INVITED REVIEW

Clinicopathologic abnormalities associated with snake envenomation in domestic animals

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Abstract: Envenomation of domestic animals by snakes occurs frequently in certain geographic areas. However, reports describing clinical signs, clinicopathologic abnormalities, therapeutic approaches, and outcomes are sparse. This review summarizes various snake families, venom types associated with harmful snakes, and the significant hematologic, hemostatic, and biochemical abnormalities associated with envenomation. Hematologic abnormalities include RBC membrane abnormalities, hemolysis, hemoconcentration, leukogram changes, and platelet abnormalities, specifically thrombocytopenia. Coagulopathies associated with snake envenomation are well described in human medicine, and many studies have demonstrated properties of venoms that lead to both procoagulation and anticoagulation. As expected, similar abnormalities have been described in domestic animals. Biochemical abnormalities are associated with the effects of venom on tissues such as liver, skeletal and cardiac

muscle, vascular endothelium, and kidney as well as effects on protein components and cholesterol. This comprehensive review of clinicopathologic abnormalities associated with envenomation and their relationship to characterized venom constituents should be useful both in the diagnosis and management of envenomation and serve as a foundation for future research in this field.

Key Words

Coagulopathy, colubrids, elapids, hemoconcentration, venom, viperids

Introduction

Snake activity increases during the spring and summer months when snakes reproduce and eggs hatch, and it is during these seasons that most domestic animals are envenomed.[1-4] Additionally, a consistent annual peak of envenomation in late summer to early autumn has been described in South Africa and is thought to result from increased pre-hibernation activity.[3] Snake envenomation of domestic animals occurs frequently, has a worldwide distribution, and has been reported in dogs,[5] cats,[5,6] horses,[7,8] sheep,[9,10] cattle,[11] and New World camelids.[12] Despite the high prevalence of snake envenomation and the large number of reports in domestic animals, the relevant veterinary literature is limited, especially with regard to associated clinicopathologic abnormalities. This review summarizes the clinicopathologic data available from reports of snake envenomation in domestic animals.

Classification of venomous snakes and venoms

Venomous snakes

A comprehensive classification of venomous snakes is beyond the scope of this review; however, a simplified overview is warranted as different snake species have been reported in cases of

envenomation in domestic animals (Table 1).[13,14] The family Colubridae includes roughly two-thirds of the snake species,[13,14] and, although the majority of colubrids are venomous, they are mostly harmless to people owing to small venom glands, weak venoms, or inefficient venom delivery systems. The family does, however, include venomous snakes, such as the boomslang (*Dispholidus typus*) and vine snake (*Thelotornis capensis*, also known as twig or bird snake), that have caused fatalities in both people and animals.[13,14] The Elapidae family includes venomous snakes like the cobras and related species. The Viperidae family includes the adders (Viperinae or vipers) and pit vipers (Crotalinae, eg, rattlesnakes and lanceheads).[13,14]

Snake venoms

Snake venom can be defined as highly modified saliva that has undergone evolutionary adaptation to immobilize the prey and aids its digestion by means of the actions of protein-degrading enzymes.[13,15] The composition of venom from each type of snake is species-specific with variable components and amounts of toxic and non-toxic compounds.[15] Snake venoms are composed of at least 90% dry-weight protein. Most of the protein is a mixture of enzymes; some aid in digesting the prey whereas others paralyze or kill it. About 25 different enzymes have been isolated from snake venoms and most have been characterized.[13,15] The classification and mechanisms of action of snake venoms are important and relevant to an understanding of the laboratory abnormalities induced by snake envenomation (Table 2).[13-16]

Snake venoms were initially classified into 3 groups: cytotoxic (tissue-dissolving and blood cell-lysing), neurotoxic (nerve-damaging), and coagulopathic (inducing bleeding).[2] However, it has become increasingly evident that this classification is oversimplified, as some snake venoms possess a combination of these actions (Table 2).[17] *Neurotoxic components* adversely affect the central nervous

system by blocking neurotransmission (ie, acetylcholine cleavage). *Cardiotoxic components* adversely affect the myocardium and cardiac conduction by increasing cellular membrane permeability to ions, thus altering fiber excitability. *Hemorrhagic toxins (hemotoxins,* including *hemorrhagins)* cause considerable bleeding into tissues near and distant from the bite site by damaging vascular endothelium in capillary walls. Endothelial damage promotes coagulation, and thus bleeding is often accompanied by clotting. Some of these components also lead to hemolysis. *Thrombins (procoagulants)* induce coagulation and disrupt the normal hemostatic balance and induce intravascular coagulation. *Cytolysins* lyse tissue cells and leukocytes.[14,15]

The venom of elapids, eg, cobras and mambas, consists primarily of neurotoxins, cardiotoxins, and hemolytic agents. Viperid venom consists of hemorrhagic elements, thrombins, and cytolysins, of which cytolysins are responsible for most of the observed clinical signs.[14,15] The toxic venom component in colubrids, eg, boomslang and vine snakes, is mainly hemorrhagic, and exsanguination is the main observed complication.[18-20] The clinicopathologic changes observed in domestic animals with snake envenomation include hematologic, biochemical, and coagulation abnormalities.

