



Epidemiological, clinical and hematobiochemical studies on hemotoxic snakebite in bovines

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ABSTRACT

Snakebite is a conflict between venomous snakes and humans as well as livestock and considered as neglected tropical disease. The present study aimed to investigate the hospital prevalence, clinical and hemato-biochemical aspects of hemotoxic snakebite in bovines. The overall hospital prevalence of hemotoxic snakebite in bovines was found to be 0.93%. Highest prevalence of hemotoxic snakebite in bovines was observed in August month during monsoon season in female bovines with higher cases in buffaloes. The highest occurrence was reported in native Marathwadi buffalo, non-descript and Deoni cattle of > 4 years and 1-4 years age group in forelimbs and hindlimbs during morning and evening hours. Ascending swelling over limbs extending upward and lameness in case of bite on limbs while asymmetrical swelling in case of bite over face, occasional signs of bleeding like melena, hematuria, epistaxis and bleeding from the site of bite were the prominent clinical signs observed in hemotoxic snakebite affected bovines. Highly significant increase in heart rate and respiration rate, while decrease in rumen motility was observed in snakebite affected bovines. Hemato-biochemical analysis showed highly significant increase in leukocyte count, erythrocyte count and PCV, significant increase in granulocyte and monocyte count, highly significant decrease in platelet count while increase in capillary blood clotting time, bilirubin, blood urea nitrogen, creatinine, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and creatine kinase levels compared to healthy bovines.

Keywords: Bovine, Envenomation, Hemato-biochemistry, Hemotoxic, Snakebite, Venom

Since ancient times, snakes are being considered as deadliest enemies of both human and animals due to their highly venomous nature and considered as a well-known veterinary emergency (Singh 2002). Snakebite is an occupational disease of agricultural workers, farmers, cattle herders (Gutierrez *et al.* 2017). Snakebite is way more neglected in animals and data on bovine snakebite is scarce in India. The venomous snakebite can be categorized as neurotoxic, myotoxic, hemotoxic or cytotoxic depending on the species of snake involved and the kind of venom it produces (Slagboom *et al.* 2017). Russell's viper, Saw scaled viper, as well as 15 different species of pit vipers in India are known to cause hemotoxicity (Menon and Menon 2015). The hemotoxic effects in viper bites are attributed to the activation of factor V, inhibition of factor X and sometimes activation of factor X, activation of prothrombin, inhibition of thrombin, fibrinolysis, activation of plasminogen and inhibition of plasmin and also causes inhibition or aggregation of platelets (Slagboom *et al.* 2017).

Hemotoxic snakebite in cattle is characterized by the progressive ascending type of swelling and lameness in affected limbs, asymmetrical swelling of the face with dyspnea in case of bite over face. Increased bleeding tendencies have been reported along with increased blood clotting time in hemotoxic snakebites (Goddard *et al.* 2011, Rodriguez *et al.* 2016, Bhikane *et al.* 2020). Diagnosis of snakebite is difficult in livestock and criteria like definite evidence of snakebite by owner, jumping out of fear or pain while grazing in dense grassland followed by the onset of signs of local bleeding at the site of bite, ascending swelling on bitten limb, lameness in affected limb could be used in the diagnosis of hemotoxic snakebite (Goddard *et al.* 2011, Bhikane *et al.* 2020). Venomous snakebite in bovines causes economic losses due to loss of production, expenditure on treatment and mortality in affected animals (Bhikane *et al.* 2020). Owing to considerable prevalence of hemotoxic snake envenomation in bovines in study area, the present study was planned with objective to study the epidemiological, clinical and hemato-biochemical aspects of hemotoxic snakebite in bovines.

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MATERIALS AND METHODS

The present study was conducted in the Veterinary

Clinical Complex and Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, College of Veterinary and Animal Sciences, Udgir, district Latur from November 2020 to October 2021. The clinical cases reported from Latur, Parbhani and Nanded districts of Marathwada region of Maharashtra state and Bidar district of Karnataka state with clear history of snakebite or history of sudden excitation while grazing followed by unilateral ascending swelling were screened for hemotoxic snakebite. All the hemotoxic snakebite affected bovines (n=36) were enrolled in diseased group and 12 disease free bovines were enrolled to healthy group. The prevalence of hemotoxic snakebite was estimated based on data related to age, breed, sex, month, season, feeding patterns and management practices.

