Haemorrhagic septicaemia in cattle and buffaloes

M.C.L. DE ALWIS**

Summary: Haemorrhagic septicaemia (HS) is a highly fatal disease in cattle and buffaloes, caused by specific serotypes of Pasteurella multocida. It is a primary pasteurellosis reproducible in susceptible animals with the specific organism alone. The accepted classification of Pasteurella multocida is based on the identification of its somatic and capsular antigens. On this basis two serotypes designated 6:B and 6:E have been found to cause HS.

The disease is prevalent in most countries in Asia, the Near and Middle East, Southern Europe and in the North, Central and East Africa. In Asia, the disease is caused by serotype 6:B and in Africa 6:E, while a few countries have recorded both. In most Asian countries, it is recognised as a disease of utmost economic importance.

In a few countries where epizootiological studies have been done, it has been found that definite patterns of incidence have been established, presumably through the interaction of climatological, immunological and epizootiological factors. The disease is more common in buffaloes than in cattle, and in enzootic areas greater losses occur among young animals. In such areas, a variable proportion of adult animals have acquired natural immunity to the disease. Among these, some are carrier animals, and such animals are believed to be the focal point of fresh outbreaks.

All countries where HS is enzootic adopt vaccination. Broth bacterins are most popular, but immunity is of short duration. The oil adjuvant vaccine gives the longest immunity but only a few countries use it as it is thick and difficult to inject. Recent research on vaccines has been directed towards producing thinner emulsions, isolating antigenic fractions and the production of avirulent mutants for use as live vaccines. It is believed that a more effective control of the disease can be achieved with a better understanding of the epizootiology, the production of better vaccines, and by attaining a higher vaccination coverage.

INTRODUCTION

With the advent of methods of serotyping Pasteurella multocida, the recognition of haemorrhagic septicaemia (HS) as a distinct disease entity has
become increasingly clear. It is an acute, septicaemic disease caused by the specific serotypes 6:B or 6:E (Namioka-Carter), in buffaloes and cattle. Unlike most other pasteurelloses, HS is a primary pasteurellosis, reproducible in susceptible animals with pure cultures of the specific organism alone.

**OCCURRENCE AND DISTRIBUTION**

Haemorrhagic septicaemia occurs in Southern and Southeast Asia including Indonesia, Philippines, Thailand and Malaysia, in the Near and Middle East, Southern Europe (including the U.S.S.R.) and in North, Central and East Africa. It has also been reported in the Republic of South Africa (15). In the U.S.A., the disease was reported among bison in National Parks in the years 1912, 1922 and 1967. Since then, apart from one explosive outbreak among young dairy cattle in New Jersey in 1969, the disease has not been reported anywhere in the U.S.A. (15). The disease does not occur in Australia, Oceania, Japan, Canada and Western Europe. There is reference to its occurrence in South America in the F.A.O.-W.H.O.-O.I.E. Animal Health Yearbook, but no supporting literature on serotype identification is available for confirmation. Table I summarizes the distribution of the disease.

Distribution of the disease bears some relationship to the type of animal reared and the system of management. This is amply borne out in the 65,000 km² island of Sri Lanka, having a variety of agroclimatic zones and cattle husbandry practices, where distinct enzootic and disease-free areas exist. In the hill country where the climate is mild, and temperate breeds of cattle and their crosses are reared under intensive systems, HS is rare. In the dry plains, on the other hand, where indigenous cattle and buffaloes roam freely, the disease is enzootic.

**Seasonal incidence.**

The disease is normally associated with wet, humid weather, and in most countries incidence increases early in the wet season. Exhaustive epidemiological studies in Sri Lanka have shown that the disease occurs at all times of the year but, whereas dry season outbreaks are contained, outbreaks occurring during the wet season tend to spread. This is presumably due to longer survival of the organism under moist conditions, and the movement of animals associated with the rains.

**Distribution of serotypes.**

In Asian countries, the only serotype recorded so far is serotype 6:B. There is one report of a single isolation of a type D strain from what was believed to be a case of HS in Malaysia (21). While type D in cattle is normally associated with a pneumonic syndrome, under certain circumstances it may produce a septicaemia, and the condition could be mistaken for classical HS.
## Table I

