SESSION 2: THE DISEASE — PATHOGENESIS, PATHOLOGY, EPIDEMIOLOGY
Pasteurellosis: The Disease

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Abstract

The epidemiology and pathogenesis of pneumonic pasteurellosis in cattle and sheep caused by Pasteurella haemolytica biotype A, systemic pasteurellosis in sheep caused by P. haemolytica biotype T, and haemorrhagic septicaemia in cattle caused by P. multocida are compared with the aim of highlighting features of each that may be relevant in future research.

PNEUMONIC and systemic pasteurellosis and haemorrhagic septicaemia (HS) have been described comprehensively (Gilmour and Gilmour 1989; Frank 1989; Carter and De Alwis 1989), so it is not necessary to elaborate here on the descriptions of these diseases; rather similarities and differences will be discussed in an attempt to define gaps in our knowledge and indicate lines of future research.

Pneumonic Pasteurellosis

Epidemiology

Pasteurella pneumonia is caused by P. haemolytica biotype A serotypes and occurs in most of the sheep-rearing countries of the world. P. haemolytica is present in the upper respiratory tract of sheep of all ages. A crucial question is: What converts these healthy carriers into cases of clinical disease? Predisposing factors fall into two main categories: first, management and environment, in which proof is circumstantial; and second, infectious agents that have been incriminated epidemiologically in association with outbreaks of pneumonic pasteurellosis. Experimental production of the disease with a predisposing virus (e.g. parainfluenza virus type 3) followed by aerosols of P. haemolytica of biotype A has demonstrated the link between virus infections and pasteurellosis.

Pneumonic pasteurellosis occurs in sheep of all ages. In lambs less than 3 weeks old the disease is hyperacute, with a generalised infection; between 3 and 12 weeks the disease is an acute infection lasting 2 or 3 days, characterised by pleurisy and pericarditis. The signs of acute pneumonia — hyperpnoea and dyspnoea — are present in older sheep. In flock outbreaks, the first manifestations are sudden deaths and very ill sheep that die quickly. Within a few days acute cases disappear, to be replaced by cases with the more obvious signs of pneumonia. Mortality is typically about 5%, and morbidity up to 10%. Other flock signs are oculo-nasal discharges and coughing. Untreated, subacute cases that survive develop chronic pneumonia with the lesions in the apical parts of the lungs, and pleural adhesions.

The prevalence of pneumonic pasteurellosis within flocks varies from year to year, as does the prevalence in districts or countries. This may be due to rising and falling immunity to the predisposing viral infection and to the pasteurellae themselves. Although it has been shown that lambs that have recovered from acute pneumonic pasteurellosis are immune to reinfection, nothing is known of specific immunity in the carriers. Immunity from vaccination appears to be serotype specific. As the spectrum of serotypes in nasal carriers is wide and constantly changing, the investigation of this aspect has been little researched, as has the phenomenon of innate resistance.

Pneumonic pasteurellosis in calves has both similarities to and differences from pneumonic pasteurellosis in sheep. It is caused by P. haemolytica, almost always of serotype A1, in contrast to the disease in sheep, which is predominantly caused by serotype A2, although serotypes A1, 6, 7 and 9 are also involved, with other A serotypes more rarely.

In cattle the carrier state differs from that in sheep. P. haemolytica is isolated in culture from nasal swabs

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of fewer healthy cattle than of sheep. This may be because in cattle the organism forms only a small proportion of the nasal bacterial flora, and is therefore less readily detected. The prevalence and numbers of *P. haemolytica* in the nasopharynx increase during stress conditions that predispose to pneumonic pasteurellosis.

The disease in calves most often occurs in newly weaned animals that have been through markets and mixed together. As in sheep, the causes are multifactorial—a mixture of stress and predisposing infection, with the final, acute illness caused by *P. haemolytica*. A combination of environmental stress and non-lethal viral infections compromise the defence mechanisms. In North America, the syndrome, called shipping fever because of its association with relocation as described above, is a prime cause of economic loss to the beef industry.

Pneumonia in adult cattle is also caused by *P. multocida* serotypes A and D. It tends to be a more sporadic disease, affecting individual animals.

**Pathogenesis and pathology**

In the normal, healthy, unstressed animal the cellular defence mechanisms of the lungs and the mucociliary ladder serve to clear pasteurelles, which are deposited there in aerosols from the nasopharynx. When the defence mechanisms are compromised, the organisms in the alveoli multiply. It has been postulated that initial multiplication occurs in the nasopharynx, and that the lungs are subsequently deluged with large numbers of bacteria they cannot cope with. Once multiplication in the alveoli has begun, the virulence determinants exert their influence to produce alveolar oedema, inflammatory cell exudate and interalveolar haemorrhage.

Spindle-shaped macrophages or 'oat-cells', which form whorls and streams between adjacent alveoli, are pathognomonic in both cattle and sheep.

**Experimental production**

Research into pneumonia caused by *P. haemolytica* was impeded for many years by the absence of reproducible and standardisable methods for experimental infection. For studies into pathogenesis and immunity, it is desirable to produce a disease similar to the natural one via the natural route, in this case via the respiratory tract, with a similar number of organisms to that which may realistically be expected to occur in nature. Some compromises have to be made. It is advantageous to produce disease in a higher proportion of experimental animals than occurs under natural conditions, so that group sizes in vaccine and therapy trials can be minimised. Production of pneumonia proved almost impossible in conventional sheep, unless very high numbers of organisms were given intratracheally. Specific pathogen free (SPF) lambs infected with para-influenza virus type 3 and 7 days later exposed to aerosols of *P. haemolytica* have proved to be a generally reliable model of the natural disease. Takes of 80–90% are possible.

**Systemic Pasteurellosis in Sheep Caused by P. haemolytica Biotype T Strains**

Systemic pasteurellosis is an important disease of sheep in Britain. However, although it has been reported in other countries (e.g. the USA and Hungary), it appears to be less common worldwide than pneumonic pasteurellosis. In Britain, it causes sudden death in weaned lambs between September and December. Its occurrence in flocks is sporadic, and mortality is typically 3–5%. Asymptomatic carriage of biotype T strains occurs in the tonsils of many sheep, rather than in the nasopharynx, as with biotype A strains. Stressful environmental and management factors have been implicated as predisposing causes, but clinical disease does not invariably follow exposure to these.

**Pathogenesis and pathology**

A model for the pathogenesis of the disease was derived from in-depth, quantitative bacteriology and from histopathology of natural cases of the disease. Usually the shepherd is presented with one or two dead sheep. Any sheep seen alive are recumbent and comatose, with copious, frothy, sometimes blood-stained, nasal and oral discharges. The duration of illness does not exceed 3–4 hours in most cases.

At necropsy there is extensive subcutaneous haemorrhage over the neck and thorax, acute congestion of the pleurae, pericardium and lungs and straw-coloured pleural and pericardial effusions. The lungs are oedematous and often uniformly plum-coloured. Petechiae are present in heart muscle, liver and spleen. The tonsils are inflamed, with ulceration of the epiglottis and the pharynx in the region of the tonsillar crypts. Ulceration is common in the oesophagus, and may be found also in the abomasal mucosa. *P. haemolytica* can be cultured from these sites, but not in large numbers. Histologically the characteristic lung lesions, apart from capillary congestion and oedema, are the result of bacterial emboli. These occur with or without, depending on the age of the lesions, a halo of inflammatory cells. Large numbers of organisms (up to 10¹⁰/g) are cultured from affected lungs. Counts from liver, spleen and kidneys are always lower. *P. haemolytica* is always isolated from the blood of clinical cases,
but there is no evidence that the disease is a true septicaemia (i.e. with organisms multiplying preferentially in the blood).

It is possible that the bacteria enter the body by way of the pharyngeal, tonsillar, oesophageal or abomasal ulcers, and then move via lymphatic or venous drainage to the terminal capillaries of the lungs. It is not known if significant bacterial multiplication takes place at these portals of entry. However, massive multiplication takes place in the lungs, with subsequent spillover into other organs and the blood. The bacteria multiplying in the lungs produce the toxins that elicit the lung changes and lead to anoxia and death.

**Experimental production**

While it is possible to kill sheep with large numbers of *P. haemolytica* of biotype T, the disease produced is not the same pathologically as the natural one, and administration of organisms in aerosols to SPF lambs has failed to cause disease. There has been only one report of production of the natural disease by manipulation of the diet (Suarez-Guemes et al. 1985).

As SPF lambs could not be infected by the respiratory route, our aim was to administer the organisms at an equivalent stage to that which occurs between tonsil and lung in the natural situation. Graded doses in 1/2 log10 steps were given to 9-week-old SPF lambs subcutaneously in the prescapular region. The outcome was highly dose dependent: fewer than 5 × 10^8 organisms killed only the occasional lamb, but 10^9 organisms killed 100% of the lambs within 5 hours. In the latter lambs, the disease was not bacteriologically or pathologically typical of the natural one. However, in lambs given the smaller dose, typical systemic pasteurellosis was produced consistently in about 75%. Clinically apparent disease occurred 9–12 hours after infection, and seldom as late as 24 hours after infection. The results confirm the original hypothesis for the pathogenesis of the disease, namely a non-respiratory-tract portal of entry with the disease following from multiplication of the organisms in embolic lesions in the lungs. The precise trigger mechanisms for the initial alimentary tract ulceration is not known, and neither is the question of whether or not this ulceration occurs only in those animals that subsequently develop systemic T disease.

**Haemorrhagic Septicaemia**

HS is a specific form of pasteurellosis in buffalo and cattle and occasionally in other species and is caused by *P. multocida* serotype 6:B in Asia and serotype 6:E in Africa. HS was the subject of a recent review (De Alwis 1992), and it is intended here only to highlight aspects of the disease that emphasise how it is similar to or differs from the other forms of pasteurellosis described above.

**Epidemiology**

As with *P. haemolytica* in pneumonic pasteurellosis, HS-causing *P. multocida* occurs in the nasopharynx of apparently healthy animals, especially in-contacts in recent outbreaks. This carriage tends to be transient, and does not explain adequately the epidemiology of HS. The recent discovery of a more-prolonged tonsillar carriage (latent carriage), with intermittent flare-ups of subclinical infection with nasopharyngeal carriage (active carriage), did much to provide a reasonable hypothesis for the pathogenesis of the disease.

It is thought that HS occurs when an active carrier (i.e. excretor) comes in contact with a susceptible animal (i.e. one with neither innate resistance nor active immunity acquired through a previous latent carrier - active carrier cycle). The introduction of an active carrier into an area where the disease is not endemic and the population is therefore highly susceptible leads to an explosive outbreak, with spread of infection from clinical cases to in-contact animals. In areas where the disease is endemic and seasonal, the low prevalence can be explained by the fact that there is more active immunity. Clinical disease will occur only when immunity wanes, or when an unexposed host is encountered.

It is difficult to explain how an unexposed host can be encountered in endemic areas. Even if it has been born after a previous outbreak, it must have been in contact with survivors of that outbreak. These survivors must include latent and occasionally active carriers, from which infection is possible. It is more likely that the susceptible host is one in which other factors have compromised immunity. At the moment these factors can only be defined as 'stress'.

**Pathogenesis and pathology**

As with *P. haemolytica* T disease in sheep, there is no evidence that HS is in fact a true septicaemia. Since the earliest clinical sign noticed is oedematous submandibular swelling, it is tempting to suggest that initial bacterial multiplication occurs in the nasopharyngeal region, with subsequent spread to the lungs and ultimately to other organs and the blood, as in T disease in sheep.

