RESEARCH ON HAEMORRHAGIC SEPTICAEMIA IN INDIA AND ITS CONTROL

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Disease position

Haemorrhagic septicaemia is taking a heavy toll of livestock population in India. The average annual death rate was reported to be more than 40,000 in 1958-59 (Dhanda, 1959). Since then, there has been a considerable fall in the mortality rate. It came down to 18,265 during the period 1968 to 1973, 11,690 in the period 1973 to 1977 and 9,625 in the year 1978. Although morbidity and mortality rate due to the disease has decreased significantly in the last four years, the number of outbreaks continues to remain substantially high i.e. above 4,000 (Table 1).

Table 1   Incidence of haemorrhagic septicaemia in India

S. N.	Year	Outbreaks	Attacks	Deaths
1	1974	3,072	12,496	7,571
2	1975	5,465	22,088	13,941
3	1976	4,658	22,642	13,011
4	1977	4,242	18,682	12,267
5	1978	4,356	15,253	9,625

The disease is mainly caused by *P. multocida* serotype 6:B as reported from other parts of S.E. Asia, though other capsular types probably play a minor role. Besides 83 type I strains out of 102 haemorrhagic septicaemia strains from cattle and buffaloes in India, six were reported to be type of IV and 13 as untypable (Dhanda, 1959). A fresh appraisal of the exact serotypes involved and their regional distribution, if any, will be helpful in controlling the disease.

Carrier problem

The endemic nature of the disease and its seasonal incidence have been attributed to the presence of healthy carriers. Studies revealed that the carrier rate of *P. multocida* (Robert's type I) wad directly related to the incidence of the disease with 0% in non-endemic zones to 1.9% in a zone with moderate incidence and 5.0 to 6.0% in enzootic areas with high incidence of disease. The carrier rate was found to be higher in buffaloes i.e. 2.5 to 6.15% than in cattle i.e. 1.25 to 2%. The overall carrier rate in areas where outbreaks of the disease occurred to a varying extent was found to be 2.8% i.e. 10 out of 355. The examination of upper respiratory tract of cattle and buffaloes, in an endemic area during the outbreak, revealed 7.5% of the animals to carry *P. multocida* (Robert's type I). A reexamination of the nasopharyhx of animals in the same village, 40 days after the outbreak was controlled by vaccination, revealed the absence of any carrier (Gupta, 1962). Similar findings on carrier rate have been reported in Sudan (Mustafa *et al.*, 1978).

Out of the 20 isolates of *P. multocida* from healthy carriers, as many as 10 were of Robert's type I, two of type II, three of type IV and the remaining could not be typed. It was interesting to note that out of 10 type I isolates eight were capsulated and pathogenic to mice and hill bulls and two

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were non-pathogenic to either. One of the non-pathogenic strain was cocobacillary and non-capsulated and the other capsulated but filamentous (Gupta, 1962). The isolation of pathogenic type I strains from apparently healthy animals obviates the necessity to postulate the increase in virulence of carrier strains for initiation of disease. The transmission of organisms from the resistant carrier to the in-contact animal with lowered resistance results in disease condition. The resistance of cattle already stressed due to debilitating effects of long summer drought, falls considerably at the onset of monsoon due to sudden exposure to inclement weather. Hot humid weather optimal for the multiplication and spread of microbes concomitant with the general fall in resistance of the susceptible population presents an ideal milieu for the disease to assume an outbreak form. When the organism is transferred to a partially immunized animal, the latter becomes a carrier and a potential danger for the initiation of the next outbreak. The presence of naturally resistant animals as reported by Bain in other S.E. Asian countries in commonly encountered in India. A rapid slide agglutination test developed by Dhanda *et al.* (Dhanda *et al.*, 1959) has been found to be very useful and is being extensively used to detect these naturally immune animals.