Vital parameters like body temperature, heart rate, respiration rate, rumen motility as well as clinical parameters of affected animals were recorded. About 2 ml of whole blood samples collected in EDTA vials from study animals were subjected to complete blood count analysis on automated hematology analyser (Diatron Abacus Junior Vet 3.11). About 2 ml of whole blood was collected in clot activator tube and harvested serum samples were subjected to analysis of biochemical parameters like total bilirubin, direct bilirubin, indirect bilirubin, blood urea nitrogen, creatinine, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and creatine kinase using standard diagnostic kits manufactured by Span Diagnostics, Surat, Gujarat on semi-automated biochemical analyser (Star 21 plus Semiautomatic Biochemistry Analyser from Rapid Diagnostic Pvt. Ltd. Delhi). Additionally, whole blood was collected in anti-coagulant free, plain glass capillaries for estimation of blood clotting time. The data generated from hemotoxic snakebite affected and healthy bovines were analysed with student 't' test using IBM SPSS software version 20.

RESULTS AND DISCUSSION

Over a study period of November 2020 to October 2021, total 36 cases of bovines suffering from hemotoxic snakebite were confirmed out of 3889 bovines admitted to college hospital, indicating overall prevalence rate of 0.93%. Month-wise highest number of cases of hemotoxic snakebite were observed in August (27.78%) followed by September and December (13.89% each), July (11.11%) while least cases were observed in January (8.33%), November, April, June and October (5.56% each), May (2.78%) with no cases of snakebite reported in months of February and March. Highest prevalence of hemotoxic snakebite in bovines was observed during monsoon (June to September) (58.33%) season followed by winter (December to February) (22.22%), post-monsoon (October to November) (11.11%) and least in summer (March to May) (8.33%) season. Suraweera *et al.* (2020) recorded that 10% of cases of snakebites in human beings in India occurred during the rainy season. Glaudas (2021) observed that *Daboia russelii* (Russell's viper) specimens implanted with radio-transmitters for the purpose of radio-telemetry

were least active during March. The findings of seasonal prevalence of snakebite in bovines are in agreement with Bhikane *et al.* (2020) who recorded highest occurrence of viperine snakebite in zebu cattle during monsoon (58.16%) followed by post-monsoon (24.48%) and winter (9.18%) while least cases were reported during summer (8.16%). The high occurrence of snakebite during monsoon, winter and post-monsoon season might be attributed to growth of dense vegetation and shrubs in grazing land during the same period as well as hot and humid climate during monsoon and post monsoon season.

The prevalence of hemotoxic snakebite was higher in female (69.45%) bovines as compared with male (30.55%). Adult male cattle are frequently used for draught work in the study area. On the contrary, the buffalo population is primarily maintained for milk production and hence automatically, the population of male buffalo in study area is very less, with mostly young male below 1 year of age and few breeding bulls. The high occurrence of snakebite in female bovines in the present study might be attributed to higher number of she buffaloes in the study area and no use of male buffaloes for draught work. Highest occurrence of hemotoxic snakebite was observed in buffaloes (61.11%) followed by cattle (38.89%). Highest breed-wise occurrence of hemotoxic snakebite in cattle has been observed in non-descript (47.86%) cattle followed by Deoni (35.71%) and least (14.29%) in Red Kandhari cattle. Similarly, highest breed-wise occurrence of hemotoxic snakebite was observed in Marathwadi (45.45%) buffaloes followed by non-descript (31.82%) and least in Murrah (22.73%) buffaloes. Bhikane *et al.* (2020) observed highest incidence of viperine snakebite in zebu cattle as compared to crossbred cattle owing to extensive rearing practices in indigenous animals as opposed to intensive rearing followed in crossbred cattle. Highest occurrence of hemotoxic snakebite in Marathwadi buffalo breed, non-descript cattle and Deoni cattle might be attributed to study area being native track of the Marathwadi buffalo and Deoni cattle.