Occurrence of haemorrhagic septicaemia in different countries*

### Category A. Countries where the disease is reported to occur, on clinical or bacteriological grounds

<table>
<thead>
<tr>
<th>Country</th>
<th>Country</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Afghanistan</td>
<td>Gambia</td>
<td>Nicaragua</td>
</tr>
<tr>
<td>Angola</td>
<td>Ghana</td>
<td>Niger</td>
</tr>
<tr>
<td>Argentina</td>
<td>Guatemala</td>
<td>Nigeria</td>
</tr>
<tr>
<td>Bahrain</td>
<td>Guinea</td>
<td>Pakistan</td>
</tr>
<tr>
<td>Bangladesh</td>
<td>Haiti</td>
<td>Panama</td>
</tr>
<tr>
<td>Belize</td>
<td>Honduras</td>
<td>Paraguay</td>
</tr>
<tr>
<td>Bhutan</td>
<td>India</td>
<td>Philippines</td>
</tr>
<tr>
<td>Bolivia</td>
<td>Indonesia</td>
<td>Portugal</td>
</tr>
<tr>
<td>Brunei</td>
<td>Iraq</td>
<td>Saudi Arabia</td>
</tr>
<tr>
<td>Burma</td>
<td>Italy</td>
<td>Sierra Leone</td>
</tr>
<tr>
<td>Cameroon</td>
<td>Ivory Coast</td>
<td>Somalia</td>
</tr>
<tr>
<td>Central African Republic</td>
<td>Kampuchea</td>
<td>South Africa</td>
</tr>
<tr>
<td>Chad</td>
<td>Kenya</td>
<td>Sri Lanka</td>
</tr>
<tr>
<td>Colombia</td>
<td>Laos</td>
<td>Sudan</td>
</tr>
<tr>
<td>Comoros</td>
<td>Lebanon</td>
<td>Tanzania</td>
</tr>
<tr>
<td>Costa Rica</td>
<td>Liberia</td>
<td>Thailand</td>
</tr>
<tr>
<td>Djibouti</td>
<td>Libya</td>
<td>Uganda</td>
</tr>
<tr>
<td>Dominican Republic</td>
<td>Mali</td>
<td>Upper Volta</td>
</tr>
<tr>
<td>East Timor</td>
<td>Malawi</td>
<td>Venezuela</td>
</tr>
<tr>
<td>Egypt</td>
<td>Malaysia</td>
<td>Vietnam</td>
</tr>
<tr>
<td>El Salvador</td>
<td>Mauritania</td>
<td>Zaire</td>
</tr>
<tr>
<td>Ecuador</td>
<td>Mexico</td>
<td>Zambia</td>
</tr>
<tr>
<td>Equatorial Guinea</td>
<td>Namibia</td>
<td></td>
</tr>
<tr>
<td>Ethiopia</td>
<td>Nepal</td>
<td></td>
</tr>
</tbody>
</table>

### Category B. Countries where HS is reported as an exceptional occurrence, or is suspected to occur but not confirmed**

<table>
<thead>
<tr>
<th>Country</th>
<th>Country</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brazil</td>
<td>Jordan</td>
<td>Spain</td>
</tr>
<tr>
<td>Denmark</td>
<td>Mozambique</td>
<td>Syria</td>
</tr>
<tr>
<td>Fed. Rep. of Germany</td>
<td>Poland</td>
<td>Togo</td>
</tr>
<tr>
<td>Finland</td>
<td>Qatar</td>
<td>Turkey</td>
</tr>
<tr>
<td>Greece</td>
<td>Romania</td>
<td>U.S.A.</td>
</tr>
<tr>
<td>Guinea-Bissau</td>
<td>Rwanda</td>
<td>U.S.S.R.</td>
</tr>
<tr>
<td>Iran</td>
<td>Samoa</td>
<td></td>
</tr>
</tbody>
</table>


** It is possible that some countries in this group may be reporting other forms of bovine pasteurellosis, and not specifically haemorrhagic septicaemia.

The serotype prevalent in Africa has been identified as 6:E. A few countries, notably Egypt and Sudan, have recorded both. The North American isolate from bison, which still exists in type culture collections, belongs to 6:B.
ECONOMIC LOSSES

With the eradication of rinderpest from most Asian countries, HS has emerged as a disease of considerable economic importance, and most countries rank HS as the major fatal disease of the water buffalo. Its economic importance is particularly felt in the Asian region, where the water buffalo, which is important both for food and draught, is particularly susceptible.

Haemorrhagic septicaemia occurs mostly in areas where animal husbandry practices are primitive, and where Veterinary Services (and consequently disease reporting systems) are poorly developed. Reported losses due to HS probably reflect no more than the general trend, the actual losses being considerably higher. This fact became evident in an epidemiological survey in Sri Lanka, and is probably true of other countries where HS is enzootic.

SPECIES AFFECTED

Under natural conditions, the disease occurs principally in buffaloes and cattle. A few reports are available of its occurrence in other species. Several sporadic outbreaks of acute septicaemic pasteurellosis in pigs associated with the HS serotype 6:B have been reported in Sri Lanka, and one of these was associated with the feeding of pigs with bovine blood from an abattoir. This isolate reproduced typical HS in cattle (De Alwis, unpublished report). Similar reports of sporadic incidence of septicaemic pasteurellosis in pigs caused by organisms serotyped as 6:B have originated from Thailand (1) and Malaysia (2). Similar reports have come from India, where the organism has been identified as Roberts’ type I or Carter’s type B (34, 46).

Sporadic outbreaks of disease associated with *P. multocida* have also been reported in goats (1, 2, 3, 4, 34) and in poultry (38, 39). The organisms were identified as Roberts’ type I, Carter’s type B or serotype 6:B in different instances.

Septicaemic pasteurellosis caused by serotype 6:B has been recorded in elephants in Sri Lanka, in conjunction with outbreaks of HS in cattle and buffaloes (31, 24, 62).

Thus, sporadic outbreaks of septicaemic disease associated with *P. multocida* and typed as Roberts’ type I or Carter’s type B (either of which could be 6:B or 11:B) or serotype 6:B itself, depending on the serotyping system used, have been recorded in species other than cattle or buffaloes. Such disease has been referred to as « septicaemic pasteurellosis », the term haemorrhagic septicaemia being usually reserved for the disease in buffaloes and cattle.

CLINICAL SYNDROME AND PATHOLOGICAL LESIONS

Haemorrhagic septicaemia occurs mostly in regions where husbandry practices are primitive, and where animals are reared under semi-wild condi-
tions. Under such conditions, animals are not under constant observation, and the only reported sign may be sudden death. Illness usually lasts 1-3 days. In order of occurrence, the visible signs are elevated temperature, submandibular oedema spreading to the brisket region, followed by progressive respiratory distress. The animal then becomes prostrate and dies.