Hematologic changes

Alterations in RBCs, WBCs, and platelets have all been reported in snake envenomations of domestic animals. In addition to their direct effects on circulating blood cells, venom constituents cause fluid and electrolyte shifts with resultant changes in cell concentrations and structure.

RBC abnormalities

Hemoconcentration has been reported frequently in *Vipera palaestinae* envenomation of dogs. HCT > 50% were recorded in 48% of 109 and 47% of 320 dogs envenomed by this viper.[1,21] In one of these

studies, 11% of the dogs presented with HCT > 60%. Dehydration was eliminated as a prominent cause of increased HCT based on lack of clinical evidence and because a significant number of dogs had decreased total plasma protein (TPP).[1] Similarly, hemoconcentration was observed at admission, prior to any treatment, in 14% of cases envenomed by puff adders (*Bitis arietans*) as well as cobras (*Naja* spp.).[3] Hemoconcentration was also present in 2 of 3 cats envenomed by *V. palaestinae*.[6] As localized swelling develops, protein and fluids shift from circulation to the inflamed tissue, which serves as a third space, and may cause transient hemoconcentration. Venom hemorrhagins induce vasculitis and capillary leaking, resulting in loss of fluid, proteins, and cells to the bite site.[3] Catecholamine-induced splenic contraction associated with excitement, pain, and stress and subsequent release of stored erythrocytes into circulation may also contribute to hemoconcentration.[1,21]

Anemia has been observed less frequently in dogs with snake envenomation. A survey of dogs envenomed by *Vipera berus* in Sweden listed anemia as a common hematologic sign[22]; however, decreased RBC counts were reported infrequently in dogs with *V. palaestinae* envenomation and were present in only 12% and 4% of the cases in these 2 studies.[1,21] Anemia was present in 1 of 3 cats envenomed by *V. palaestinae*.[6] Marked swelling from a puff adder bite is rapid and progressive and is associated with local hemorrhage and edema. Up to 0.5 L of whole blood might be present in a swelling on the neck of a 20-kg dog following a puff adder bite. HCT is not initially affected, although TPP might decrease. With time, the physiologic response to blood loss and decreased blood pressure results in fluid retention, hemodilution, and a later fall in HCT[2] with a further decrease expected with intravenous fluid therapy. Decreased HCT and hemoglobin concentration were reported in horses immunized subcutaneously with multivalent crotalin venom for production of antivenom.[23] Thus, the effect of venom on HCT appears to be dynamic. Although many animals may be presented initially with increased HCT, anemia may develop as the body compensates for the ensuing fluid shifts that occur during the days following a bite.

An increase in MCHC has been shown to occur after envenomation by *V. palaestinae* and is suggested to result from intravascular hemolysis owing to the actions of a hemolysin identified in the viper's venom. Other mechanisms could include indirect hemolysis by the action of phospholipase A_2 on the RBC membrane, degradation of trapped RBCs in fibrin clots, and severe vasculitis.[21] Red-colored urine has also been reported in dogs envenomed by *V. palaestinae* and was a significant risk factor for mortality likely owing to its association with a systemic bleeding disorder. However, the cause of the red-colored urine was not investigated and may have resulted from frank bleeding, hemoglobinuria, or myoglobinuria; thus, the possibility that hemolysis, rhabdomyolysis, or renal lesions played a role could not be excluded.[21] Hemolysis in elapid envenomation has been shown to result from either direct or indirect action on RBC membranes and is mediated by a polypeptidic "direct lytic factor" or phospholipase activity, respectively.[16] Membrane change lead to an influx of water resulting in RBC swelling and, ultimately, hemolysis. RBC sensitivity to venom-induced hemolysis varies among species, with canine RBCs being more sensitive to hemolytic action than those of other species, such as cats and people.[16] In animals with decreasing HCTs, the presence of free hemoglobin in plasma or urine should be investigated. Hemoglobinuria is seen occasionally following severe puff adder bites and most likely results from intravascular hemolysis.[2] Hemolysis and increased peripheral nucleated RBCs were present in a cat envenomed by *V. palaestinae*.[6] Occurrence of hemolytic anemia has been reported as a frequent finding in horses envenomed by rattlesnakes.[8]

