Myocardial injury in dogs with snake envenomation and its relation to systemic inflammation

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Running title: Myocardial injury in dogs with snakebites
Abbreviations

CRP: C-reactive protein

cTnI: Cardiac troponin I

VPC: Ventricular premature contraction
Abstract

Objective – To investigate the presence of myocardial injury in dogs hospitalized for snake envenomation and to examine its relationship with systemic inflammation.

Design – Prospective case-control study.

Setting – University teaching hospital and small animal referral hospital.

Animals – Dogs naturally envenomed by the European viper (Vipera berus) (n=24), African puff adder (Bitis arietans) (n=5), or snouted cobra (Naja annulifera) (n=9).

Interventions – Blood was collected from dogs envenomed by V. berus at admission, 12-24 hours post-admission, and 5-10 days post-admission. Blood was collected from dogs envenomed by B. arietans or N. annulifera at admission, and 12, 24, and 36 hours post-admission.

Measurements and Main Results – Concentrations of cardiac troponin I (cTnI), a marker of myocardial injury, and C-reactive protein (CRP), a marker of systemic inflammation, were measured in each blood sample. Evidence of myocardial injury was found in 58% of dogs envenomed by V. berus at one or more time-points. A significant correlation between cTnI and CRP concentrations was found at all time-points. Evidence of myocardial injury was found in 80% of dogs envenomed by B. arietans at one or more time-points; however, no correlation was found between cTnI and CRP concentrations. Evidence of myocardial injury was found in 67% of dogs envenomed by N. annulifera at one or more time-points. A significant correlation between cTnI and CRP concentrations was found at admission, but not at other time-points.

Conclusions – Myocardial injury frequently occurred in dogs with snake envenomation. While the degree of systemic inflammation was significantly correlated with degree of myocardial injury in V. berus envenomation at all time-points, this was not the case in dogs envenomed by N. annulifera or B. arietans. This could be due to differences in the toxic substances of the snake venoms or to differences in the cytokines induced by the venom toxins.
Keywords
Biomarker, cardiac troponin I, companion animals, toxins

Introduction
In many countries, snake envenomation poses a health risk to both people and animals. The type of snake, amount of venom injected, and site of envenomation all influence the initial hazard and the progression of the intoxication.\textsuperscript{1,3} In some cases, only a local reaction occurs with edema and tissue necrosis,\textsuperscript{4} but often the toxic insult progresses to a systemic reaction that may lead to specific or multiple organ dysfunction and death.\textsuperscript{5-7} Snake venoms are known to contain a complex mixture of toxins. Some are proteolytic causing tissue destruction in order to enhance local venom uptake and spread. Others target specific organ systems (e.g. nervous system, cardiovascular system, coagulation system).\textsuperscript{1} In addition to the direct effects of the toxins, a systemic inflammatory response may also contribute to disease progression and further tissue damage.\textsuperscript{8}

The development of myocardial injury in patients with critical illness has been investigated in both human and veterinary medicine as measured through increases in circulating cardiac troponin I (cTnI) concentrations, a highly specific biomarker of cardiomyocyte injury and necrosis.\textsuperscript{9-12} The presence of myocardial injury has been found to be significantly associated with mortality in people and more recently in dogs.\textsuperscript{10-13} Myocardial injury has also been described in association with snake envenomation.\textsuperscript{8,14,15} However, while some snake venoms (i.e., the venom of cobras) are known to contain specific cardiotoxins,\textsuperscript{5} it is possible that others cause myocardial injury secondarily through induction of systemic inflammation.\textsuperscript{8} Inflammatory disease states are
thought to cause myocardial injury through many mechanisms including hemodynamic changes, micro-vascular thrombosis, and toxic effects of cytokines.\textsuperscript{10,13,16-18}

