf clinically affected with haemorrhagic septicaemia.](image)

Some descriptions of the syndrome arising from experimental transmission have been made in Sri Lanka. These include experimental transmission of the disease by subcutaneous inoculation of in vitro cultures, intranasal or oral transmissions, or natural infections occurring in animals housed in close contact with clinically affected animals, the last being the closest equivalent to naturally occurring disease (M.C.L. De Alwis, unpublished data; Horadagoda et al. 1991). The results of these studies are described below.

### 3.4.1 Incubation period

On exposure to experimental or natural infection, clinical signs appear after a brief incubation period. The following incubation times were observed in Sri Lanka for indigenous buffalo calves, 4–10 months of age (M.C.L. De Alwis, unpublished data):

- **subcutaneous infection** (n=1) — around 12–14 hours (i.e. when observed at about 12 hours the animal appeared normal and when observed at 14 hours it was sick);
- **aerosol or oral infection** (n=7 and 2, respectively) — average of 30 hours;
- **naturally exposed animals** (n=4) — 46–80 hours after initial contact.

In a further study (Horadagoda et al. 1991) using 18 indigenous buffalo calves aged between 7 and 12 months, the incubation period recorded was approximately 24 hours for oral infection (n=2) and ranged from 18 to 66 hours for aerosol infection (n=12).

### 3.4.2 Duration

The duration of the clinical course of disease is highly variable. In experimental infections by the subcutaneous route, the clinical course lasted only a few hours. In experimental transmissions by natural routes (oral or aerosol), and in natural exposure experiments, M.C.L. De Alwis recorded a clinical course ranging from two to five days (unpublished data). Horadagoda et al. 1991 recorded a clinical course of 14–19 hours after experimental oral infection (n=2), 25–110 hours after aerosol infection (n=12) and 19–70 hours after natural exposure (n=4).

In field observations of five outbreaks of disease involving 37 buffaloes and 7 cattle, Saharee and Salim (1991) recorded a clinical course of 4–12 hours in per acute cases and 2–3 days in acute and subacute cases. On a herd basis, the outbreaks usually occur very fast and do not persist for long. The observations of Saharee and Salim (1991) in west Malaysia indicated that 75% of outbreaks lasted for less than 15 days within a herd.

### 3.4.3 Progression of the disease

The clinical syndrome may broadly be divided into three phases as follows.

- **Phase 1** is dominated by increased temperature, loss of appetite, general apathy, and depression. If closely monitored, a rise in rectal temperature to 40–41°C is recorded, which lasts throughout the course, dropping to subnormal levels during the terminal phase, a few hours before death.
- **Phase 2** is dominated by a respiratory syndrome. There will be an increased respiration rate (40–50/minute), laboured breathing, clear nasal discharge and salivation. Submandibular oedema
may also begin to show during this phase. As the disease progresses, the nasal discharge becomes opaque and mucopurulent.

• **Phase 3** is dominated by recumbency. The respiratory distress becomes more acute, the animal lies down, terminal septicaemia sets in and death follows: case fatality is nearly 100%.

In many instances, there are varying degrees of overlap between the phases, and the shorter the course, the less distinct are the three phases. Unless a close vigilance is maintained, the first phase may be easily overlooked. The course of the disease arising from field reports is often shorter than experimental observations, probably due to the failure to detect the first phase under free-range conditions.

In general, it has been observed that the disease is more acute and has a shorter course in buffaloes than in cattle. Graydon et al. (1993) observed that upon experimental subcutaneous inoculation, buffaloes died 24–31 hours after inoculation, whereas the time of death for cattle was 60 hours.

### 3.4.4 Atypical syndromes

Atypical syndromes caused in the natural hosts by HS-causing pasteurella serotypes have been recorded from time to time. An outbreak of pneumonia was reported in a batch of buffalo calves 4–10 months of age in Sri Lanka (De Alwis et al. 1975). The course of the disease was somewhat longer than usual and a few cases lingered on for up to 10 days. Pneumonia was the dominant feature. Out of 33 affected animals, 30 died and all showed a terminal septicaemia. It was postulated that this protracted pneumonic syndrome was the result of a heavy burden of infection on buffalo calves with low levels of immunity. Syndromes have been described in literature as 'septicaemic form', 'respiratory form' or 'cutaneous, pectoral or oedematous forms' (presumably based on the dominant symptoms).

