HAEMORRHAGIC SEPTICAEMIA IN SRI LANKA

Malcom C.L. de ALWIS*

Background information

The Republic of Sri Lanka is an island situated within the tropics between the Northern Latitudes 5°55′ and 9°50′ and the Eastern Longitudes 79°42′ and 8°51′. The island covers an area of 65,000 sq. KM (25,322 sq. miles), with a maximum North South length of 435 KM (270 miles) and an East West width of 225 KM (140 miles). It has a mountainous area, rising to a peak of 2500 M (8280 ft) in the South Central region, surrounded by plains, narrow in the Western, Southern and Eastern areas, but widening into a vast expanse in the North of the mountains.

The mean temperatures in the plains are in the region of 80 - 82°F, dropping steadily by 1°F for every 300 ft. rise in elevation to give a low temperature range of 50°F to 75°F in the hills and mid-country.

The island's annual rainfall varies from 1000 mm (40″) approximately in the driest plains to 5000 mm (200″) in the south-western slopes. Its rainfall is dependent mainly on the two monsoons, the South West Monsoon from mid-April to September, and the North East Monsoon from November to March. The dry zone receives rainfall only during the North East Monsoon, while the wet zone receives rainfall during both monsoons.

On the basis of the rainfall and elevation, and from the standpoint of the cattle industry, the country may be broadly divided into different agro-climatic zones. The types of cattle reared and the management practices vary with the different zones and are indicated in Table 1.

Sri Lanka has a total cattle population of 1.1 million and a buffalo population of 0.4 million, approximately.

In the cooler climes of the hill country, high yielding exotic breeds of cattle, such as the Friesian, Ayreshire, Jersey and Shorthorn are reared. Each farmer will own only a few animals and these are reared under a zero grazing system, where grass is cut and fed.

In the intermediate areas a few exotic animals are still found but the vast majority of animals are crosses between the exotic breeds and the zebu or indigenous cattle. This area also harbours a sizeable buffalo population. Here too, the animals are either stall fed, or managed under a limited grazing system such as in the coconut plantations in the Western coastal plains where the animals are tethered under the coconut palms for grazing by day and are housed by night.

In the dry zone large herds of cattle and buffaloes are collectively grazed on natural pasture, parklands and on tank and river beds. Some animals may be paddocked at night. These animals provide a source of draught power for ploughing the rice fields and also serve as a source of beef. In areas where a marketing facility exists the cows may be milked.

The incidence of haemorrhagic septicaemia (HS) was conclusively established for the first time in 1955 when a major epizootic broke out, killing thousands of cattle and buffaloes. Since that time, a definite pattern has been established in the incidence and distribution of the disease in the country.

The highest incidence of the disease is in the low country dry zone. In these areas the disease is enzootic and outbreaks occur regularly. In the dry zone of the hills and the mid-country and in the low country wet zone, sporadic outbreaks occur from time to time. Except in a few localised areas the disease is not enzootic. In the hill country wet zone haemorrhagic septicaemia has occurred only exceptionally and this area is generally considered to be free of this disease. Fig. 1 illustrates broadly the low, moderate and high incidence areas in Sri Lanka.

* Veterinary Research Officer, Veterinary Research Institute, Dept. of Animal Production & Health,Peradeniya, Sri Lanka.

Table 1 Types of cattle in the different agro-climatic zones, their management practices and the incidence of HS