The extent of the gross pathological lesions depends on the course of the disease. Infection experiments (28) have shown that animals which died within 24-36 hours of infection had only widespread petechial haemorrhages, most pronounced at the base of the heart, abomasal wall and to a lesser extent on the intestines. There was also generalized congestion of the lungs. When the course of the disease extended to 48 hours, the haemorrhages were more severe and ecchymotic, with fibrinous pericarditis. When the illness lasted for over 72 hours, there was extensive consolidation of the lungs, with lobulation due to marked thickening of the interlobular septa. There was serofibrinous pericarditis and pleurisy, with adhesions between the pericardium and pleura.

In general, buffaloes die quicker and consequently show fewer lesions. Cattle may linger on for a few days, resulting in more pronounced pathological lesions. While most outbreaks of HS are typical, a few noteworthy instances of atypical syndromes caused by serotype 6:B have been recorded. In Sri Lanka, a pneumonic pasteurellosis syndrome caused by serotype 6:B has been reported (28). A syndrome associated with oedema and lameness of the forelimbs has been reported in Burma. Lameness in young, otherwise healthy buffaloes in enzootic areas during the HS season has been frequently reported in Sri Lanka. These reports have yet to be investigated. They may be animals which have responded to infection with subclinical or «arrested» infections, and have become immune (23).

**EPIDEMIOLOGY**

1. Morbidity and mortality pattern.

   In areas where HS occurs, early detection and treatment is not feasible, and consequently the case fatality rate is near 100%. Thus, morbidity and mortality are nearly the same.

2. Factors influencing morbidity and mortality.

   (a) *Species.*

   While most countries, particularly in Asia, recognize that losses due to HS are higher in buffaloes than in cattle, few countries have produced evidence to support this. In Malaysia, it is reported that 73% of all losses due to HS occur in buffaloes, despite the fact that their buffalo population is only half that of cattle (2). One survey in Sri Lanka indicated that the herd infection rate was much the same in cattle and buffaloes, but the morbidity rate
within affected herds was considerably higher in buffaloes. Another survey put buffalo mortality at three times that of cattle (32, 22). A notable exception is the Bali breed of cattle in Indonesia, which is reported to be highly susceptible.

(b) Age.

It is also agreed in most countries that greater losses occur in young animals than in adults, but quantitative estimates are scarce. Surveys in Sri Lanka have shown that 65% of all losses due to HS in buffaloes and 77% in cattle involved animals under 2 years old (32). Another study in that country indicated that the most vulnerable age was between 6 months and 2 years (29). Further studies have shown that this correlation between age and mortality applies to enzootic areas only.

In areas where regular seasonal outbreaks occur, mortality in individual outbreaks is low and is confined to young animals. When occasional sporadic outbreaks occur outside the enzootic areas, mortality is high and animals of all ages die (22). This relationship has an immunological basis, since in enzootic areas, where the frequency of HS is high, a large percentage of animals (particularly adults) possess naturally acquired immunity (32, 30).

(c) Enzootic and non-enzootic areas.

Morbidity and mortality due to HS have been shown to vary between enzootic and other areas in Sri Lanka. Losses in enzootic areas occur regularly, mostly during a certain season, and occur in small numbers which sometimes do not attract attention. When, on the other hand, occasional outbreaks occur in non-enzootic areas, they can reach alarming proportions.

Massive epizootics may occur when the disease is first introduced into a country, or has escaped from an enzootic pocket to other areas. Remarkably similar epidemiological patterns have been observed in Sri Lanka and in Zambia. In the former country, a few cases of HS have been reported annually in certain pockets since 1915. The disease broke out in the dry plains for the first time in 1955, and took a very heavy toll of cattle and buffaloes of all ages. Since then, a plateau has been reached in the pattern of incidence. In these areas, the disease has become enzootic and small numbers of young animals continue to die of HS annually. In Zambia, the disease had been confined to certain enzootic pockets since the 1930’s, but it broke out as a violent epizootic in 1978. Thereafter, the trend was similar to that in Sri Lanka.

(d) System of management.

The incidence of HS appears to be related to the system of management, since it is lowest in small stall-fed herds and highest in large, nomadic herds. Both situations have been demonstrated in the same country (32). Further, within an enzootic area the incidence of HS may be 4-5 times greater in large herds of over 50 animals than in small herds of under 10 animals, and this is clearly related to differences in management practices.
3. Naturally acquired immunity.

Bain (9) found that some buffaloes examined in Thailand were naturally immune to HS. The importance of this finding in the epidemiology of HS was not realised at the time, and it was concluded that about 10% of cattle and buffaloes in Asia may be naturally immune to HS. Investigations made in Sri Lanka in the late 1970's (30, 23) indicated that the proportion of naturally immune animals varied from herd to herd, and from time to time in the same herd. This phenomenon was related to recent occurrence of HS in the herd or locality. While a few instances of cattle naturally immune to HS have been found in Australia (10), U.S.A. (26) and in Chad (Perreau, cited in 11) where natural exposure was not evident, sufficient evidence has been adduced by De Alwis (23) in a study of the immune status of buffaloes exposed to HS, that subclinical or « arrested infection » is the chief source of naturally acquired immunity in enzootic countries. In situations where no vaccination is practised, it is clear that the morbidity and the mortality patterns are largely governed by the proportion of naturally immune to non-immune animals at the time of the outbreak. Thus, in enzootic areas where frequent outbreaks occur, the proportion of naturally immune animals is high, and consequently only a small number of hitherto unexposed susceptible animals die. When the disease occurs after a long interval, the proportion of non-immune susceptible animals is high, and explosive outbreaks occur.