RBC membrane abnormalities have been described in animals with snake envenomation. Echinocytes are RBCs with uniformly-sized, regularly-spaced projections from their membranes. Transient echinocytosis has been reported in association with rattlesnake envenomation in people and dogs within 24 hours of envenomation and largely resolved within 48 hours.[4,24,25] Echinocytosis in snakebite victims possibly results from ATP depletion of membrane cation pumps by ATPases and alteration of RBC membrane composition by phospholipases, specifically phospholipase A_2.[26] Both

ATPases and phospholipase A_2 are present in virtually all snake venoms; thus, echinocyte formation in rattlesnake envenomation can be secondary to both ATP depletion and phospholipase activation.[25] Phospholipase A_2 is a calcium-dependent enzyme that converts lecithin to lysolecithin, which is subsequently incorporated in the outer layer of the RBC membrane. The disproportionate expansion of the outer layer leads to formation of echinocytes and spheroechinocytes.[26] Dehydration of RBCs as the result of systemic electrolyte depletion, or RBC cation loss, has also been suggested as contributing to formation of echinocytes.[25,26] A large percentage of snakebite victims have been shown to be hypokalemic.[25] This reduction may occur secondary to membrane changes produced by the action of the venom phospholipase A_2.[26] An in vitro study reported that venom-induced echinocytosis is dose-dependent. Low venom concentrations induced type I and type II echinocytosis, whereas type III echinocytosis, spheroechinocytosis, and spherocytosis were induced with increasing venom concentration.[26] Echinocytosis is considered an indicator of rattlesnake envenomation in dogs, and spherocytosis in dogs with a history suggestive of snakebite should point to severe envenomation, particularly in small breed dogs.[26]

Unusual RBC membrane-like structures, or "erythroid loops", were observed on blood smears of dogs within hours of viper envenomation and resolved after 72 hours.[27] The erythroid loops were mostly angular in shape but did occasionally appear as thin, linear, sometimes convoluted, pale blue bands. The exact mechanism for their occurrence is still undetermined, but they may be membrane fragments secondary to RBC lysis or detached disrupted membrane fragments resulting from alteration in the outer layer of the RBC membrane during echinocyte formation.[27]

Leukocyte abnormalities

Leukograms reported in dogs with snakebites indicate an acute inflammatory response based on the presence of leukocytosis, neutrophilia, and a left shift.[1-3,21] Stress, pain, and excitement may contribute

to leukocytosis and neutrophilia, but these are usually not associated with a left shift. Leukocytosis was identified as a risk factor for mortality in dogs with *V. palaestinae* envenomations.[28] The overall absence of lymphopenia and eosinopenia also suggests that stress is a less important mechanism.[1,2] Neutrophilia was frequently present in New World camelids envenomed by rattlesnakes; however, in contrast to dogs lymphopenia was concurrently present, and thus stress may have played a role in the leukogram abnormalities in these animals.[12] Horses immunized subcutaneously with crotalin venoms for production of a polyvalent antivenom developed mild leukocytosis owing to increased neutrophils and lymphocytes.[23]

Leukopenia has been reported in snake envenomation; it mostly occurs in acute envenomation and appears to resolve within 30 to 40 minutes of presentation. This acute leukopenia is thought to result from leukocyte entrapment in thrombi formed as a consequence of the prothrombin activator component of snake venoms.[16]

Platelet abnormalities

Venom-induced thrombocytopenia is frequently observed in animals and people with moderate to severe viper envenomation. Possible mechanisms leading to thrombocytopenia in viper envenomation include vasculitis, sequestration of platelets in inflamed tissue, and consumption of platelets with potential development of disseminated intravascular coagulation (DIC).[21] Thrombocytopenia is frequently reported in puff adder bites[2]; puff adder venom is known to contain an irreversible platelet aggregation-inducing component that has been associated with active hemorrhage in baboons, people, and dogs.[2,29,30] Thrombocytopenia has also been reported in people, dogs, horses, and New World camelids envenomed by rattlesnakes (*Crotalus* spp.) and may result from both enzymatic and non-enzymatic venom components leading to platelet aggregation and adherence to damaged endothelium, as well as a variety of other mechanisms.[8,12,24,31] Phospholipase A_2 cleaves arachidonic acid from

platelet membranes leading to 1) formation of thromboxane A_2, 2) induction of conformational changes in platelet glycoproteins and fibrinogen-binding with subsequent platelet aggregation, and 3) activation of prothrombin or generation of thrombin-like proteases that induce platelet aggregation and sequestration at the site of actual tissue injury.[24,32] Antivenom administration after rattlesnake envenomation has been shown to increase platelet counts.[24]