Zone	Elevation	Rainfall	Temperature	Type of Animal	Husbandry Practice	H. S. Incidence
Wet Zone Hill Country	Over 1200m (4000′)	Over 2000mm (80″)	10°C–24°C (50°F–75°F)	Pure Exotic* Breeds & Crosses	Zero grazing, small herds	Low(1) or Absent
Mid-Country	450m–1200m (1500′–4000′)	1875mm–5000mm (75″–200″)	21°C–32°C (70°F–90°F)	Pure Exotic* Breeds & Crosses	Zero grazing, small herds	Low(1)
Low Country	0m–450m (0′–1500′)	1875–2500mm (75″–100″)	24°C–35°C (75°F–95°F)	Crosses of Exotic* Breeds, Zebu types, Indigenous cattle and buffaloes	Limited grazing. medium-sized herds.	Moderate(2)
Dry Zone Hill Country	Over 1200m	1250mm–1500mm (50″–60″)	10°C–30°C (50°F–85°F)	Crosses of Exotic* Breeds, Zebu types Indigenous cattle and buffaloes	Limited grazing. medium-sized herds.	Moderate(2)
Mid-Country	450m–1200m	1250mm–1500mm (50″–60″)	21°C–32°C (70°F–90°F)	Crosses of Exotic* Breeds, Zebu types Indigenous cattle and buffaloes	Limited/Free grazing. medium-sized herds.	Moderate(2)
Low Country	0m–450m	1000mm–1750mm (40″–70″)	21°C–38°C (70°F–100°F)	Indigenous cattle, Zebu cattle and their crosses. Buffaloes	Free grazing Large herds	High(3)

* Friesians, Jersey, Ayreshire and Shorthorns.

1. Low – Non-enzootic areas – Exceptional incidence only.
2. Moderate – Non-enzootic – Occasional sporadic outbreaks.
3. High – Enzootic areas – Annual seasonal outbreaks. Sporadic outbreaks at other times.

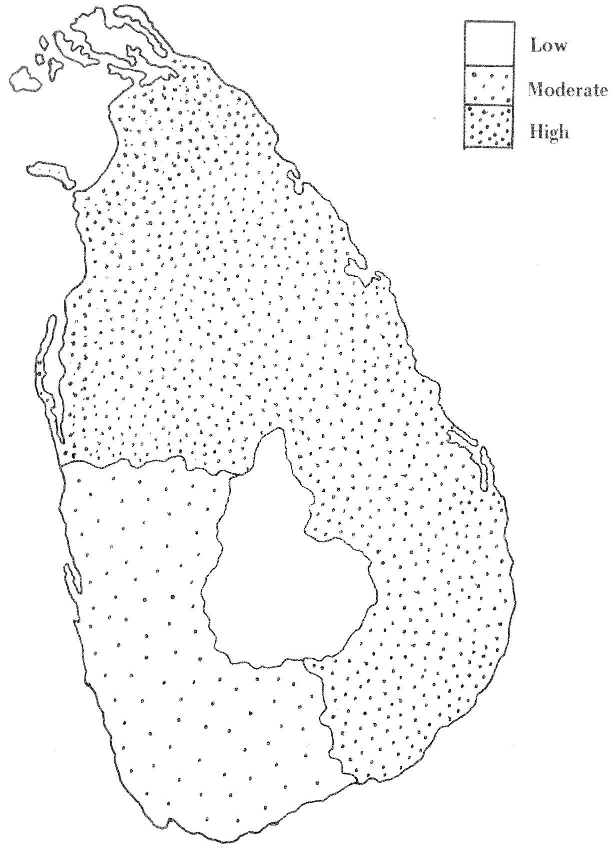

Fig. 1 Haemorrhagic septicaemia incidence in Sri Lanka

The causative organism

During the epizootic in 1955, the causative organism was identified as *Pasteurella multocida* Roberts type 1.[8] In 1968 in an investigation carried out in an abattoir where cattle from the dry zone are slaughtered, 45 isolations of *P. multocida* were made from the nasopharynx of clinically normal cattle.

Of these, 43 were found to be of serotype 6:B or the Asian type 1,whilst two isolates were found to belong to serotype 11:B or the Australian type 1[10].

