

Hemorrhagic Septicemia in Buffalo (*Bubalus bubalis*) Calves Under Sub-Tropical Conditions in Pakistan

Ahrar Khan,^{1*} Muhammad Kashif Saleemi,¹ Muhammad Zargham Khan,¹ Shafia Tahseen Gul,¹ Muhammad Irfan¹ and Muhammad Shahbaz Qamar²

¹Department of Pathology, University of Agriculture, Faisalabad, Pakistan.

²Institute of Animal Nutrition and Feed Technology, University of Agriculture, Faisalabad, Pakistan

Abstract.- In an outbreak of hemorrhagic septicemia in buffalo calves in Pakistan, 31.48% mortality was recorded. Mortality significantly peaked on 8th day (37%). Elevated body temperature (40.63±0.70°C), profuse salivation, dyspnea, respiratory sound “rales”, ocular discharge, mucus nasal discharge and restlessness were observed. Grossly, head, neck and inter-mandible region were congested and edematous. Subserosal petechial hemorrhages throughout the body and blood-tinged fluid in thoracic and abdominal cavities were seen. Lungs showed pneumonic changes. Kidneys were swollen and hyperemic. There were petechial hemorrhages on the epicardium. Histopathologically, extensive lung edema, emphysema, thickened alveolar septa, congestion, and edema fluid in bronchioles were observed. Kidneys exhibited glomerulus atrophy, congestion, presence of casts in tubules along with pyknosis, condensation of nuclei and necrosis of tubular epithelium.

Key words: Hemorrhagic septicemia, buffalo calves, morbidity, mortality.

INTRODUCTION

Black gold, the buffalo (*Bubalus bubalis*) is known as the world’s second most important milch animal as it shares more than 95% of the milk produced in the South Asia (Javaid *et al.*, 2009). There are about 29.9 million heads of buffaloes in Pakistan (Afzal, 2010); out of these, 76.7% belong to Nili-Ravi breed, which is the most popular of buffaloes in Pakistan (Khaliq and Rahman, 2010).

Calves play an important role in the development of the dairy sector, as the future of the dairy herd depends upon the successful raising of young calves. Female calves are especially kept for herd replacement. The male calves are usually kept up to weaning (Ahmad *et al.*, 2009). Calf-care is not only essential for sustenance of the dairy industry but is also essential for preserving and maintaining good quality germ plasm, however, some of diseases such as hemorrhagic septicemia, foot and mouth disease and brucellosis threaten health very badly in Pakistan. There is dire need to launch national program for the control and eradication of such frequently prevalent diseases (Afzal, 2009).

Hemorrhagic septicemia (HS) is an acute and often fatal disease primarily occurring in water buffaloes and cattle, but occasionally other domesticated and wild mammals (De Alwis, 1992). Buffaloes are more susceptible to HS than cattle (De Alwis, 1999). Hemorrhagic septicemia is caused by the gram-negative bacterium *Pasteurella multocida* (Wijewardana, 1992; Tabatabaei *et al.*, 2007). So far, various serotypes (A, B, C, D and E) of *P. multocida* have been detected among the livestock population (Ghandrasekaran *et al.*, 1981; Borkowska-Opacka and Kedrack, 2003; Kedrak and Borkowska-Opacka, 2003; Kumar *et al.*, 2004). Serotypes B: 2 and E: 2 are two common serotypes of *P. multocida* associated with disease in animals in Asia and Africa, respectively (Benkirane and De Alwis, 2002). The disease has a major impact on the livestock industry in countries of South and Southeast Asia, where HS associated with serotype B: 2 is distributed widely (Dagleish *et al.*, 2007; Hajikolaei *et al.*, 2008; Ataei *et al.*, 2009). It results in severe economic losses and is ranked as the most important contagious disease of cattle and buffaloes (Benkirane and De Alwis, 2002), with young animals being most at risk of successive outbreaks (De Alwis and Sumanadasa, 1982).

Importance of HS to buffalo rearing in Pakistan is highlighted by a recent survey which indicated its prevalence up to 49% (Farooq *et al.*,

* Corresponding author: ahrar1122@yahoo.com
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2007). Pakistan, being a sub-tropical region of South Asia has an early summer dry hot environment temperature and late summer is hot and humid, such conditions favor the explosive occurrence of HS (De Alwis, 1999). This manuscript deals with such an outbreak which took place at a farm in buffalo calves.