Thrombocytopenia has been observed at the time of presentation in > 50% of dogs envenomed by *V. palaestinae*.[1] In another study of dogs envenomed by this viper, thrombocytopenia was present in 32% of the dogs at presentation, and its incidence increased to 52% at 24 hours post-presentation.[21] In that study, thrombocytopenia at presentation with progressive worsening over time was found to be a significant risk factor for mortality. Thrombocytopenia was positively correlated with mortality in coral snake (elapid) envenomation in dogs.[33] Thrombocytopenia is also frequently found in dogs envenomed by *V. berus* and was present in all 3 cats envenomed by *V. palaestinae*.[6,22] A retrospective study of snake envenomation in South Africa also identified thrombocytopenia as a frequent laboratory abnormality[3]; dogs envenomed by puff adders and the Mozambique spitting cobra (*Naja mossambica*) with cytotoxic venom had more severe thrombocytopenia (median platelet count, 66×10^9/L; reference interval, 200-500 $\times 10^9$/L) compared with those envenomed by other snakes of the Viperidae and Elapidae families (median platelet count, 243×10^9/L).

Hemostatic changes

Coagulopathies represent some of the most important effects of snakebites and occurs in many viper, elapid, and colubrid envenomations. The initial clinical signs associated with these envenomations is often mild and onset of signs can occur later than in cases of neurotoxic or cytotoxic snakebites.[18-20] Most animals are presented to a veterinarian after a considerable delay following envenomation.

Hemostatically active components are distributed widely in venoms of many snake species, particularly vipers, pit vipers, and elapids, and may exert both procoagulant and anticoagulant properties, causing concurrent thrombosis, consumptive coagulopathy, and bleeding.[2,34] The components that affect hemostasis can be classified into several categories: 1) enzymes that cleave fibrinogen; 2) enzymes that degrade fibrin(ogen); 3) plasminogen activators; 4) prothrombin activators; 5) factor V activators; 6) factor X activators; 7) anticoagulants, including inhibitors of prothrombinase complex formation, thrombin inhibitors, phospholipases, and protein C activators; 8) enzymes with hemorrhagic activity affecting blood vessel wall integrity; 9) enzymes that degrade plasma serine proteinase inhibitors; 10) platelet aggregation inducers; and 11) platelet aggregation inhibitors.[34]

Although many snake venoms contain several components, no single venom contains all these factors. Pathophysiologic mechanisms of snakebite-induced coagulopathies have been extensively investigated in human medicine and in laboratory animals. However, people are not the natural prey for venomous snakes, and the effect of any venom component in natural prey may be different than that in people. Additionally, snakebite coagulopathies are not necessarily similar to other forms of coagulopathies, an assertion supported by the almost uniform failure of standard treatment in these cases.[17] Studies have shown that coagulopathies induced by snakebites are attributed to multiple venom components affecting human hemostasis. There are several associated clinical problems: 1) decreased blood coagulability with an increased bleeding tendency; 2) frank bleeding resulting from endothelial damage; 3) secondary deleterious effects of hemorrhagic diatheses, such as hypovolemic shock and secondary organ damage, including intracranial or anterior pituitary hemorrhage and renal damage; and 4) macro- and microthrombosis, such as pulmonary thromboembolism, and secondary organ damage. The effects of snake venom on hemostasis can be ranked according to the type of toxin.[17]

Hemorrhage after envenomation can occur as a result of abnormal functioning of coagulation factors, but also from the effects of factors in venom on capillary endothelium and platelets.[35] Viperid

and crotalid (rattlesnake) venoms are rich in metalloproteinases, which are responsible for rapid development of local hemorrhage following intradermal or subcutaneous injection. This effect can be attributed to the capacity of metalloproteinases to degrade extracellular matrix proteins, particularly type-IV collagen, a major component of the basement membrane, leading to weakened adhesion of endothelial cells to the basement membrane and compromised integrity of blood vessels.[35,36] Patients envenomed by viperid or crotalid snakes often suffer systemic hemorrhage without evidence of coagulation factor abnormalities, suggesting that systemic bleeding in certain snakebites could be attributed to a venom-induced platelet disorder.[35] Studies have shown that platelet interactions with collagen and von Willebrand's factor are inhibited by various mechanisms through targeting of platelet receptors or their ligands by certain venom components.[35] In addition, thrombocytopenia, although an inconsistent finding in envenomation by specific coagulopathic snakes, eg, boomslang and vine snakes, is a contributing factor and may have delayed onset in some cases.[18-20,37,38] The underlying mechanism of delayed thrombocytopenia may be the occurrence of early platelet sparing because venom-induced thrombin is different from native thrombin and its induction of platelet aggregation is less efficient.[20] Snake venoms contain large amounts of phospholipase A_2 in many isoenzymatic forms; several hundred phospholipase A_2 enzymes have been purified and characterized in snake venom.[39] They induce a wide spectrum of pharmacologic effects, including neurotoxic, cardiotoxic, myotoxic, hemolytic, convulsive, anticoagulant, antiplatelet, edema-inducing, and tissue-damaging actions. Some phospholipase A_2 enzymes hydrolyze negatively charged phospholipids that are cofactors of the prothrombinase complex, thereby accounting for their anticoagulant properties. Others hydrolyze platelet membrane phospholipids, releasing platelet activating factor or arachidonic acid, a precursor of thromboxane A_2, thereby inducing platelet aggregation.[39]