In 1974, a detailed study was made of 50 strains of *P. multocida* associated with outbreaks of haemorrhagic septicaemia and collected over a period of 10 years. In this study, it was found that the isolates were biochemically and serologically uniform and belonged to serotype 6:B.[1]

Clinical signs

In a typical outbreak of haemorrhagic septicaemia, the first reports are those of animals being found dead without any previously observed symptoms. Other affected animals will first show a rise of temperature. This is followed by a swelling under the jaw, gradually spreading to the brisket region and occasionally down the fore limbs, with a resultant lameness. There is profuse salivation and a nasal discharge. In the next stage respiratory distress becomes evident with laboured breathing. The body temperature may drop to sub-normal levels. Finally the animal will fall on its side and die. The course of the disease may range from a few hours to about two days.

A pneumonic from of Pasteurellosis caused by the same serotype has also been reported in buffalo calves and is believed to be an atypical syndrome in calves having low levels of immunity[2].

Necropsy findings

In the hyperacute form where the animals die suddenly without showing any symptoms very few pathological lesions are evident macroscopically. There is consistently a marked oedema in the sub-mandibular region. Widespread petechial haemorrhages may be present, particularly on the base of the heart, abomasal wall, omentum and on the intestines. In the more protracted pneumonic form fibrinous pericarditis, pleurisy with adhesions and extensive consolidation of the lungs with lobulation, resulting from marked thickening of the interlobular septa become evident.[2] It has also been shown in experimental infections that extensive lesions of pericarditis, pleurisy and pneumonia are usually observed only when the course of the disease is at least three days.

Morbidity and mortality patterns

In large herds, under field conditions, case fatality is almost 100 per cent. Rarely towards the end of an epizootic an occasional animal may recover spontaneously. In organised dairy farms, however it is possible to save some animals if, when the first cases are reported, all in-contact animals are regularly examined for any rise of body temperature and treated immediately with a suitable antibiotic.

The morbidity rate is very variable and depends on a variety of factors. It is established that the buffalo is more susceptible than cattle. In one study involving 36 outbreaks of disease in buffaloes and 26 in cattle, the mean morbidity was found to be 39 per cent for buffalo and 16 per cent for cattle. In the highly enzootic areas of the dry zone, where the disease breaks out annually, the morbidity rate is low. It has also been observed that in such outbreaks the animals that die are the very young ones, particularly those born after the previous outbreak of disease. In some herds where the disease broke out after a lapse of several years the morbidity was higher and extended to older animals. Morbidity is highest when sporadic outbreaks occur in non-enzootic areas, and deaths occur in all age groups. Table 2 shows the relative morbidity in cattle and buffaloes, and Table 3 illustrates the relative morbidity in buffaloes, in enzootic and non-enzootic areas.

Table 2 Morbidity patterns due to haemorrhagic septicaemia in buffaloes and cattle

	No. of herds	Herd size			Morbidity %		
		Min.	Max.	Mean	Min.	Max.	Mean
Buffalo	36	20	170	52	08	88	39
Cattle	**26**	20	200	59	3.3	50	16

Table 3 Morbidity due to haemorrhagic septicaemia among buffaloes in enzootic and non-enzootic areas

	No. of herds	Morbidity %		
		Min.	Max.	Mean
Enzootic areas	24	08	64	29.5
Non-Enzootic areas	12	24	88.8	59

Some epizootiological aspects

1 Transmission of the disease

The HS causing *Pasteurellae* do not survive outside the animal body for long periods. They are highly susceptible to drying and heat. Thus, fairly close contact with infected animals or infected pasture, bedding etc., is necessary for the spread of the disease. In Sri Lanka there is considerable evidence to indicate that rivers, tanks, irrigation channels and waterways are important sources of infection, when contaminated with virulent organisms from sick animals and carcasses.

