



Epidemiological Aspects, Clinicopathology, Hemato-biochemistry and Therapeutic Management of Viperine Snake Envenomation in Buffaloes (*Bubalus bubalis*)

A.U. Bhikane¹, R.K. Jadhav², S.G. Chavhan³, P.S. Masare²

10.18805/IJAR.B-5382

ABSTRACT

Background: Poisonous snakebite is a neglected tropical disease, frequently reported in animals including buffaloes. Snakebites are responsible for considerable morbidity and mortality in animals causing economic losses to dairy farmers.

Methods: To establish the baseline data of epidemiology, clinical syndrome, hemato-biochemistry and diagnosis of viper snakebites and to standardize the treatment of viper snakebite in buffaloes.

Result: The highest incidence of viper envenomation was observed during the monsoon and post-monsoon season in female buffaloes above 4 years of age. The clinical signs such as tachycardia, increased respiration rate, ascending swelling of affected limbs, asymmetrical swelling with dyspnea in case of bite over face and bleeding at the site of bite were observed in envenomed buffaloes. Hemato-biochemical analysis revealed neutrophilic leukocytosis, thrombocytopenia, prolonged capillary blood clotting time, elevated values of blood urea nitrogen (BUN) and creatinine. Post-mortem examination showed hemorrhages and blood-stained serous fluid in subcutaneous tissue and skeletal muscles of the affected limbs. The histopathological changes such as extensive myonecrosis and hemorrhages with marked edema in skeletal muscles, widespread interstitial and intra-alveolar hemorrhages and fibrinous microthrombi in pulmonary vasculature, multifocal to coalescing areas of massive hemorrhages and coagulative necrosis within hepatic parenchyma and marked degeneration of renal tubular epithelium were evident. Therapeutic regimen consisting of polyvalent anti-snake venom, with antibiotics, diuretics, vitamin B complex, styptics, fluids and corticosteroids in non-pregnant while non-steroidal anti-inflammatory drugs in pregnant buffaloes was used. The present treatment strategy showed subsidence of clinical signs, restoration of platelet count, blood clotting time, urea nitrogen and creatinine values depicting clinical recovery with 88.15% survival rate in treated buffaloes.

Key words: Buffaloes, Epidemiology, Hemato-biochemistry and Treatment, Viper snakebite.

INTRODUCTION

India is a land to several venomous snakes with around 60 species abundant across the country responsible for severe life-threatening envenomation (Whitaker and Captain, 2004). Similar to human beings, poisonous snakebite is responsible for considerable morbidity and mortality in animals, especially among grazing cattle and buffaloes in geographical locations with habitat of venomous snakes. Snakebite is an occupational hazard common in rural labourers and farmers, who are dealing with various agricultural operations (Pandey *et al.*, 2016). Similarly, grazing animals are more prone to snake envenomation due to an increased predisposition to encounter snake populations in dense grasslands of the region (Bhikane *et al.*, 2020). Except few case studies and one retrospective study of snakebite in cattle, data on only one case report on snakebite in buffaloes is available (Sasikala *et al.*, 2016, Kachhawa *et al.*, 2016; Senthil Kumar *et al.*, 2018; Bhikane *et al.*, 2020; Sawane *et al.*, 2023). Considering the prevalence of viper snakes in the region, frequent occurrence of snakebites in buffaloes and economic losses to dairy farmers, the present study was planned with the objective to establish a baseline data on viper snakebites

¹Maharashtra Animal and Fishery Sciences University, Nagpur-441 108, Maharashtra, India.

²Department of Veterinary Medicine, College of Veterinary and Animal Sciences, Maharashtra Animal and Fishery Sciences University, Udgir-413 517, Latur, Maharashtra, India.

³Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Maharashtra Animal and Fishery Sciences University, Udgir-413 517, Latur, Maharashtra, India.

Corresponding Author: R.K. Jadhav, Department of Veterinary Medicine, College of Veterinary and Animal Sciences, Maharashtra Animal and Fishery Sciences University, Udgir-413 517, Latur, Maharashtra, India. Email: ravindrajadav@mafsu.ac.in

How to cite this article: Bhikane, A.U., Jadhav, R.K., Chavhan, S.G. and Masare, P.S. (2024). Epidemiological Aspects, Clinicopathology, Hemato-biochemistry and Therapeutic Management of Viperine Snake Envenomation in Buffaloes (*Bubalus bubalis*). Indian Journal of Animal Research. doi: 10.18805/IJAR.B-5382.

Submitted: 06-03-2024 **Accepted:** 28-09-2024 **Online:** 25-10-2024

in buffaloes with respect to epidemiology, clinical syndrome, hemato-biochemistry and diagnosis in order to standardize the therapeutic protocol for improving the survival of victims.

MATERIALS AND METHODS

The present study was carried out in buffaloes admitted to Teaching Veterinary Clinical Complex, College of Veterinary and Animal Sciences, Udgir, Maharashtra, India. The cases of buffalo admitted to hospital were examined for snake envenomation over a period of 10 years from January 2010 to December 2019. Out of 110 cases of buffalo suspected for viperine bite, 80 cases were confirmed based on diagnostic criteria used. The cases with the history of grazing on heavily grown tall pasture or area having dense vegetation, evidence of snake in the vicinity of buffalo shed or known snake habitat, violent shaking of the head, sudden bellowing, running, jumping, kicking during grazing and breaking of rope at rest followed by progressive swelling and bleeding from the site of the bite with low platelet count ($<100 \times 10^3/\mu\text{l}$) were considered as viper bites and included in the present study. The data pertaining to age, sex, breed, month, season, time of snakebite, feeding system *etc.* was collected for calculating the incidence of snakebite. Data pertaining to probable time of bite was categorized as morning (5-12 hours), afternoon (12-16 hours), evening (16-19 hours) and night (19-5 hours).