MATERIALS AND METHODS

Study area

Faisalabad, third largest city of Pakistan is situated at latitude 31° – 26' N, longitude 73° – 06' E and altitude 184.4m. It has long summer episode that extends from May to September. In summer, ambient temperature ranges from 30 to 45°C and even sometimes may go to 48°C. A severe hot and humid (humidity 45.38±13.11%) period begins from mid July and extended up to mid September (Fiaz *et al.*, 2010).

Case history and treatment

At Livestock Experiment Station, University of Agriculture, Faisalabad, a urea feeding trial started in June 2009 on 54 buffalo calves with an average age 6-11 months. These calves were kept on 75% Vanda (concentrate diet) and 25% wheat straw treated with 4% urea. Overall dry ration was having 16% protein contents. Each calf was vaccinated with NIAB (Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan) HS oil based vaccine @ 2 ml subcutaneous in mid June 2009. In August and September, 2009, weather became harsh with environmental temperature (28-45°C) and humidity 65.8%. Suddenly in September, all the calves became ill (morbidity 100%) and started showing signs of hemorrhagic septicemia. The morbid calves were treated with injections of Triox (Amoxicillin LA 150 mg/ml; Orient Animal Health, Karachi, Pakistan), Excenel (Ceftiofur Sodium – 50 mg/ml; Pfizer Inc, Pakistan), Predicort (Prednisolone Acetate 7.5mg/ml + Dexamethasone sodium phosphate 2.5 mg/ml; S J & G Fazal Ellahi Sons, Lahore, Pakistan) and Vetafenic (Meloxicam 7.5 mg/ml; Diclofenac sodium; S J & G Fazal Ellahi Sons, Lahore, Pakistan) for three days.

Gross pathology and histopathology

When mortality started it was thought that it

is due to urea toxicity. Determination of serum urea concentration of morbid animals revealed that it is within normal range. Moreover, lesions were also not supportive of urea toxicity.

All the dead animals were subjected to postmortem examination. Morbid tissues were collected, preserved in 10% neutral buffered formalin and processed for histopathological studies using routine method of paraffin embedding. Sections of 5 µm thickness were stained with hematoxylin and eosin for microscopic study.

Data analysis

For analysis purpose, calves were divided in three age groups, *i.e.*, 6-7, 8-9 and above 9 months. Data thus collected on various clinical signs in relation to morbidity and mortality, mortality in relation to age and postmortem lesions were subjected to Chi square analysis.

RESULTS

Clinical signs, morbidity and mortality

All the calves became morbid (100%) and started showing signs of hemorrhagic septicemia. Out of 54 buffalo calves, 17 died (31.48%). Of the dead calves, more calves (47.05%) belonged to 8-9 months followed by above nine months (29.41%) and 6-7 months (23.53%) ($\chi^2 = 1.111$; $P = 0.574$). Course of the disease spread over nearly ten days. Mortality significantly peaked at 8th day where it was 37% (Fig. 1).

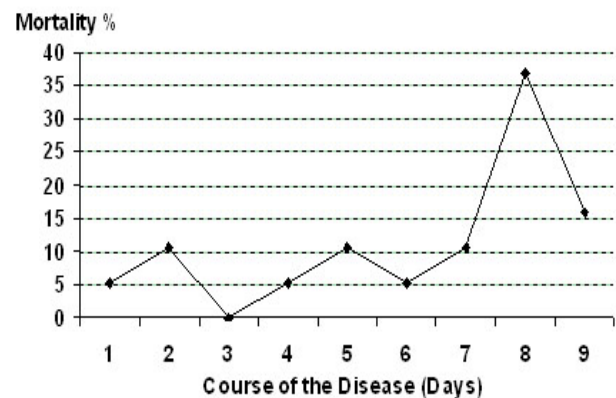


Fig. 1. Mortality of buffalo calves during the course of hemorrhagic septicemia.

Frequency of clinical signs in morbid animals varied significantly ($\chi^2=32.357$; $P=0.001$), however, when these clinical lesions analyzed in calves those died later showed non-significant variation ($\chi^2=3.190$; $P=0.956$). Elevated body temperature ($40.63\pm 0.70^\circ\text{C}$) in 18/54 (33.3%) calves whereas moderately ($38.61\pm 0.50^\circ\text{C}$) and slightly ($37.85\pm 1.75^\circ\text{C}$) raised body temperature was recorded in 22 (40.74%) and 14 (25.92%) affected buffalo calves. Affected calves showed variable clinical signs. All the calves were off feed, depressed and showing restlessness. Respiratory sounds “rales” were present in 90.74% calves. Other clinical signs included were profuse salivation, dyspnea, mucoid nasal discharge, tympany, etc. Respiratory sounds, dyspnea, mucous nasal discharge, high temperature, reduced appetite, restlessness, profuse salivation were present in 100% calves those died of HS (Table I).