2 Seasonal incidence and distribution of the disease

In the enzootic areas of the dry zone a definite seasonal incidence has been established. Most epizootics occur during the end of the drought period or with the first monsoon rains. Unexpected showers of rain due to depressions that occur during the dry season also precipitate the disease. The movement of animals that takes place during the rainy season, contributes largely to the spread of the disease. Large herds of cattle and buffalo that normally graze along the banks of the rivers, are driven to highland areas during the rainy season, on account of the floods. There is also a considerable movement of animals on account of their use in ploughing the rice fields during the rainy season. At the end of the drought season the animals are in the lowest phase of nutrition. With the onset of the rains the animals in an already debilitated condition, besides being exposed to inclement weather conditions are also worked in the rice fields. All these events impose a considerable stress on the animals and thereby help to precipitate the disease.

It is difficult to explain fully why the disease is enzootic in the dry zone only and not in the hill country of Sri Lanka. There is, however, much room for speculation in this regard. The climatic conditions in the hill country are more uniform throughout the year and not subject to such extremes of wet and dry weather as in the dry zone. Also there is very little movement of animals. Most animals are stall fed or graze in localised areas. The mixing of large herds in common grazing land as in the dry zone does not occur. It is interesting to note that in the Jaffna peninsula situated in the Northern extremity of the island which has a dry zone climate but where the husbandry practices are more like those of the hill country, the incidence of HS is considerably low. It is also noteworthy that most hill country areas have only a few or no buffaloes at all.

3 Naturally acquired immunity to haemorrhagic septicaemia

It has been found that a certain proportion of cattle and buffaloes in Sri Lanka are naturally immune to HS. The incidence of such animals is high in enzootic areas and low or absent in the hill country. Within an enzootic area its incidence varies from one herd to another and even in the same herd from time to time. This feature is illustrated in Table 4.

In a State-owned buffalo farm during a period when there was a high incidence of HS and pneumonic pasteurellosis caused by the HS organism (*P. multocida*, serotype 6:B), 70 percent of 50 buffalo calves that were left unvaccinated against HS, and survived up to 16 months of age were naturally immune. In the same farm during the following year when the incidence of the disease was controlled by a more effective vaccination programme, none out of the 50 buffalo calves that were left unvaccinated became naturally immune at the same age. The information presented in Table 4 also indicates that the incidence of naturally acquired immunity is higher in older animals, than in young calves.

All these findings point to a relationship between recent incidence of disease and naturally acquired immunity. An outbreak of HS that occurred in an organised buffalo farm afforded an excellent opportunity to study this phenomenon in greater detail. During this epizootic a group of young calves 4 to 6 months of age were left unvaccinated and housed in the same shed as the diseased animals. About a third of the animals died of HS. Of the balance approximately 80 percent of the animals developed high indirect haemagglutination titres against HS 3 to 4 weeks after exposure, rising to a peak at three months (1 in 320 to 1 in 640) and then declining to very low levels at six months. They, however, continued to show mouse protective antibody in their sera. Eight months after the natural exposure, these calves showed the mouse protective antibody in serum dilutions ranging from 1 in 4 to 1 in 64 when challenged with a 100 LD50 of virulent organisms. Such a high level of immunity was never attainable by vaccination with any of the vaccines presently used. The probable cause of this naturally acquired immunity is a sub-clinical infection resulting from exposure to sub-infective numbers of virulent organisms.

Table 4 Naturally acquired immunity to HS in cattle & buffaloes in Sri Lanka

Province	H. S. Status	Young animals Under 2 yrs.		Adult animals Over 2 yrs.	
		Number tested	Number immune	Number tested	Number immune
Central	Non-enzootic Low incidence or absent	82	0	30	01
Western	Non-enzootic Occasional sporadic outbreaks	34	0	125	15
North-Central	Enzootic High incidence	93	04	110	22
Eastern	Enzootic High incidence		–	215	159

4 Carrier status

It is generally believed that when a fresh outbreak of disease occurs after a long interval the carrier animal is the source of the organisms—hence the importance of the carrier in the epizootiology of the disease. In a survey carried out in an abattoir where cattle from the dry zone are brought for slaughter a carrier rate of 15 per cent was recorded.[10] In another study in Sri Lanka yet in progress[7], it was observed that the carrier rate is high in herds with a recent incidence of HS, whereas in herds which have been free of disease for some time very few or no carriers are detected. Thus, in a herd during the period following an outbreak of disease there is both a high incidence of naturally immune animals and a high incidence of carriers.