Envenomation by snakes of the family \textit{Viperinae} (e.g. vipers, adders) can induce myocardial injury and cardiac arrhythmias,\textsuperscript{8,14,19,20} and the cardiac involvement has been confirmed through histopathology with findings of edema, hemorrhage, necrosis, interruption of the sarcolemma, and granular degeneration in the myocardium.\textsuperscript{21,22} It is unknown, however, whether a direct cardiotoxic substance exists in the venom, or whether the myocardial injury is due to a secondary deleterious effect on the myocardium from a systemic inflammatory response induced by the venom toxins.\textsuperscript{3,8,14} In Scandinavia, the European viper is the only poisonous snake, and every year a large number of dogs are hospitalized after envenomation.\textsuperscript{23} While almost all of these dogs recover,\textsuperscript{3,7,23} some develop more severe systemic signs which may delay the recovery. Cardiac injury could be contributing to this protracted recovery phase.\textsuperscript{14} Serious complications and death are more frequently seen with envenomation by snakes such as the \textit{Elapidae} (e.g. cobras),\textsuperscript{5} and while cobra venom is known to contain cardiotoxins, the occurrence of myocardial injury in dogs with cobra envenomation and its relation to systemic inflammation and disease progression has not been examined.

The purpose of this study was to investigate the presence of myocardial injury in dogs hospitalized for envenomation by the European viper (\textit{V. berus}), the African puff adder (\textit{B. arietans}), or the snouted cobra (\textit{N. annulifera}) and to examine its relation with systemic inflammation as measured by C-reactive protein.\textsuperscript{24} It was hypothesized that myocardial injury would be related to the degree of systemic inflammation induced by the snake envenomation. Since arrhythmias are commonly found in dogs envenomed by \textit{V. berus}, it was also investigated
whether a significantly higher degree of myocardial injury was present in dogs with arrhythmias compared to dogs with normal ECGs.

**Materials and methods**

The present study was approved by the respective local ethical committees at the involved institutions, and written consent was obtained from all dog owners. Healthy dogs for a control group were recruited from staff-owned dogs and elective patients presenting to the Blue Star Animal Hospital, Gothenburg, Sweden, and were deemed healthy through medical history, clinical examination, and hematologic and biochemical profiles. Blood was obtained from these dogs only once and handled as for the *V. berus* group.

Dogs envenomed by *V. berus* were included prospectively as they presented to the Blue Star Animal Hospital, Gothenburg, Sweden, from July to August 2011. Dogs were excluded if there was uncertainty of the diagnosis of snake envenomation, for example if bite marks were not localized or the dog lacked signs strongly indicative of snakebite (i.e., sudden development of edema in the area of the suspected bite, lethargy, and a history suggestive of snakebite) or if the dogs were not considered healthy prior to the envenomation. Dogs were also excluded if the clinical exam revealed a cardiac murmur in order to exclude primary cardiac disease as a cause of cardiac troponin release. Information regarding the interval from envenomation to presentation and possible treatment prior to presentation was obtained, and a full clinical examination was carried out.

Blood samples were obtained at admission, 12-24 hours post-admission, and 5-10 days after envenomation. Full admission hematologic and biochemical profiles were analyzed, and, as renal disease has been associated with increased cTnI concentrations in previous studies,\textsuperscript{25,26} dogs were excluded if they had increased serum creatinine concentrations (>130 µmol/L [1.47 mg/dL])
based on the normal reference interval of the Central Laboratory at the Department of Veterinary Clinical and Animal Sciences, University of Copenhagen, Denmark. Blood was collected in plain tubes for analysis of serum cTnI as a marker of myocardial injury and serum C-reactive protein (CRP) as a quantitative marker of systemic inflammation. Samples were centrifuged at 665 G for 9 minutes, separated, and stored at -80°C within 2 hours of blood collection, except for 1 dog for which the serum sample was stored for 24 hours at -20°C before being moved to -80°C. Samples were shipped on dry ice to the Department of Veterinary Clinical and Animal Sciences, University of Copenhagen, Denmark, and stored for a maximum of 3 months until batch analysis.

A two-minute ECG was recorded at admission and 12-24 hours post-admission. At the control visit 5-10 days after envenomation, an ECG was recorded again if arrhythmias had been noted during hospitalization.

Dogs envenomed by *B. arietans* or *N. annulifera* were included prospectively as they presented to the Department of Companion Animal Clinical Sciences, University of Pretoria, South Africa, from November 2010 to March 2011. Dogs were excluded if there was uncertainty of the diagnosis, or if they were not considered healthy prior to the envenomation. Dogs were also excluded if the clinical exam revealed a cardiac murmur in order to exclude primary cardiac disease as a cause of cardiac troponin release. Information regarding the interval from envenomation to presentation and possible treatment prior to presentation was obtained, and a full clinical examination was carried out.