4. Carrier animals.

It is well known that a certain proportion of healthy cattle and buffaloes harbour pathogenic pasteurellae in their nasopharynx (58, 63). In the early 1960's, Gupta (37) in India found a correlation between incidence of HS and the carrier rate. During an epizootic of HS in a village, 7.5% of healthy animals were carriers, but none was detected in the same village 40 days later. The proportion of carriers varied with the incidence of HS, ranging from none in disease-free areas to 6.1% in high incidence areas. These findings were corroborated by Mustafa et al. (47) in Sudan and by Hiramune and De Alwis (43) in Sri Lanka. The latter workers also found that the carrier rate was highest among clinically unaffected animals immediately after an epizootic, and it declined rapidly in 6-9 weeks. The proportion of carriers detected by several workers is shown in Table II. Naturally immune animals may be carriers, or animals which have passed through a carrier stage, both phenomena being related to recent incidence (see Table III).

It has been stated that regular vaccination will eliminate the carrier status. An eradication programme with intensive annual vaccination was attempted on an Indonesian island. After the first year of vaccination there were still 2.3% of carriers, but none was detected after the second year. More recent work in Sri Lanka (Wijewardana and De Alwis, unpublished report) has indicated that the nasopharyngeal carrier state is transient, and that different animals are carriers at different times in the first few weeks following an outbreak. The same workers have also detected animals harbouring virulent pas-
### Table II

**Detection of haemorrhagic septicaemia carriers by different workers**

<table>
<thead>
<tr>
<th>Author</th>
<th>Country</th>
<th>Findings</th>
<th>Method of identification of organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Singh (1948)</td>
<td>India</td>
<td>3.5% in living animals, 7.0% in slaughtered animals.</td>
<td>Agglutination with serum prepared against virulent strain.</td>
</tr>
<tr>
<td>Wijewanatha and Karunaratne (1968)</td>
<td>Sri Lanka</td>
<td>15% carriers. 43/45 isolates were 6:B. Two were 11:B.</td>
<td>Capsular and somatic typing.</td>
</tr>
<tr>
<td>Gupta (1962)</td>
<td>India</td>
<td>7.5% in healthy living animals during outbreak. 0% in same animals, 40 days later.</td>
<td></td>
</tr>
<tr>
<td>Mustafa et al. (1968)</td>
<td>Sudan</td>
<td>0%, 3.84% and 5.55% in three herds unassociated with HS. 44.4% among healthy animals in an HS-affected herd.</td>
<td>Capsular typing.</td>
</tr>
<tr>
<td>Hiramune and De Alwis (1982)</td>
<td>Sri Lanka</td>
<td>22% in herds one week after outbreak diminishing to 1.9% 6 weeks after.</td>
<td>Somatic and capsular typing.</td>
</tr>
</tbody>
</table>

### Table III

**Carrier status and naturally acquired immunity in relation to incidence of haemorrhagic septicaemia**

<table>
<thead>
<tr>
<th>Carrier animals in India* (%)</th>
<th>Naturally immune animals in Sri Lanka** (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low incidence areas</td>
<td>0</td>
</tr>
<tr>
<td>Moderate incidence areas</td>
<td>1.9</td>
</tr>
<tr>
<td>High incidence areas</td>
<td>5.0-6.0</td>
</tr>
</tbody>
</table>

** De Alwis and Sumanadasa (1982).

teurellae in their retropharyngeal lymph nodes, without their being present in the nasopharynx. While it is clear that the nasopharyngeal carrier status is only transient, it is not known how long the organism persists in the retropharyngeal lymph nodes, and whether such persistence could occur in any
animal, or only in certain immunodeficient animals. The detection of animals which are nasopharyngeal negative but lymph node positive has totally changed the concept of a carrier. It seems reasonable to designate the nasopharyngeal positives as «active carriers» or «shedders» and the nasopharyngeal negative, lymph node positives as «latent carriers». Whether a latent carrier can change to an active carrier, and the circumstances under which such change could occur has yet to be determined.

5. The epidemiological cycle.

It may be a common occurrence that when an outbreak occurs spontaneously within a closed herd, a latent carrier becomes active, and the animal begins to shed virulent pasteurellae. Alternatively, an active carrier may be introduced into a herd. The outbreak would commence only when the shed organism is picked up by a susceptible animal in sufficient numbers. The magnitude of the outbreak will depend on the proportion of susceptible animals in the herd or village. Figure 1 shows such a cycle.

**FIG. 1**
The epidemiological cycle in haemorrhagic septicaemia

A clinical field diagnosis is based on the history, signs and lesions. The history includes the mortality pattern in relation to the surrounding circumstances, such as the previous occurrence of HS in the herd, whether it is an enzootic area, the age group and species affected, and vaccination history. Mortality rates can vary from 5% in a herd of cattle in an enzootic area with regular annual epizootics, to near 100% among buffaloes in a disease-free area experiencing a sporadic outbreak after 10 years. The signs and lesions observed should also be viewed from the aspect of the surrounding circumstances.

2. Laboratory diagnosis.

Biochemically, strains of *P. multocida* causing HS are no different from other members of the group, and they can be differentiated only by serology. The earliest serological tests to gain some acceptance were the passive mouse protection test, the plate agglutination test of Little and Lyon (45), and the mouse protection test of Roberts (56). Roberts defined 4 different serological types, designated types I-IV. All HS strains belonged to type I. Carter (13, 14) devised an indirect haemagglutination test and identified 4 different types, designated A, B, D and E, based on the capsular antigens. The limitations of both techniques became evident when Namioka and Murata (50) described 11 « somatic » types. There were 2 somatic types within Roberts’ type I or Carter’s type B, one of which caused HS (type 6) and the other did not (type 11). Namioka and Murata (49) also described a simplified capsular typing technique. Heddleston, Gallagher and Rebers (42) described an agar gel precipitation test (AGPT) using a heat-stable antigen and identified 16 different types of *P. multocida*. These types are sometimes also referred to as « somatic » types, presumably because the HS serotypes belonging to Heddleston’s type 2 corresponded closely to Namioka’s somatic type 6.