All the suspected buffaloes were subjected to detailed clinical examination. About 2 ml of blood was collected from the suspected buffaloes by jugular venipuncture in EDTA vials for hematological analysis on automated hematology analyzer (Model: Abacus Junior Vet, Diatron GMBH, Austria). The ear vein of the affected buffaloes was aseptically prepared and punctured with a 20-gauge needle. The blood oozing from the needle was directly collected in plain capillaries without any anticoagulant. The time required for clotting of blood in capillaries was estimated by breaking the capillary at an interval of one minute and noted as blood clotting time when coagulated blood formed string after breaking the capillary. About 5 ml of heparinized blood samples were collected from the affected buffaloes and subjected to centrifugation at 3000 rpm for 10 minutes. The harvested plasma was used for the estimation of blood urea nitrogen (BUN) and creatinine on biochemistry analyzer (Model: Chemistry Analyzer-CA 2005 B4B Diagnostic division, China, Model no. CA 2005).

The detailed post-mortem examination of two buffaloes died of viperine snake envenomation was performed as per the standard protocol for bovine necropsy (King *et al.*, 2013). The gross post-mortem lesions were recorded and various tissue samples were collected in 10% neutral buffered formalin for histopathological examination. After proper fixation, the tissue samples were embedded in paraffin wax. The blocks were cut to obtain 4-5 μm thick sections and subjected for hematoxylin and eosin (H and E) staining technique as described by Bancroft and Gamble (2007).

All 80 viper bite cases in buffaloes were treated using treatment protocol based on the pilot study as per our past experiences. The buffaloes were treated with polyvalent anti-snake venom (PASV) 20-40 ml IV within the first 24 hours

depending upon the severity of signs and time since the snakebite. Supportive treatment comprised of Amoxycillin + Cloxacillin @10 mg/kg IV for 7-9 days, Dextrose 5% 4-6 L IV for 5 days, Furosemide @1 mg/kg IV for 5 days, Carbazochrome salicylate 10 ml IM daily for 3-5 days, Vitamin B-complex 5-10 ml IM for 5-9 days and Dexamethasone 80 mg IV on the first day, 40 mg on 2nd and 3rd day and 20 mg on 4th and 5th day in non-pregnant buffaloes, Meloxicam 0.5 mg/kg IV daily for 5-9 days in pregnant buffaloes and from 6th day onwards for next 4 days in non-pregnant buffaloes. Oral hematonic bolus (ferrous fumerate 1500 mg, vitamin B₁₂ 75 mcg and folic acid 7500 mcg per bolus) 1 PO BID for 10 days from 5th day onwards was advised in recovered buffaloes.

Statistical analysis

All the data pertaining to vital clinical parameters, hemato-biochemical investigations and capillary blood clotting time was recorded before treatment as well as after clinical recovery. The data of the same parameters in normal healthy buffaloes was compared with snakebite affected buffaloes to estimate the level of significance at 99% and 95% confidence interval using paired 't-test' by using software IBM SPSS version 20.

RESULTS AND DISCUSSION

Incidence

Snakebite is one of the most important acute life-threatening medical emergency conditions affecting both humans and animals, recognized and declared as a neglected tropical disease by World Health Organization (Ahmad *et al.*, 2016). Ample data on companion animal and equine snakebite is available as compared to ruminants (Bolon *et al.*, 2019). During the present study 80 cases of viper snakebites were confirmed in buffaloes admitted over a period of 10 years from 2010 to 2019, with an overall incidence of 0.98%.

The month-wise incidence of viper bites in buffaloes revealed the highest occurrence in August (18.75%) and July (16.25 %) months. Season wise highest incidence was reported in monsoon (56.25%), followed by post-monsoon (21.25%), winter (16.25%) and least in summer (6.25%). The present findings agree with higher rates of snakebites and deaths during the same season due to snakebite in humans and zebu cattle (Mohapatra *et al.*, 2011; Bhikane *et al.*, 2020). Monsoon commences with rainfall, rapid growth of tall, lush and dense pasture in the grasslands, which is an important habitat for snakes. Age-wise highest incidence of viper snakebite was observed in buffaloes aged > 4 years (58.75%), followed by 1-4 years (36.25%) and least in young buffaloes below < 1 year (5%). Higher incidence in > 4 year and 1-4-year age groups might be attributed to complete dependence of adult buffaloes on grazing for meeting nutritional requirements compared to young bubaline population, which are generally kept indoors and stall fed.

Sex-wise incidence of viper bites was almost 96.25% in female buffaloes while 3.75% in male buffaloes. Highest incidence in female buffaloes could be attributed to sole rearing of female animals for milk production which are more prone to come across snakes while grazing. While male buffaloes are generally reared up to the end of lactation or age of 1 year, during which they are mostly stall fed and rarely allowed for grazing reducing their risk of exposure to the snakes. Out of the total affected buffaloes with viper snake envenomation, 91.25% were of the local Marathwadi breed, owing to its native tract while 8.75% were graded Murrah buffaloes. When the data pertaining to the probable time of bite was analyzed, it was found that maximum bites occurred during the morning (41.25%) followed by afternoon (26.25%) and evening (23.75%) hours while least in night hours (8.75%). Almost 91.25% (73) buffaloes were bitten during grazing, while about 8.75%

(7) buffaloes were bitten while resting in the stall/shed during night hours which was in agreement with the findings of Bhikane *et al.* (2020).