**Immunity**

Vaccination is the preferred method of control for all three forms of pasteurellosis described. However, there are few, if any, highly efficient vaccines. A brief consideration of some aspects of immunity is worthwhile.
First, innate immunity. Not all naive animals exposed to pasteurellae and to the same stresses as their companions develop the disease. The immunologists should seek to determine what factors in the immune system differ in resistant and susceptible animals. Second, active immunity. Again, little is known about active immunity. It is clear that all carriers are not immune in pneumonic and systemic pasteurellosis in sheep, since carriers become clinical cases under the influence of environmental stresses or other infectious agents. True active immunity may occur following subclinical infection, and it has been shown that sheep that recover from acute pneumonic pasteurellosis as a result of antibiotic therapy are solidly immune to further disease from experimental challenge. Further, it was shown that antigens produced in vivo were important in this immunity.

Populations of calves with higher titres of leukotoxin-neutralising antibodies have a lower susceptibility to shipping fever than do those with lower titres. It is not known whether these antibodies result solely from the carrier state, or from previous subclinical infections. In HS, De Alwis has postulated that subclinical nasopharyngeal recrudescences of tonsillar infections maintain immunity. It is not known which antigenic determinants stimulate this immunity. An understanding of the nature of this immunity is probably crucial to the development of improved vaccines for HS. The similarities and differences in the epidemiology, pathogenesis, pathology and immunity of pneumatic pasteurel-losis, systemic pasteurellosis and haemorrhagic septicaemia are worthy of study in looking for leads for future research into the prevention of all three diseases.

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References


Bovine Tonsils as Reservoirs for *Pasteurella haemolytica*: Colonisation, Immune Response, and Infection of the Nasopharynx

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Abstract

Studies were conducted to determine whether *Pasteurella haemolytica*-colonised tonsils could serve as a reservoir for *P. haemolytica* infection of the nasopharynx. Calves' tonsils were inoculated with *P. haemolytica* by instillation into the palatine tonsillar sinuses. Pasteurellae were counted in tonsil washes and aspirated nasal secretions. All calves shed from the tonsils for at least several weeks, whereas only 14 of 28 calves shed *P. haemolytica* in the nasal mucus, usually for less than 1 week. Colonised calves responded serologically to *P. haemolytica* and to leukotoxin. Most calves did not exhibit clinical disease. However, some younger calves (3-5 months) had severe clinical disease ranging from acute submandibular oedema with pneumonia to invasion and destruction of the medial retropharyngeal lymph nodes. Some older calves (7-15 months) exhibited more chronic systemic invasion with joint involvement. After nasal shedding had ceased for at least 1 week, calves were infected with infectious bovine rhinotracheitis virus intranasally. Numbers of *P. haemolytica* in tonsils did not increase, but 7 of 21 calves began shedding *P. haemolytica* in their nasal secretions. Nasal shedding usually lasted for more than 3 days.

These studies show that bovine tonsils can harbour *P. haemolytica* for long periods and can be reservoirs for *P. haemolytica* infection of the nasopharynx. Knowledge of the ecological and physiopathologic relationship of the tonsil with *P. haemolytica* could improve understanding of the pathogenesis of pneumonic pasteurellosis and lead to new means of protection.

FIELD and laboratory studies on bacterial colonisation of the nasopharynx suggest that *P. haemolytica* serotype 1 can survive for long periods in healthy calves in sites other than the main nasal passages (Frank and Smith 1983; Frank 1986, 1988; Frank et al. 1986, 1989). These sites would serve as reservoirs of *P. haemolytica* in healthy calves, and colonise the nasopharynx during times of stress or respiratory viral infection. Heavy colonisation of the nasopharynx often occurs prior to the development of pneumonic pasteurellosis. Since *P. haemolytica* has been isolated from tonsils of sheep and cattle at necropsy, they appeared to be a likely reservoir.

We conducted three experiments to follow *P. haemolytica* colonisation in calves' tonsils after inoculation, and to determine the nasal shedding pattern in tonsil-colonised calves. We had previously observed calves whose nasal mucus was culture-negative for *P. haemolytica*, later begin shedding during an infectious bovine rhinotracheitis (IBR) virus infection. Therefore, our second objective was to determine whether IBR virus infection would elicit an increase in the *P. haemolytica* population in the tonsils and shedding of *P. haemolytica* in nasal mucus.

Materials and Methods

Experimental design

Calves were infected with *P. haemolytica* serotype 1 by instilling a culture (approximately $10^9$ colony-forming units, CPU) into their palatine tonsillar sinuses with a modified pipette (Fig. 1) inserted through a speculum (Fig. 2). All calves were culture-negative for *P. haemolytica* in pre-exposure tonsil wash and nasal mucus specimens. Serial tonsil washes and nasal mucus and sera were collected. The numbers of *P. haemolytica* in tonsil washes and nasal

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mucus were determined. Right and left tonsil washes of each calf were assayed separately, while nasal mucus aspirates from each nostril were pooled.

Antibody titres were determined by an indirect haemagglutination procedure and by leukotoxin neutralisation (Frank and Briggs 1992). One week to 1 month after \textit{P. haemolytica} infection, calves were inoculated intranasally with IBR virus using an aerosol apparatus (Frank et al. 1986).

Calves were observed for signs of respiratory tract disease, and rectal temperatures were recorded daily.

\textbf{Collection of specimens}

Tonsillar washings were collected by instilling 3 mL of Dulbecco’s buffered saline \textit{P. haemolytica} 7.5 into each palatine tonsillar sinus, and immediately aspirating the fluid with the same type of modified pipette used for bacterial instillation. Nasal mucus was aspirated by suction from each nostril through a modified pipette into a trapping tube (Fig. 3).

\textbf{Quantitation of \textit{P. haemolytica} from specimens}

The tonsil wash and nasal mucus samples were sonified to disperse \textit{P. haemolytica} throughout the specimens (Frank et al. 1986). Sonified samples were serially diluted and spread onto blood agar plates. After overnight incubation at 37°C, the \textit{P.}
haemolytica colonies were identified (Frank and Wessman 1978; Frank 1982) and counted.

**Results**

Extreme variations were often found in the numbers of *P. haemolytica* in washes from the two tonsils of individual calves. In some cases, one tonsil was culture-negative while the other contained large numbers of *P. haemolytica*.

Calves had varying levels of pre-exposure serum indirect haemagglutination titres to *P. haemolytica*, and leukotoxin-neutralisation titres. All calves tested responded to tonsillar colonisation with increases in both types of antibody.

**Experiment 1**

Ten steers weighing 250-300 kg were inoculated with *P. haemolytica* — two with serotype 2, and eight with serotype 1. Calves were exposed to IBR virus on day 25. Tonsil wash and nasal mucus specimens were collected twice weekly, beginning at day 0.

**Clinical observations**

After *P. haemolytica* inoculation, calves did not have fevers or clinical signs of disease. The IBR virus exposure elicited signs of mild respiratory tract disease. All calves had febrile responses (rectal temperature > 39.4°C) following IBR virus exposure, beginning 2-3 days after exposure and lasting 4-6 days.

**P. haemolytica isolation**

After *P. haemolytica* instillation, the organism was recovered from tonsil wash specimens for at least 3 weeks. The numbers of *P. haemolytica* serotype 1 were constant for approximately 2 weeks, then began decreasing. The numbers of serotype 2 isolate began decreasing immediately (Fig. 4). The nasal mucus of one calf inoculated with serotype 1 contained *P. haemolytica* on days 4 and 7, and that of another calf contained *P. haemolytica* on day 4. All other nasal mucus samples were culture-negative for the 25-day period before IBR virus inoculation.

The *P. haemolytica* population in tonsil wash specimens was not increased after IBR virus exposure. No isolations were made from the nasal mucus of calves that had been inoculated with serotype 1. Two of the serotype 1 calves were re-exposed by intranasal instillation on day 32 to determine whether their nasal passages could be colonised with *P. haemolytica*. They were susceptible to colonisation, as they shed *P. haemolytica* in their nasal mucus for at least 7 days. One *P. haemolytica* serotype 2-inoculated calf shed in the nasal mucus from days 29 to 35. The other shed from day 29 to day 32.

**Experiment 2**

Twelve Holstein and Jersey steers, aged 3–15 months and weighing 75–260 kg, were used. Eight calves were exposed to *P. haemolytica* serotype 1 on day 0, and 4 were exposed on day 7. All calves were exposed to IBR virus on day 14. Tonsil washings and nasal mucus were collected twice weekly.

**Clinical observations**

Infection of the tonsils with *P. haemolytica* caused clinical illness in some calves. Response to infection was varied and included acute submandibular oedema with pneumonia; invasion and destruction of the draining medial retropharyngeal lymph nodes; a more chronic systemic invasion with joint involvement; and the absence of clinical disease. Of the 8 calves exposed on day 0, 4 had acute infections. One calf died and another was euthanased on day 1. Two calves were euthanased on day 2 because of acute respiratory distress. One calf with a more chronic infection was euthanased on day 9.

The four calves with acute infections were febrile by day 1. They had visible, submandibular swelling and severe dyspnoea with forced, audible expiration. There was massive oedema in the soft tissues around the larynx and trachea. The lungs of two calves were pneumonic, while those of two others were grossly normal.

The more chronically infected calf was febrile, and its appetite suppressed. Its tonsils were swollen and there was submandibular swelling and nasal discharge. When euthanased on day 9, the submandibular swelling was reduced, but the carpal and hock joints were swollen. There were small, multifocal lung abscesses and pleural adhesions. The retropharyngeal lymph nodes were necrotic. The tonsils contained fibrin casts. Joint fluids were cloudy and contained extensive fibrin deposits.

Three of the seven remaining calves had clinical signs of infection. These included fever, suppressed appetite, swollen tonsils, and occasionally swollen joints.

After IBR virus exposure, five of the seven calves had fevers for at least 4 days. Three calves necropsied on days 21 and 23 had swollen, abscessed tonsils and/or retropharyngeal lymph nodes.

**P. haemolytica isolation**

After *P. haemolytica* inoculation, *P. haemolytica* was recovered from tonsil washes of all calves. The numbers of *P. haemolytica* began decreasing
immediately, and two calves were culture-negative by day 14 (Fig. 4). Only two of eight calves shed *P. haemolytica* in the nasal mucus after *P. haemolytica* exposure and before IBR virus exposure (Fig. 5).

After IBR virus infection there was no measurable increase in the *P. haemolytica* population of the tonsils. Four of seven calves, all of which had ceased shedding *P. haemolytica* prior to IBR virus exposure, began to shed *P. haemolytica* in the nasal mucus (Fig. 5).

**Experiment 3**

Ten calves, 10–11 months old and weighing 180–230 kg, were used. Four calves were infected with *P. haemolytica* serotype 1 by tonsillar instillation (conventional exposure). Four others were exposed by inserting cotton soaked in a *P. haemolytica* culture into their palatine tonsillar crypts (implant-exposed), and two were exposed conventionally with *P. haemolytica* serotype 2. Calves were tranquillised during exposure, and tonsillar washes were not collected in order to minimise the risk of tonsillar trauma. Conventionally exposed calves were infected with IBR virus at 11 days and implant-exposed calves were infected at 9 days.

**Clinical observations**

After *P. haemolytica* instillation, serotype 2-inoculated calves had no clinical signs of disease. Of the four conventionally exposed serotype 1 calves, two were febrile and one of those had submandibular swelling and dyspnnoea. Necropsy of that calf on day 3 revealed enlarged tonsils and necrotic areas in the retropharyngeal lymph nodes and oedema in the soft tissue around the larynx and trachea. The lungs and trachea were grossly normal. One clinically normal calf euthanased on day 9 exhibited no gross lesions.

Of the four implant-exposed calves, three were febrile, and two of those had submandibular swelling. One became dyspnoeic and was euthanased on day 8. The calf had severe bilateral pneumonia with fibrinous adhesions and the trachea was hyperaemic. The tonsils were grossly normal.