Table IV shows the different serological techniques that have been developed and the place of HS serotypes within each system. In each typing system, a small proportion of strains, biochemically designated pasteurellae and associated with disease, remain untypable. Brogden and Packer (12) compared *P. multocida* serotyping systems and concluded that serotypes determined by one system did not correlate with those determined by another system. This is to be expected, as the type of antigen preparation used and the techniques adopted differ in each case.

(a) A practical serotyping system.

For the purpose of designating cultures, a standard, practical serotyping system has to be adopted in all countries. The F.A.O./A.P.H.C.A. Workshop on haemorrhagic septicaemia held in Colombo in 1979 recommended the use of a « somatic-capsular » (Namioka-Carter) combination. The capsular
TABLE IV

<table>
<thead>
<tr>
<th>Authors</th>
<th>Technique</th>
<th>No. of types</th>
<th>Position of HS types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Little and Lyon (1943)</td>
<td>Slide agglutination and passive mouse protection test.</td>
<td>1, 2 and 3</td>
<td>2</td>
</tr>
<tr>
<td>Roberts (1947)</td>
<td>Passive mouse protection test.</td>
<td>I-V</td>
<td>I</td>
</tr>
<tr>
<td>Carter (1955)</td>
<td>Capsular typing by indirect haemagglutination test using heat-labile (56°C for 30 min) antigen.</td>
<td>A, B, D and E</td>
<td>B and E</td>
</tr>
<tr>
<td>Namioka and Murata (1961)</td>
<td>Simplified capsular typing by slide agglutination test using fresh cultures.</td>
<td>do.</td>
<td>B and E</td>
</tr>
<tr>
<td>Namioka and Murata (1961)</td>
<td>« Somatic » typing by agglutination test using HCl-treated cells.</td>
<td>I-11</td>
<td>6</td>
</tr>
<tr>
<td>Heddleston et al. (1972)</td>
<td>Gel-diffusion test using heat-stable (100°C for 1 hour-supernate) antigen.</td>
<td>1-16</td>
<td>2 and 5</td>
</tr>
</tbody>
</table>

typing procedure is relatively simple, but somatic typing necessitates the production of type-specific antisera, involving a complicated absorption system (48) shown in Table V. Capsular typing is, therefore, practised in most countries and relatively few resort to somatic typing. At the Veterinary Research Institute in Sri Lanka, somatic typing is done with unabsorbed rabbit antisera produced against whole cells, using HCl-treated extracts of the test strain (6:B — Asian HS serotype) in tube agglutination tests with different dilutions of serum: the African HS strain (6:E) and an Australian non-HS strain (11:B) are used as controls. Such a simplified procedure is possible with capsular type B, which has only two somatic types (6 and 11) and with type E which has only one somatic type 6, both of which are HS serotypes.

Carter and Chengappa (19) suggested a combination of the Heddleston and Carter systems. Heddleston’s typing (by AGPT), sometimes referred to as somatic typing, employs a heat-stable (100°C for 1 hour) supernate antigen instead of the HCl-treated residual cells of the Namioka system.
TABLE V
Strains and antisera used for preparing factor sera for somatic typing*

<table>
<thead>
<tr>
<th>«O» group</th>
<th>Antisera (ml)</th>
<th>Strain used for absorption</th>
<th>No. of YPC plates</th>
<th>Dilution with saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>3397 (0.3); Kobe 5 (0.5); M 4 (0.2)</td>
<td>P 8</td>
<td>15</td>
<td>1:10</td>
</tr>
<tr>
<td>2.</td>
<td>P 27 (0.6); Kobe 6 (0.2)</td>
<td>Kobe 5</td>
<td>15</td>
<td>1:10</td>
</tr>
<tr>
<td>3.</td>
<td>P 8 (1.0)</td>
<td>M 17</td>
<td>15</td>
<td>1:10</td>
</tr>
<tr>
<td>4.</td>
<td>M 17 (0.6); M 11 (0.4)</td>
<td>M 4</td>
<td>15</td>
<td>1:10</td>
</tr>
<tr>
<td>5.</td>
<td>TS 8 (0.5); VA 3 (0.5)</td>
<td>Kobe 6</td>
<td>10</td>
<td>1:10</td>
</tr>
<tr>
<td>6.</td>
<td>R 479 (1.0)</td>
<td>Kobe 6</td>
<td>10</td>
<td>1:10</td>
</tr>
<tr>
<td>7.</td>
<td>PM (1.0)</td>
<td>TS 8</td>
<td>15</td>
<td>1:10</td>
</tr>
<tr>
<td>8.</td>
<td>147 (1.0)</td>
<td>Kobe 5</td>
<td>10</td>
<td>1:10</td>
</tr>
<tr>
<td>9.</td>
<td>Liver</td>
<td>P 8</td>
<td>10</td>
<td>1:10</td>
</tr>
<tr>
<td>10.</td>
<td>TS 9 (1.0)</td>
<td>P 27</td>
<td>10</td>
<td>1:10</td>
</tr>
<tr>
<td>11.</td>
<td>989 (1.0)</td>
<td>R 479</td>
<td>10</td>
<td>1:10</td>
</tr>
</tbody>
</table>

* From Namioka, 1978.

(b) Rapid laboratory diagnostic tests.