Clinical signs

No significant changes were observed in body temperature (100.31 ± 0.56 vs. $100.41 \pm 0.15^\circ\text{F}$) while highly significant ($P < 0.01$) increase in heart rate (68.00 ± 3.14 vs. 54.14 ± 0.79 bpm) and significant ($P < 0.05$) increase in respiration rate (29.78 ± 3.06 vs. $21.42 \pm 0.70/\text{min}$) was observed in viper bite affected buffaloes compared to healthy control buffaloes. The most characteristic sign of viper envenomation observed in buffaloes was ascending edematous swelling with pain and lameness over limbs (Fig 1a and 1b). In most of the cases of bite over limbs, the swelling was found extending upward towards dewlap and brisket in forelimb bites. Asymmetrical edematous swelling



Fig 1a: Ascending swelling over left forelimb extending towards brisket in buffalo with viper bite. **Fig 1b:** Ascending swelling over right forelimb extending towards brisket and dewlap in buffalo with viper bite.



Fig 2a: Asymmetrical swelling over face in buffalo bitten by viper snake on face region. **Fig 2b:** Ascending swelling of tail due to viper bite on tail of affected buffalo. **Fig 2c:** Ascending swelling over right forelimb in buffalo heifer with local bleeding from the site of bite.

over the face with pain and severe dyspnea was a consistent finding in viper bite over the face region (Fig 2a). In one case of viper bite over the tail region in buffalo, ascending swelling was observed over the tail (Fig 2b). Analysis of data based on the site of snakebite revealed maximum cases of viper bites on forelimbs (42.50%) followed by hind limbs (32.75%), face (23.75%) and tail (1.25%). The findings were in agreement with those reported by Bhikane *et al.* (2020) and Bolon *et al.* (2019). The highest number of bites observed on limbs (75.00%) might be attributed to their direct and closer contact with ground during either walk or grazing in dense grassland followed by face (23.75%) during grazing time.

The mucous membranes showed variable changes *viz.*, congestion and petechiae in the initial stage while pallor in the late stage. Signs of local and mucosal bleeding were prominent in most of the affected buffaloes. Variable degree of local bleeding (Fig 2 c.) was observed at the site of bite in 56.25% of cases while mucosal bleeding in the form of hematochezia/ melena (8.75%, Fig 3 a), epistaxis (6.25%, Fig. 3 b), vaginal bleeding (1.25%) and hematuria (1.25%) was also evident. The general signs observed in all envenomed buffaloes were inappetence to anorexia, suspended rumination, dullness, depression and decreased rumen motility.

Snake venoms are responsible for several toxic actions in the body of human and animal *viz.*, necrotizing, anticoagulant and procoagulant fractions, neurotoxic, cardiotoxic, myotoxic, nephrotoxic, cytotoxic, hemolytic and hemorrhagic fractions producing edema, inflammation, hemorrhages, necrosis and altered blood coagulation (Goddard *et al.*, 2011; Kumar *et al.*, 2022). The clinical signs observed in the present study are in agreement with the findings of Rodriguez *et al.* (2016); Altug and Isler (2019);

Bhikane *et al.* (2020) and Sawane *et al.* (2023). In the present study, fang marks were visible only in 13.75% (11) cases on unpigmented (white) areas upon shaving of the bite site in early hours while fangs were not visible in 86.25% (69) cases due to pigmented dark and thick skin with hair coat.

Hemato-biochemistry

Mean (\pm S.E.) values of hemato-biochemical parameters revealed a significant ($P<0.05$) increase in WBC count (9.77 ± 0.82 vs. $7.57\pm 0.37\times 10^3/\mu\text{l}$) and PCV (35.52 ± 1.76 vs. $30.52\pm 1.13\%$). The highly significant ($P<0.01$) increase in neutrophil count (51.31 ± 3.03 vs. $32.52\pm 4.90\%$) and highly significant ($P<0.01$) decrease in platelet count (37.4 ± 10.87 vs. $203.08\pm 15.04\times 10^3/\mu\text{l}$) was observed in the viper bite affected buffaloes as compared to healthy buffaloes with non-significant changes in RBC count, hemoglobin concentration and lymphocyte count. Highly significant ($P<0.01$) increase in capillary blood clotting time (26.16 ± 2.30 vs. 7.11 ± 0.11 minutes) was observed in viper bite affected buffaloes compared to healthy counterparts. Hematological findings in the present study are in agreement with the findings of Rodriguez *et al.* (2016) and Bhikane *et al.* (2020) in cattle, Armentano and Schaer (2011) in dogs and Lenchner *et al.* (2014) in cats. Mucosal or local bleeding in viper bite affected buffaloes might be attributed to abnormal function of coagulation factors, effects of venom on capillary endothelium and platelets leading to severe thrombocytopenia and prolonged capillary blood clotting time (Goddard *et al.*, 2011). The bleeding syndromes in the form of epistaxis, melena, hemoglobinuria or hematuria could be attributed to venom-induced platelet disorders (Kamiguti, 2005). Assessment of kidney function revealed a highly significant ($P<0.01$)



Fig 3a: Severe hematochezia in buffalo suffering from viper envenomation. **Fig 3b:** Buffalo heifer with epistaxis due to viper snakebite.

increase in BUN (37.08 ± 5.11 vs. 16.63 ± 0.92 mg/dl) and creatinine (2.46 ± 0.36 vs. 0.68 ± 0.05 mg/dl) as compared to healthy buffaloes which was also reported previously by several authors in snakebite affected animals (Goddard *et al.*, 2011; Bhikane *et al.*, 2020; Sawane *et al.*, 2023).