The highest number of cases of hemotoxic snakebite were observed in bovines above 4 years of age (58.33%) followed by bovines in 1-4 year age group (41.67 %) while no case was reported in bovines below 1-year age group. In the study area, young animals below 1-year age are not allowed to go out for grazing which could be the reason for young animals not being affected by snakebite. The findings of present study are in agreement with Bhikane *et al.* (2020) who reported highest number of snakebites in cattle aging > 4 years (69.38%) followed by 1-4 years (27.53%) and least in cattle < 1-year age (3.06%). The bovines below 1-year age are generally kept indoors while adult bovines go outdoors for grazing and draught work thereby increasing the possibility of snakebite in adult bovines. In the present study, all the affected animals were allowed to graze in open pasture while no animal was completely stall fed.

Highest number of cases of hemotoxic snakebite in bovines were observed on forelimbs (44.44%) followed

by hind limbs (36.11%) and least in head (19.44%) region. The findings of the present study are in agreement with those of Bhikane *et al.* (2020) who observed highest number of snakebites on forelimbs (48.97%) followed by hind limbs (33.67%), head (15.30%) while few cases over tail or scrotum. Bolon *et al.* (2021) also observed highest incidence of snakebite on limbs of animals from Nepal and Cameroon. Highest occurrence of hemotoxic snakebite was observed in pregnant (38.88%) bovines followed by male bovines (30.55%), lactating bovines (22.22%) and least in heifers (8.33%). Literature on prevalence of hemotoxic snakebite in bovines with respect to stage of pregnancy or lactation is not available hence it is difficult to justify the findings of present study.

Highest number of hemotoxic snakebites in bovines occurred during morning (7 AM to 12 PM) hours (50%) followed by evening (4 PM to 9 PM) hours (47.22%), while least snakebites were observed during afternoon (12 PM to 4 PM) hours (2.78%). The findings of the study are in agreement with those of Bhikane *et al.* (2020) who observed that most of the viperine snakebites in zebu cattle took place during early morning (28.75%) and evening (26.53%) hours followed by afternoon (18.36%) while least bites were recorded during night (7.14%). The maximum occurrence of viper and cobra bites in India occur during the day or early darkness while watering the plantation or walking barefoot in grown grass or soybean crops (Sinha and Sharma 2018). The high occurrence of snakebite during early morning hours and evening hours might be attributed to snake movements during predisposed time in



Fig. 1. Ascending edematous swelling over left hind limb in Deoni cow suffering from hemotoxic snakebite.

the dense grassland.

The values (mean±S.E.) of vital parameters, hematological and biochemical parameters have been depicted in Table 1. The prominent clinical signs observed in snakebite affected bovines were ascending swellings over bitten limbs (Fig. 1) with lameness, extension of swelling

Table 1. Values (Mean±S.E.) of vital parameters, hematology and biochemistry in hemotoxic snakebite affected and healthy bovines