After IBR virus infection all seven of the remaining calves were febrile for at least 4 days and developed white IBR virus-induced plaques on the nasal mucosa. Three calves were later euthanased. One of them had chronic lung lesions and haemorrhagic retropharyngeal lymph nodes.

**P. haemolytica isolation**

Tonsil washes were not collected during this experiment, but after *P. haemolytica* instillation all calves shed *P. haemolytica* in the nasal mucus for an extended time (Fig. 5). The seven IBR virus-exposed calves ceased nasal shedding of *P. haemolytica* prior to exposure. Four of the calves began shedding *P. haemolytica* in the nasal mucus after IBR virus exposure (Fig. 5).
Discussion and Conclusion

Recovery of *P. haemolytica* from tonsil wash specimens for several weeks indicates that the tonsil is a site in which *P. haemolytica* can be carried for long periods by healthy calves. Nasal mucus specimens collected from calves with colonised tonsils were usually culture-negative for *P. haemolytica*, but sporadic isolations were made from some calves. This indicates that small numbers of *P. haemolytica* reaching the nasopharynx were not able to establish colonisation. Our past studies indicate that *P. haemolytica* reaching the nasal passages of healthy calves are cleared rapidly (Frank et al. 1986, 1989).

However, cattle with culture-negative nasal mucus can become culture-positive for *P. haemolytica* during IBR virus-induced respiratory tract disease (Frank et al. 1986) and during transport-induced stress (Frank and Smith 1983; Frank 1985; Frank et al. 1989). This indicates that the nasopharynx is susceptible to colonisation at these times. Because of their proximity to the upper respiratory tract, tonsils are an ideal reservoir for *P. haemolytica* in the healthy calf for colonisation of the nasopharynx during stress or viral respiratory tract disease.

In experiment 1, the nasal passages did not become colonised as a result of an IBR virus-induced respiratory tract disease. Even so, these same calves were susceptible to colonisation of the nasopharynx by *P. haemolytica*, as evidenced by colonisation of the nasal mucus after being re-exposed to *P. haemolytica* by intranasal instillation on day 32.

In experiment 2, however, four of the seven calves that survived to be exposed to IBR virus shed *P. haemolytica* in the nasal mucus. All of the calves had high serum indirect haemagglutination and leukotoxin-neutralisation titres at the time of nasal shedding. Therefore, the *P. haemolytica*-colonised tonsil can be a source of inoculation for the nasopharynx, even in seropositive cattle. Immune status of the calves may be influenced by prior experience with *P. haemolytica* serotype 1, but this has relatively minor effect on the level of nasal colonisation (Frank 1988).

Considering experiment 1 in which *P. haemolytica* did not cause evident clinical disease after tonsillar instillation (Frank and Briggs 1992), the systemic disease encountered in experiments 2 and 3 was unexpected. The clinical effects appear to be related to age, since calves that became acutely infected were among the youngest, while some of the older calves became chronically infected. The smaller calves used in experiment 2 may have been more susceptible than the larger calves used in experiment 1.

The calves that were clinically affected had *P. haemolytica* in the tonsillar tissue, *P. haemolytica* invasion to the draining medial retropharyngeal lymph nodes, and oedema of the surrounding tissues. Whether the invasion of the tonsil and surrounding tissues occurred as a result of trauma during instillation of *P. haemolytica* into the tonsillar sinuses or because of the ability of *P. haemolytica* to invade the tonsil is unknown.

The invasion and soft tissue response likely, led to lung involvement in two of the acutely infected calves in experiment 2. There was *P. haemolytica*-containing fluid present in the pharynx and swelling of the tissues anterior to the larynx caused by oedema of the surrounding tissues. The swelling caused forced inspiration and expiration, which appeared likely to have caused aspiration of *P. haemolytica*-containing fluids into the lung. Alternatively, *P. haemolytica* invasion of the tonsils could have led to pneumonia via the vascular route.

Calves with necrotic retropharyngeal lymph nodes and lung and hock-joint involvement indicated that *P. haemolytica* can become systemic after entry through the tonsils. Lung involvement after *P. haemolytica* invasion has been shown to occur experimentally in calves after intravenous inoculation of *P. haemolytica* (Thomas et al. 1989).

In the bovine tonsil the epithelium of the tonsillar sinus is infiltrated with lymphocytes and degenerated epithelial areas are normally present (Pelagalli et al. 1983). It is possible that *P. haemolytica* could invade such an area, since it produces a leukotoxin that will kill lymphocytes (Benson et al. 1978). Also, there is evidence that *P. haemolytica* could damage the epithelial cells, because *P. haemolytica* has been shown to produce a soluble factor (probably lipo polysaccharide) that is toxic to bovine pulmonary endothelial cells in vitro (Breider et al. 1990). Tonsillar trauma, however, is a natural possibility when rough forages are consumed (Frank and Briggs 1992). Therefore, several possible mechanisms exist for *P. haemolytica* invasion of tonsillar tissue. In some cases after invasion it is possible that the *P. haemolytica* could proceed to cause pneumonia Pasteurellosis.

The anatomic structure of bovine tonsils (Pelagalli et al. 1983) and techniques for collecting a biopsy specimen of the tonsils in live cattle have been described (Sweat et al. 1963). Others have isolated *P. haemolytica* from bovine and ovine tonsils. Tonsils from adult sheep collected at an abattoir carried mostly T biotypes of *P. haemolytica*, whereas mostly A biotypes were carried in the nasopharynx (Gilmour et al. 1974). In a study in which sequential tonsil swab specimens were collected from ewes and their lambs,
it was concluded that tonsils of lambs could be colonised shortly after birth by *P. haemolytica* acquired from their dams (Al-Sultan and Aitken 1985). *P. haemolytica* serotype 2 or serotype 1 has been isolated previously from the tonsils and/or retropharyngeal lymph nodes of calves from which the nasal conchae, trachea and lung tissues were culture-negative (Shoo and Wiseman 1990).

*P. haemolytica* has been isolated from the tonsils of naturally infected calves (Shoo and Wiseman 1990). We have shown that *P. haemolytica* can be carried for long periods in the tonsils of healthy, unstressed calves without causing disease (Frank and Briggs 1992). Experiments 2 and 3 demonstrate that tonsillar infections with *P. haemolytica* can develop into systemic infections. We have also shown that IBR virus infection does not cause an increase in the *P. haemolytica* population in the tonsil, but can result in shedding of *P. haemolytica* in the nasal mucus. Since IBR virus can elicit nasal shedding of *P. haemolytica* in calves with colonised tonsils, the tonsil can serve as a reservoir for *P. haemolytica* infection of the nasopharynx.

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Haemorrhagic Septicaemia Carriers Among Cattle and Buffalo in Malaysia

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Abstract

*Pasteurella multocida* Carter type B, the haemorrhagic septicaemia (HS) serotype, can be passively carried in the lymph nodes of the upper respiratory tract of cattle and buffalo. A survey was conducted in Malaysia on cattle and buffalo in abattoirs to isolate *P. multocida* from the nasopharynx and lymph nodes and to establish the serotypes present in endemic and nonendemic areas. Types A, B and D, and some untypeable organisms, were isolated from carrier animals. Three percent of the animals from HS-endemic areas were positive for *P. multocida* Carter type B. Except for one isolate from the nasopharynx, all isolates were from lymph nodes. The movement of carriers from endemic to nonendemic areas may be important in the spread of haemorrhagic septicaemia, and other diseases caused by *P. multocida*.

*Pasteurella multocida* Carter type B, the haemorrhagic septicaemia (HS) serotype, has been shown to be passively carried in lymph nodes of the upper respiratory tract of cattle and buffalo (Singh 1948; Omar et al. 1962; Wijewantha and Karunaratne 1968; Mohan et al. 1968; Mustafa et al. 1978; Hiramune and De Alwis 1982; Wijewardana et al. 1986). These passive or dormant carriers do not show clinical signs or excrete the organisms through their nasal passages, and are thus not infective. The state of dormancy can only be confirmed by culture of lymph nodes obtained at slaughter. However, dormant carriers can become active carriers when they are stressed. At this stage the animals are infective, and pasteurellae are shed in the nasal passages and can be isolated from nasopharyngeal swabs.

Singh (1948) found 3.5% carriers in a group of live animals swabbed through the external nares and 7% carriers from lymph nodes of slaughtered animals. Wijewantha and Karunaratne (1968) found 15% carriers among slaughtered animals. Mustafa et al. (1978) found carrier rates of 0–5% in nonendemic areas and 44.4% in endemic areas. Hiramune and De Alwis (1982) found no carriers in nonendemic areas and 2.7% in endemic areas.

Wijewardana et al. (1986) obtained results similar to Hiramune and De Alwis (1982). It is likely that the number of carrier animals in cattle and buffalo populations influences the frequency of HS outbreaks in endemic areas. The HS carrier in Malaysia was demonstrated by Omar et al. (1962) by the isolation of *P. multocida* Robert type 1 from the tonsil of a clinically healthy buffalo. Subsequently, pasteurellae of Carter types A, B and D were isolated from cattle and buffalo slaughtered in an abattoir. Since further information on HS carriers in endemic and nonendemic areas is important to develop appropriate prevention and control programs, a survey was conducted on cattle and buffalo in abattoirs and in the field to isolate and establish the serotypes of *P. multocida* from the nasopharynx and lymph nodes of cattle and buffalo from endemic and nonendemic areas.

Materials and Methods

The abattoir survey was conducted in an endemic area (Kelantan), 2 weeks after an outbreak had occurred; and in four nonendemic areas (Ipoh, Shah Alam, Seremban and Melaka). A total of 325 cattle, comprising Kedah–Kelantan, local Indian Dairy and crossbreds, and 46 buffalo (*Bubalus bubalis*, swamp type) were examined. The animals were mostly adult males of unknown vaccination history.

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Pre- and post-slaughter nasopharyngeal and pharyngeal swab samples were taken to screen for *P. multocida*. Each sample was washed in 5 mL tryptose broth in bijour bottles. Retropharyngeal lymph nodes and tonsils were collected in clean plastic bags. All samples were transported to the laboratory immediately, in an icebox.

In addition, a field survey was conducted on 205 cattle and 15 buffalo in Kota Baru and Trengganu, representing endemic areas, and Ipoh and Shah Alam, representing nonendemic areas. Nasopharyngeal swabs were taken in a manner similar to that used in the abattoir survey.

Screening for *P. multocida*
Pieces of tonsil and retropharyngeal lymph nodes were removed aseptically and crushed in about 3 mL of tryptose broth to allow the organism to elute into the broth. A 0.5-mL aliquot of well-shaken broth containing lymph nodes and tonsil and a 0.5 mL aliquot of broth containing swab samples, were inoculated intraperitoneally into a mouse. Broth that killed mice within 72 hours was suspected of containing pasteurellae.

Isolation and identification of *P. multocida*
Heart blood from dead mice was cultured in tryptose blood agar and MacConkey agar and the plates were incubated at 37 °C for 12–24 hours. Smears of heart blood were made and the organisms identified by their staining reaction to Gram’s and methylene blue stains. Bacteria that grew on tryptose blood agar, but not on MacConkey Agar, were Gram-negative coccobacilli characterised by a bipolar appearance with methylene blue stain, and were oxidase- and catalase-positive. They were considered to be *P. multocida* (Carter 1967; Namioka 1978).

The slide agglutination test (Namioka and Murata 1961) was used for the capsular typing of isolates. All *P. multocida* type B isolates identified by the simplified capsular typing method were confirmed by IHA (Carter 1955).