Dhanda and Nilakanthan (1961) reported the occurrence of paraplegia in 189 cattle from 28 villages in India following a vaccination campaign against rinderpest, where a total of 48,603 animals in 71 villages in the Andhra Pradesh were vaccinated. Of these animals, 135 died. The cerebrospinal fluid was collected from two animals, and *P. multocida* was isolated from cultures. The authors typed the isolate as Roberts type I but it is now known that Roberts type I includes the Asian and African strains B:2 and E:2, respectively, as well as the Australian strain 989 (11:8 by Namioka-Carter; B:3,4 by Carter-Heddleston). It is therefore not certain whether the report is one of an atypical syndrome caused by the Asian serotype, or one with a variant serological configuration. It is also significant that in this outbreak, there was no terminal septicaemia, a feature characteristic of infection with the Asian B:2 serotype.

### 3.5 Pathology

#### 3.5.1 Gross pathology

Upon opening a carcase of an animal that has died of HS, the most obvious lesion is subcutaneous oedema — subcutaneous infiltration with yellow serosanguinous fluid, particularly in the submandibular and brisket regions. Subcutaneous petechial haemorrhages are also evident. There are also widespread petechial haemorrhages in the thoracic cavity, particularly on the base of the ventricles and the auricles. There may be excessive fluid in the pericardial sac and pericarditis with marked thickening of the pericardial wall may be present. The lungs may be congested with varying degrees of consolidation and with a marked thickening of the interlobular septa. In the abdominal cavity, petechial haemorrhages are widespread in all tissues. Massive ecchymotic or petechial haemorrhages may be

![Figure 3.2. Heart of an animal that died of haemorrhagic septicaemia showing ecchymotic and petechial haemorrhages on the subepicardial adipose tissue.](image)
Figure 3.3. Heart and pericardium of an infected buffalo. The parietal pericardium is markedly thickened. Note the fibrinous strands between the two layers of pericardium and the excessive pericardial effusion.

Figure 3.4. A lateral view of the thoracic and abdominal viscera of a buffalo calf that died of haemorrhagic septicaemia.

Figure 3.5. Abomasum of a buffalo calf that died of haemorrhagic septicaemia, showing severe diffuse haemorrhages on the mucosa.

Figure 3.6. A slice of the affected cardiac lobe of the lung in a buffalo calf that died of haemorrhagic septicaemia. Note the demarcation between the pneumonic (arrow) and congested areas.

seen on the abomasal wall, as well as on the mesentery. The lymph nodes are usually enlarged. Figures 3.2 to 3.6 show typical lesions.

In experimental transmissions, De Alwis et al. (1975) observed that the pathological picture depended upon the duration of the syndrome. In animals that died within 24–36 hours of experimental inoculation, the gross pathology was limited to widespread petechial haemorrhages and generalised congestion of the lungs. When the duration was 36–72 hours, haemorrhages were petechial or ecchymotic, and more pronounced. Fibrinous pericarditis was present.

When the course was longer than 72 hours, there was extensive consolidation of the lungs, with marked lobulation, pleuritis, pericarditis with marked thickening of the pericardial wall and, in later stages, pleural adhesions (De Alwis et al. 1975). The pleura overlying the pneumonic lungs are sometimes thickened, forming a sheet of fibrin, often adhering to the costal wall and pericardium (De Alwis et al. 1975; Horadagoda et al. 1991). Rhoades et al. (1967) found similar lesions, and further reported that the nature of the lesions depended on the route of infection.
Figure 3.7. Histological appearance of the lung of a buffalo calf that died of haemorrhagic septicaemia. (a) Thickened pleura and interlobular septa stained with haematoxylin and eosin (HE)(X25). Note the congested alveolar capillaries and alveolar oedema. (b) Congestion of alveolar capillaries, oedema fluid and infiltration of polymorphonuclear leucocytes in an area of early pneumonia (HEx100). (c) An area of acute inflammation of the lung (HEx100). The alveolar capillaries are infiltrated with numerous polymorphonuclear leucocytes. (d) Vacuolated alveolar macrophages in alveolar spaces (arrow) stained with immunoperoxidase/haematoxylin (X400). Some of the alveoli demonstrated a strong immunoreaction.

3.5.2 Histopathology

Early reports on the histopathological changes in HS include that of Rhoades et al. (1967) who produced the disease experimentally in a calf using the bison B:2 strain. The histopathological lesions observed included small haemorrhages involving the peritracheal adventitia and submucosa, generalised interstitial pneumonia, hyperaemia, oedema and cellular responses most prominent in the anteroventral portions of the lung, increased lymphocytes and macrophages in the thickened alveolar septa. Lymph nodes were hyperaemic, and there were subserous haemorrhages and hyperaemia in the spleen. In the gastrointestinal tract, hyperaemia and subserous haemorrhages were evident. The liver showed hyperaemia, cloudy swelling and fatty degeneration. The kidney showed cloudy swelling and pyknosis of tubular epithelial cells. Sections of the heart showed marked hyperaemia with subepicardial and subendocardial haemorrhages. Figure 3.7 shows the histological appearance of the lung of a buffalo that died of HS.