Material reaching a laboratory is often contaminated, and direct culture usually fails to isolate Pasteurella. Mouse inoculation may serve as a « biological screen » to purify the culture, while simultaneously ascertaining its mouse virulence, which is of diagnostic significance. The purified culture may be subjected to simplified capsular typing by the slide agglutination test, using the fresh cultures and hyperimmune rabbit sera. The above procedures enable a conclusive diagnosis to be made within 48 hours of receiving the material. Rapid double diffusion tests in agar gel using rabbit antisera and the Carter antigen (supernatant fluid heated at 56°C for 30 min), and radial immunodiffusion tests using hyperimmune calf antisera have also been described (5, 64). The former essentially identifies what is described as the « capsular » antigen and has, in fact, been used to distinguish encapsulated from unencapsulated strains (26).

More rapid tests for identifying HS serotypes of *P. multocida* have been developed recently. The production of hyaluronidase has hitherto been considered a property confined to certain Gram-positive bacteria. Carter and Chengappa (17) examined 74 isolates of *P. multocida* of different serotypes, and found that serotype 6:B consistently produced hyaluronidase. All isolates of the African HS serotype 6:E and the Australian non-HS serotype 11:B were negative. These workers used the streak method as well as the rapid plate method, in which test cultures were spotted onto a medium containing sodium hyaluronidate and bovine albumin fraction V. The undegraded substrate was tested by flooding the plate with acetic acid. A wider range of
cultures need to be tested for this property which, if verified, will become a useful rapid diagnostic test for this serotype.

Carter and Chengappa (18) described the use of counter-immunoelectrophoresis for rapid identification of types B and E. The IHA antigen (56°C for 30 min) and rabbit antisera were used. Distinct bands of precipitation were produced by types B and E with homologous sera within 30 minutes, with no cross reactions.

TREATMENT

Treatment is rarely attempted, as the success rate is very poor if treatment is carried out once visible signs have appeared. Thus under the conditions prevailing in HS-enzootic areas, case fatality is almost 100%.

Treatment is effective only in the very early stages. The only practical procedure is to check regularly (twice daily) the rectal temperature of all in-contact animals after the first case has been reported, and to commence antibiotic therapy immediately. The old practice of using sulphonamides still continues in many countries. International nonproprietary name: sulfadimidine sodium 33% solution administered intravenously at a dosage of 1 ml per 5 pounds body weight is effective, but this is certainly not the best drug. The practical difficulties of intravenous therapy and the consequences of leakage into subcutaneous tissues weigh against this treatment. The author has found intramuscular administration of streptomycin or oxytetracycline convenient and effective. There are few reports of antibiotic resistance in HS strains of *P. multocida*. Strains from Malaysia, Indonesia, Thailand, Burma, India and Sri Lanka, when tested in Sri Lanka, were sensitive to all of 10 common antibiotics tested, except that the Thai strain showed partial resistance to streptomycin. The antibiotics tested were penicillin, ampicillin, streptomycin, tetracycline, chloramphenicol, erythromycin, neomycin, sulphadiazine and a sulphonamide-trimethoprim combination. Treatment with hyperimmune serum is hardly attempted. Experimentally, 60-100 ml of hyperimmune serum administered to 2-year-old (400 lb weight) buffaloes at varying periods ranging from 6 hours before to 18 hours after infection had no therapeutic effect (44).

CONTROL

In all countries where HS occurs, vaccination is accepted as the method of control. Most vaccines for this purpose are produced in the countries in which they are used.

Most countries use broth bacterin or alum-precipitated vaccine (APV), while a few use oil adjuvant vaccine (OAV). Thailand alone uses an aluminium hydroxide gel vaccine. Most of the vaccines used are not standardized
products. Vaccines produced in different countries differ in the strain of organism used, the method of cultivation and the bacterial content per dose (a critical factor in immunization).

1. Selection of vaccine strain.

India uses a selected vaccine strain « P 52 » which is believed to possess special immunogenic properties. Indonesia uses the Burmese « Katha » strain for the same reason. Most other countries use indigenous strains. Malaysia uses 5 strains from 5 different regions of the country.

Bain (10) carried out active immunity tests in mice using Pasteurella multocida propagated in bovine cells, and with laboratory sub-cultures of same. He found that cross-protection existed between Asian HS strains (6:B), African HS strains (6:E) and the non-HS Australian strain « 989 » (11:B), when cell-cultured bacteria were used, but the cross-protection declined and only homologous protection remained when sub-cultures were used. De Alwis (unpublished data) comparing the immunogenicity of strains from Malaysia, Indonesia, Thailand, Burma, India and Sri Lanka found variations between and within strains in the dry weight yield of bacteria when grown under the same conditions. When active cross-protection tests were done using doses standardized on the basis of dry weight, no consistent immunogenic differences were demonstrable between strains in active cross-protection tests in mice.

2. Preservation of seed culture.

It is widely accepted that freshly isolated cultures are the best immunizing agents. Vaccine seed cultures are, therefore, periodically passaged in susceptible cattle or buffaloes, which are bled during the septicaemic phase, and the blood is stored in the frozen state. Tryptose agar with 0.3% yeast extract and 5% ox or buffalo blood is used as a medium for sub-culture from infected, frozen blood. Sub-culturing can continue as long as large (2 mm) encapsulated colonies are formed within 24 hours at 37°C. Spontaneous dissociation may occur at any stage in the process of sub-culture, resulting in the formation of minute, pinpoint colonies, and such cultures should be discarded.

3. Culture of bacteria for vaccine production.

(a) Method.

Various culture methods have been adopted in different countries, ranging from simple static broth cultures and aerated dense cultures to the use of complex fermentors. Ordinary broth cultures may be used for the production of simple bacterins, or for the alum-precipitated vaccine (APV). Dense cultures, appropriately diluted if necessary, are suitable for producing APV and particularly the oil adjuvant vaccine (OAV).