Reservoir status

Pasteurella multocida Roberts type I has been reported to cause septicaemic conditions in pigs, sheep and goats (Dhanda, 1959). They have also been isolated from the upper respiratory tract of healthy and diseased pigs (Gupta, 1969, Singh, 1974). Four strains of poultry origin, at least one of which was reported to have been isolated from an outbreak of chronic fowl cholera from an endemic zone of haemorrhagic septicaemia, were found to belong to 6:B serotype (Gupta, 1973, Gupta and Kumar, 1978). The isolation of 6:B strain from chicks in an endemic region points out its significance as a reservoir for haemorrhagic septicaemia. Cross-reactivity of these strains with '0' group factor sera other than '6' suggests that certain strains could possess a complex somatic antigenic structure and could possibly affect a wider host-range. Such strains could play an important role in transmission of disease from one host species to an other. It will be worthwhile to know if some type I isolates from various other domestic animals in India also belong to serotype 6:B to elucidate their role as reservoir for haemorrhagic septicaemia in cattle.

Vaccines

Mainly three types of vaccines e.g. (1) oil-adjuvant (Dhanda *et al.*, 1956) (2) alum precipitated (Iyer *et al.*, 1955) and (3) broth bacterin are produced using highly immunogenic local strain P-52, serotype 6:B. The average yearly production of various vaccines during the last five years is given in Table 2. More than 35.7 million doses of vaccines were produced in the year 1978 of which about 62% consisted of alum precipitated vaccine, 34.2% of broth bacterin and 2.8% of oil-adjuvant vaccine. The oil-adjuvant vaccine is produced from agarwash suspension of the organisms. The yield is, therefore, very small. Some trials have been conducted with the production in vortex aerated tanks (Ahooja *et al.*, 1979), but the method is yet to be adopted for large scale manufacture. It is essential to step up the production of oil-adjuvant vaccine harnessing the latest technology.

Table 2 Production of different vaccines (in doses)

	1974	1975	1976	1977	1978	Total
Oil-Adjuvant	719,799	2,286,966	754,489	1,389,894	999,215	6,150,363
Alum Precipitated	13,911,460	17,898,750	20,402,910	19,848,780	22,342,650	94,404,550
Broth Bacterin	12,718,190	12,440,970	11,232,430	12,808,150	12,391,770	61,591,510
Total	27,349,449	32,626,686	32,389,829	34,046,824	35,733,635	162,146,423

The currently used oil-adjuvant vaccine has not gained much popularity in the field as it is difficult to inject due to its thick consistency. A multi-emulsion vaccine has been developed by secondary emulsification of the oil-adjuvant vaccine with Tween 80, which as free flowing milk-like liquid, can be easily injected due to its low viscosity and is quite stable on storage. The multi-emulsion vaccine was found to be protective in mice, rabbits, cattle and buffaloes as assessed by direct challenge and mouse protection tests (Mittal *et al.*, 1977). The vaccine was found to afford 100, 80 and 50 percent protection in calves, respectively, when challenged at 3, 6, and 9 months post-immunization with 50 million minimum mouse infective doses of *P. multocida* (P-52) str. (Mittal, *et al.*, 1979). The duration of immunity was found to be lower than that afforded by oil-adjuvant vaccine in the limited study carried out so far. However, in view of its great advantages over the conventional oil-adjuvant vaccine, large scale field trials are necessary to assess its usefulness.

Various other vaccines such as capsular protein vaccine (Dhanda, 1959b), sodium thiocyanate extract vaccine (Singh, 1963) have also been tried. A mixed vaccine with *Cl. chauvei* has also been found useful (Srivastava *et al.*, 1976). Trials are also under way to assess the usefulness of Streptomycin-dependent live *P. multocida* vaccine as described by Wei and Carter (1978) for prevention of haemorrhagic septicaemia in cattle.

In accordance with the Drugs Act of India the potency of oil-adjuvant vaccine is tested by challenge in buffalo-calves. Studies have been undertaken to develop a testing procedure for this vaccine in mice (Nagrajan *et al.*, 1972, Gupta and Sareen, 1976), and rabbits (Nanjia *et al.*, 1966, Mittal and Jaiswal, 1976). Ose and Muenster (1968) have suggested that a vaccine giving two log protection in mice could be considered satisfactory. However, all the batches of oil-adjuvant vaccine, which protected buff-calves to effective challenge, when tested by this method in mice have given protection greater than four logs. Studies are under way to determine whether the batches of vaccines which give mice protection between 2 to 4 logs are protective for buff-calves.