In experimental transmission, Horadagoda et al. (1991) found close similarities between microscopic lesions in animals infected experimentally by the intranasal route and those infected by natural exposure. There was fibrinous bronchopneumonia, with a marked dilatation of the pleura, interlobular septa and lymphatics with fibrinous exudate. Fibrin clots were present within the distended lymphatics. The alveoli were filled with fibrin with varying amounts of inflammatory cells consisting mainly of polymorphonuclear leucocytes.
With immunoperoxidase labelling, it was possible to demonstrate *P. multocida* antigens in subpleural lymphatics and interlobular septae, and within blood vessels of visceral organs.

The only available report on the pathological lesions in HS outbreaks occurring naturally in Africa is that of Bastianello and Jonker (1981).

### 3.6 Pathogenesis

Upon entry of the pasteurella organism into the animal, it is believed that the initial site of multiplication is the tonsillar region. The outcome of this infection depends on an interaction between the virulence of the organism and its rate of multiplication in vivo, on the one hand, and the specific immune mechanisms and nonspecific resistance factors of the host animal, on the other. Thus, the dose of infection is a vital factor and if the organism overcomes the host's defence mechanisms, clinical disease will result. If the defence mechanisms dominate over the organism, what is described as an 'arrested infection' occurs, and the animal becomes an immune carrier. Such animals possess solid immunity, and the presence of large numbers of such immune animals following an outbreak of disease contributes to 'herd immunity' (De Alwis et al. 1986).

There is currently no evidence that the HS-causing strains of *P. multocida* produce any exotoxins. It has however been observed that serotype B:2 strains that cause HS produce hyaluronidase (Carter and Chengappa 1980), whilst a few known B:2 strains not associated with HS fail to produce this enzyme. It is equally true that other type B strains such as B:3,4, which are known to produce a syndrome indistinguishable from the typical HS, also do not produce hyaluronidase. It is, therefore, uncertain whether this enzyme is of any significance in the pathogenesis.

It is significant that with some type B (B:2) and type E strains (E:2), disease can be predictably reproduced experimentally. Other type B strains (B:3,4, B:1, B:4) have been associated with sporadic outbreaks of disease, but pathogenicity cannot always be demonstrated experimentally. It is still uncertain whether the former strains possess any specific virulence factors, and, if so, whether they are merely phenotypic characters or have a genetic base.

Whatever the virulence factor(s), it is logical to expect it to allow the organism to initially multiply against the defence mechanisms of the body and then produce the lesions that are characteristic of the disease. At present, there is some circumstantial evidence to indicate what these factors might be.

It has been found that the classical Asian serotype (B:2) is capable of causing vacuolation and eventually lysis of macrophages, a property that will diminish phagocytosis and promote multiplication of invading bacteria. This activity of cytoplasmic vacuolation leading to macrophage lysis and death has been demonstrated using HS-causing type B strains, but not with the non-HS B strains, in a model using mouse peritoneal macrophages and in vitro studies using a mouse macrophage cell line (Shah et al. 1996). This activity has also been demonstrated using culture supernatants of the same strains, but not for those of the non-HS strains. In the absence of a true exotoxin, it might be expected that free endotoxin would be found in the culture supernatants, a feature also common to other gram-negative bacteria.

Using an ovine mammary neutrophil system and [*H]-labelled type B strain of *P. multocida*, Muniandy et al. (1993) found that capsular polysaccharide extracts known to contain 20% lipopolysaccharides (LPS), potassium thiocyanate extracts and Westphal type LPS extracts inhibited phagocytosis. These workers also found that when encapsulated cells and de-encapsulated cells were used, the percentage of de-encapsulated cells phagocytosed was significantly higher than when encapsulated cells of *P. multocida* were used. These observations indicated that HS-causing strains of *P. multocida* appeared to possess a factor in their capsule that inhibited the ability of phagocytes to engulf and destroy invading bacterial cells.

It is well established that the endotoxins of gram-negative bacteria consist predominantly of LPS. The toxic effects of the LPS of *P. multocida* associated with HS have been amply demonstrated (Rebers et al. 1967; Rhoades et al. 1967). These workers produced experimental HS in calves and pigs by different routes using type B strains. They also administered endotoxin prepared from this strain to a calf. The symptoms and lesions produced in the calf given endotoxin resembled those of experimental infection. More recently, N.U.