The most popular method of producing dense cultures is to use a vortex tank, but this has the disadvantage of frequent contamination (6). India and Iraq have reported the use of an agar wash suspension (3, 7). This is a labo-
rious process and consequently only 2.8% of India's total production is reported to be of the oil adjuvant type. Sri Lanka prefers to use 10 or 20 litre Pyrex bottles, or a simple culture vessel called the lift fermentor (Bellco Glass Inc., U.S.A.), with sparger aeration. The latter vessel, fitted with two side arms and an improvised siphon arrangement, has been used for continuous culture too.

**TABLE VI**

*Some growth media used in haemorrhagic septicaemia vaccine production*

<table>
<thead>
<tr>
<th>Medium recommended in the F.A.O. haemorrhagic septicaemia monograph (Bain, De Alwis, Carter and Gupta, 1982)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxoid tryptone or Difco casitone 120 g</td>
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</table>

<table>
<thead>
<tr>
<th>Medium used in Malaysia (Thomas, 1968)</th>
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<tbody>
<tr>
<td>Peptone (Oxoid) 150 g</td>
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</table>

<table>
<thead>
<tr>
<th>Medium used in Egypt (Geneidy et al., 1967)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casamino acids 500 g</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Medium used in Sri Lanka (Arawwawela et al., 1981)</th>
</tr>
</thead>
<tbody>
<tr>
<td>* Casein hydrolysate (BDH) 0.2 %</td>
</tr>
<tr>
<td>Peptic digest of blood may be added up to 0.5%</td>
</tr>
<tr>
<td>* Prepared as a concentrate, filter sterilized and added</td>
</tr>
</tbody>
</table>
(b) Culture media.

Basal media with various forms of enrichment are being used, and some of them are listed in Table VI.

(c) Yields of bacteria.

Yield is important because it determines the potency of the vaccine and the economy of vaccine production. It is widely accepted that about 2 mg of dry bacteria is required to immunize a bovine and must, therefore, be the minimum bacterial content per dose.

Few countries express vaccine potency in terms of dry bacterial content. The Malaysian broth bacterin is reported to contain an average yield of 0.297 mg/ml and the OAV an average of 3.88 mg/dose (2). In Sri Lanka, dry weight yields of 1.5-2 mg/ml dense culture are obtained within 12-16 hours.

Other parameters used to express the density of culture are turbidity and the total and viable counts. As the amount of capsular substance can vary between strains, turbidity appears to be more meaningful than cell counts.

(d) Bacterin.

This is the most popular vaccine used in most countries, but little has been done to determine its potency. The duration of immunity claimed is variable, ranging from 10-12 weeks after 2 inoculations 2-3 weeks apart (reported in Zambia) to the 9 months' immunity claimed in Sudan.

(e) Alum-precipitated vaccine.

This appears to be the most popular vaccine used in Asian countries. Immunity lasting for 5-6 months is claimed. Controlled trials in Sri Lanka using a standardized vaccine containing 2.5 mg bacteria per dose gave 3-4 months' immunity to challenge (33).

(f) Aluminium hydroxide gel vaccine.

This is used only in Thailand. Immunity up to 4 months is claimed.

(g) Oil adjuvant vaccine.

This is the most potent of the available vaccines, but is produced in relatively few countries (Malaysia, Indonesia, Sri Lanka, Egypt, Iraq and a small proportion of the total production in India and Bangladesh). Its potency is very variable. Controlled trials in Indonesia and Sri Lanka report the duration of immunity as 6-9 months and in Malaysia as 1 year. Bangladesh claims 21 months and India 28 months (4, 3). The Sri Lanka trials were performed on calves 4-6 months old, given a single dose of OAV and reared in an environment completely free of HS, in contact with unvaccinated controls. In similar trials made in HS enzootic areas, animals were still immune 14 months after vaccination, the longest period tested. Some unvaccinated controls also acquired immunity during this period. In enzootic areas, the immunity of vaccinated animals seemed to be prolonged by exposure to infection.
The high viscosity and the consequent difficulty in administration weighs heavily against the OAV. Some reduction in viscosity has been effected in Sri Lanka by using only 4% lanoline instead of the 8-12% used in some formulations.

4. Evaluation of vaccines.

Most countries accept absence of disease following vaccination as an indication of the efficacy of a vaccine. Routine testing of vaccines in cattle or buffaloes, or in any laboratory animal other than mice, does not seem feasible in most laboratories. Potency testing in mice has been attempted in Malaysia, India and Sri Lanka (52, 41, 20, 60). Various vaccination-challenge schedules have been developed for quick results. All these workers have adapted the technique of Ose and Muenster (54). It must, however, be noted that the last-named did not use HS serotypes of Pasteurella. They arbitrarily adopted a protection of 2 log units in vaccinated mice (as compared with unvaccinated controls) as an index of adequate protection in cattle, but were unable to establish a correlation between mouse potency and cattle potency. Thus, while the present tests provide a rough guide to the certification of a vaccine as suitable, there is a need to develop a realistic correlation between potency for mice and potency for cattle or buffalo, with a standardized haemorrhagic septicaemia vaccine.

The mouse protection test of Ose and Muenster, when performed most economically, requires about 50 mice per batch of vaccine. This may not be within the reach of the average laboratory in Asia or Africa, except for testing occasional batches. For routine testing of all batches of vaccine, reliance has to be placed on purity, agglutinability and the antigen content as measured by rapid, turbidimetric methods.