Blood samples were obtained at admission and at 12, 24, and 36 hours post-admission. Full admission hematologic and biochemical profiles were analyzed, and dogs were excluded if they had increased serum creatinine concentrations (>130 µmol/L [1.47 mg/dL]). Blood was collected in plain tubes for analysis of serum cTnI and CRP. Samples were centrifuged at 2100 g for 8 minutes, separated, and stored at – 80°C within 2 hours of blood collection. Samples were
shipped on dry ice to the Department of Veterinary Clinical and Animal Sciences, University of Copenhagen, Denmark, and stored for a maximum of 14 months until batch analysis.

C-reactive protein and cTnI were analysed at the Central Laboratory at the Department of Veterinary Clinical and Animal Sciences, University of Copenhagen, Denmark. Cardiac troponin I was analysed using a commercially available high-sensitivity immunoassay recently validated for use in dogs. C-reactive protein was analysed using a commercially available turbidimetric immunoassay validated for canine use and calibrated with purified canine CRP. Evidence of systemic inflammation was defined as a concentration of CRP > 35 mg/L (3.5 mg/dL).

Statistical analysis
All statistical analyses were conducted using commercial statistical software. Data were assessed for normality using D’Agostino-Pearson omnibus test. Logarithmic transformation was applied to assure a Gaussian distribution of otherwise non-parametric data. A two-tailed t-test was used to compare Gaussian data, and the Mann-Whitney-U test was used to compare non-Gaussian data. Correlations between cTnI and CRP concentrations were assessed graphically as well as by Spearman’s correlation coefficient. Statistical significance was defined as \( P < 0.05 \).

Results
Healthy dogs:
Nine healthy dogs were included in the study and consisted of 5 female intact, 1 female neutered, 2 male intact, and 1 male neutered dog and ranged in age from 0.5-5.0 years (mean 3.2 years). One dog was a mixed breed; all other dogs were purebreds of 6 different breeds, the most frequent being Border Collie (n=3). The cTnI concentrations in healthy dogs were (median
Median concentration of CRP was 0 [0-0.75] mg/L (0 [0-0.075] mg/dL). Thus, all healthy dogs had cTnI and CRP concentrations within established reference intervals.

**European viper (V. berus):**

Twenty-four dogs envenomed by *V. berus* were included in the study. In 20 of these dogs, bite marks were identified during the clinical examination. For the remaining 4 dogs, the history and clinical signs were strongly indicative of snake envenomation. Included dogs consisted of 12 female intact, 1 female neutered, 8 male intact, and 3 male neutered dogs ranging in age from 0.5-11 years (mean 4.1 years). Six dogs were mixed breeds; all other dogs were purebreds of 15 different breeds, the most frequent being Labrador Retriever (n=2), German Shepherd (n=2), and Danish-Swedish Farm Dog (n=2). Twenty dogs returned for the planned follow-up visit 5-10 days later.

CtNI concentrations were (median [range]) 0.02 [0.004-27.2] µg/L (0.02 [0.004-27.2] ng/mL) at admission, 0.08 [0.007-26.4] µg/L (0.08 [0.007-26.4] ng/mL) at 12-24 hours post-admission, and 0.03 [0.004-4.4] µg/L (0.03 [0.004-4.4] ng/mL) at the follow-up visit 5-10 days later (Figure 1). Nine of 24 dogs (37.5%) had cTnI concentrations above the reference limit at admission, 13 of 24 (54%) at 12-24 hours post-admission, and 6 of 20 (30%) still had increased cTnI concentrations at the follow-up visit. All in all, 14 of the 24 dogs (58%) had increased cTnI concentrations at one or more time-points. CRP concentrations were (median [range]) 5.3 [0-137.5] mg/L (0.5 [0-13.8] mg/dL) at admission, 73.5 [5.6-120.3] mg/L (7.4 [0.6-12.0] mg/dL) at 12-24 hours post-admission, and 3.3 [0-65.4] mg/L (0.3 [0-6.5] mg/dL) at the follow-up visit.
Figure 1. Cardiac troponin I (cTnI) concentrations in 24 dogs envenomed by the European viper (*V. berus*) at admission (0h), 12-24 hours post-admission (12-24h) and 5-10 days later (followup) as well as 5 dogs envenomed by the African puff adder (*B. arietans*), and 9 dogs envenomed by the snouted cobra (*N. annulifera*) at admission (0h) and 12, 24, and 36 hours (h) postadmission. Groups assigned with an asterisk have median (*V. berus* and *B. arietans*) or mean (*N. annulifera*) concentrations significantly different from the control group (clinically healthy dogs).
Five of 24 dogs (20.8%) had CRP concentrations > 35 mg/L (3.5 mg/dL) at the time of admission, 20 of 24 (83%) 12-24 hours after, and 2 of 20 (10%) at the follow-up visit.