Control of haemorrhagic septicaemia

1 Vaccines

Haemorrhagic septicaemia is controlled by vaccination. In Sri Lanka two types of vaccines are used. These are the alum precipitated vaccine and the oil-adjuvant vaccine. Field isolates of *P. multocida* whose identity is confirmed by serotyping are used in the vaccine production. Using a highly nutrient broth medium and aeration techniques, dense cultures are produced in vortex tanks. Formalin is used to inactivate the bacteria and either the appropriate amount of alum is added or an emulsion made using a mineral oil and lanoline. The vaccine is standardised so that each dose of vaccine contains 2.5 mg bacteria by dry weight.

Extensive field trials carried out in Sri Lanka have shown that a single shot of the alum precipitated vaccine protects cattle and buffaloes for 3 - 4 months only. Under similar conditions the oil-adjuvant vaccine gave complete protection for 6 months and partial protection for up to 9 months. It was also found that buffalo calves under 3-1/2 months of age responded poorly to vaccination[5].

Some veterinarians and vaccinators however, prefer the alum vaccine, since this vaccine is easier to administer as compared with the thick viscous oil-adjuvant vaccine. The usefulness of the alum vaccine, however, is limited to protection over the high risk period, in enzootic areas, by vaccination just before the HS season or for vaccination in the face of an outbreak of disease.

2 Vaccination programmes

Two types of vaccination programmes are practiced viz, routine prophylactic vaccination and barrier vaccinations in and around outbreak areas.

1) Routine prophylactic vaccination

About 80 percent of the cattle and buffalo population of Sri Lanka live in areas where the risk of haemorrhagic septicaemia justifies a routine, prophylactic vaccination programme. In these vaccination programmes, the oil-adjuvant vaccine is administered annually. Whilst such a vaccination programme is sufficient to immunize adult cattle and buffaloes, annual vaccination is insufficient to immunize young calves. This became very evident when HS broke out in two organised buffalo farms, where annual vaccination with oil-adjuvant vaccine was practiced. Table V gives an analysis of the mortality on an age basis. The maximum mortality was seen in the 6-to-8-month age group.

Table 5 Age distribution of deaths due to haemorrhagic septicaemia

Age Group in Months	% Deaths in Farm A*	% Deaths Farm B*
0 – 3	0	0
3 – 6	3.8	0
6 – 12	27.5	12.8
12 – 18	41.0	8.8
18 – 24	19.2	2.1
2 – 4 yrs.	0.7	1.9
over 4 yrs.	0	0

* The mortality in each age group was calculated as a percentage of the number at risk in that age group.

The same study also indicated that 91.4 per cent and 84.2 per cent of the total deaths in the two farms, respectively, were among animals that had received a single shot of vaccine on reaching the age of 4 months and were nearly reaching the age of 16 months at which the next vaccination was due. [3]

To overcome this problem we now recommend primary vaccination at 4 months followed by booster vaccinations at 6-7 months and at one year. This we believe, will provide adequate cover for young calves and annual vaccination will suffice thereafter.

2) Barrier vaccinations

It is also recommended that irrespective of the previous vaccination history all cattle and buffaloes in and around areas in which the disease has broken out be vaccinated as soon as the incidence of disease is reported. It is generally observed that the number of deaths declines markedly within 4 to 5 days of such vaccination.

In addition to the vaccination of the immediate in-contact animals in infected areas, a barrier vaccination programme is carried out round such an area. This vaccination together with restriction of the movement of animals in and out of the infected area helps to contain and thereby control the disease.