Table I.- Clinical signs observed in morbid and died buffalo calves due to hemorrhagic septicemia.

Clinical Signs	Morbid calves (n=54)		Died calves (n=17)	
	No	%	No	%
Reduced appetite	54	100	17	100
Restlessness	54	100	17	100
Respiratory sounds	49	90.74	17	100
Profuse salivation	43	79.63	17	100
Dyspnea	43	79.63	17	100
Lethargicness	43	79.63	13	76.47
Rough hair coat	32	59.26	10	58.82
Mucous nasal discharge	32	59.26	17	100
Tympany	21	38.89	13	76.47
High temperature	18	33.33	17	100
Ocular discharge	10	18.52	10	58.82
χ^2	32.357		3.190	
Df	9		9	
P value	0.001		0.956	

Gross and histopathology

At necropsy, edema of head, neck and inter-mandible region, congested trachea having froth, swollen, congested and consolidated lungs exhibiting picture of bronchopneumonia and lymphadenitis were present in all carcasses of calves died of HS (Table II). Grossly head, neck and inter-

mandible region were congested and swollen/edematous (Fig. 2A). This edematous swelling consisted of a coagulated serofibrinous mass (Fig. 2B). Subserosal petechial hemorrhages were present throughout the body. Thoracic and abdominal cavities contained blood-tinged fluid. Scattered petechial hemorrhages were visible in the tissues and lymph nodes, particularly in the pharyngeal and cervical lymph nodes. These nodes were swollen and hemorrhagic. Trachea was congested and contained froth (Fig. 2C). Kidneys were swollen and hyperemic. Liver seemed to be normal. There were petechial hemorrhages on the epicardium.

Table II.- Frequency of gross lesions observed in buffalo calves died of to hemorrhagic septicemia.

Gross Lesions	Frequency	
	No	%
Head, neck and inter-mandible region		
Edema	17	100
Congestion	13	76.47
Trachea		
Congested	17	100
Froth	17	100
Pneumonic Lungs	17	100
Lymphadenitis	17	100
Subserosal petechial hemorrhages	15	88.23
Hydrothorax	15	88.23
Hemorrhages on Epicardium	14	82.35
Hydroperitonium	13	76.47
Kidneys		
Swollen	13	76.47
Hyperemic	13	76.47
χ^2	1.304	
df	11	
P value	1.000	

Histopathological examination revealed extensive edema fluid accumulation in alveoli along with emphysema (Fig. 3A). Thickened alveolar septa, congestion and edema fluid in bronchioles were also observed (Fig. 3B). Mononuclear cells were infiltrating in the lung tissue and blood vessels contained rod shaped bacteria. Kidneys showed variable degrees of degeneration. Glomeruli were atrophied and nearly all the renal tubules were studded with casts. Tubular epithelium showed pyknosis, condensation of nuclei and necrosis (Fig. 3C). Congestion was also obvious.



