



### Experimental animals

Ten villages with the infection/outbreak of HS in Tandojam were randomly selected to study surveillance in order to estimate the prevalence. A total of n=4248 animals (2963 buffaloes and 1285 cattle) from these villages were further divided into young (below 1 year) and adult (above 1 year) animals both for buffalo and cattle. Each homestead was visited from door to door in all villages and the relevant information regarding the affected animals (morbidity, mortality and case fatality rates) was recorded on a questionnaire. For comparison purposes, 10 villages (control) free of infection in the same district were also surveyed.

**Table 1 - Comparative GMT values against HS in buffaloes and cattle**

Species	Group	Distribution of animals on basis of HS titer										GMT
		2 <sup>-0</sup>	2 <sup>-1</sup>	2 <sup>-2</sup>	2 <sup>-3</sup>	2 <sup>-4</sup>	2 <sup>-5</sup>	2 <sup>-6</sup>	2 <sup>-7</sup>	2 <sup>-8</sup>	2 <sup>-9</sup>	
Buffaloes	Affected	11	15	19	31	17	5	2	0	0	0	5.7
	Healthy	0	0	0	9	23	28	32	5	3	0	34.3
Cattle	Affected	8	11	24	22	25	7	3	0	0	0	6.1
	Healthy	0	0	2	11	19	31	29	8	0	0	32.0

### Blood collection and serological analysis

Blood samples (6 ml) without anticoagulant were collected from HS affected 100 buffaloes and cattle each. Matched samples from 100 healthy buffaloes and cattle each were also collected which served as control. Serum from each sample was decanted and stored at -20°C until analysis. Antibodies against *Pasteurella multocida* were measured by indirect haemagglutination test (IHA) using human blood group 'O' (Bain et al., 2002). Briefly, two fold dilutions of the test sera starting from 1:5 to 1:640 were made in normal saline using micro titer plates (96 wells) and added 25 µl amounts to all the wells of plate except those of column 11 and 12 which served as control. First four wells (A-D) of column 11 were added with known negative serum and last four wells (E-H) with the known positive serum. Sensitized RBC's (1%) were added in equal amounts (25 µl) to all the wells of the plate, so that column 12 served as control for the RBC's.

The plates were incubated at room temperature for two hours and the observations were recorded. Thereafter, the plates were kept re-incubated under refrigeration for overnight, shaken vigorously, allowed to resettle and were read again. Results were interpreted as positive (no bead formation) or negative (bead formation with sharp clear margins). The IHA antibody titers against HS were converted into geometric mean titers (GMT) for each group (Burgh, 2008).

### Statistical analysis

All data were analyzed by using Statistical Analysis System package software (SAS version 9.2, SAS Institute Inc., Cary, NC, USA). The animals (buffalo and cattle) were divided into two age groups i.e. young and adult. Data thus collected regarding mortality, morbidity and case fatality were subjected to Chi square analysis.

## RESULTS AND DISCUSSION

Hemorrhagic septicemia is a disease of utmost economic importance particularly in Asia where the susceptible animal population consists of 432 million cattle and 146 million buffaloes, which constitutes 30 and 95% of the world's cattle and buffalo population, respectively (FAO, 2016). In India, during the past four decades, HS is documented to be responsible for 45-55% of all bovine deaths. During the 18 years period from 1998 to 2016, it accounted for 58.7% of the aggregate of deaths due to five endemic diseases, viz. foot-and-mouth disease (FMD), rinderpest, black quarter, anthrax and HS (Dutta et al., 2016). In an active surveillance study in Sri Lanka, it was shown that in the 1990's, around 15% buffaloes and 8% cattle died of HS annually (Alwis and Vipulasiri, 2001). Similarly, 34.4% of all deaths in susceptible stock (Sheikh et al., 2016) and 31.48% mortality have been reported in buffalo calves (Khan et al., 2011) in Pakistan. The results of this study also clearly indicate that HS is a vital hurdle in the economic uplift of the livestock sector with high incidence rates and alarming morbidity, mortality and case fatality rates. In the present study, the comparative values of GMT against HS, deduced through IHA test, both for buffaloes and cattle are presented in table 1. It was noticed that the GMT value was 5.7 for affected buffaloes in comparison to 34.3 in healthy ones. Similarly, it was 6.1 in affected cattle in contrast to 32.0 in healthy ones. Hence, in diseased buffaloes, the titer was lesser as compared to diseased cattle making them more susceptible to the disease. These results are in line with the findings of Alwis et al. (2001) who have reported a higher GMT values for cattle as compared to those for buffaloes.