Both boomslang and vine snake venoms are potent procoagulants shown to activate prothrombin (factor II) and factor X in people, resulting in consumptive coagulopathy with subsequent

hemorrhage and microthrombosis.[32,37,38] The same is believed to be true in dogs.[18-20] Although hemorrhage can be profound in these cases, it is the occurrence of coagulation, ie, microvascular thrombosis, rather than hemorrhage that has the greatest impact on morbidity and mortality, leading to multiple organ failure. The venoms of the boomslang and vine snakes have also been shown to accelerate fibrinolysis by activating plasminogen. This might improve the prognosis in such cases that develop thrombotic microangiopathy. Therefore, fibrinolysis should not be prevented by treatment with fibrin-stabilizing drugs.[18,20]

The venom of the Gaboon viper (*Bitis gabonica*) has been shown to interfere with thromboplastin through defective thrombin generation and prothrombin consumption, inhibition of thromboplastin generation, and accelerated loss of formed thromboplastin.[40] Despite the proteolytic activity of the venom, activation of plasminogen could not be demonstrated. ADP-induced platelet aggregation has also been shown to be inhibited by L-amino acid oxidase (LAAO) from the venoms of *Vipera berus berus* and *Vipera lebetinae*, both close relatives of *V. palaestinae*.[41,42]

Hemorrhage is one of the main causes of death following envenomation by *V. palaestinae*. The exact mechanism of action has not yet been determined, but the metalloproteinases hemorrhagin 1 (HR1) and 2 (HR2) are both present in the venom and have been shown to exhibit protease activity (gelatinase and caseinase), which can lead to platelet and coagulation factor dysfunction.[28] As previously described, platelet function has been shown to be adversely affected by viperid and crotalid metalloproteinases through several mechanisms.[35] Viperid snake venoms are rich in serine proteases, phospholipases, and metalloproteinases that affect both coagulation and fibrinolysis as well as platelet aggregation and can potentially lead to hypercoagulability and DIC. Prothrombin time (PT) and activated partial thromboplastin time (aPTT) have both been shown to be prolonged at the time of presentation in the majority of dogs envenomed by *V. Palaestinae* and remained prolonged 24 hours after presentation.[21] In a more recent study, increased d-dimer concentrations, prolonged PT and aPTT,

thrombocytopenia, and low antithrombin activity suggested that a hypercoagulable/procoagulant state was present early after envenomation and also developed later in the course of disease. Both hyper- and hypofibrinogenemia were observed in a significant number of dogs during hospitalization. Of 39 dogs included in the study, coagulation abnormalities were absent in only 2 dogs during the disease course; DIC played an important role in the mortality of dogs envenomed by *V. palaestinae* and DIC had developed in all fatal cases.[28]

Venom-induced consumptive coagulopathy

Snakebites have been considered a cause of DIC for decades based on the current diagnostic criteria for DIC, including thrombocytopenia, increased concentration of d-dimers or fibrin degradation products (FDPs), prolonged PT and aPTT, and fibrinogen depletion.[43,44] More recently, the coagulopathy associated with snakebites has been referred to as a venom-induced consumptive coagulopathy (VICC).[43,44] VICC is characterized by prolonged clotting times, depletion of fibrinogen and cofactors V and VIII, and high concentrations of FDPs; however, it is not characterized by the other important features of DIC, such as evidence of systemic microthrombi and end-organ failure.[43,44] VICC results from the activation of the coagulation pathway by a procoagulant toxin(s) in the snake venom that acts at a specific position in the pathway, depending on the specific toxin. These toxins include procoagulants, such as thrombin-like enzymes, prothrombin activators, and factor X activators.[43] The pathogenesis and mechanism of initiation of coagulation activation differ in DIC, in which activation is usually mediated by the tissue factor/factor VIIa pathway.[43] The venoms of brown snakes (*Pseudonaja* spp.) and taipans (*Oxyuranus* spp.), both extremely dangerous Australian snakes, have been shown to contain group C prothrombin activators that closely resemble the mammalian prothrombinase (Xa:Va) complex responsible for converting prothrombin to thrombin. Exposure to these venom components results in activation of coagulation, leading to consumptive coagulopathy.[44] The main complication in