Parameter	Affected (n=27)	Healthy (n=12)	't' value
Heart rate (bpm)	67.41±2.63	56.33±1.03	3.919**
Respiration Rate (/minute)	28.96±1.66	21.83±0.41	4.174**
Rectal Temperature (°F)	101.37±0.31	101.43±0.11	-0.203 ^{NS}
Ruminal motility (/minute)	0.05±0.02	0.65±0.49	-12.289**
RBC ($\times 10^{12}/L$)	6.74±0.56	5.33±0.31	2.193**
Hb (gm/ dL)	10.69±0.36	9.63±0.55	1.635 ^{NS}
PCV (%)	30.20±1.34	26.04±1.49	-6.299**
WBC ($\times 10^9/L$)	12.50±1.23	9.73±0.56	2.051**
Granulocyte ($\times 10^9/L$)	5.29±0.64	3.99±0.33	1.816*
Monocyte ($\times 10^9/L$)	1.31±0.35	0.59±0.06	1.881*
Lymphocyte ($\times 10^9/L$)	51.06±4.82	52.45±3.18	-0.297 ^{NS}
Platelet count ($\times 10^9/L$)	25.04±5.12	194.0±26.33	-6.299**
BCT (min)	25.07±1.59	6.25±0.49	11.296**
Total bilirubin (mg/ dL)	1.26±0.07	0.69±0.04	7.180**
Direct Bilirubin (mg/ dL)	0.58±0.04	0.35±0.05	-3.789**
Indirect bilirubin (mg/ dL)	0.69±0.05	0.35±0.08	5.671**
BUN (mg/ dL)	38.52±2.41	5.52±0.63	13.425**
Creatinine (mg/ dL)	1.91±0.13	1.11±0.19	3.168**
AST (U/ L)	135.48±3.31	75.17±3.20	13.101**
ALT (U/ L)	55.93±1.81	12.49±0.90	21.513**
ALP (U/ L)	231.89±5.92	31.24±2.80	30.621**
CK-MB (U/ L)	375.04±36.43	41.42±4.84	9.0780**

NS, Non-significant; *, Significant ($P<0.05$); **, Highly significant ($P<0.01$).

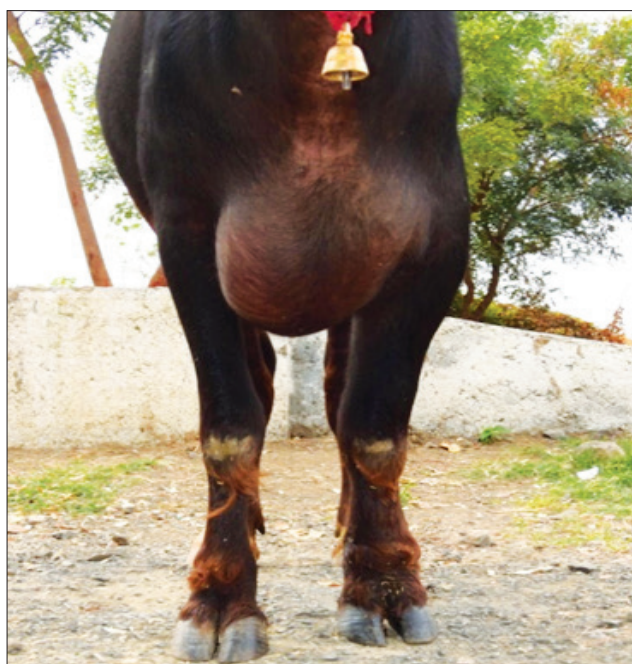


Fig. 2. Ascending edematous swelling over left forelimb extending towards brisket in buffalo suffering from hemotoxic snakebite.

towards brisket (Fig. 2) has been observed in case of bites on forelimbs, difficulty in sitting, asymmetrical swelling in case of bite over face with dyspnea (Fig. 3), anorexia and occasional signs of melena (Fig. 4), hematochezia, epistaxis, hematuria etc. Similar clinical signs were also



Fig. 3. Asymmetrical swelling with respiratory distress in buffalo bitten by hemotoxic snake on face.