Mucoid types of *P. multocida* were grown in the presence of hyaluronidase to remove capsular hyaluronic acid (Carter and Rundell 1975). Treated organisms were then subjected to IHA or simple agglutination tests (simplified capsular typing) for serotyping. Known hyaluronidase-positive *P. multocida* types were used as sources of hyaluronidase.

*P. multocida* organisms not of type B or A were typed by the acriflavine flocculation test (Carter and Subronto 1973).

Results
A total of 49 (13%) of *P. multocida* isolates were obtained from 371 cattle and buffalo samples. Only one (0.3%) of the 44 isolates from cattle was isolated from the nasopharynx, while the rest were from lymph nodes. All five buffalo isolates were obtained from lymph nodes. No isolates were obtained from pharyngeal swabs of slaughtered cattle or buffalo, or from the nasopharynx of live animals in the field.

Of the isolates typed, 44 (11.8%) were from cattle, of which 6 (2.1%) were of type A, 11 (3%) type B, and 5 (2%) type D. Of the five (11%) isolates from buffalo, 1 (2%) was of type B and 1 (2%) of type D. Twenty-one (6%) of the isolates from cattle and 3 (7%) from buffalo could not be typed. No type E isolates were made from either cattle or buffalo.

The 12 (3%) type B isolates from cattle and buffalo were from an endemic area and were collected in Kelantan 2 weeks after an outbreak of haemorrhagic septicaemia. No isolates were made from the nonendemic areas.

Discussion
Abattoirs are convenient and relatively inexpensive sources of samples for epidemiological studies. The selection of abattoirs in this study was based on the availability of laboratory facilities for processing samples. The survey confirmed that HS carriers are present in cattle and buffalo in Malaysia. The *P. multocida* isolates from carrier animals were of types A, B and D. Some of the *P. multocida* organisms isolated could not be typed into any of the known serotypes.

From the 371 abattoir samples studied, 49 (13%) yielded *P. multocida*. No isolates reacted to type E antiserum, while 12 (3%) reacted to type B antiserum. It is known that *P. multocida* type E organisms exist only in the African continent (Bain et al. 1982). The isolation of *P. multocida* types A and D, along with nontypeable *P. multocida*, suggested that cattle and buffalo could succumb to *P. multocida* infections other than haemorrhagic septicaemia.

The findings that all type B isolates were from a district in Kelantan, where an outbreak of HS occurred 2 weeks before the survey was conducted, and that no isolates were obtained from the nonendemic areas of Ipoh, Shah Alam, Melaka and Seremban, confirm the belief that carrier animals are found close to outbreak areas (Mustafa et al. 1978; Hiramune and De Alwis 1982; Wijewardana 1986).
The tonsil and retropharyngeal lymph nodes were sampled because they were the most consistent sites for the isolation of *P. multocida* in carrier animals (Wijewardana 1986). Animals that carried *P. multocida* organisms in their lymph nodes were shown to be passive carriers (Wijewardana et al. 1986; Carter and De Alwis 1989). These animals may discharge the organisms to the environment when stressed, causing the next outbreak of disease. However, the current methods of detecting carriers are based only on nasal swabs in the case of active carriers and culture of lymph nodes from slaughtered animals in the case of passive carriers. The inability to detect carriers in the field poses a problem when moving animals from endemic to nonendemic areas.

Active carriers are those animals from which *P. multocida* organisms could be isolated from the nasopharynx (Wijewardana et al. 1986; Carter and De Alwis 1989). In this study, only one active carrier of type B was detected in cattle from endemic areas (Kelantan) and none from nonendemic areas, confirming previous studies (Hiramune and De Alwis 1982; Wijewardana et al. 1986).

The results of the abattoir survey demonstrated that cattle and buffalo in HS-endemic areas in Malaysia harbour *P. multocida* type B in the lymph nodes of the upper respiratory tract. The movement of these carriers from endemic to nonendemic areas may be important in the transmission of the disease.

References


Epidemiology of Pasteurella Pneumonia in Pigs

M.P. White, T.K.S. Makkur, R.D. Cameron and R.J. Love

Abstract

Enzootic pneumonia is an important problem affecting the pig industry worldwide. Generally it is regarded as being caused by Mycoplasma hyopneumoniae. While Pasteurella multocida is also commonly recovered from lesions, little is known of the role this organism plays in the disease process.

Two field surveys were conducted in New South Wales (NSW), Australia, during 1989-90. P. multocida was recovered from 49% and 61% of lungs respectively. The organism was present in at least some lungs from all 31 herds examined. There was a strong association between lesion severity and frequency of recovery of the organism. Positive cultures were made from approximately 10% of normal lungs, 35% of lungs with low-grade lesions, 60% of lungs with medium-grade lesions, and 80% of lungs with high-grade lesions. There were similar recovery rates from active lesions and chronic lesions; and the recovery rate from younger (14-18-week-old) pigs was similar to that from older (22-26-week-old) pigs. Sequential nasal swabbing of pigs from weaning to slaughter showed that upper respiratory tract infection with P. multocida was very common and that most nasal isolates were capsule type D. However, the appearance of the capsule type A strain in nasal-swab cultures coincided with the onset of clinical signs of pneumonia. Most pulmonary isolates were capsule type A. Growth-rate depression was evident only when there was a combination of high lung pneumonia scores and pulmonary infection with P. multocida.

Mycoplasma hyopneumoniae is generally considered to be the primary cause of enzootic pneumonia. However, P. multocida is also commonly recovered from lesions (Osborne et al. 1981; Pijoan et al. 1984; Morrison et al. 1985). Despite the significance of this organism in a number of important diseases of livestock, little is known of its role in porcine enzootic pneumonia. Two field surveys were carried out in New South Wales (NSW), Australia, during 1989-90, in order to determine more clearly the significance of P. multocida infection in this disease complex.

Materials and Methods

Design of surveys

The first survey consisted of a case series study of 1283 pigs from 31 herds with enzootic pneumonia. From each herd, a single batch of pigs consigned to slaughter was monitored through one of eight abattoirs involved in the study. Farms selected were convenience sampled from commercial herds with greater than 50 sows in the central-western and mid-coastal areas of NSW. Herd size varied from 50 to
2500 sows. From information supplied by the abattoirs, pigs were classified as either pork or bacon animals. In Australia, pork pigs are generally younger, lighter animals (14–18 weeks old), while bacon pigs tend to be older, heavier animals (20–25 weeks old). Farms sampled specialised in the production of either pork or bacon pigs, and no cull batches of pork pigs were included in the survey.

Most abattoirs dehaired pigs using the traditional immersion in a hot water tank. However, one abattoir used a hot steam method of scalding, in which pigs were suspended head down and sprayed. This second method avoided microbial contamination of lungs with faecal and skin organisms during immersion.

The second survey consisted of a cohort study involving 110 pigs from a single commercial piggery. Nasal swabs and blood samples were taken at 2-weekly intervals from weaning to slaughter using thin-tipped aluminium-shafted swabs inserted 6–8 cm into the left nares, after being premoistened in Hanks 199 medium. Nasal swabs were directly inoculated onto selective 7% horse-blood agar containing neomycin sulphate (2 μg/mL) and bacitracin (3.5 μg/mL). From 60 lungs, 174 lobes were also cultured for the presence of Haemophilus spp., using chocolate agar with a Staphylococcus nurse colony under microaerophilic/high carbon dioxide conditions in candle jars.

Results

The prevalence of pneumonia in the two surveys was 46% and 71% respectively. This was within the range observed by most authors. The prevalence of high-grade lesions — i.e. those of lung score 10 or greater — was 22% in the first survey and 35% in the second. The prevalence of pleurisy in the first survey was 6%, however lungs from three herds accounted for most of these lesions, and the rest of the consignment batches examined were relatively pleurisy free. In the second survey, 22% of lungs showed some pleurisy, although most had only low-grade lesions.

P. multocida was recovered from 49% and 61% of all lungs in the first and second surveys respectively. These results refer to all lungs cultured, regardless of extent or quality of lesion, and include results from normal lungs. In the second survey, but not the first, all lobes with pneumonic lesions were cultured. This resulted in a 20% increase in the isolation rate of the organism. There was no apparent association between the recovery of P. multocida and the presence of pleurisy.

P. multocida was recovered from at least some lungs from all 31 herds included in the surveys; i.e. there was a herd prevalence of 100%.

There was a strong association between the recovery of P. multocida and lesion score (Table 1). The organism was much more likely to be recovered from high-grade lesions than from lungs without lesions or with low-grade lesions, and there was a step-wise increase in isolation rate as lesion score increased, from approximately 10% of normal lungs to approximately 80% of high-grade lesions.

The recovery of P. multocida was similar from younger pork pigs (48%) and older bacon pigs.
Serial nasal-swab cultures showed that nearly all pigs became infected with *P. multocida* between weaning at 3 weeks and slaughter at 25 weeks (Table 4), and some pigs were infected as early as 3 weeks of age. Most nasal isolates belonged to capsule type D. Capsule type A organisms did not appear in nasal-swab cultures until the pigs were 15 weeks old, and coincided with the onset of coughing in the herd.

The growth performance of pigs was assessed in relation to the presence of pneumatic lesions and the recovery of *P. multocida* from those lesions (Table 5). Only pigs with high-grade lesions of pneumonia from which *P. multocida* could be isolated showed a marked decrease in growth performance. Pigs with lungs from which *P. multocida* was not recovered had a growth rate similar to that of pigs with normal lungs, regardless of the severity of those lesions.

### Table 1. Association between recovery of *P. multocida* and severity of enzootic pneumonia lesion.

<table>
<thead>
<tr>
<th>Lesion score</th>
<th>Survey 1</th>
<th>Survey 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal lobes</td>
<td>12.8</td>
<td>10.7</td>
</tr>
<tr>
<td>Low-grade lesions (score 1-3)</td>
<td>31.0</td>
<td>36.4</td>
</tr>
<tr>
<td>Medium-grade lesions (score 4-7)</td>
<td>58.7</td>
<td>58.3</td>
</tr>
<tr>
<td>High-grade lesions (score 8-10)</td>
<td>79.7</td>
<td>77.8</td>
</tr>
</tbody>
</table>

(44%). Active lesions of pneumonia were as likely to be *P. multocida* positive on culture as more chronic lesions in both surveys (Table 2). There was no apparent association between the recovery of *P. multocida* from lungs and the sex of the pig or the season of year in which the lungs were cultured.

### Table 2. Association between recovery of *P. multocida* and stage of lesion.

<table>
<thead>
<tr>
<th>Stage of lesion</th>
<th>% of lobes positive for <em>P. multocida</em></th>
<th>Survey 1</th>
<th>Survey 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active</td>
<td></td>
<td>53.9</td>
<td>62.1</td>
</tr>
<tr>
<td>Chronic</td>
<td></td>
<td>48.6</td>
<td>42.0</td>
</tr>
</tbody>
</table>

### Table 4. Cumulative percentage of pigs with at least one nasal swab positive for *P. multocida*, according to age at sampling.

<table>
<thead>
<tr>
<th>Age of pigs (weeks)</th>
<th>Cumulative % of pigs' nasal-swab positive for <em>P. multocida</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>4.8</td>
</tr>
<tr>
<td>5</td>
<td>16.1</td>
</tr>
<tr>
<td>7</td>
<td>33.8</td>
</tr>
<tr>
<td>9</td>
<td>41.9</td>
</tr>
<tr>
<td>11</td>
<td>54.8</td>
</tr>
<tr>
<td>13</td>
<td>67.7</td>
</tr>
<tr>
<td>15</td>
<td>72.6</td>
</tr>
<tr>
<td>17</td>
<td>74.2</td>
</tr>
<tr>
<td>20</td>
<td>77.4</td>
</tr>
<tr>
<td>23</td>
<td>88.7</td>
</tr>
</tbody>
</table>

Of the 372 pulmonary isolates, 92% of strains were capsule type A, 1% capsule type D, and 7% were untypeable. Of the 138 nasal isolates, 73% were type D, 11% were type A, and 16% were untypeable.