3 Practical difficulties encountered in vaccination programmes

The attitude of the villagers to vaccination is an important consideration. The co-operation extended by the average villager to a routine prophylactic vaccination programme is limited. In the face of an outbreak, on the other hand, livestock owners enthusiastically present their animals for vaccination.

Most dry zone animals are accessible, even to the owner, only during the cultivation season, when the animals are used for draught purposes. During other times of the year they are merely let loose for free grazing and unless the owners make a special effort to round them up, they are not within reach of the vaccinators.

Restraint of individual animals, particularly buffaloes, is also a problem. As a result, many animals get left out when vaccinating.

As a result of the above constraints, taking the number of recorded vaccinations as a percentage of the cattle population, the vaccination coverage is under 50 per cent. Since in organised farms several vaccinations are done on each animal annually, the actual coverage may be only in the region of 40 per cent.

Since, free grazing animals cannot be rounded up for a rigid vaccination programme as outlined above, where primary and booster vaccinations are made in the first year of life, young calves also receive only one annual vaccination and such incompletely protected animals may die in outbreaks of disease. Such deaths in vaccinated animals discourage owners from presenting their animals for vaccination thereafter.

All these problems point to the need for a vaccine that will confer a minimum of one year reliable immunity and which is easy to transport, store and administer under the conditions prevailing in the enzootic areas.

4 Research into new vaccines

Many other adjuvants have been used in other countries on an experimental basis but none have been established as superior to the oil adjuvant vaccine.

The observation made in Sri Lanka, that, on exposure to infection with virulent live bacteria, some animals developed a level of immunity not attainable by vaccination with the killed vaccines, gave rise to the belief that a live vaccine may confer better immunity than the formalin killed vaccines. The superiority of the live *Pasteurella* vaccines over killed ones in fowl cholera has also been established.

5 Recent work on live HS vaccines

Recently a streptomycin-dependent mutant was developed from a type B strain of *P. multocida* of Egyptian origin.[9] This mutant was found to protect mice against challenge with type B *Pasteurellae* causing HS. Subsequently a number of streptomycin-dependent mutants were produced from type B strains isolated in Sri Lanka.[4] Field trials on immunizing cattle and buffaloes using one of these mutants are in progress. Immunity has been successfully induced without the risk of disease.[6] The duration of immunity is now being investigated.

Investigations are also being done using atypical *Pasteurella* cultures which are incapable of producing disease, but are sufficiently immunogenic. One such culture which has given promising results in preliminary investigations is *P. multocida*, ATCC 19427. Further work with this organism is in progress.

The ideal strain for use in a live vaccine would be one which is totally avirulent, but possesses all the antigenic components of the naturally occurring strain. It should be able to multiply sufficiently within the animal body so as to provide an adequate immune response, when inoculated in relatively small numbers. If a suitable mutant could be found which could immunize cattle and buffaloes in doses of 10^8 viable organisms, it is estimated that one 5 litre batch of vaccine would be sufficient to produce the same number of doses as the quantity of oil-adjuvant and alum precipitated vaccine presently produced in Sri Lanka annually, by growing weekly batches of dense cultures all year round. This in itself is a distinct advantage for a live vaccine, over the killed vaccine presently in use.

The eventual solution to the control of HS may lie in the isolation of a suitable strain, which could adequately immunize cattle and buffaloes even by the intra-nasal route. Such a vaccine could be administered by an aerosol spray thus eliminating the need for restraining animals for individual vaccination.