Dogs envenomed by *V. berus* had significantly higher cTnI concentrations than healthy dogs at admission (*P*=0.049) and 12-24 hours post-admission (*P*<0.001), but not at the follow-up visit 5-10 days later (*P*=0.056) (Figure 1).

Envenomed dogs with CRP concentrations > 35 mg/L (3.5 mg/dL) at admission and 12-24 hours post-admission had significantly higher cTnI concentrations than envenomed dogs with CRP concentrations < 35 mg/L (3.5 mg/dL) (*P*=0.005 and *P*=0.013) and healthy control dogs (*P*=0.003 and *P*<0.001) (Figure 2). No difference in cTnI concentrations were seen between envenomed dogs with CRP concentrations < 35 mg/L (3.5 mg/dL) and healthy control dogs at any time-point (*P*=0.2 and *P*=0.19). Cardiac troponin I concentrations were significantly correlated with CRP concentrations at admission (*r*=0.5, *P*=0.014), 12-24 hours post-admission (*r*=0.67, *P*<0.001), and 5-10 day post-admission (*r*=0.55, *P*=0.013).

Six dogs presented with arrhythmias at the time of admission (Table 1), 2 of which had increased cTnI concentrations at this time. Ten dogs had arrhythmias 12-24 hours post-admission (Table 1) at which time 5 of these had increased cTnI concentrations. No difference was observed between cTnI concentrations in dogs with or without arrhythmias at admission (*P*=0.65) or 12-24 hours post-admission (*P*=0.26). None of these dogs had cardiac arrhythmias at the follow-up visit 5-10 days after envenomation.

**African puff adder (*B. arietans)*:

Five dogs envenomed by *B. arietans* were included in the study. In all cases the owners witnessed the bite and either were able to identify the snake based on pictures or killed the snake and brought it to the hospital along with the dog. Included dogs consisted of 2 female intact, 2
Table 1. Arrhythmias in dogs with *V. berus* envenomation at admission (n=6/24) and 12-24 hours post-admission (n=10/24).

<table>
<thead>
<tr>
<th>Arrhythmia</th>
<th>Admission</th>
<th>12-24 hours</th>
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<tbody>
<tr>
<td>Sinus bradycardia</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>ST coving</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>ST segment depression</td>
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<td>2 (1*)</td>
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<tr>
<td>Occasional VPCs</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Frequent VPCs</td>
<td>1*</td>
<td>2*</td>
</tr>
<tr>
<td>Sustained idioventricular rhythm</td>
<td></td>
<td>1*</td>
</tr>
<tr>
<td>Ventricular tachycardia</td>
<td>1*</td>
<td>1*</td>
</tr>
</tbody>
</table>

* indicates increased cTnI concentrations.
Figure 2. Relation between presence of systemic inflammation (SI = serum C-reactive protein concentration > 35 mg/L) and myocardial injury assessed by serum concentration of cardiac troponin I in 24 dogs envenomed by the European viper (*V. berus*) at admission (0h) and 12-24 hours post-admission (12-24h). Groups assigned with an asterisk have median concentrations significantly different from the control group (clinically healthy dogs).
male intact, and 1 male neutered dog ranging in age from 1.4-12 years (mean 5.9 years). The dogs were purebreds of 4 different breeds, the most frequent being Boerboel (n=2). One dog died in the hospital before 36 hours post-admission, and 36 hour samples were, therefore, available for only 4 dogs.