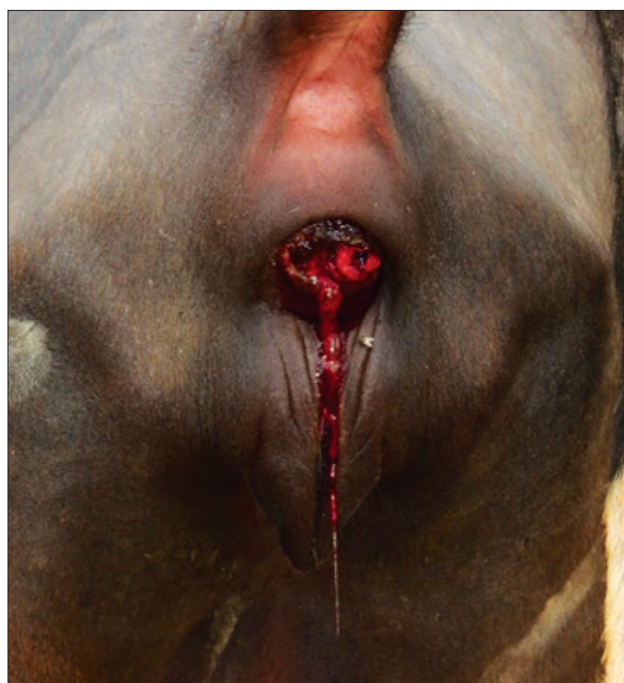


Fig. 4. Frank melena in buffalo suffering from hemotoxic snakebite.

observed in viper bite affected cattle by Altug and Isler (2019), Bhikane *et al.* (2020) and Jadhav *et al.* (2021). Out of 36 cases of hemotoxic snakebite, typical fang marks were visible in only one case (2.78%). Clinical signs like reduced appetite, dullness and depression were observed in all the ailing animals. Highly significant ($P < 0.01$) increase in heart rate (67.41 ± 2.63 bpm vs. 56.33 ± 1.03 bpm) and respiration rate (28.96 ± 1.66 /min vs. 21.83 ± 0.41 /min), while decrease in rumen motility (0.05 ± 0.02 /min vs. 0.65 ± 0.49 /min) was observed in snakebite affected bovines as compared to healthy bovines. Neurological signs were not observed in any case of hemotoxic snakebite in the present study.

Highly significant ($P < 0.01$) increase in RBC (6.74 ± 0.56 vs $5.33 \pm 0.31 \times 10^{12}$ /L) and PCV (30.20 ± 1.34 vs $26.04 \pm 1.49\%$) was observed in bovines affected with hemotoxic snakebite compared with normal healthy counterparts. The occurrence of hemoconcentration in cases of hemotoxic snakebite might be attributed to capillary leak syndrome leading to the loss of fluids in the form of edema and catecholamine induced splenic contraction induced by pain and stress (Goddard *et al.* 2011). Highly significant ($P < 0.01$) increase in WBC count (12.50 ± 1.23 vs $9.73 \pm 0.56 \times 10^9$ /L) while significant ($P < 0.05$) increase in granulocyte count (5.29 ± 0.64 vs $3.99 \pm 0.33 \times 10^9$ /L) and monocyte count (1.31 ± 0.35 vs $0.59 \pm 0.06 \times 10^9$ /L) was observed in bovines suffering from hemotoxic snakebite as compared to healthy bovines. Altug and Isler (2019) reported granulocytosis (8.58×10^9 /L and 14.67×10^9 /L), leukocytosis (16.84×10^9 /L and 14.67×10^9 /L) and monocytosis (0.95×10^9 /L and 0.73×10^9 /L) in two cases of viperine snake envenomation in cattle. Bhikane *et al.* (2020) observed leukocytosis ($12.78 \pm 0.72 \times 10^9$ /L), neutrophilia

(68.36±3.27%) and lymphopenia (29.07±3.39%) in zebu cattle suffering from viperine snake envenomation. Increase in leukocyte and neutrophil count in snakebite has been attributed to acute inflammatory response. However, variability between differential leukocyte counts can be attributed to variability in snake venom composition between different genera of snakes, different species of snakes, and different bio-geographical distribution of same species of snake as well as different age group of snakes (Goddard *et al.* 2011, Casewell *et al.* 2014, Slagboom *et al.* 2017, Laxme *et al.* 2021).