### Table 3. Association between recovery of *P. multocida* and other organisms and scalding system used during slaughter.

<table>
<thead>
<tr>
<th>Scalding system</th>
<th>% of lobes positive for each organism</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pasteurella</td>
</tr>
<tr>
<td>Hot water tank</td>
<td>45.1</td>
</tr>
<tr>
<td>Steam</td>
<td>55.8</td>
</tr>
</tbody>
</table>
The widespread involvement of *P. multocida* in enzootic pneumonia across herds. Origin of lungs examined, and did not demonstrate lesions. The true recovery rate of the organism may be substantially higher — pigs in which *P. multocida* pneumonia contributed to mortality or to early culling would not have been included in the surveys. Additionally, only a small amount of lung fluid was used to inoculate plates; i.e. the cultural technique used was a simple one, suitable for screening a large number of samples.

Other workers have used a variety of approaches to increase the recovery rate of *P. multocida*. Pijoan et al. (1984) used a combination of broth and mouse inoculation to increase the recovery rate from pig pneumatic lesions by 17%. The recovery rate was higher in the second survey, although this was influenced by the different system used to select lobes for culture; i.e. several lobes per lung were cultured in the second survey, compared to only one per lung in the first survey. Culturing the lobe with the largest lesion only was shown to give 80% of the recovery rate of culturing all pneumatic lobes.

*P. multocida* was shown to be widespread, rather than being a problem of particular farms, in that at least some lungs from each herd were culture-positive. Previous microbiological surveys of pig pneumonia (Osborne et al. 1981; Pijoan et al. 1984; Morrison et al. 1985) did not identify the herd of origin of lungs examined, and did not demonstrate clearly the widespread involvement of *P. multocida* in enzootic pneumonia across herds.

The strength of the association between lesion size and *P. multocida* recovery rate suggests that the organism significantly exacerbates the condition by extending the size and duration of lesions, thereby contributing to the pathology, clinical signs and economic loss of enzootic pneumonia.

The recovery of *P. multocida* from approximately 10% of normal lungs was somewhat higher than expected. It is probable that normal lungs are constantly clearing inhaled or aspirated *P. multocida*, and this would account for some normal lungs being *P. multocida*-positive on culture. It is also possible that grossly normal lungs may contain areas of microconsolidation, visible only on histopathological examination, and these areas may harbour the organism. Such areas have been noted in experimentally induced *P. multocida* pneumonia in pigs (White et al., unpublished).

There is some evidence that *P. multocida* becomes involved in the development of enzootic pneumonia lesions at an earlier, rather than later, stage. The organism was recovered with a similar frequency from younger pork pigs and older bacon animals, and from earlier active lesions and older, more chronic lesions. Early involvement would allow the organism to make a greater contribution to the development of the disease. Further studies on the timing of *P. multocida* involvement in enzootic pneumonia may require serial slaughtering experiments, to determine the recovery rate of the organism at different stages of lesion development. Serological studies are currently in progress.

Many organisms besides *P. multocida* and *M. hyopneumoniae* have been recovered from porcine enzootic pneumonia (Gois et al. 1975, 1980) and this has made interpretation of the relative importance of *P. multocida* difficult. However, when the contamination of lungs with hot water from the scalding tank was avoided by the use of a steam scalding system, the recovery rate of other organisms markedly declined, whereas that of *P. multocida* rose moderately. *P. multocida* was the predominant organism isolated from lungs from steam-scaled pigs, and the moderate increase in isolation rate may have been due to the lack of overgrowth of pasteurellae by coliforms etc., which sometimes occurred in the lung cultures from tank-scaled pigs. The role of *Streptococcus* spp. in the enzootic pneumonia complex requires further elucidation.

Most animals appeared to acquire a *P. multocida* infection of the upper respiratory tract. There was little correlation between successive swabs from the same animal, and this probably reflects the relative insensitivity of nasal swabbing as a diagnostic test for pasteurella infection. ELISA studies also suggest

### Table 5. Association between growth rate of pigs, recovery of *P. multocida*, and severity of enzootic pneumonia lesions.

<table>
<thead>
<tr>
<th>Lung score and lung culture</th>
<th>Mean dressed weight (kg)</th>
<th>Mean growth rate (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All pigs with normal lungs</td>
<td>64.5</td>
<td>371</td>
</tr>
<tr>
<td>All pigs with low-grade lung score (&lt;10)</td>
<td>64.3</td>
<td>370</td>
</tr>
<tr>
<td>All pigs with high-grade lung score (&gt;10)</td>
<td>62.2</td>
<td>357</td>
</tr>
<tr>
<td>Pigs with high-grade lung score and lung culture negative for <em>P. multocida</em></td>
<td>65.0</td>
<td>374</td>
</tr>
<tr>
<td>Pigs with high-grade lung score and lung culture positive for <em>P. multocida</em></td>
<td>60.4</td>
<td>347</td>
</tr>
</tbody>
</table>

**Discussion**

In both surveys the prevalence of pneumonia was typical of the industry, and *P. multocida* was shown to be associated with pneumatic lesions frequently. The true recovery rate of the organism may be somewhat higher — pigs in which *P. multocida* pneumonia contributed to mortality or to early culling would not have been included in the surveys. Additionally, only a small amount of lung fluid was used to inoculate plates; i.e. the cultural technique used was a simple one, suitable for screening a large number of samples.

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that most pigs in the study became infected with *P. multocida* by the time of slaughter (White et al., unpublished). The organism has also been recovered more frequently from the tonsillar crypts than from the anterior nares of pigs (van Leengoed et al. 1986). The pigs that were nasal-swab positive at weaning probably acquired infection from their dams. This was followed by a gradual increase in the proportion of pigs with at least one positive nasal-swab culture during the period of survey (Table 4), presumably due to horizontal transmission among cohorts. The appearance of capsule type A strains in nasal-swab cultures occurred at the same time as the onset of clinical signs of pneumonia. Whether this increase in the population of type A strains in the upper respiratory tract preceded or followed the initial development of pneumonia lesions is unclear.

Two factors appeared to be required in order for pigs to suffer a marked decline in growth performance: first, the presence of larger lesions of lung score 10 or more; and second, the presence of *P. multocida* in those lesions. It is likely that *P. multocida* is responsible for much of the economic loss associated with porcine enzootic pneumonia, and that *M. hyopneumoniae* is the most common initiator of the lung damage that allows *P. multocida* to become established. It is interesting to speculate that, under adverse environmental conditions (e.g. high ammonia levels), *P. multocida* may be able to colonise the lungs without the assistance of other microorganisms. It has been well demonstrated, experimentally at least, that a variety of insults other than *M. hyopneumoniae* can be used to induce pneumatic pasteurellosis in pigs, including an adenovirus (Smith et al. 1973), a live attenuated swine fever vaccine (Pijoan and Ochoa 1978), the pseudorabies virus (Fuentes and Pijoan 1987), embryonated *Ascaris suis* eggs (Raynaud et al. 1977), and *P. multocida* in conjunction with large doses of saline (Hall et al. 1988).

Further work in this project is concentrating on characterisation of the Australian pig pneumonia isolates of *P. multocida* with respect to somatic serotypes, toxigenicity, outer membrane protein profiles and biochemical profiles, and serological and protection studies.

**Acknowledgments**

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**References**


The Epidemiology of Haemorrhagic Septicaemia in Sri Lanka

M.C.L. De Alwis¹

Abstract

Clinical haemorrhagic septicaemia (HS) has been reported in Sri Lanka since the beginning of the present century. Serological confirmation was made only in 1955. The disease is endemic in buffaloes and cattle that are mainly free-roaming on the dry plains covering about two-thirds of the land area. It is seldom seen in intensively reared exotic animals in the hill country. More losses occur in buffaloes than in cattle. Few reports are available of the disease in pigs and wild elephants. Goats are highly resistant to HS serotypes. Morbidity in cattle and buffaloes is highly variable, and the pattern varies in endemic and non-endemic areas. Case fatality is near 100%. In endemic areas, mostly young animals are affected. The spread of outbreaks is related to rainfall.

Varying proportions of animals in endemic areas are immune carriers, a state that results from non-lethal natural exposure. The persistence of the organism in the tonsils has been demonstrated both experimentally and under natural conditions. A prolonged latent carrier state with intermittent shedding has also been demonstrated. Currently, the changing epidemiological patterns following intensive vaccination are being studied.

Sri Lanka is a tropical island of 65 000 km², which displays a range of climatic conditions based on altitude and rainfall. Broadly, hill country (>1000 m above mean sea level), mid country (300 m–1000 m) and low country (<300 m) are recognised; as well as wet (>2000 mm), intermediate (1250–2000 mm) and dry (<1250 mm) zones. Agricultural patterns in the country are governed by these climatic conditions, thus giving rise to a number of 'agro-climatic' zones. The incidence and distribution of animal diseases are related to the types and numbers of livestock and their husbandry practices, which are in turn related to the agro-climatic zones.

History

A disease clinically resembling haemorrhagic septicaemia (HS) has been recorded in Sri Lanka since the beginning of the 20th century. The first occasion the disease broke out in epidemic proportions was in 1955–56, when nearly 10 000 deaths were recorded. It was during this epidemic that the organism was first identified by R.V.S. Bain as Roberts Type I (Dassanayake 1957; Perumalpillai and Thambiayiah 1957), and vaccination was introduced.

In the early years, vaccination was carried out in an ad hoc manner, with no strategically planned program supported by definite epidemiological knowledge. Vaccination failures were therefore common. This situation gave rise to considerable speculation that different types and strains may exist. In the early 1970s a collection of 50 isolates from different parts of the country were serotyped and conclusively identified as Carter's type B and Namioka's type 6; i.e. serotype 6:B (De Alwis and Panangala 1974). More recently, the Sri Lankan HS isolates were identified as Heddleston’s type 2 (Wijewardana, unpublished). No variant cultures have been associated with outbreaks of HS.

Since the major epidemic of the mid-1950s, a definite pattern of incidence and distribution of HS has been established. In the dry plains in the north-western, north-central, south-eastern and eastern regions of the country, which cover approximately two-thirds of the land area, the disease has become endemic, and regular annual outbreaks occur. In these areas, moderate to large herds of indigenous

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animals roam freely. In a restricted land area in the hill country, where exotic animals or their crosses are reared intensively, the occurrence of HS is exceptional. In the mid and low country, wet and intermediate regions of the south-western part of the island, sporadic outbreaks occur from time to time, often resulting from spread of infection from endemic areas, by transport of animals (Fig. 1).

Species Affected
The main species affected are buffalo and cattle. Epidemiological studies have shown that buffalo are more susceptible than cattle. A study in selected endemic areas showed that there was no significant difference between the herd infection rates over a 3-year period in the 870 herds of buffalo and 803 herds of cattle investigated. The mean mortality, however, was higher in buffalo than in cattle, and this difference was highly significant (De Alwis and Vipulasiri 1980). Another study of outbreaks in both endemic and non-endemic areas showed that mortality in buffalo was three times higher than in cattle (De Alwis 1981).

Disease caused by HS serotypes have been reported in Sri Lanka in other species of animals. There have been rare, sporadic outbreaks in pigs (De Alwis, unpublished), and two reports of the disease in wild elephants (De Alwis and Thambithurai 1965; De Alwis 1982a; Wickremasuriya and Kendaragama 1982).