Control

Preventive inoculations in endemic regions and containment of outbreaks by vaccination in affected herds are usually practiced. A strategy to control and eliminate the focus of infection from endemic area was carried out in Madhya Pradesh with spectacular results (Gupta, 1974). It consisted of locating priority villages I, II and III having disease outbreaks for three or more, two and one times respectively, in the preceding five years, in a particular endemic focus. Mass vaccination was carried out with HS oil-adjuvant vaccine covering the majority of the cattle population before the break of monsoon every year in priority I villages and villages contiguous to them within five-mile radius. Priority II and III villages were also covered with oil-adjuvant vaccine while the villages contiguous to them were vaccinated with alum precipitated vaccine. District Ujjain having had the highest average incidence of HS in Madhya Pradesh of more than 80 outbreaks annually from 1960 to 1965 with a cattle population of 1,000,000, was covered under this programme. The disease incidence, mortality and vaccinations carried out in the district are shown in Table 3. In the year 1965-66 the vaccination was concentrated in the outbreak area and was increased from 28,433 to 45,592 with a resultant fall in number of outbreaks from 88 to 41. The planned vaccination following the strategy was carried out in April through June 1966 and then followed every year. It is noticed that there was a sudden fall in number of outbreaks from 41 to 9 in the first year and to 3 in the next year. The number of outbreaks rose to 11 in the year 1968-69. Thereafter the incidence remained at a low level of 3 to 4 annually with a rise to 11 in the year 1971-72, till it became nil in the year 1974-75. The rise in incidence of outbreaks in the year 1968-69 and 1971-72 was due to attacks in new foci. In the first year of the programme 88,195 e.g. 8.8% of the total cattle population of the district was vaccinated. In the year 1968-69 when there were some new foci of infection the number of vaccinations was increased to 111,923 to cover the new villages. About 15% of the vaccinated animals received oil-adjuvant vaccine while the rest the alum precipitated vaccine. Implementation of similar programme in other endemic areas may prove effective in combating the disease.

Table 3 Incidence of haemorrhagic septicaemia mortality and
vaccinations carried out in District Ujjain, M.P. India.

Year	Number of outbreaks	Number of deaths	Number of vaccinations done
1963—64	77	325	19,883
1964—65	88	478	28,433
1965—66	41	322	45,592
1966—67	9	44	88,195
1967—68	3	23	85,743
1968—69	11	94	111,923
1969—70	4	10	112,715
1970—71	4	12	81,684
1971—72	11	61	90,101
1972—73	7	27	65,551
1973—74	3	9	85,700
1974—75	NIL	NIL	58,296

Recent studies on toxins and toxic fractions

Attempts to detect the production of soluble toxin during *in vivo* or *in vitro* growth of *P. multocida* have not been fruitful (Dhanda, 1959a, 1960, Bain, 1963). Recent studies revealed the presence of an aggressive factor in the culture filtrates of a pathogenic avian strain of *P. multocida*. A non pathogenic strain inoculated along with the filtrate from 6-hour broth culture of pathogenic strain killed 13 mice out of a total of 18 in 24 hours, while none out of three when inoculated alone. The fraction was probably identical with the beta-antigen described by Prince (1969) which was a lipopolysaccharide associated with protein.

In vivo growth of *P. multocida* in diffusion chambers implanted in the peritoneal cavity did not result in the death of chicks or mice. This was in conformity with the observations of Bain (1963) who failed to demonstrate any lethal activity in the culture filtrate or plasma and tissue exudates from animals dying of haemorrhagic septicaemia.

A soluble toxic fraction lethal to mice released in normal saline suspension during the process of harvesting bacterial growth from the surface of yeast extract agar and subsequent over night storage at 4°C has been described (Gupta, 1973). The gel diffusion and immunoelectrophoretic studies revealed that it contained at least two fractions. It could be purified by centrifugation at $105,000 \times G$ or by filtration through sephadex gel (G-200). The purified fraction formed a line of identity with "Particulate antigen" prepared as described by Rebers *et al.*, (1965), against *P. multocida* whole cell antiserum in agar gel. It gave positive reaction for tests for protein and carbohydrate and was highly immunogenic.

Other studies

Cytophilic and opsonin-adhering antibodies against *P. multocida* have been reported in the sera of vaccinated buffalo-calves. Highest titres were found in sera from vaccinated animals after challenge infection with *P. multocida* (P-52 str.) (Sarmah, 1976).