Highly significant ($P<0.01$) decrease in platelet count (25.04 ± 5.12 vs $194.0\pm26.33\times10^9/L$) was observed in the bovines suffering from hemotoxic snakebite as compared to normal healthy counterparts. Bhikane *et al.* (2020) observed thrombocytopenia ($27.35\pm6.71\times10^9/L$) in zebu cattle suffering from viperine snake envenomation. Thrombocytopenia in animals and humans with moderate to severe viper envenomation has been attributed to effects of venoms injected through initiation of pathological cascades of vasculitis, sequestration of platelets in inflamed tissue and platelet consumption with development of disseminated intravascular coagulation (Goddard *et al.* 2011). Highly significant ($P<0.01$) increase in blood clotting time (25.07 ± 1.59 min vs 6.25 ± 0.49 min) was observed in bovines suffering from hemotoxic snakebite in comparison to healthy bovines. Rodriguez *et al.* (2016) observed prolonged clotting time >60 min in experimentally induced *Bothrops asper* snake envenomation in bovines. Prolonged clotting time in snakebites has been attributed to venom induced consumption coagulopathy (VICC). It is characterized by prolonged clotting time, depletion of fibrinogen and cofactors V and VIII and high concentration of fibrinogen degradation products (Isbitser 2010, Goddard *et al.* 2011). Delayed clotting time is an indicator of deficiency of factors involved in blood clotting and it is used throughout the world for detection of snake venom induced consumption coagulopathy (Dormandy and Hardisty 1961, Sano-Martins *et al.* 1994, Benjamin *et al.* 2018).

Biochemical analysis showed highly significant ($P<0.01$) increase in values of total bilirubin (1.26 ± 0.07 vs 0.69 ± 0.04 mg/dL), direct bilirubin (0.58 ± 0.04 vs 0.35 ± 0.05 mg/dL) and indirect bilirubin (0.69 ± 0.05 vs 0.35 ± 0.08 mg/dL), BUN (38.52 ± 2.41 vs 5.52 ± 0.63 mg/dL) and creatinine (1.91 ± 0.13 vs 1.11 ± 0.19 mg/dL) in hemotoxic snakebite affected bovines compared to healthy counterparts. Bhikane *et al.* (2020) also observed increased BUN (13.03 ± 3.83 mmol/L) and creatinine (212.15 μ mol/L) in zebu cattle suffering from viperine snake envenomation. Elevated values of bilirubin in snakebite have been attributed to hemolysin/hemotoxins in snake venom as well as combined pathology of hemolysis and ischemic hepatopathy (Aroch and Harrus 1999, Segev *et al.* 2004). The elevation of BUN and creatinine in cases of snakebite has been attributed to nephrotoxic effects caused by snake venoms (Heller *et al.* 2006, Patil and Bansod 2012, Aye *et al.* 2017). Highly significant ($P<0.01$) increase in values of aspartate

aminotransferase (135.48 ± 3.31 vs 75.17 ± 3.20 U/L), alanine aminotransferase (55.93 ± 1.81 vs 12.49 ± 0.90 U/L), alkaline phosphatase (231.89 ± 5.92 vs 31.24 ± 2.80 U/L) and creatine kinase (CK) (375.04 ± 36.43 vs 41.42 ± 4.84 U/L) was observed in hemotoxic snakebite affected bovines compared to healthy bovines. The elevated values of AST, ALT and ALP in snakebite affected bovines could be attributed to the hepatocellular damage and cholestasis secondary to hypoxemia as well as direct hepatic damage and muscle injury by cytotoxins in snake venom (Aroch and Harrus 1999, Segev *et al.* 2004). Ali *et al.* (2020) also observed increased levels of alanine aminotransferase (260 IU/dl) in a Jersey crossbred cow suffering from hemotoxic snake envenomation.

Hemotoxic snakebite in bovines is mainly observed in grazing bovines during monsoon and post-monsoon season in limbs and face region characterized by ascending edematous swelling over limbs with lameness, asymmetrical swelling over face with dyspnea, tachycardia and tachypnea. Prominent abnormalities like granulocytic leukocytosis, thrombocytopenia, prolonged blood clotting time, elevated values of bilirubin, blood urea nitrogen, creatinine and enzymes like aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and creatine kinase indicative of hemotoxicity, liver and kidney damage as well as muscle damage have been observed in hemotoxic snakebite affected bovines.

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