Since goats are found roaming freely in HS endemic areas, they were for long suspected to be reservoir hosts. In an abattoir study (Wijewardana et al. 1986a), no isolations were made from the nasopharynx or associated lymph nodes of goats. None of these goats showed any antibody against HS. Attempts to transmit the disease naturally to goats by tying them in close contact with clinically diseased buffalo failed, and no antibody response was detected in any of the goats. Direct experimental infection of goats by the sub-cutaneous or intranasal routes using doses of up to $10^4$-$10^6$ cattle lethal doses proved futile, except in a very few animals (Wijewardana et al. 1986b). The overall conclusion was that goats were highly resistant to infection with HS-causing pasteurella.

![Fig. 1. (a) Climatic zones in Sri Lanka; (b) Incidence of haemorrhagic septicaemia in Sri Lanka.](image-url)
Morbidity, Mortality and Case Fatality

In general HS occurs in situations where early disease detection is difficult. In such situations, once clinical disease is established, death is almost certain, with case fatality being near 100%. Morbidity however, is highly variable and will range from less than 5% to nearly 90%. Many factors appeared to influence morbidity; e.g. the species affected (cattle or buffalo), age, endemicity of the area, and herd size. The higher susceptibility of the buffalo has already been dealt with.

Age

Studies in HS-endemic areas have shown that, in cattle and buffalo, while morbidity among under 2-year-olds was 30-32%, the corresponding figure for over 2-year-olds was 3-5% for cattle and 8-9% for buffalo (De Alwis and Vipulasiri 1980). An analysis of the mortality patterns in two outbreaks showed that 91% and 84% respectively of all HS deaths occurred in the 6-18-month age group (De Alwis et al. 1976).

Endemicity

Studies showed that, in endemic areas, outbreaks occurred frequently, but the morbidity in each outbreak was low and only young animals were affected. When occasional sporadic outbreaks occurred in nonendemic areas, however, all age groups were affected and morbidity was high (De Alwis and Vipulasiri 1980).

Herd size

In Sri Lanka, the herd size in cattle and buffalo is related to the system of management. Large herds usually roam freely, grazing on common grasslands in the village and drinking in common village tanks. The smaller herds are mostly confined. It has been found that in small herds of fewer than 10 animals, incidence is low, but when the disease does occur, morbidity is high. The larger herds, on the other hand, experience more frequent outbreaks, but in each outbreak morbidity is low. One study showed that, in endemic areas, the percentage of herds of more than 50 animals infected over 3 years was four to five times higher than the figure for small herds of less than 10 animals (De Alwis and Vipulasiri 1980).

Seasonal Incidence

Outbreaks of HS have generally been associated with the rainy season. An epidemiological study that reviewed HS status in the 1970s, when immunisation programs were weak, found that outbreaks occurred throughout the year. The distribution of outbreaks was fairly constant from January to July, but a steep rise in the number of outbreaks began in August and reached a peak in October. This trend roughly corresponded to levels of the organism and presumably denotes its degree of dissemination (De Alwis and Vipulasiri 1980). Since 1984 the immunisation programs have been strengthened and rationalised and mass vaccination is carried out during the period June to August, with the resultant abolishing of the peak (Hettiarachchi 1991) (Fig. 2).

Fig. 2. Distribution of haemorrhagic septicaemia outbreaks in buffalo and cattle as related to the annual rainfall pattern.
Naturally Acquired Immunity

Studies on the antibody status of unvaccinated cattle and buffalo in Sri Lanka in high-, moderate- and low-incidence areas showed that 36%, 7% and 0.47% of animals respectively had antibodies to the indirect haemagglutination assay (IHA) (De Alwis and Sumanadasa 1982). In another study, where 26 unvaccinated buffalo calves were kept exposed to an outbreak of HS, it was found that those that were unaffected developed antibodies detectable by IHA in 2–3 weeks and the antibodies were detectable for at least 8 months (De Alwis 1982b). A similar study in three field outbreaks in unvaccinated herds showed that 80–100% of animals that survived developed high antibody levels (De Alwis et al. 1986).

It is evident that different morbidity patterns in endemic and nonendemic areas are due to the different proportions of naturally immune animals. The morbidity pattern is thus governed by the phenomenon of naturally acquired immunity, which in turn is dependent on recent exposure to disease. During the rainy season, outbreaks spread. At the end of this season, therefore, most surviving animals have acquired natural immunity. By the next year’s annual rainy season, a substantial number of new animals would have reached the susceptible age, when the next outbreak occurs. The cycle thus continues.

The Carrier Status

The earliest study on HS carriers in Sri Lanka was by Wijewantha and Karunarathne (1968). In nasal and pharyngeal swabs of cattle originating from HS-endemic areas, typical *P. multocida* ‘Asian type I’ was isolated from 14% and the ‘Australian type I’ from 0.6% of animals. The latter non-HS serotype is designated 11:B (Namioka:Carter) or B:3,4 (Carter:Heddleston). In a more recent survey in a group of cattle at the same abattoir, Wijewardana et al. (1986a) isolated typical HS-causing *pasteurellae* from 0.6% of swabs from the external nares; from 1.4% of swabs from the nasopharynx; and from 2.2% of cultured retropharyngeal lymph nodes.

Hiramune and De Alwis (1982) found no carriers among 250 animals examined in a HS nonendemic area, and nearly 3% carriers among 589 animals examined in endemic areas. They further found that, in the latter group, the carrier rate varied considerably from one location to another, and was in general related positively to recent outbreaks of HS. In small groups recently exposed to HS, up to 23% of animals were carriers.

More recently, De Alwis et al. (1986) swabbed the nasopharynx of animals that had survived recent outbreaks repeatedly, at regular intervals. They found that the carrier status, as evidenced by the presence of the *pasteurellae* in the nasopharynx, was a transient one. Further, different animals showed up as carriers on different days. In order to further elucidate the nature of the carrier status, carriers were produced experimentally by controlled exposure and observed for up to 1 year. Using a marked organism, the intermittent appearance of the organism in the nasopharynx was confirmed. By slaughter of these carrier animals, *pasteurellae* were isolated, not only from the nasopharynx, but also from the lymph nodes associated with the respiratory tract, the most consistent site being the tonsils, from which site they were recovered even 8 months after a single exposure (De Alwis et al. 1990).

A current survey of the presence of HS-causing *pasteurellae* in the tonsils of abattoir animals has confirmed the tonsils as a site of persistence under natural conditions (Wijewardana et al., unpublished). Thus the presence of ‘latent carriers’ that harbour *pasteurellae* in their tonsils only, and ‘active carriers’ where the organism is also present in the nasopharynx, has been established. It also became evident that, in most earlier studies, the carriers detected had only been those in the ‘active’ state, and not the ‘latent’ ones. Attempts to clear carriers by antibiotic therapy failed, although the organism was sensitive to the antibiotics used in vitro. This phenomenon was explained by Hordagoda and Belak (1990). These workers, using a peroxidase-antiperoxidase technique, established that the *pasteurellae* in the tonsils were lodged in the crypts, and not in the tonsillar tissue, and hence not exposed to parenterally administered antibiotics.

Conclusions

The epidemiological studies on HS in Sri Lanka carried out over the past two to three decades have:

- established the identity of the single serotype involved;
- recognised the presence of endemic and nonendemic areas;
- established that buffalo have higher susceptibility than cattle;
- recognised the seasonal incidence pattern and its possible dependence on climatic and immunological interactions;
- recognised morbidity patterns in endemic and nonendemic areas, and noted a possible relationship to the phenomenon of naturally acquired immunity;
- recognised species-specific and age-specific morbidity patterns;
- recognised the presence of latent and active carriers, and identified the site of persistence in latent carriers;
postulated that the initiation of an outbreak results from a latent carrier becoming active, shedding pasteurellae through nasal secretions, and infecting in-contact susceptible animals. (It is unlikely that a carrier itself will break down into a clinical case as was earlier believed, on account of its immune status.)

A few grey areas remain to be investigated; e.g. the factors that cause a latent carrier to become active are as yet unknown. In addition, to reproduce HS experimentally by infection through the natural routes of infection, large numbers of organisms are required. How an active carrier could transmit such large numbers of organisms is still uncertain. It is not known whether intrinsic or extrinsic factors cause variation in the infective dose under natural conditions. Current and future epidemiological studies on HS in Sri Lanka will be directed towards these areas.

References


The Effect of the *Pasteurella multocida* Toxin in Calves

W. Erler and D. Schimmel

Abstract

Twenty-seven *Pasteurella multocida* strains were isolated from affected lungs of calves. Of six strains belonging to serotype D, three strains produced toxin. This suggested that toxigenic *P. multocida* strains participate in the formation of pneumonic lesions in calves. The intratracheal application of different toxin preparations to calves subsequently showed that it is possible to induce lung lesions with purified toxin. The lesions arelobular to lobularly confluent in distribution and catarrhal in character. The intramuscular application of a crude toxin preparation to five calves resulted in atrophy of the nasal conchae in four animals. On the basis of these results, it would appear that toxigenic *P. multocida* plays an important role in both pneumonia and atrophic rhinitis of calves.

In recent years a number of papers have been published on the importance of the dermonecrotic toxin of *Pasteurella multocida* in the pathogenesis of progressive atrophic rhinitis of pigs. The triggering of this disease by the toxin is now undisputed (Rutter and Mackenzie 1984).

In this paper, we report on isolating toxigenic *P. multocida* (as well as non-toxic *P. multocida* and *P. haemolytica*) from pneumonic lungs of calves; and on the importance of the toxin in producing lung lesions and atrophic rhinitis in calves.

Materials and Methods

Calves aged 3–8 weeks from different herds, with clinical signs of enzootic pneumonia, were transported to the Institute and euthanased. Parts of macroscopically altered lungs were taken for microbiological examination and the isolation of *P. multocida*. The *P. multocida* strains isolated were differentiated serologically by the method of Carter, and toxin production was determined using the guinea pig skin test, tissue culture with embryonic bovine lung cells, and an ELISA with monoclonal antibodies.

A crude toxin was prepared from a toxigenic strain by ultrasonic extraction. From this extract, the toxin was purified by size-exclusion chromatography on Sephadex G 200 and ion-exchange chromatography with DEAE-Sephacel. The purity of the toxin was checked by PAGE. The amount of toxin was determined by an ELISA.

For the animal experiments we used calves of 4–6 weeks of age. The animals were challenged intratracheally with different toxin preparations. One week later they were euthanased and the extent of lung lesions was assessed on the scale: 0 = without a change, to 3 = very marked changes.

In addition, a crude toxin preparation (3 x 50 μg) was administered intramuscularly to five calves.

Results and Conclusion

Results of isolation, typing and toxin production of *P. multocida* strains from affected calf lungs are given in Table 1. From the 27 strains isolated, 1 belonged to type A, 7 to type D or A/D, and 12 strains were not typeable (nt). Fifty percent of the D strains examined produced toxin, but none of the nt strains. These findings demonstrate that toxigenic *P. multocida* strains participate in the formation of pneumonic lesions in calves.

The results of intratracheal application of purified toxin preparations to calves are shown in Table 2. From these, it is evident that it is possible to induce lung lesions with the purified toxin. The lesions are lobular to lobularly confluent, and catarrhal in character.
Table 1. Isolation, serotyping and toxin production of *P. multocida* strains from affected lungs of calves.

<table>
<thead>
<tr>
<th>Serotype</th>
<th>A</th>
<th>D</th>
<th>A/D</th>
<th>nt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strains isolated</td>
<td>27</td>
<td>1</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Toxin examined</td>
<td>12</td>
<td>6</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Toxin produced</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Percent</td>
<td>33.3</td>
<td>50</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

The crude toxin preparation (3 × 50 µg) given intramuscularly to five calves resulted in swelling of the liver and spleen. In addition, four of the calves developed atrophic changes in the nasal conchae.