Viperine envenomation in buffaloes was diagnosed by using circumstantial evidences obtained from animal owner, dead snake presented at the time of admitting the patient by few owners, set of clinical signs supported by bleeding profile (thrombocytopenia, prolonged capillary blood clotting time) and renal function tests (elevated BUN and creatinine). As per the data pertaining to circumstantial evidences regarding snakebite, 47.50% cases of bites were witnessed by farmers during grazing followed by signs of fear and excitement, while 8.75% cases were bitten during the resting period. In the rest of the cases (43.75%), the

animal owners reported evidence of jumping, bellowing and sudden restlessness followed by the onset of progressive swelling over limbs or face.

Post-mortem findings

The post-mortem examination of buffaloes died due to viperine snake envenomation revealed excessive ascending swelling of the affected limb as compared to the unaffected limb (Fig 4a and 4b). The external skin surface of the affected limb frequently showed cyanosis or blue discoloration (Fig 4c), exudation of blood-stained serous fluid (Fig 4d.) and cracks due to overstretching. On incision to affected limb, the heavy hemorrhages were evident within underlying subcutaneous tissue and skeletal muscles (Fig 5a, 5b and 5c). The gross lesions observed in other visceral organs include the presence of



Fig 4: a: Postmortem findings in buffaloes died due to viperine snake envenomation. b: Excessive ascending swelling of affected right hind limb (asterisk) as compared to left hind limb. c: Cyanotic or blue discoloration of skin of affected hind limb. d: Exudation of blood-stained serous fluid from skin surface of affected hind limb.

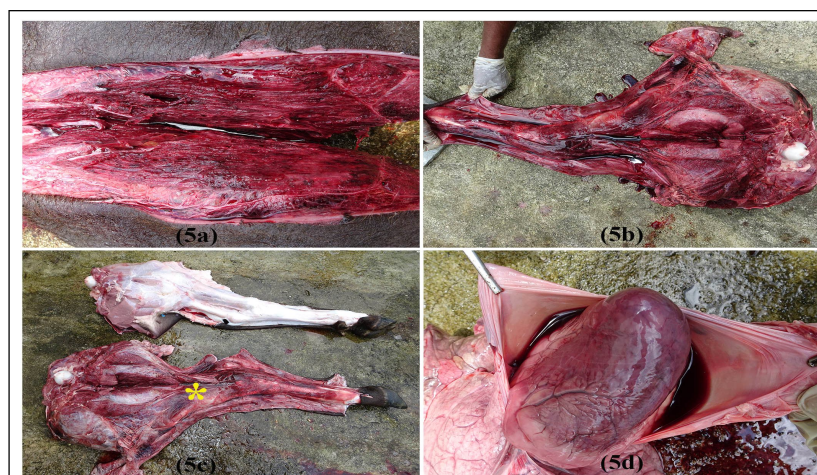


Fig 5: a-c: Postmortem findings in buffaloes died due to viperine snake envenomation. Heavy hemorrhages within underlying subcutaneous tissue and skeletal muscles of affected right hind limb (asterisk) as compared to left hind limb of same animal. d: Heart: Presence of blood-tinged pericardial fluid within pericardial sac.

blood-tinged pericardial fluid (Fig 5d), patchy or ecchymotic hemorrhages in lungs (Fig 6a, 6b and 6c) and moderate hepatomegaly along with mild icteric discoloration (Fig 6d).

The histopathological examination of skeletal muscles from affected limb revealed the presence of extensive myonecrosis (evident from hypereosinophilic dissociated or fragmented myofibers and loss of striations), widespread hemorrhages (Fig 7a and 7b), mild to moderate mononuclear cell infiltration and edema (Fig 7c). The marked intradermal or hypodermal hemorrhages were also

evident in skin sections of the affected limb (Fig 7d). Microscopic lesions observed in lungs were the frequent presence of fibrinous microthrombi within pulmonary vasculature, widespread interstitial and intra-alveolar hemorrhages and edema, as well as presence of proteinaceous fluid within bronchial lumens (Fig 8a, 8b, 8c and 8d). Multifocal to coalescing areas of massive hemorrhages (Fig 9a, 9b and 9c) and coagulative necrosis (Fig 9d) were evident in hepatic parenchyma of viperine envenomated animals causing marked distortion of hepatic

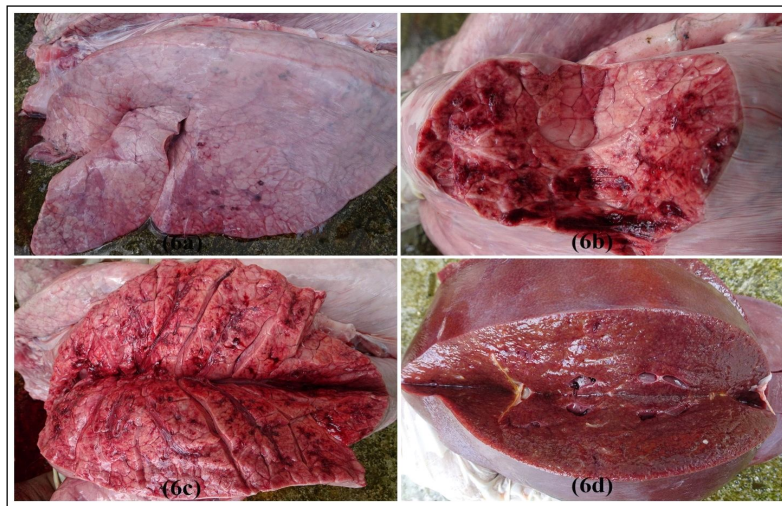


Fig 6: a-c: Postmortem findings in buffaloes died due to viperine snake envenomation. Lung: Patchy or ecchymotic hemorrhages. d: Liver: Moderate hepatomegaly along with mild icteric discoloration.