CtN1 concentrations were (median [range]) 0.06 [0.003-0.24] µg/L (0.06 [0.003-0.24] ng/mL) at admission, 0.7 [0.008-4.1] µg/L (0.7 [0.008-4.1] ng/mL) at 12 hours post-admission, 0.7 [0.01-30.1] µg/L (0.7 [0.01-30.1] ng/mL) at 24 hours post-admission, and 0.3 [0.002-5.0] µg/L (0.3 [0.002-5.0] ng/mL) at 36 hours post-admission (Figure 1). Two of 5 dogs (40%) had increased CtN1 concentrations at admission, 3 of 5 (60%) at 12 hours post-admission, 4 of 5 (80%) at 24 hours post-admission, and 2 of 4 (50%) at 36 hours post-admission. CRP concentrations were (median [range]) 9.0 [1.7-62.1] mg/L (0.9 [0.2-6.2] mg/dL) at admission, 86.3 [72.8-117.7] mg/L (8.6 [7.3-11.8] mg/dL) at 12 hours post-admission, 81.8 [60.0-133.8] mg/L (8.2 [6.0-13.4] mg/dL) at 24 hours post-admission, and 57.6 [43.6-127.1] mg/L (5.8 [4.4-12.7] mg/dL) at 36 hours post-admission. Two of 5 dogs (40%) had CRP concentrations > 35 mg/L (3.5 mg/dL) at the time of admission, and 5 of 5 (100%) at 12, 24, and 36 hours post-admission.

Dogs envenomed by *B. arietans* had significantly higher CtN1 concentrations than healthy control dogs at 24 hours post-admission (P=0.039), but not at any other time-point (P=0.083-0.88) (Figure 1). It was not possible to evaluate the difference between envenomed dogs in respect to CRP concentrations due to the small sample size and the fact that every dog had CRP concentrations > 35 mg/L (3.5 mg/dL) at all time-points from 12-36 hours post-admission. No correlation between CRP and CtN1 concentrations was found at any time-point (P=0.13-0.75).

One dog envenomed by *B. arietans* died suddenly in the hospital between 24 and 36 hours post-admission. It had severe subcutaneous hemorrhage and suspected hemolysis, and multiple
organ failure due to severe hypovolemia was considered a likely cause of death. This dog had CRP concentrations > 35 mg/L (3.5 mg/dL) at 12 and 24 hours post-admission and the highest cTnI concentration of any dog in the group at both these time-points (4.1 µg/L (4.1 ng/mL) at 12 hours post-admission and 30.1 µg/L (30.1 ng/mL) at 24 hours post-admission). Necropsy revealed several small paintbrush hemorrhages in the endocardium of the left ventricle. A grade 2 endocardiosis of the mitral valves was also reported.

**Snouted cobra (*N. annulifera*):**

Nine dogs envenomed by *N. annulifera* were included in the study. In all cases the owners witnessed the bite and either were able to identify the snake based on pictures or killed the snake and brought it to the hospital along with the dog. Included dogs consisted of 3 female intact, 2 female spayed, and 4 male intact dogs with an age span of 1.6-9 years (mean 3.7 years). Two dogs were mixed breeds; all other dogs were purebreds of 5 different breeds, the most frequent being Smooth-haired Dachshund (n=3). For 2 dogs 12-hour samples were not obtainable.

cTnI concentrations were (median [range]) 0.03 [0.008-1.9] µg/L (0.03 [0.008-1.9] ng/mL) at admission, 0.8 [0.02-3.9] µg/L (0.8 [0.02-3.9] ng/mL) at 12 hours post-admission, 0.08 [0.03-1.8] µg/L (0.08 [0.03-1.8] ng/mL) at 24 hours post-admission, and 0.07 [0.02-5.5] µg/L (0.07 [0.02-5.5] ng/mL) at 36 hours post-admission (Figure 1). Two of 9 dogs (22.2%) had increased cTnI concentrations at admission, 5 of 7 (71%) at 12 hours post-admission, 6 of 9 (67%) at 24 hours post-admission, and 4 of 9 (44%) at 36 hours post-admission. CRP concentrations were (median [range]) 7.9 [0-160.4] mg/L (0.8 [0-16.0] mg/dL) at admission, 95.0 [18.6-127.0] mg/L (9.5 [1.9-12.7] mg/dL) at 12 hours post-admission, 97.8 [0-157.8] mg/L (9.8 [0-15.8] mg/dL) at 24 hours post-admission, and 71.3 [0-99.9] mg/L (7.1 [0-10.0] mg/dL) at 36 hours post-admission. Three of 9 dogs (33.3%) had CRP concentrations > 35 mg/L (3.5 mg/dL) at the time
of admission, 6 of 7 (86%) at 12 hours post-admission, 7 of 9 (78%) at 24 hours post-admission, and 6 of 9 (67%) at 36 hours post-admission.