On the basis of the results published by Frymus et al. (1991) and Chrisp and Foged (1991) on the effects of the toxin of *P. multocida* in rabbits, and our own results (Schimmel and Erler 1991), it can be concluded that toxigenic *P. multocida* plays an important role in both atrophic rhinitis and pneumonia of pigs, calves and rabbits.

Table 2. Results of intratracheal administration of *P. multocida* toxin to calves.

<table>
<thead>
<tr>
<th>Application</th>
<th>Number of animals</th>
<th>Severity of pneumonia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purified toxin (25 µg)</td>
<td>10</td>
<td>0 1 3 6 2.5</td>
</tr>
<tr>
<td>Buffer control</td>
<td>10</td>
<td>7 3 0 0 0.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
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<tr>
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<td>10</td>
<td>7 3 0 0 0.3</td>
</tr>
</tbody>
</table>

References


The Pathology of Experimental Haemorrhagic Septicaemia in Cattle and Buffalo

R.J. Graydon, B.E. Patten and H. Hamid

Abstract

Two buffalo and two cattle that had not been vaccinated against haemorrhagic septicaemia (HS) were challenged with $8 \times 10^7$ colony-forming units of Pasteurella multocida type B:2 (Carter:Heddleston) by subcutaneous injection. In addition, one bovine that had been vaccinated with a commercial oil adjuvant HS vaccine (PusVetma, Surabaya, Indonesia) and eight cattle that had been vaccinated with four other strains of P. multocida isolated from cattle were challenged with the same organism. The non-vaccinated animals died 24–60 hours post challenge, with clinical and pathological changes similar to those described in natural cases of acute HS. The other nine cattle remained clinically normal. They had no significant lesions at necropsy and no organisms were cultured from their organs or blood. It was concluded that each of the strains used as a vaccine was able to protect against a subsequent challenge with the B:2 strain of P. multocida.

The published literature on haemorrhagic septicaemia (HS) contains relatively little on the pathology of the disease, despite its importance in many countries around the world (Losos 1986). HS is regarded as one of the most economically important diseases of cattle and buffalo in Indonesia, yet little material from HS outbreaks is received by the veterinary diagnostic laboratories.

The experiments described here were designed to reproduce HS in un-vaccinated animals and to determine the cross-protection between P. multocida strains that normally affect cattle. The paper also outlines the pathological features of the experimental disease in cattle and buffalo in Indonesia.

Materials and Methods

Two cattle and two buffalo that had not been vaccinated against HS, and one bovine that had been vaccinated with a commercial oil adjuvant HS vaccine containing P. multocida Katha strain (PusVetma, Surabaya, Indonesia), were challenged with $8 \times 10^7$ colony-forming units (CFU) of P. multocida type B:2 (Carter:Heddleston) by subcutaneous injection in the left side of the neck. An identical challenge was given to eight cattle that had been vaccinated by multiple injections of a formalin-killed bacterin of one of four strains of P. multocida — type 14 (Heddleston), type 7:A (Namioka:Carter), type 11:B (Namioka:Carter), or type B:2 (Heddleston:Carter) — previously isolated from cattle.

Following challenge, the animals were observed at regular intervals for the duration of the experiment. At each observation period, venous blood was collected from the jugular vein and the clinical condition and rectal temperature of the animals was recorded. Animals were necropsied at the time of death or when the animal was moribund. The vaccinated cattle were euthanased at the end of the experiment by the intravenous injection of sodium pentobarbitone 325 mg/mL (Lethabarb, Arnolds of Reading, Melbourne, Australia).

Results

Clinical findings

The clinical findings in the non-vaccinated cattle consisted of extensive swelling at the injection site and
a rise in body temperature. The swellings were first observed 4 hours after the injection, and the febrile response was seen after 12-18 hours. A temperature rise was noted in one buffalo, but the other, which died after 24 hours, had neither of these clinical signs. Apart from these specific signs, the clinical syndrome was one of lethargy, recumbency and finally death.

The time to death of the non-vaccinated animals ranged from 60 hours in the cattle to 24 and 31 hours respectively in the buffalo.

**Gross pathology**

The gross pathological changes were similar in all of the non-vaccinated animals examined in this study. However, the lesions were most severe in the buffalo. There was extensive, subcutaneous oedema in the tissues surrounding the injection site. The epidermis and dermis were thickened, and the underlying muscle was pale and oedematous. The oedema extended from the brisket to the neck, and to the sub-mandibular region and larynx in the buffalo that died after 24 hours, but was more localised in the other animals. The left precapsular lymph nodes, which drained the injection sites, were swollen and oedematous, with thickened capsules. In cross section the lymph nodes were diffusely reddened and contained numerous 3-5-mm-diameter pale, necrotic foci. The other lymph nodes examined were of normal appearance.

There were no other gross lesions in the cattle, but both buffalo had congested lungs, especially in the cranial lobes, stable foam in the larger airways, thread-like pleural adhesions, and numerous ecchymotic haemorrhages on the epicardial and endocardial surfaces. In the buffalo that survived for 31 hours, the larynx was diffusely hyperaemic.

**Histopathology**

The microscopic lesions at the injection site consisted of severe oedema and fibrin deposition in the subcutaneous tissues and between and surrounding the muscle fibre bundles. This was accompanied by necrosis and degeneration of muscle fibres and vasculitis and thrombosis of blood and lymphatic vessels. The associated inflammatory cell response was made up of neutrophils and macrophages, with occasional bacterial colonies and micro-abscesses scattered throughout.

The lymph nodes draining the injection site had fibrin and oedema in the perinodal tissues. This was accompanied by thrombosis and vasculitis of capsular blood vessels. The nodes were very oedematous, with fibrin deposition throughout, and the subcapsular and medullary sinuses were packed with neutrophils. There were numerous foci of necrosis scattered throughout both cortex and medulla. There were no histopathological changes in the other lymph nodes examined.

Large numbers of bacteria were a feature of tissue taken from the site of the injection and from the lymph nodes of the cattle, but not the buffalo. However, bacterial emboli in the adrenal glands and spleen were a feature in the buffalo.

The lungs showed relatively mild purulent interstitial pneumonia, congestion and haemorrhage, and a variable amount of oedema. Lymphatics were dilated, and sometimes contained neutrophils.

There were no significant gross or microscopic changes in any of the vaccinated bovines.

**Bacteriology**

*P. multocida* was re-isolated from all non-vaccinated animals from a variety of samples including blood, tonsils, parotid and sub-maxillary lymph nodes, lung, spleen and oedema fluid. *P. multocida* was not isolated from blood or any other tissues from the vaccinated animals.

**Discussion**

The clinical and pathological findings in these animals were similar to those reported in reviews by Losos (1986) and De Alwis (1992). As expected, the buffalo were more severely affected than the cattle and had a shorter survival time. Pyrexia, which occurred after 10-12 hours, was a feature in the cattle and in the longest surviving buffalo, but not in the severely affected buffalo that died after 24 hours. This animal's survival time was the same as that given by Bain (1963), but shorter than the survival times given by other authors (Losos 1986; De Alwis 1992). A surprising finding was the lack of observable swelling at injection sites in the buffalo, even though the oedema was very severe at necropsy. This was in contrast to the cattle, which had a detectable swelling from 4 hours onward. Our findings are the opposite of those reported by Losos (1986), where subcutaneous oedematous swellings were more common in buffalo than cattle.

The lesions observed in the lymph nodes in the animals in this study were more severe than those recorded by other authors, who reported swelling, congestion and hyperaemia, but not frank necrosis (Siew et al. 1970; Bastionello and Jonker 1981; Losos 1986). Experimental cases described by Rhoades et al. (1967) had little or no lymph node enlargement.
In addition, there was more necrosis and haemorrhage at the injection site in our animals than in the experimental infection following subcutaneous injection reported by these authors. In this respect our cases resemble the naturally occurring cases recorded by Bastianello and Jonker (1981), which had similar lesions in the vicinity of the joints and lungs. These differences may represent strain variations or be due to method of infection. The lung lesions found in our cases were mild, but similar to those reported elsewhere. Gross and microscopic changes were not observed in the gastrointestinal tract. All animals in this study developed septicaemia, as determined by the presence of bacteria in a variety of tissues and re-isolation of the challenge organism from several locations.

The failure to produce significant pathological changes or bacterial colonisation in the vaccinated animals suggests that each of the strains of HS used as a vaccine provided protection against subsequent challenge with virulent B:2 organisms.

Finally we would suggest that more effort be placed on attempting to confirm the clinical field diagnosis of HS by bacterial isolation. We believe that the disease is easily confused with other acute fulminating diseases of cattle and buffalo, and that laboratory confirmation of diagnosis will assist in establishing the true incidence of the disease.

Acknowledgments

The authors wish to acknowledge the support of the Director, Research Institute for Veterinary Science, Bogor, Indonesia, for the conduct of this work. The financial assistance of the Australian International Development Assistance Bureau (to Dr Graydon) and the Australian Centre for International Agricultural Research (to Dr Patten) is also gratefully acknowledged.

References


**Pasteurella multocida and Pasteurella haemolytica Infections in Ruminants and Pigs in Southern New South Wales**

I.J. Links,1 J.E. Searson,t J. Godwin,t J.R. Glastonbury,1 A.P. Philbey1 and L.M. Matthews

**Abstract**

*Pasteurella* spp. were isolated from 219 disease incidents in southern New South Wales over a 5-year period. *Pasteurella haemolytica* was recovered on 108 occasions (sheep 65, beef cattle 33, dairy cattle 5 and other animal species 5) and *Pasteurella multocida* also on 108 occasions (pigs 65, sheep 19, beef cattle 12, dairy cattle 4 and other species 8). Other *Pasteurella* spp. (including *P. gallinarum* 4 isolations, and *P. ureae* 1) were recovered on 11 occasions. Eight submissions yielded two strains of *Pasteurella* spp.

Disease incidents were classified on the basis of the pathological findings. *P. haemolytica* and *P. multocida* infections in sheep were associated with pneumonia as the principal pathological condition (26 and 11 submissions respectively), pneumonia as an incidental pathological finding (15 and 2), mastitis (16 and 1), perinatal septicaemia/stillbirth (8 and 2), and abnormal semen (1 and 2). In beef cattle, the two species were associated mainly with pneumonia as the principal diagnosis (24 and 7). In dairy cattle they were associated with pneumonia (2 and 3) and mastitis (2 and 1). In pigs *P. multocida* was associated with pneumonia as the principal pathological condition (31 submissions), incidental pneumonia (6), and atrophic rhinitis (24). Toxigenic strains of *P. multocida* were isolated from 5 submissions (4 type D and 1 type A) and non-toxigenic strains from 7 submissions derived from piggeries with clinical atrophic rhinitis while non-toxigenic strains were isolated from 12 submissions derived from piggeries free of the disease. Pleurisy (12) and pericarditis (6) were commonly seen in association with pneumonia.

The antibiotic sensitivity patterns of the isolates are presented.