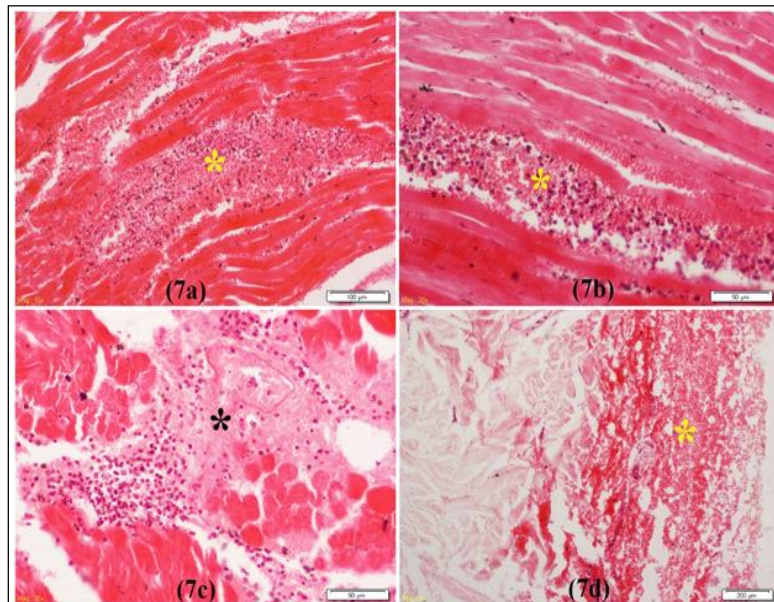


Fig 7: a-c: Histopathological changes of viperine snake envenomation in buffaloes. Skeletal Muscle: Extensive myonecrosis, hypereosinophilic dissociated or fragmented myofibers, loss of striations along with widespread hemorrhages (yellow asterisks) and Mild to moderate mononuclear cell infiltration and oedema (black asterisk). d: Skin: Marked intradermal or hypodermal hemorrhages (asterisk). (H and E Stain) (Fig 7a: Bar= 100 μ m, Fig 7b and 7c: Bar = 50 μ m, Fig 7d: Bar= 200 μ m).

architecture. The lesions such as marked degeneration of tubular epithelium, presence of casts or cellular debris within tubular lumens and Bowman's space and atrophy of glomerular tufts were evident in kidneys of envenomed buffaloes (Fig. 10a, 10b, 10c and 10d).

Post-mortem lesions due to snake envenomation are specific to each snake. Localized swelling at the site of the bite is attributed to exudation of serous fluid and inflammatory reaction to venom components, which is often deeply blood stained (Constable *et al.*, 2017). Post-mortem

examination of a cow presumed to have died from the viper bite would show petechial and ecchymotic to frank hemorrhages in the lung, liver, tracheal lumen, peritoneum, epicardial and sub-endocardial surfaces and also pale linear streaks in the right ventricular myocardium (Banga *et al.*, 2009). Additionally, reported the histopathological changes like hemorrhages at the site of bite as well as in various organs including lungs along with necrotic changes in skeletal and cardiac muscles. These gross and microscopic changes reported by various earlier research

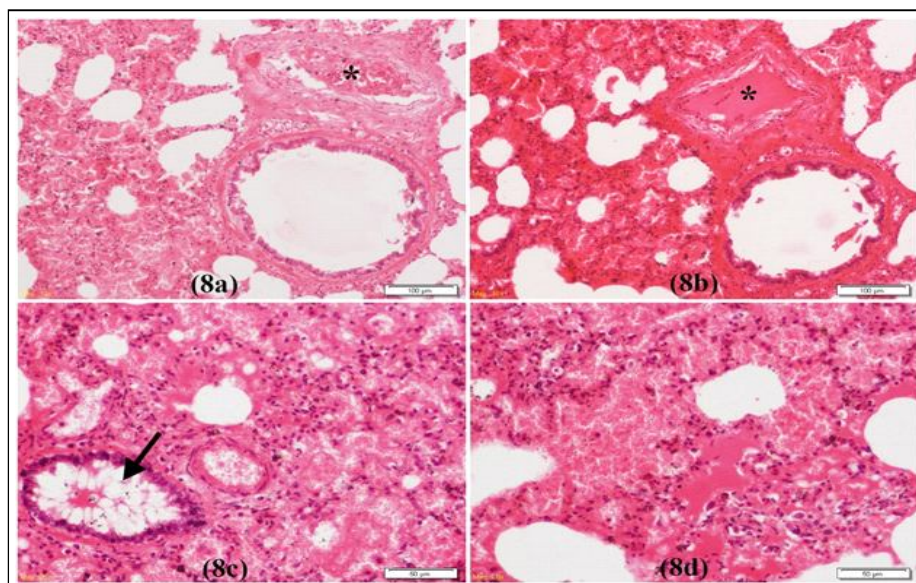


Fig 8:a-d: Histopathological changes of viperine snake envenomation in buffaloes. Lung: Fibrinous microthrombi within pulmonary vasculature (asterisks), widespread interstitial and intra-alveolar hemorrhages and oedema as well as presence of proteinaceous fluid within bronchial lumens (arrow). (H and E Stain) (Fig 8a and 8b: Bar= 100 μ m, Fig 8c and 8d: Bar= 50 μ m).