VICC is hemorrhage without obvious fibrin deposition, microvascular thrombotic obstruction, and resultant end-organ damage or failure, which are found in DIC and explain the significantly higher mortality rate in DIC compared with VICC.[43] In a small proportion of patients with VICC, a clinical syndrome consistent with thrombotic microangiopathy (TMA) has been reported. The etiology of snakebite-associated TMA remains unclear and has been suggested to result from a specific venom toxin. However, TMA has been reported to occur with bites of different snake species that have different procoagulant toxins, making this speculation unlikely. TMA does not appear to occur in the absence of VICC, so it most likely results from VICC or from some other process that occurs in conjunction with VICC, but is only manifested in a subgroup of patients. Concurrence of TMA and VICC in these patients may underlie the erroneous diagnosis of DIC in these indivduals.[43]

Coagulation testing

If available, extended coagulation studies are appropriate in cases of snakebite envenomation, especially if the snake species is known to induce hemostatic defects. These tests should include PT and aPTT, fibrinogen and FDP or d-dimer concentrations, and antithrombin activity. Thromboelastography (TEG) may prove useful as an assessment of global coagulation and has been shown to identify early hemostatic abnormalities in dogs in the pro-inflammatory and hypercoagulable phase of DIC.[45] However, TEG studies in cases of snakebites in domestic animals have not been published yet. Serial assays are essential even if abnormalities are not indentified initially. For example, late-developing coagulopathy has been shown to occur frequently in dogs envenomed by *V. palaestinae*.[28] Apart from determining the presence of a coagulopathy, identification of the type of coagulopathy can be diagnostically and therapeutically important. Consumptive coagulopathy is characterized by prolonged PT and aPTT as well as low to absent fibrinogen and increased FDP and d-dimer concentrations. In coagulopathy resulting from anticoagulant action PT and aPTT will be

prolonged, but fibrinogen concentration should be normal and FDPs and d-dimers undetectable. The presence of a coagulopathy, measured by prolonged PT and aPTT, has been shown to be a prime discriminator in envenomation of people by venomous Australian snakes.[16,17] Similar results were seen in dogs, but the same tests were poorly predictive of envenomation in cats.[16] Although FDPs are discussed in several case reports, controlled studies to assess the efficacy of measuring FDP and d-dimer concentrations in snakebites have not been reported.[16]

Snake venom toxins that affect hemostasis have been used extensively in basic coagulation research, facilitating routine assays of hemostatic variables. Snake venom thrombin-like enzymes (SVTLE) are used for fibrinogen or fibrin(ogen) breakdown product assays, as well as for detection of fibrinogen dysfunction. Because SVTLE are not affected by heparin, antithrombin and other hemostatic factors and functions can be analyzed using heparin-containing samples in the presence of SVTLE. Snake venom also contains prothrombin activators, and these are used in prothrombin assays to investigate dysprothrombinemias. Russell's viper (*Daboia russelli*) venom contains toxins which have been used to assay clotting factors such as factors V, VII, X, platelet factor 3, and lupus anticoagulants. Snake venom toxins also have therapeutic anticoagulant effects and have proven useful in therapeutic defibrination, the total removal of plasma fibrinogen from the circulation by means of SVTLE. The SVTLE acts by converting fibrinogen into a non-clottable form of fibrin which is then removed by various enzymatic processes.[46]

Biochemical changes

Various biochemical abnormalities have been documented in snake envenomation and may be helpful in detecting and quantifying the pathologic effects. Previous reports of biochemical alterations, mostly in dogs envenomed by *V. palaestinae*, suggested multiple organ pathology.[1,21] High activities of alanine

aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), gamma-glutamyltransferase (GGT), and alkaline phosphatase (ALP) have been documented with envenomation by *V. palaestinae*, suggesting hepatocellular Damage and cholestasis secondary to hypoxemia in cases of acute hypovolemia as well as direct damage by cytotoxins.[1,21] Increased AST activity and, to some degree, increased ALT activity may also occur from concurrent muscle damage.[1] A hemolysin/hemotoxin, shown to cause hemolysis in people envenomed by various species of snakes, was hypothesized to be the cause of moderate hyperbilirubinemia observed in a number of dogs envenomed by *V. palaestinae*.[1,21] However, hyperbilirubinemia may also have resulted in part from a combination of hemolysis and ischemic hepatopathy.[21]

Myotoxins are defined as venom components that have specific actions on skeletal muscle, and those found in elapid venom are phospholipases that act by damaging the integrity of the sarcolemma, resulting in local hemorrhage, necrosis, and even systemic rhabdomyolysis.[16] Increased serum activities of creatine kinase (CK), as well as AST and LDH, with *V. palaestinae* envenomation in dogs were attributed to muscle damage at the site of the snakebite; however, increased activity of these enzymes from concurrent myocardial damage could not be ruled out.[1,21] Increased muscle enzyme activity has been reported in New World camelids envenomed by rattlesnakes.[12] Mild increases in CK and ALT activities were reported in horses immunized subcutaneously with multivalent crotalin venom for production of antivenom.