Cytophilic antibodies against *P. multocida* have also been produced in calf, rabbits, g. pigs and mice. Sera of these animals showed cytophilic antibodies to peritoneal macrophages and spleen cells of mice. Rabbits and mice sera did not show cytophilic antibodies for neutrophils of g. pigs by direct test but indirect test showed cytophilic antibodies. Treatment of mouse peritoneal macrophages with proteolytic enzymes viz. trypsin and papain increased the subsequent uptake of

cytophilic antibodies of late immune sera. An identical treatment did not sufficiently cause an increase in the uptake of early cytophilic antibodies and on the contrary there was a marked decrease in the uptake of these antibodies. These findings showed that the receptors of cytophilic antibodies are sensitive to proteolytic enzymes but are not sensitive to these enzymes for late cytophilic antibodies.

Biochemical study of the blood samples from buffaloes in the febrile stage of the disease revealed an increase in bilirubin and other bile salts, a decrease in cholesterol and changes in the pattern of serum proteins all consistent with hepatic insufficiency (Gupta *et al.*, 1977). A significant increase in platelet adhesiveness and plasma clotting time in sheep infected with *P. multocida* has been reported (Sara *et al.*, 1978). Further studies on the biochemical changes in the blood of affected animals will be helpful in throwing light on the cause of death in haemorrhagic septicaemia disease.

Various serological techniques have been used for typing of *P. multocida* isolates, with varying degree of sensitivity. Gupta (1962), while comparing various serological techniques, found haemagglutination inhibition test using crude polysaccharide extract as antigen to be highly specific, sensitive and free from cross-reactions. Baxi (1966) while typing 138 strains of *P. multocida* found indirect haemogglutination using alkaline autoclaved extract as antigen to be a most sensitive test.

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Discussion

Hiramune T. (Japan): In your paper you stated that you have isolated 4 strains of *Pasteurella multocida* belonging to 6:B group from poultry. 1) What organ were the strains isolated from? 2) Do you have any experience with experimental infection with 6:B strain in chickens? Can you indicate its pathogenicity in chickens?

Answer: 1) I did not make the isolations but I had serotyped three strains of poultry origin from the stock strains maintained at IVRI and one strain from an outbreak of chronic fowl cholera in Maharashtra State. The last isolate was from heart blood. 2) I am sorry. I have no experience of transmission of 6:B strains experimentally.

Gatapia S.L. (Philippines): You mentioned the production of toxins in *Pasteurella multocida*. Don't you think that it would be desirable to carry out studies on media to enhance toxin production for toxoid preparations in immunizing the animals against haemorrhagic septicaemia (HS)?

Answer: It is an aggressive factor and its production could be enhanced by more favorable media and growth conditions.

Watanabe M. (Japan): You mentioned that there are many carriers. Which factors cause the carriers to propagate the disease?

Answer: Animals become debilitated in the dry season (summer) and following the stress of inclement weather in the beginning of the monsoon they become more susceptible. The warm and humid climate is conducive to the multiplication and spread of organisms. At that time, the carriers transmit the infection to the in-contacts which then become ill and further spread the disease. The carriers are usually resistant to the disease. Also partial immunity can lead to the carrier status. Iridescent type of organisms which are harboured by animals with low resistance turn to the blue type which remains for a long time in the animal (carrier).

de Alwis M.C.L. (Sri Lanka) Comment: In Sri Lanka, the carriers have been found to harbour strains of the virulent type producing clinical signs. These strains disappear 6 weeks after the outbreak.

Hanafi M. (Indonesia): 1) What is the total vaccination coverage each year in India? 2) You mentioned that the oil-adjuvant vaccine gave the best results. However, why is it that you use it less than the others? 3) How about haemorrhagic septicaemia (HS) carriers among wild animals?

Answer: 1) The overall coverage is below 17% (bovine population: 200 million; doses of vaccine: 35 million). However, the overall coverage is irrelevant because vaccination is confined to endemic pockets where the disease is found. 2) The production of oil-adjuvant is very low because of the limitations associated with agar-wash method of production employed in India at present. Attempts are being made to adopt the growth in vortex tanks and continuous culture methods. 3) No authentic reports of wild animals as HS carriers are available, to my knowledge.