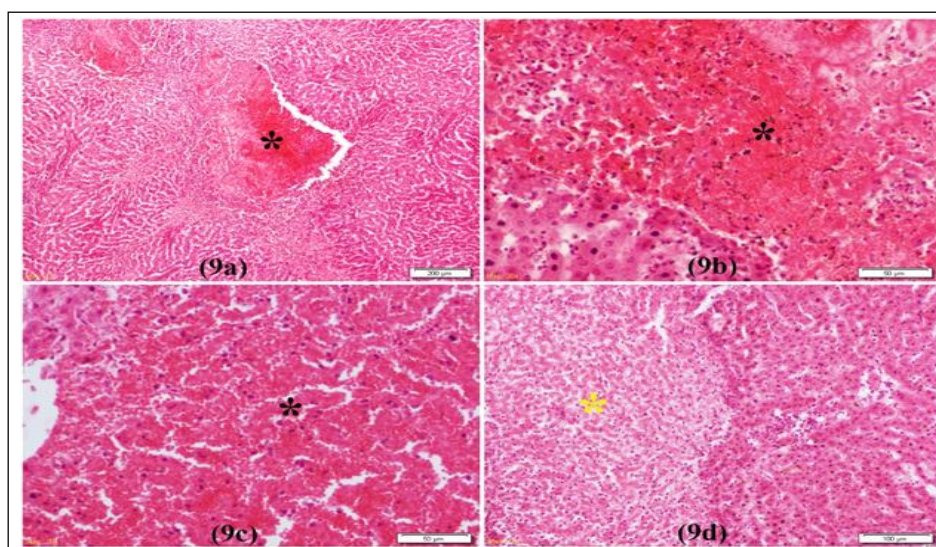


Fig 9: a-d: Histopathological changes of viperine snake envenomation in buffaloes. Liver: Multifocal to coalescing areas of massive haemorrhage (black asterisks) and coagulative necrosis (yellow asterisk) causing marked distortion of hepatic architecture. (H and E Stain) (Fig 9a: Bar=200 μ m, Fig 9b and 9c: Bar=50 μ m, Fig 9d: Bar=100 μ m).

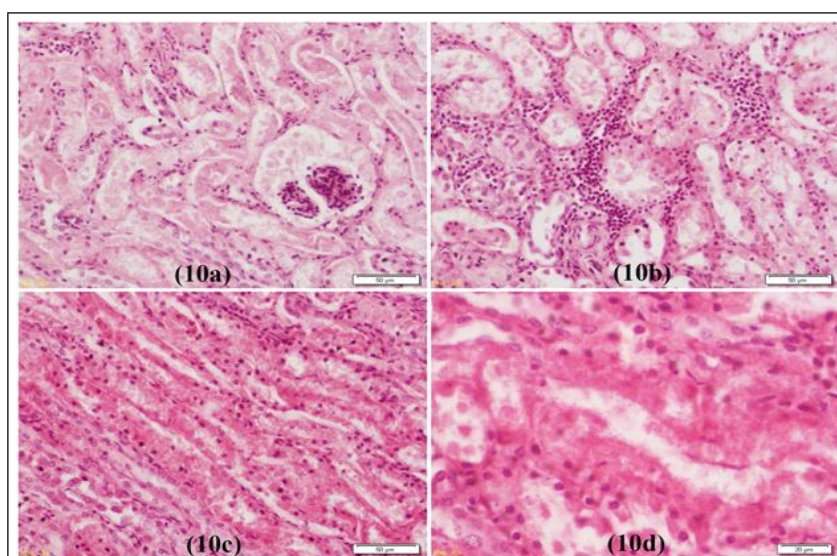


Fig 10: a-d: Histopathological changes of viperine snake envenomation in buffaloes. Kidney: Marked degeneration of tubular epithelium, presence of casts or cellular debris within tubular lumens and Bowman's space and atrophy of glomerular tufts. (H and E Stain) (Fig 10-10c: Bar= 50 µm, Fig 10d: 20 µm).

Parameter	Day 0 (n=25)	Day 6 (n=25)	't' value
Body temperature (°F)	100.32±0.44	100.24±0.36	0.136
Heart rate (/minute)	68.52±2.68	59.73±1.98	2.635*
Respiration rate (/minute)	29.05±2.68	22.63±0.66	2.313*
RBC (×10 ⁶ /µl)	6.38±0.27	4.92±0.25	5.38**
HB (gm%)	11.16±0.56	8.67±0.44	5.55**
PCV (%)	35.52±1.76	29.06±1.69	3.94**
WBC (×10 ³ /µl)	9.77±0.8	9.83±0.61	-0.054 NS
Neutrophils (%)	51.31±3.03	47.19±2.85	1.283 NS
Monocytes (%)	3.51±0.59	4.56±0.64	-1.233 NS
Lymphocytes (%)	44.22±3.05	37.40±2.71	-1.038 NS
Platelet (×10 ³ /µl)	37.40±10.87	139.45±18.64	-7.128**
BUN (mg/dl)	37.08±5.11	22.78±3.39	2.78*
Creatinine (mg/dl)	2.46±0.36	1.34±0.20	2.46*
B.C.T. (minutes)	26.50±1.87	10.27±0.75	10.82**

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

workers were also in consonance with the pathological findings observed in present study. These findings observed in present study were also in consonance with the pathological findings observed by Bhikane *et al.* (2020).