CK activity has been used in people as an indicator of the amount of venom injected and the delay from envenomation to presentation in individuals bitten by non-elapid myolysis-inducing snake species; it has also been used as a marker of the severity of lesions induced by other snake species.[47,48] The use of CK in domestic animals for these purposes is limited as the exact time of envenomation is usually unknown. Rhabdomyolysis might be responsible for hyperkalemia, hypercalcemia, and hyperphosphatemia reported in dogs envenomed by vipers. However, the mechanisms responsible for

hypokalemia and hypocalcemia, especially in the presence of normal albumin, are unclear.[1,16,21] Hypokalemia was also observed in New World camelids envenomed by rattlesnakes.[12]

Hypoalbuminemia was reported in dogs envenomed by *V. palaestinae* and is suggested to result from albumin leakage secondary to vasculitis and capillary damage, especially at the envenomation site.[21] Similarly, hypoproteinemia was present in 1 of 3 cats envenomed by *V. palaestinae* and was presumed to occur for the same reason.[6] Hypoalbuminemia was a frequent finding in New World camelids envenomed by rattlesnakes.[12] Hyperglobulinemia, observed in a large proportion of cases envenomed by *V. palaestinae*, is thought to result from increased levels of acute phase proteins rather than an increase in immunoglobulins.[1] However, in horses immunized subcutaneously with multivalent crotalin venom for production of antivenom, a significant increase in serum protein concentration was observed after the second inoculation dose, and the concentration remained increased for the duration of the study.[23] This was probably a result of both an acute phase response and increased antibody synthesis.

Mild hyperglycemia has been observed in a number of dogs envenomed by *V. palaestinae* and was probably the result of envenomation-associated stress, pain, and anxiety.[21] Hyperglycemia was also observed in New World camelids envenomed by rattlesnakes.[12]

Hypocholesterolemia with severely low cholesterol concentrations were reported in fatal cases of people envenomed by *V. palaestinae.* Cholesterol concentration was inversely correlated with envenomation severity in human patients.[49] Hypocholesterolemia was present in 2 of 16 fatal cases of *V. palaestinae* in dogs[50] and in 10 of 35 dogs envenomed by this viper.[21] Hypocholesterolemia appears to be an important risk factor for mortality with *V. palaestinae* envenomation and is hypothesized to result from lipoprotein leakage through capillaries and changes in lipoprotein transport and metabolism caused by phospholipase A_2 activity.[21]

Acute renal failure has been associated with snakebites in dogs. The primary causes include nephrotoxic effects of myoglobinuria and hemoglobinuria, DIC, toxic nephropathy, and hypovolemic shock with renal ischemia. Ischemic necrosis is also mediated by both vasoconstriction and procoagulant microthrombotic effects.[16,51] Severe intravascular hemolysis, rhabdomyolysis, and anuric renal failure have been reported in a dog envenomed by a red-bellied black snake (*Pseudechis porphyriacua*), an elapid snake.[52] The dog had a progressively declining HCT with dark discoloration of the serum and urine and hyperbilirubinemia, as well as moderate azotemia and hyperphosphatemia. Marked increases in CK and AST activities were consistent with rhabdomyolysis, although the activity of both enzymes may have also been increased from intravascular hemolysis. Hemolysis may interfere with the coupled reactions of CK assays which could lead to falsely increased CK activity.[53] The dog became progressively anuric and was euthanized. Necropsy revealed muscular necrosis and marked renal tubular necrosis with intraluminal occlusion secondary to pigmentary casts.[52] Another report of a German shepherd dog envenomed by *Vipera aspis* described acute intrinsic renal failure after envenomation. Necropsy revealed discrete glomerular hypercellularity, mesangial lysis, and renal tubules filled with numerous hyaline casts and necrotic cells.[54]