A

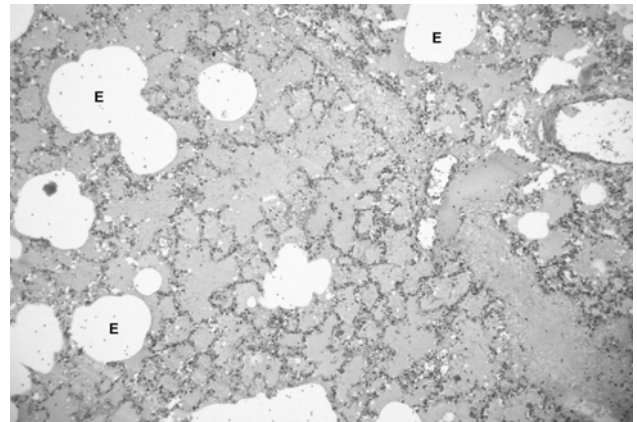


B

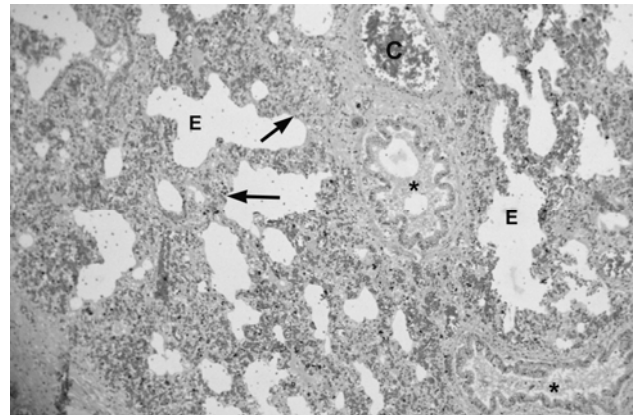


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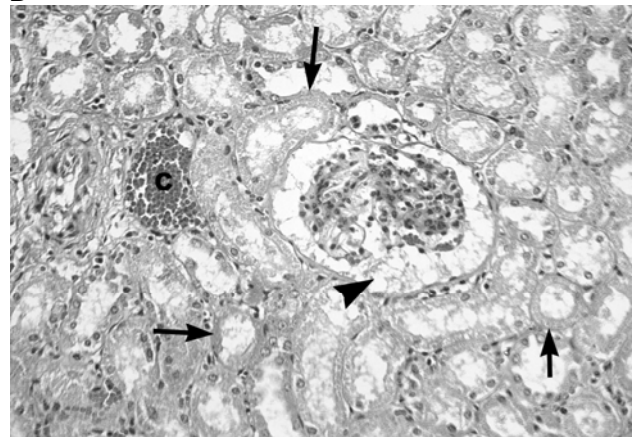
Fig. 2. Buffalo calf died of hemorrhagic septicemia showing mandibular and neck region edema and redness. Note the coagulated serofibrinous mass having straw-colored fluid (arrow) in B and congested trachea of buffalo calf containing froth in C.



A



B



C

Fig. 3. Histological structure of lungs of buffalo calf which died of hemorrhagic septicemia showing A) fluid filled alveoli (edema) and emphysema (E), B) exudate in bronchioles (*), thickened alveolar septa (arrows), congestion (C), edema, hemorrhages and emphysema (E), and C) atrophied glomerulus (arrow head), congestion (C), necrosis of tubular epithelium (arrows) and cast deposition in tubular lumens. Stain: H and E. Magnification: A and B 100X; C, 400X.

DISCUSSION

Outbreaks of HS usually occur as catastrophic epizootics in many Asian and African countries resulting in high mortality and morbidity (Bain *et al.*, 1982; De Alwis, 1992). Outbreaks of HS has been recorded in Sri Lanka (De Alwis and Vipulasiri, 1980), Zimbabwe (Lane *et al.*, 1992), South Asia, Middle East and Africa (FAO, 1989), Pakistan (Khan *et al.*, 2006; Farooq *et al.*, 2007). A wide range of mortality rate (5 to 90%) has been reported in different outbreaks in India (Saini *et al.*, 1991) and Philippines (Molina *et al.*, 1994). In the present study, 100% morbidity and 31.48% mortality due to HS has been recorded in buffalo calves. In cattle and buffaloes, morbidity and mortality due to HS has been recorded to be 95.25 and 21.19%, respectively (Khan *et al.*, 2006). This high mortality could be attributed to its acute and sometimes per acute clinical nature which does not allow treating the animals.

HS is an acute, fatal, septicemic major disease of cattle and buffaloes (Farooq *et al.*, 2007) caused by the gram-negative bacterium *Pasteurella multocida* (Tabatabaei *et al.*, 2007). Serotypes B: 2 and E: 2 are two common serotypes of *P. multocida* associated with disease in animals in Asia and Africa, respectively (Benkirane and De Alwis, 2002). The disease has a major impact on the livestock industry in countries of South and Southeast Asia, where it causes severe economic losses and is ranked as the most important contagious disease of cattle and buffaloes (Benkirane and De Alwis, 2002).

Major control measure of HS is the vaccination (Benkirane and De Alwis, 2002). In the present study, in spite of vaccination with HS oil based killed vaccine; outbreak of HS took place in buffalo calves. Current vaccines are administered parenterally, require repeated administration, and are not sufficiently efficacious (Tabatabaei *et al.*, 2007). In the present study, there could be vaccine failure or vaccination was not carried out sufficient time before the onset of high-risk season (monsoon) as vaccination of HS for prophylactic purpose must be carried out preferably two to three months before the season (Benkirane and DeAlwis, 2002). It is also recommended that during an outbreak, one should

resort to immediate whole herd vaccination, irrespective of previous vaccination history (Benkirane and De Alwis, 2002). This strategy also failed in the present outbreak as mortality reached to 31.48%. A live-attenuated vaccine, which would mimic the early stages of the natural infection, might be expected to confer more solid and long-term protective immunity (Tabatabaei *et al.*, 2007).