Treatment

A total of 80 confirmed cases of viper bite in buffaloes were treated by using therapeutic protocol mentioned above. In one case with severe dyspnea due to extensive swelling over sub-mandibular region, tracheotomy was performed. Mean (± S.E.) values of vital clinical parameters and hemato-biochemical parameters before treatment and on sixth day in viper bite affected buffaloes (n=25) are depicted in Table 1. Appreciable reduction in edematous swelling at

the site of bite was observed from second day of treatment with complete resolution by 8-10 days. Significant (P<0.05) reduction in heart rate and respiration rate was observed on 6th day of treatment compared to pre-treatment values suggestive of restoration of heart rate and respiration rate towards normal physiological range. Hematological parameters showed highly significant (P<0.01) reduction in RBC, hemoglobin and PCV with non-significant alterations in leukogram and differential leukocyte count on 6th day. Bleeding profile showed highly significant (P<0.01) increase in platelet count while decrease in capillary blood clotting time. Kidney function test showed significant (P<0.05) reduction in the values of BUN and

creatinine on 6th day of treatment as compared to pre-treatment values.

Efficacy of antivenom therapy in snakebite cases depends on antivenom specificity to snake venom, potency of antivenom, dose, severity of envenomation and the time elapsed between bite and antivenom administration (Otero *et al.*, 2002). In the present study, 20-40 ml of PASV was found effective in the therapeutic management of viper envenomation in buffaloes along with supportive therapy. The dose of PASV found effective in the present study is in agreement with the earlier reports in cattle, horses and new world camelids (Altug and Isler, 2019; Bhikane *et al.*, 2020; Fielding *et al.*, 2011; Chiacchio *et al.*, 2011; Dykgraaf *et al.*, 2006). The poor therapeutic response was observed in lately presented cases (> 24 hours) with bleeding diathesis in the present study was in agreement with one report in new world camelids (Dykgraaf *et al.*, 2006). The aggravation of envenomation and absence of free circulating venom for neutralization might be responsible for poor response to antivenom therapy in lately presented cases (Bawaskar, 2004). The emergency tracheotomy in one buffalo with bite on face and severe dyspnea due to submandibular swelling assisted in relieving the respiratory distress thereby increasing the survival chance. In the present study, out of 80 buffaloes treated for viperine envenomation with follow up in 76 cases had showed complete clinical recovery in 67 buffaloes with success rate of 88.15%, which signifies early treatment using PASV along with supportive therapy is able to produce complete clinical recovery in buffaloes with viper envenomation.

CONCLUSION

Viper envenomation was commonly reported in grazing buffaloes during monsoon and post-monsoon seasons, mostly in native Marathwadi adult female buffaloes. Snakebites were frequently reported in lower limbs with edematous swelling, pain and lameness, while bites in the head region showed asymmetrical swelling with pain and severe dyspnea. Diagnosis and monitoring of treatment of viper bite in buffaloes could be effectively done using capillary blood clotting time, platelet count and kidney function tests. Treatment comprising of polyvalent anti-snake venom in early stage once or twice along with fluids, antibiotics, steroidal or non-steroidal anti-inflammatory drugs, stypitics, diuretics, vitamin B complex for 5-7 days and hematinics for 10 days proved as most effective treatment protocol with 88.15% recovery rate.

Conflict of Interest

All the authors declare that they have no conflict of interest.

REFERENCES

Ahmad, W., Ahmad, M., Khan, R.A. and Mustaq, N. (2016). Promising inhibition of krait snake's venom acetylcholinesterase by *Salix nigra* and its role as anticancer, antioxidant agent. *Ind. J. Anim. Res.* 50 (3): 317-323. doi: 10.18805/ijar.10711.

Altug, N. and Isler, C.T. (2019). Snake envenomation in two cattle: clinical/ laboratory aspects and treatment using equine derived antivenin of viperidae. *Turkish J. Vet. Anim. Sci.* 43: 546-550. doi:10.3906/vet-1904-5.

Armentano, R.A. and Schaer, M. (2011). Overview and controversies in the medical management of pit viper envenomation in the dog. *J. Vet. Emergency and Critical Care.* 21(5): 461-470. doi: 10.1111/j.1476-4431.2011.00677.x.

Bancroft, J.D. and Gamble, M. (2007). *Theory and Practice of Histological Techniques.* 6th Edition, Churchill Livingstone, Elsevier, China.

Banga, H.S., Brar, R.S., Chavhan, S.G., Sandhu, H.S. and Kammon, A.M. (2009). Pathology of Snakebite in cow. *Toxicology international.* 16(1):69-71. [https:// www.informaticsjournals .com/index.php/toxi/article/view/20850](https://www.informaticsjournals.com/index.php/toxi/article/view/20850).

Bawaskar, H.S. (2004). Snake venoms and Antivenoms: Critical supply issues. Editorial: *J. Assoc. Physicians of India.* 52:11-13.

Bhikane, A.U., Jadhav, R.K., Masare, P.S. and Chavhan, S.G. (2020). Clinical, hematobiochemical and pathological findings and therapeutic management of viperine snake envenomation in zebu cattle. *Trop. Anim. Health Prod.* 52 (6): 3425-3437. doi: 10.1007/s11250-020-02376-6.

Bolon, I., Finat, M., Harrera, M., Nickerson, A., Grace, D., Schutte, S., Martins, S.B. and Ruiz de Castaneda, R. (2019). Snakebite in domestic animals: First global scoping review. *Preventive Vet. Med.* 170(104729):1-11. [https://doi.org/ 10.1016/j.prevetmed.2019.104729](https://doi.org/10.1016/j.prevetmed.2019.104729).