Cardiac arrhythmias have been reported in both people and dogs after *V. palaestinae* envenomation, although a specific cardiotoxin has yet to be demonstrated in this viper's venom.[55-57] Cardiac troponins are responsible for regulating myocardial calcium-mediated actin-myosin interactions. In people and dogs, cardiac troponin-I (cTn-I) and troponin-T (cTn-T) have similar structures and are sensitive and specific biomarkers of myocardial injury.[57] In a significant number of dogs envenomed by *V. palaestinae*, both cTn-I and cTn-T concentrations were increased at presentation and continued to increase over time. Dogs with increased cTn-T, but not cTn-I, had a significantly higher occurrence of cardiac arrhythmias and increased resting heart rate at presentation and the first 24 hours after presentation and were hospitalized for a significantly longer period

compared with dogs with normal cTn-T concentrations.[57] Increased CK and AST activities, reported in previous studies of dogs envenomed by this snake, may be attributed in part to myocardial damage.[1] Eight of 24 dogs envenomed by *V. berus* had evidence of myocardial damage and 6 of 24 of these dogs had increased cTn-I concentrations.[58] Thus, it appears that dogs envenomed by *V. palaestinae* and *V. berus*, and probably other viper species, sustain some degree of myocardial injury, and attending clinicians should be alerted to potential ongoing cardiac injury. Similar findings were seen in a horse several days after being envenomed by a prairie rattlesnake (*Crotalus viridis viridis*).[56] The horse developed severe bradyarrhythmias with third-degree atrioventricular block. Increased cTn-I concentration confirmed myocardial damage suggested by the electrocardiogram. At necropsy, gross examination of the heart revealed widespread myocardial necrosis of ventricular walls, atria, and interatrial and interventricular septae. Myocarditis developed several days to weeks after resolution of local inflammation caused by *V. palaestinae* bites in 2 horses.[59] One horse was presented with tachyarrhythmia and ventricular premature complexes. In both horses extensive myocardial necrosis was observed at necropsy. Thus, horses, like dogs, sustain myocardial lesions following viper bites and serum biochemical analysis, specifically CK activity and cTn concentrations, should be evaluated in such cases.

Conclusion

Information on envenomation of domestic animals in different geographic regions by various families of snakes is sparse. This comprehensive review of associated clinicopathologic abnormalities and their relationship to characterized venom constituents should be useful both in the diagnosis and management of envenomation and as a foundation for future research in this field.

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Table 1. Classification of venomous snake species and their geographic distribution and major venom types.

Family name	Common venomous species	Geographic distribution	Major venom types
Colubridae	Boomslang snake, twig or bird snake (also known as Vine snake)	Worldwide distribution (excluding Antarctica)	Hemotoxic – affects coagulation
Elapidae	Cobras and related species (eg, mambas)	Tropical and subtropical regions (ie, North and South America, Africa, Asia, and Australia)	Mostly neurotoxic (some members are mixed neurotoxic/cytotoxic or completely cytotoxic)
Viperidae	Vipers or adders (*Viperinae*) and pit vipers (*Crotalinae*, eg, rattlesnakes and lanceheads)	Worldwide distribution (excluding Antarctica, Australia, Newfoundland, New Zealand, Ireland, Madagascar, Hawaii, and the Arctic Circle)	Mostly cytotoxic (some venoms are partially neurotoxic)

Table 2. Constituents of snake venom, their distribution in snake families and their effects on the prey

Venom constituents	**Snake type**	**Major mechanism of action**	**Net effect on the prey**
Cholinesterase	Most dangerous species (particularly elapids)	Blocks neuromuscular transmission by splitting acetylcholine to choline and acetic acid	Flaccid paralysis
Phospholipases (eg, PLA_2)	Virtually all venomous snakes, especially elapids	PLA_2 cleaves platelet membrane arachidonic acid and thromboxane A_2 is formed	Coagulation abnormalities, vascular and muscle damage, edema, and tissue necrosis
L-amino acid oxidase (LAAO) (gives some venoms their distinctive yellow coloring)	Vipers	Digest tissues; triggers other enzymes	Increased prey solubility, promoting intestinal absorption by the snake
Hyaluronidase	All venomous snakes	Dissolves intercellular matrix	Facilitation of rapid absorption of other venom enzymes by the prey
Proteinase	Vipers	Accelerates protein breakdown in the prey	Increased prey solubility, promoting intestinal absorption by the snake
Adenosine triphosphate	Most snakes, especially vipers	Lowers blood pressure through enzyme catabolism of ATP into 2 toxic substances	Immobilization of smaller prey, induction of shock
Phosphodiesterase	Virtually all venomous snakes	Induces negative cardiovascular effects	Rapid decrease in blood pressure
Metalloproteinases	Vipers (high quantities)	Degrade prey's extracellular matrix; induce vascular endothelial lesions	Local and systemic coagulation abnormalities