Hot and humid weather is a major contributory factor in the outbreak of HS. High environmental temperature (37°C) does favor the growth of the bacteria (Hajikolaie *et al.*, 2008). Weather also poses stress on young calves. Scarcity of green fodder in these days is another stress factor.

HS is a bacterial disease and, theoretically, could be effectively treated by wide range of antibiotics currently available. However, treatment is constrained by a host of practical considerations. According to Benkirane and De Alwis (2002), animals can be cured only if they have been treated in the very early stage of the disease. Under field conditions this is not possible, however, in organized farms early detection and effective treatment is possible. Penicillins are widely used in the treatment of HS because the causative organism is sensitive to this β -lactam antibiotics (Kristinsson and Adam, 2007; Pedersen *et al.*, 2009). In the present study, amoxicillin was used as a major drug for treatment and 68.52% HS morbid calves could be recovered as a result of treatment. *P. multocida* are resistance to streptomycin, tetracycline, oxacillin and trimethoprim (Wassenaar and Silley, 2008), therefore, while treating HS case such drugs be avoided.

Early treatment depends on the early diagnosis of the disease (De Alwis, 1999). Under field conditions, HS is usually diagnosed on the basis of clinical signs and symptoms (Khan *et al.*, 2006; Farooq *et al.*, 2007; De Alwis, 1999). Most cases of HS in cattle and buffalo are acute or peracute (Anonymous, 2003). It is obvious from the clinical signs recorded in the present study that respiratory sounds, dyspnea, mucous nasal discharge, high temperature, reduced appetite, restlessness profuse salivation were present in 100% calves died of HS (Table I). Such clinical signs with variable degree have already been reported along with distinctive respiratory sound "rales", and

typical swellings in the submandibular region in HS cases (Sheikh *et al.*, 1996; De Alwis, 1999; Farooq *et al.*, 2007).

Pasteurella multocida remains as commensal in bronchi, terminal bronchioles, and alveoli. This pathogen cannot invade lungs due to defense mechanism but stresses as climatic change, malnutrition, transport, etc. trigger the organism and lungs are unable to clear the pathogens (Harper *et al.*, 2006). Organisms are proficient in destructing bovine blood mononuclear leukocytes and lung macrophages. Pharmacologically active substances like histamine, prostaglandins, hyaluronidase, chondroitinase (inflammatory substances) as well as fibro-elastic elements are released with the death of the macrophages (Carter and Chengappa, 1980; Rimler and Rhoades, 1994). Other than release of these active substances, organism leads to septicemia which has the most severe effect in the respiratory tract, heart and gastro-intestinal tract (Radostits *et al.*, 2007). Trachea, part of respiratory is large enough in animals that travels along the neck where acute inflammatory reaction takes place as a result of released inflammatory substances which in return lead to development of submandibular edema (Harper *et al.*, 2006; Shafarin *et al.*, 2009). The changes produced in the lungs comprises of bronchopneumonia which appear as consolidated lungs (Shafarin *et al.*, 2009) that in return produces high bronchial/respiratory sounds (rales). Other than the release of pharmacologically active substances, there is another school of thought in the development and consequences of HS that the *Pasteurella multocida* produces endotoxins which are responsible for all manifestations (Horadagoda *et al.*, 2001; Zafar *et al.*, 2010). Death of the animals occurs due to hypoxia and toxemia (Khin *et al.*, 2010).

At necropsy, it was observed in the present study that edema in head, neck and inter-mandible region, congested trachea having froth; pneumonic lungs and lymphadenitis were present in all carcasses of calves died of HS (Table II). Such type of lesions has been reported (Sheikh *et al.*, 1996; De Alwis, 1999; Benkirane and De Alwis; 2002; Farooq *et al.*, 2007). HS also leads to fatal peritonitis in calves (Catry *et al.*, 2005).

It can be concluded from this study that HS is

highly fatal disease, can provisionally be diagnosis based on a combination of clinical signs, gross pathological lesions and a consideration of hot and humid season. Treatment of the affected cases is only effective when treated at an early stage.

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