Dogs envenomed by *N. annulifera* had significantly higher cTnI concentrations than healthy dogs at admission (*P* = 0.04), 12 (*P* = 0.005), 24 (*P* < 0.001), and 36 hours (*P* = 0.0014) post-admission (Figure 1). It was not possible to examine the difference between envenomed dogs in respect to CRP concentrations due to the small sample size of the study and the fact that almost every dog had CRP concentrations > 35 mg/L (3.5 mg/dL) at all time-points from 12-36 hours post-admission. C-reactive protein concentrations were significantly correlated with cTnI concentrations at admission (*r* = 0.77, *P* = 0.025), but not at any other time-point (*P* = 0.21-0.91).

One dog envenomed by *N. annulifera* was euthanized due to poor prognosis. The dog developed severe infection and necrosis of the bite wound, had clinical symptoms of sepsis, and needed extensive surgery. Euthanasia was therefore elected by the owner. At 36 hours post-admission this dog’s CRP concentration was < 35 mg/L (3.5 mg/dL), but it had the highest cTnI concentration of any dog at this time-point (5.5 µg/L (5.5 ng/mL)). Necropsy revealed no abnormal macroscopic cardiac lesions.

**Discussion**

This study documents myocardial injury, as measured by increased cTnI, as well as systemic inflammation, as measured by increased CRP, occurring in dogs envenomed by 3 different snake species, *V. berus*, *B. arietans*, and *N. annulifera*. In addition, a correlation between degree of myocardial injury and systemic inflammation in the cases of *V. berus* and, at admission, *N. annulifera* envenomation, is documented.

A total of 58% of dogs envenomed by *V. berus* had increased cTnI concentrations at one or more time-points. This percentage is nearly twice as high as that described in an earlier study of
"V. berus" envenomation in dogs by Pelander et al, (32%). The lack of a high-sensitivity cTnI assay in the previous study may, however, account for this difference. A study of a different viper species, "V. palaestinae", reported a percentage of dogs with myocardial injury very similar to the one found in our study (65%). These earlier studies of viper envenomation investigated the occurrence of myocardial injury around the time of admission, and found the highest cTnI concentrations at 36 and 72 hours post-admission. Therefore, in the present study, we investigated the presence of cardiac injury as late as 5-10 days after "V. berus" envenomation and found persistently increased troponin concentrations in 28.6% of dogs. Several of the dogs with increased cTnI concentrations 5-10 days after envenomation were dogs with initial mild and moderate troponin concentration increases. As the half-life of troponin is short, this indicates that there was some ongoing cardiac injury even 5-10 days after envenomation in some dogs.

Cardiac arrhythmias have previously been reported in 9-25% of viper-envenomed dogs. Whether cardiac arrhythmia is a result of direct cardiac injury or extra-cardiac pathology has been a matter of debate. Arrhythmias may, in fact, be due to toxin-induced altered fiber excitability of the cardiac conduction system, possibly occurring without direct myocyte injury. This may be the case in some of the dogs with viper envenomation. In accordance with a previous study we did not find a higher degree of cardiac injury in those "V. berus"-envenomed dogs that presented with or developed arrhythmias. Interestingly, however, dogs with systemic inflammation had significantly higher concentrations of cTnI than dogs without systemic inflammation, and no difference in cTnI concentrations were observed between envenomed dogs without systemic inflammation and healthy control dogs. These findings indicate that the pathogenesis of myocardial injury in dogs envenomed by "V. berus" may be due to an inflammatory injury to the myocardium rather than a direct cardiotoxic effect of the venom.
Similarly, another study suggested that cardiac injury in *V. aspis*-envenomed rats was cytokine-mediated, and cytokine-mediated cardiac dysfunction has also been suspected in cases of scorpion envenomation. Cytokines, such as tumor necrosis factor-α, are believed to cause myocardial injury by increasing cardiomyocyte membrane permeability leading to leakage of troponins into the extracellular fluid. This injury is thought to be reversible, but may still have prognostic significance.