References

1) De ALWIS, M.C.L. and PANANGALA, V.S. (1974): *Ceylon vet. J.* **22**, 58.
2) ________, JAYASEKERA, M.U. and BALASUNDERAM, P. (1975): *Ceylon vet. J.* **23**, 58.
3) ________, KODITUWAKKU, A.O. and KODITUWAKKU, Susima, (1976): *Ceylon vet. J.* **24**, 18.
4) ________, CHENGAPPA, M.M. and CARTER, G.R. (1978): Unpublished data.
5) ________, GUNATILEKE, A.A.P. and WICKREMESINGHE, W.A.T. (1978): *Ceylon vet. J.* **26**, in press.
6) ________, and CARTER, G.R. (1979): Unpublished data.
7) HIRAMUNE, T. and De ALWIS, M.C.L. (1979): Unpublished data.
8) PERUMAL PILLAI, C. and THAMBIAYAH, V.S. (1957): *Ceylon vet. J.* **5**, 24.
9) WEI, B.D. and CARTER, G.R. (1978): *Am. J. vet. Res.* **39**, 1534.
10) WIJEWANTHA, E.A. and KARUNARATNE, K.G. (1968): *Cornell vet.* **58**, 462.

Discussion

Konno S. (Japan): 1) What was the examination method for haemorrhagic septicaemia (HS) carrier status in the animals? 2) Do you get a pure culture of *Pasteurella multocida* when you cultivate the nasal swabs collected from diseased animals?

Answer: 1) We perform cultures (direct) and biological tests (mouse inoculation) with nasopharyngeal swabs 20-25 cm long. Selective media are also used. 2) No. Even when selective media are used, the direct cultures are not pure. Biological tests (mouse inoculation) are more reliable for inoculation.

Sudana G. (Indonesia): You mentioned that the carrier status cannot be detected 6 weeks after the outbreak of HS (observation of 351 head of cattle) 1) What is the source of infection after the outbreak? 2) What is the most likely route of infection in buffalo and cattle? Through wounds, close contact, stress or others?

Answer: 1) In my paper, by "carrier" is meant an animal which is in good health but harbours the organism in the naso-pharynx. Within 6 weeks, the organism apparently disappears from the site. We do not know whether it is harboured in some other unknown site. Further investigations are required in this matter. 2) In natural infection, the most likely route is by ingestion (contaminated drinking water consecutive to the dumping of carcasses of infected animals into rivers) or inhalation.

Gupta B.K. (India) Comment: As regards the carriers, there are two types of *Pasteurella multocida* to consider. The fluorescent type (epidemic type) does not remain long in the respiratory tract. The blue type (endemic type) stays for a long period in the respiratory tract and is responsible for the perpetuation of outbreaks.

de Alwis M.C.L. (Sri Lanka) Comment: All the isolates were found to be pathogenic to rabbits, mice but not to birds.

Gupta B.K. (India): Have you carried out serotyping of carrier strains?

Answer: We have performed capsular typing by the indirect haemagglutination test of Carter and by the slide agglutination method of Namioka. The organisms belong to type B. No O typing has been done.

Namioka S. (Japan): Did you carry out serological tests to detect the carrier animals? For example. Haemagglutination tests, agglutination tests for antibody status?

Answer: No studies of antibody status associated with carrier status have been conducted.

However, we have found that following an outbreak of HS, among the surviving animals immunity status is very high. We have also found independently that in similar situations, the carrier status was high. The two different studies have not been made in the same animals.

Gatapia S.L. (Philippines): It appears from the discussion that vaccination against HS with alum precipitated or with oil-adjuvant vaccines affords a short immunity. Therefore, more emphasis should be placed on vaccination with live vaccine. You also mentioned that immunity is of longer duration in naturally infected calves. Do you have any experience with the use of anti-sera in high risk areas, particularly in the case of young animals? Subsequently, live vaccine application should be recommended.

Answer: In Sri Lanka we have no experience with the use of hyper-immune serum either prophylactically or therapeutically. I believe that the use of such serum would be of very little practical value in the field. In situations where the disease can be detected early enough, antibiotic treatment is preferable. The problem is that the course of the disease is so short that there is no time usually for any treatment.

Gupta B.K. (India) Comment: In India, we have used anti-sera for therapeutic purpose, during the phase of an outbreak only, for immediate protection. Thereafter, we vaccinate the animals.