**PASTEURELLA infection occurs commonly in many animal species (particularly sheep, pigs and cattle) in Australia. There are a range of *Pasteurella* spp. involved, particularly *P. multocida*, and *P. haemolytica* types A and T. They are associated with infection of the respiratory tract (pneumonia, pleurisy and rhinitis) and the reproductive system (mastitis and metritis). There are, however, few reports of pasteurellosis in Australia in the literature.**

There are reports on *P. multocida* and atrophic rhinitis in pigs (Love et al. 1985, Mercy et al. 1986, Eamens et al. 1988, Gardner et al. 1989); *P. haemolytica* infection in neonatal foals (Peet et al. 1977); *P. multocida* septicaemia in fallow deer (Carrigan et al. 1991); *P. haemolytica* mastitis in ewes (Kabay and Ellis 1989; Philbey 1990); *P. haemolytica* type A mastitis in a goat (Ryan and Greenwood 1990); *P. multocida* (Walker et al. 1979; Reece and Coloe 1985; Ireland et al. 1989) and *P. anatipestifer* (Munday et al. 1970; Grimes and Rosenfeld 1972; Rosenfeld 1973; Reece and Coloe 1985) in birds. A comprehensive review of pasteurellosis has been recently published (Eamens 1990).

This study reports the isolation of pasteurella from pathological material submitted to the Regional Veterinary Laboratory (RVL), Wagga Wagga, New South Wales, Australia. **Materials and Methods**

Over the period July 1987 – June 1992, pathological material from a wide range of animal species submitted to RVL, Wagga Wagga, from the southwestern region of New South Wales was routinely...
cultured for *Pasteurella* spp. on 7% sheep blood agar (Oxoid, Basingstoke, Hants., UK, Blood Agar Base No 2) and incubated at 37°C in air with 10% CO₂. Between January 1988 and April 1989, in the course of a survey on atrophic rhinitis, a number of pig herds were sampled for *P. multocida*, using nasal swabs cultured onto Pedersen’s NB medium and tested for toxigenicity (Eamens et al. 1988). From November 1991 to June 1992, sick or dead animals from selected pens in two large beef feedlots were sampled for *Pasteurella* spp. using nasal swabs or by culturing other tissues. Isolates were confirmed as Pasteurella by standard methods (Cowan 1974). Isolation of *P. multocida* from a number of pigs in a piggery was regarded as one isolation for the purposes of this report.

Tissues for histopathology were fixed in neutral buffered formalin, sectioned, and stained with haematoxylin and eosin by standard methods.

Antibiotic sensitivities were performed by the Oxoid Multodisk diffusion technique according to the method of Simmons and Craven (1980). Antibiotics tested were neomycin 30 μg, compound sulphonamide 300 μg, tetracycline 30 μg, sulphamethoxazole/trimethoprim 25 μg, ampicillin 10 μg, furazolidone 100 μg, lincospectin 150 μg and streptomycin 10 μg.

### Results

*Pasteurella* spp. were recovered from 219 submissions, which yielded a total of 227 isolations. Two distinct strains were isolated from 4 sheep, 2 beef cattle, 1 dairy cattle and 1 pig submission. The distribution of these isolates among the animal species is shown in Table 1.

Isolates included under *P. haemolytica* included 95 identified as type A (57 from sheep, 31 from beef cattle, 3 from dairy cattle, 3 from birds and 1 from a laboratory rabbit) and 8 identified as type T (4 from sheep, 1 from beef cattle, 2 from dairy cattle and 1 from an alpaca). Five isolates were not typed beyond *P. haemolytica*.

### Table 1. Number of isolates of *Pasteurella* spp. recovered from various animal species (227 isolates).

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Animal species*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pasteurella</em></td>
<td>1 2 3 4 5 Total</td>
</tr>
<tr>
<td><em>P. multocida</em></td>
<td>19 12 4 65 8 108</td>
</tr>
<tr>
<td><em>P. haemolytica</em></td>
<td>65 33 5 0 5 108</td>
</tr>
<tr>
<td>Other species</td>
<td>2 0 1 1 7 11</td>
</tr>
<tr>
<td>Total</td>
<td>86 45 10 66 20 227</td>
</tr>
</tbody>
</table>

* 1 sheep, 2 cattle (beef), 3 cattle (dairy), 4 pigs, 5 other species

The other animal species from which *P. multocida* was isolated included poultry and dogs (3 isolates each) and a laboratory rabbit and a cat (1 isolate each).

Three isolates included under other *Pasteurella* spp. were confirmed as *P. gallinarum* (from birds) and *P. ureae* (from mastitis in a goat). Six isolates could not be identified at the species level.

For sheep, beef cattle, dairy cattle and pigs, the clinical disease categories from which the *Pasteurella* spp. were derived are shown in Table 2.

Perinatal mortality in lambs due to pasteurellosis was confirmed on 10 occasions (9 from one property, which was intensively monitored during a research project) yielding *P. haemolytica* type A on 6 occasions, *P. haemolytica* type not determined on 2 occasions and *P. multocida* on 2 occasions.

The principal pathological conditions diagnosed in the various species are shown in Table 3.

### Table 2. Clinical syndrome or other reason reported for the various animal species yielding *Pasteurella* spp. (227 isolations).

<table>
<thead>
<tr>
<th>Species</th>
<th>Clinical syndrome/other reason*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1  2  3  4  5  6  7  Total</td>
</tr>
<tr>
<td>Sheep</td>
<td>31 12 16 10 6 5 6 86</td>
</tr>
<tr>
<td>Beef cattle</td>
<td>13 17 1 1 2 1 10 45</td>
</tr>
<tr>
<td>Dairy cattle</td>
<td>2 5 3 0 0 0 0 10</td>
</tr>
<tr>
<td>Pigs</td>
<td>13 23 1 1 4 18 6 66</td>
</tr>
<tr>
<td>Other species</td>
<td>7 9 1 0 0 1 2 20</td>
</tr>
<tr>
<td>Total</td>
<td>66 66 22 12 12 25 24 227</td>
</tr>
</tbody>
</table>

* 1 death, 2 respiratory signs, 3 mastitis, 4 perinatal mortality, 5 illthrift, 6 monitoring, 7 other
A was involved on 20 occasions, type T on 2 occasions, and *P. multocida* on 11. Sheep with mastitis yielded *P. haemolytica* type A on 16 occasions, while *P. multocida* was recovered on only 1 occasion.

**Table 3.** Principal pathological abnormality observed in the various animal species yielding *Pasteurella* spp. (227 isolations).

<table>
<thead>
<tr>
<th>Species</th>
<th>Pathological condition*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Sheep</td>
<td>39</td>
</tr>
<tr>
<td>Beef cattle</td>
<td>32</td>
</tr>
<tr>
<td>Dairy cattle</td>
<td>6</td>
</tr>
<tr>
<td>Pigs</td>
<td>32</td>
</tr>
<tr>
<td>Other species</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>114</td>
</tr>
</tbody>
</table>

* 1 pneumonia, 2 mastitis, 3 upper-respiratory-tract conditions, 4 septicaemia with hepatitis, 5 gastroenteritis, 6 other pathological conditions

In beef cattle with pneumonia, *P. haemolytica* type A was isolated 23 times, while *P. multocida* was recovered 7 times. Pyrexia in feedlot cattle was associated with both *P. haemolytica* type A (4 isolates) and *P. multocida* (2).

In dairy cattle, pneumonia was associated with *P. multocida* (3 cases) and *P. haemolytica* types A and T (1 case each). Mastitis in dairy cattle was associated with *P. haemolytica* type A (2) and *P. multocida* (1).

*P. multocida* was virtually the only strain isolated from pigs. Toxigenic strains of *P. multocida* were isolated from 5 submissions (4 type D and 1 type A) and non-toxigenic strains from 7 submissions derived from piggeries with clinical atrophic rhinitis, while non-toxigenic strains were isolated from 12 submissions derived from piggeries free of the disease.

Histopathological examination was performed on lungs from 48 ovine cases. *P. haemolytica* type A and type T, and *P. multocida*, were associated with fibrinous pneumonia (13, 0 and 1 case respectively out of a total of 16) and with bronchopneumonia (15, 1 and 5 out of 22 cases). There were 3 cases of verminous pneumonia, 2 cases of suspected mycoplasmal pneumonia, 2 cases of inhalation pneumonia and 1 case of interstitial pneumonia.

Examination of 26 beef cattle lungs revealed *P. haemolytica* type A and *P. multocida* associated respectively with fibrinous pneumonia (15 and 6 out of 21) and bronchopneumonia (2 and 1 out of 4). Only 4 dairy cattle lungs were examined, 2 with fibrinous pneumonia and 2 with bronchopneumonia.

A total of 34 pig lungs were examined. *P. multocida* was associated with 15 cases of bronchopneumonia, 9 cases of enzootic pneumonia, 9 cases of fibrinous pneumonia, and 1 case of interstitial pneumonia.

Stress of shipment is considered a factor in precipitating pneumonia in cattle — so-called 'shipping fever'. There were 7 cases with a history of recent transport: 1 dairy (with fibrinous pneumonia due to *P. multocida*) and 6 beef (2 with bronchopneumonia with either *P. haemolytica* type A or *P. multocida*; and 4 with fibrinous pneumonia, 3 caused by *P. haemolytica* type A and 1 by *P. multocida*).

The results of antibiotic sensitivity testing of *P. haemolytica, P. multocida* and other *Pasteurella* spp. are reported in Table 4.

**Table 4.** The in vitro sensitivity of 154 *Pasteurella* spp. isolates to a range of antibiotics.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Pasteurella haemolytica</th>
<th>Pasteurella multocida</th>
<th>Pasteurella other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>100*</td>
<td>98</td>
<td>80</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>100</td>
<td>97</td>
<td>90</td>
</tr>
<tr>
<td>Sulphmethoxazole/</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>99</td>
<td>88</td>
<td>90</td>
</tr>
<tr>
<td>Lincospectin</td>
<td>98</td>
<td>88</td>
<td>90</td>
</tr>
<tr>
<td>Furazolidone</td>
<td>92</td>
<td>45</td>
<td>90</td>
</tr>
<tr>
<td>Neomycin</td>
<td>71</td>
<td>66</td>
<td>100</td>
</tr>
<tr>
<td>Sulphonamide</td>
<td>68</td>
<td>57</td>
<td>80</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>43</td>
<td>34</td>
<td>80</td>
</tr>
<tr>
<td>No. isolates</td>
<td>79</td>
<td>65</td>
<td>10</td>
</tr>
</tbody>
</table>

* % of isolates sensitive to the antibiotic

**Discussion**

Submissions received at RVIL, Wagga Wagga, average approximately 4000 per year, with 50% from sheep, 34% from cattle, and 5% from pigs. Because of the bias in selection of pathological material submitted to the laboratory (some specimens being derived from research projects and surveys), the results of this study are not a true indication of the prevalence of the various *Pasteurella* spp. or the clinical or pathological conditions associated with infection. Nonetheless, *pasteurellae* were found to be a relatively common cause of disease in sheep, cattle and pigs.

Mortality, respiratory disease and mastitis were by far the most common clinical entities detected, while
pneumonia, mastitis, hepatitis and atrophic rhinitis were the most common pathological abnormalities. This is consistent with the expected pattern of disease associated with this group of bacteria in other countries (Eamens 1990).

*P. multocida* was essentially the only strain isolated from pigs. *P. haemolytica* type A was most common in beef cattle and sheep, with type T being relatively rare, and *P. multocida* represented about 30% of isolates in these two animal species.

Mastitis in sheep is a more common problem than is generally recognised, with the vast majority of cases being attributable to *P. haemolytica* type A. Perinatal mortalities in lambs due to pasteurella septicaemia were also quite common, although most were derived from one property. The incidence of this infection is probably underestimated, because of the infrequency with which newborn lambs are examined and cultured.

With the stress of transport and mixing of animals from a large number of sources considered a major factor in the initiation of respiratory disease, the major expansion in the beef feed-lotting industry in recent years is likely to greatly increase the incidence of pasteurellosis. Although relatively rare in New South Wales to date, 7 cases of pasteurellosis following transport were identified in the current study.

No attempt has been made in this report to analyse the age of the animals involved or the economic impact of pasteurellosis.

**Acknowledgments**

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**References**