Similarly, the mean GMT values of 4.12 and 64.41 for affected and recovered animals have been reported by Khan et al. (2006). In the total population of 4248 animals from 10 infected/outbreak villages, the overall morbidity, mortality and case fatality rates were 17.39, 14.66 and 84.30%, respectively with buffaloes having significantly higher values as compared to cattle (Table 2). In buffalo population, the morbidity, mortality and case fatality rates were 22.30, 19.64 and 88.04%, respectively; however, for the cattle population, these values were 6.07, 3.19 and 52.56%, respectively. These results are in accordance with those of Alwis (2001), who documented overall mortality rate of 45.2 and 15.8% for buffaloes and cattle, respectively. Similarly, Sheikh et al. (2016) have also documented 9% mortality and 78% case fatality rates of HS in buffaloes, whereas these values were 2.5 and 62% in cattle. A mortality rate of 31% has been reported in buffaloes by Suhail et al. (2003) in North Waziristan Agency, Pakistan. Radostits et al. (2005) have reported that the overall mean case fatality for buffaloes is nearly three times as

high as in cattle. Buffalo has been considered the most susceptible animal to HS throughout the world with highest incidence, morbidity, mortality and case fatality rates. Perhaps, the genetic makeup of the buffalo makes it an ideal host for the causative parasite hence increasing its susceptibility to the disease. Young stock of both buffaloes and cattle was more affected as compared to the adult ones (Table 3). The morbidity, mortality and case fatality rates were significantly higher in young stock. In buffaloes, these values were 57.58, 52.30 and 90.83% for calves; and 3.17, 1.92 and 60.65%, for adult buffaloes, respectively.

Similarly, in case of cattle, the calves had the morbidity, mortality and case fatality rates of 8.63, 5.27 and 61.11%, respectively in comparison to the values of 4.83, 2.18 and 45.23% for adult cattle. These findings coincide with those of Khan et al. (2006), who have also reported that the young stock of both buffaloes and cattle have higher morbidity, mortality and case fatality rates as compared to the older ones. The exhaustion of the maternal immunity against HS after the 60th day of life and delayed vaccination might be attributed to the higher susceptibility of the young calves (Mahmood et al., 2007).

Species	Population (n)	Morbidity	Mortality	Case fatality
Buffalo	2963	661 (22.30%)	582 (19.64%)	79 (88.04%)
Cattle	1285	78 (6.07%)	41 (3.19%)	37 (52.56%)
Total	4248	739 (17.39%)	623 (14.66%)	116 (84.30%)
P Value		0.05	0.05	0.001

Species	Age	Population (n)	Morbidity	Mortality	Case Fatality
Buffalo	Young	1042	600 (57.58%)	545 (52.30%)	55 (90.83%)
	Adult	1921	61 (3.17%)	37 (1.92%)	24 (60.65%)
Cattle	Young	417	36 (8.63%)	22 (5.27%)	14 (61.11%)
	Adult	868	42 (4.83%)	19 (2.18%)	23 (45.23%)
P Value			0.20	0.70	0.50

## CONCLUSIONS

It can be concluded that HS is endemically occurring in cattle and buffaloes. Buffaloes are more susceptible to the disease as compared to the cattle. However, the young stock of both is highly affected in terms of morbidity, mortality and case fatality of HS as compared to the adult ones. It is highly recommended that department of livestock should give attention toward such disease in this area to control such huge economical loss.

## DECLARATIONS

### Competing interests

The authors have declared that no competing interest exists.

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### Author's contribution

Dr. Faiza Habib was the main researcher, Dr. Rehana Shah Nawaz was research coordinator, Dr. Azizullah Memon was study supervisor, Dr. Adnan Yousaf did all correspondence of this article, Dr. Adnan Jabbar and Dr. Muhammad Bilal Muhammad revised the article, Dr. Tahseen Jamil contributions in statistics, Dr. Rabia Khalil and Dr. Adeela Sharif assisted in results analysis and other activities related to the research.

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