5. Vaccination schedules.

Various vaccination schedules have been adopted in different countries, ranging from annual prophylactic vaccination and vaccination in the face of an outbreak, to vaccination just before the HS season. Since the disease has a seasonal distribution in most countries, the last-mentioned is the most popular schedule. The disease is most prevalent in those areas of the Third World where husbandry practices are still primitive, and so the response of farmers to vaccination is very poor. Most farmers are willing to cooperate in vaccination only in the face of an outbreak. In Sri Lanka, non-cooperation by farmers was the biggest constraint to the vaccination programme (25). Except in special intensive vaccination programmes in specified areas, vaccination coverage is very low and ranges from 20-40%. One detailed study in Sri Lanka indicated that the total coverage annually was around 60% of the susceptible stock in enzootic areas, and that about half of this number were vaccinated only in the face of outbreaks.

Scientific evaluations of the efficacy of vaccination programmes are scarce. One such evaluation in Sri Lanka indicated that routine vaccination
with APV resulted in a statistically significant drop in the percentage of herds infected, but not in the morbidity rate once the disease had become established in a herd (32). This is an indicator of the low grade and short duration of immunity conferred by the vaccines in use, protection being sufficient to prevent the first case occurring, but inadequate against the heavy burden of infection within a herd once clinical disease has become established.

6. Economics of haemorrhagic septicaemia control.

There is little information on economic aspects of HS control. One intensive immunization programme covering three enzootic districts in Sri Lanka cost only US $0.66 to immunize a single animal. Ten per cent of this was the cost of the OAV produced in a local laboratory of modest means, and with a capacity of 2 million doses per year. The remaining 90% was the cost of veterinary field services.

7. Possible improvements to the present control methods.

Prospects for improving the present control methods depend upon achieving a higher vaccination coverage, and producing a better vaccine, easier to administer and providing longer immunity.

Several new vaccines have been tried out recently but none has become established.

(a) Double emulsion vaccine.

This was developed in India by Gupta et al. (40), in order to reduce the viscosity of oil adjuvant vaccine. The original vaccine was re-emulsified with an equal volume of 0.2% polysorbate 80 (Tween 80). The resulting fluid had a free-flowing milky consistency, but the potency of the vaccine was less than that of the standard OAV.

(b) Purified capsular extract vaccine.

Attempts to identify the antigenic fractions and to use such fractions to immunize cattle were made decades ago in India by Dhanda (35), but this work did not result in a practical vaccine.

More recently, Penn and Nagy (55) and Nagy et al. (53) obtained capsular extracts by solvent precipitation from the supernatant fluid of fermentor-grown P. multocida type B. The complex technology involved in producing a vaccine of this type may be beyond the scope of many vaccine-producing laboratories in countries affected by HS. Furthermore, the vaccine was of the oil adjuvant type and this was a disadvantage because of its high viscosity.

(c) Live vaccines.

Numerous attempts have been made from time to time to produce live vaccines against various forms of pasteurellosis. The main problem has been to achieve stability of the attenuated strain or mutant. While success has been achieved with fowl cholera, no suitable mutant has been found to immunize
against HS. Recently, avirulent streptomycin-dependent mutants were produced by chemical mutagenesis of 6:B strains (61, 27). One of these mutants was successfully used to immunize cattle and buffaloes (26). The large numbers of this mutant ($10^{10}-10^{11}$) required to produce immunity did not permit the development of a practical vaccine. Successful immunization with streptomycin-independent reverse mutants and also a naturally occurring variant strain (« ATCC 19427 ») has been achieved with a smaller number of organisms, but this work has to be developed further before a practical vaccine is obtained (De Alwis, unpublished report).

With the availability of freeze-drying equipment, the opportunity exists for developing a live vaccine in lyophilized form.

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Appendix

52nd GENERAL SESSION OF THE O.I.E.

RESOLUTION No. II

HAEMORRHAGIC SEPTICAEMIA IN CATTLE AND BUFFALOES

CONSIDERING

that the recognition of haemorrhagic septicaemia as a specific disease entity is still not clear in many countries;

that many countries report the prevalence of haemorrhagic septicaemia without adequate serological identification of the causal agent;

that whilst it is recognised that the oil adjuvant vaccine is the best prophylactic agent presently available, many countries continue to use simple broth bacterins of low potency;

that the vaccines produced and used in different countries are not standardised internationally;

that effective control of the disease depends on:

(a) the identification of the serotypes prevalent in each country;

(b) a better understanding of the epizootiology of the disease in each country;

(c) the formulation and execution of strategic vaccination programmes; and

(d) the development of highly potent vaccines,

THE COMMITTEE RECOMMENDS
1. That the term « haemorrhagic septicaemia » be defined as a specific form of primary pasteurellosis of cattle and buffaloes with high mortality in clinical cases and which is uniformly caused by *Pasteurella multocida* types 6:B or 6:E.

2. That continued efforts be directed towards improving the methods for rapid diagnosis and complete serological typing of *Pasteurella multocida*.

3. That steps be taken to identify and serotype isolates from countries where this has not been done already.

4. That standards be laid down for the haemorrhagic septicaemia oil adjuvant vaccine.

5. That steps be taken to enable all countries where the disease exists to produce their own oil adjuvant vaccine conforming to the standards mentioned in paragraph 4 above.

6. That adequate facilities be made available in national or regional laboratories to enable such laboratories to provide the necessary services.

7. That research be carried out on:
   (a) the epizootiology of the disease in various countries; and
   (b) the development of vaccines of higher potency which are easier to handle in the field.

8. That hygienic measures such as the control of movement of animals from diseased areas and the proper disposal of carcasses and other infected material, and the adoption of improved husbandry practices, where possible, be carried out in order to reduce the incidence and spread of the disease.

*(Adopted by the International Committee of the O.I.E. on 23 May 1984.)*

**

REFERENCES