Chiacchio, S.B., Martins, G.T.B., Amorim, R.M., Goncalves, R.C., Barraviera, B. and Ferreira Junior, R.S. (2011). Triple bothropic envenomation in horses caused by a single snake. *J. Venomous Anim. Toxins. Tropical Dis.* 17(1): 111-117. <https://doi.org/10.1590/S1678-91992011000100016>.

Constable, P.D., Hinchcliff, K.W., Done, S.H. and Grunberg, W. (2017). Snake Bite: In *Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats.* 11th Edn. Elsevier Ltd., St. Louis, Missouri, USA. Pp: 2176-2179.

Dykgraaf, S., Pusterla, N. and Hoogmoed, L.M. (2006). Rattlesnake Envenomation in 12 New World Camelids. *J. Vet. Intern. Med.* 20: 998-1002. <https://doi.org/10.1111/j.1939-1676.2006.tb01818.x>.

Fielding, C.L., Pusterla, N., Magdesian, K.G., Higgins, J.C. and Meier, C.A. (2011). Rattlesnake envenomation in horses: 58 cases (1992-2009). *J. Am. Vet. Med. Asso.* 238(5): 631-635. doi: 10.2460/javma.238.5.631.

Goddard, A., Johan, P.S., Leisewitz, A.L., Nagel, S. and Aroch, I. (2011). Clinicopathologic abnormalities associated with snake envenomation in domestic animals. *Vet. Clinical Pathol.* 40(3): 282-292. doi: 10.1111/j.1939-165X.2011.00335.x.

Kachhawa, J.P., Sharma, A., Tanwar, T.K. and Singh, A.P. (2016). Therapeutic management of snakebite in buffalo- A case report. *Indian J. Vet. Med.* 36(2): 134-135.

Kamiguti, A.S. (2005). Platelets as targets of snake venom metalloproteinases. *Toxicon.* 45:1041-1049. doi: 10.1016/j.toxicon.2005.02.026.

King, J.M., Johnson, L.R., Dodd, D.C. and Newsom, M.E. (2013). *The Necropsy Book: A guide for veterinary students, residents, clinicians, pathologists and biological researchers.* College of Veterinary Medicine, Cornell University, Ithaca, New York, USA.

- Kumar, P.V., Jayaseelan, B.D., Dinesh, M.D., Thangavel, M. and James, T. (2022). Bioactive potential of aqueous extract of *Calotropis gigantea* against *Echis carinatus* Venom. Ind. J. Ag. Res. doi: 10.18805/IJARE.A-5992.
- Lenchner, I., Aroch, I., Segev, G., Kelmer, E. and Bruchim, Y. (2014). A retrospective evaluation of *Vipera palaestinae* envenomation in 18 cats (2006-2011). J. Vet. Emergency and Critical Care. 24(4): 437-443. doi: 10.1111/vec.12207.
- Mohapatra, B., Warrell, D.A., Suraweera, W., Bhatia, P., Dhingra, N., Jotkar, R.M., Rodrigues, P.S., Mishra, K., Whitaker, R. and Jha, P. (2011). Snakebite mortality in India: A nationally representative mortality survey. PLoS Negl. Tropical Dis. 5(4): e2018. doi:10.1371/journal.pntd.0001018.
- Otero, R., Gutiérrez, J., Mesa, M.B., Duque, E., Rodriguez, O., Arango, J.L., Gomez, F., Toro, A., Cano, F., Rodriguez, L.M., Caro, E., Martinez, J., Cornejo, W., Gomez, L.M., Uribe, F.L., Cardenas, S., Nunez, V. and Diaz, A. (2002). Complications of *Bothrops*, *Porthidium* and *Bothriechis* snakebites in Colombia. A clinical and epidemiological study of 39 cases attended in a university hospital. Toxicon. 40 (8):1107-1114. doi: 10.1016/s0041-0101(02)00104-6.
- Pandey, P.C., Bajaj, S. and Srivastav, A. (2016). A clinico-epidemiological profile of neuroparalytic snakebite: Using low dose ASV in a tertiary care centre from North India. J. Assoc. of Physicians of India. 64: 16-20.
- Rodriguez, C., Estrada, R., Herrera, M., Gomez, A., Segura, A., Vargas, M., Villalta, M. and Leon, G. (2016). *Bothrops asper* envenoming in cattle: Clinical features and management using equine-derived whole IgG anti-venom. The Vet. J. 207: 160-163. doi: 10.1016/j.tvjl.2015.08.008.
- Sasikala, K., Ponnuswamy, K.K., Vijayakumar, G., Venkatesakumar, E. and Sivaraman, S. (2016). Successful medical management of snake envenomation in a Jersey cow. Indian Vet. J. 93(12): 52-53.
- Sawane, C.S., Jadhav, R.K., Bhikane, A.U., Masare, P.S. and Kushwaha, N. (2023). Epidemiological, clinical and hematobiochemical studies on hemotoxic snakebite in bovines. Ind. J. Anim. Sci. 93 (1): 23-28. <https://doi.org/10.56093/ijans.v93i1.124168>.
- Senthil Kumar, A., Senthilrajaprabu, R. and Sribalaji, N. (2018). Therapeutic management of snake envenomation in a crossbred dairy cattle- A case report. Research J. Chem. and Envir. Sci. 6(1): 124-126.
- Whitaker, R. and Captain, A. (2004). Snakes of India: The field guide. Chennai: Draco books, Pp. 495.