Interestingly, about a third of the dogs envenomed by *V. berus* showed no signs of myocardial injury. As we found a significant correlation between cardiac troponins and CRP concentrations at all time-points, dogs without cardiac injury were also the ones without or with milder degrees of systemic inflammation, thus possibly reflecting a milder degree of or reaction to the envenomation.

In the case of *B. arietans*, a significant difference in cTnI concentrations was observed between envenomed dogs and healthy controls only at 24 hours post-admission. Nevertheless, 80% of the dogs had increased cTnI concentrations at one or more time-points. The lack of significance at other time-points may be due to a type 2 statistical error, i.e. insufficient sample size of the study to detect a difference, which is a major limitation of the study.

For dogs envenomed by *N. annulifera*, a significantly higher cTnI concentration was observed in envenomed dogs compared to healthy controls at all examined time-points. Cobra venom is known to contain specific cardiotoxic substances which are cytolytic and cause an increase in cardiomyocyte membrane permeability, and a direct cardiotoxic effect of the venom may, therefore, explain the troponin release in these dogs. It might be argued that part of the myocardial injury observed could be due to inflammatory mediators as was suspected with *V. berus* envenomation in this study. A correlation between cTnI and CRP concentrations was,
however, only observed at admission and not at any other time-point. With both cTnI and CRP concentrations increasing quickly in response to an insult,\textsuperscript{36,37} this early relationship may not necessarily reflect a link between systemic inflammation and myocardial injury, but could be due to release kinetics of both markers. The poor correlation at any other time-point may thus indicate that myocardial injury in these dogs was caused by a direct cardiotoxic effect. This is further supported by the fact that the dog that was euthanized had no sign of systemic inflammation at 36 hours post-admission, but had the highest cTnI concentration of any dog envenomed by \textit{N. annulifera}. Nevertheless, systemic inflammation was present in most of the dogs. If inflammatory mediators did not contribute to the myocardial injury, it could, therefore, be speculated that different cytokines may be induced by the different snake venoms. Finally, the small group sizes of dogs envenomed by \textit{B. arietans} and \textit{N. annulifera} may, however, also have hindered the finding of a significant correlation between CRP and cTnI concentrations.

While all dogs with \textit{V. berus} envenomation survived, 1 dog envenomed by \textit{B. arietans} died suddenly in the hospital, and 1 dog envenomed by \textit{N. annulifera} was euthanized due to poor prognosis for survival. While it was not possible to appreciate statistically higher cTnI concentrations in non-survivors with such small numbers, it is interesting to note that both these dogs had the highest cTnI concentrations in their respective groups (30.1 µg/L (30.1 ng/mL) (\textit{B. arietans}) and 5.5 µg/L (5.5 ng/mL) (\textit{N. annulifera})), indicating that myocardial injury may be a negative prognostic indicator in dogs envenomed by these snake species.

This study contains several limitations. First of all, while dogs with primary cardiac disease as indicated by an audible murmur were excluded from the study, echocardiography was not performed. Ideally, an echocardiographic examination should have been performed on each dog. Accordingly, some dogs with mild cardiac disease may have been included in the study. In fact, one of the dogs that died was diagnosed with a mild endocardiosis on necropsy. However, cardiac
disease with no clinical signs generally gives rise to little or no troponin release.\textsuperscript{38,39} The dog in question had the highest cTnI concentration of any dog in the study, and for this to be caused by a subclinical heart disease is highly unlikely. The dog was, therefore, not excluded from the study. Although echocardiography would have been ideal, we could conclude that dogs with snake envenomation had significant myocardial injury compared to healthy dogs. Secondly, while ECGs were able to detect arrhythmias in several dogs, it is possible that some arrhythmias may have been missed due to the brief nature of ECG recordings. Continuous ECG recordings on all included dogs would have been valuable as would thoracic radiographs, pulse oximetry and blood pressure measurements on each dog in order to rule out hypertension or hypoxemia as causes of myocardial injury. A final limitation was the small groups of dogs with \textit{B. arietans} and \textit{N. annulifera} envenomation. A follow-up study with larger groups of dogs would be required to further examine the presence and causes of myocardial injury in such patients.

In conclusion, myocardial injury was a frequent finding in dogs with snake envenomation in this study. In dogs with \textit{V. berus} envenomation, cTnI concentrations were correlated with CRP concentrations, which served as a marker of systemic inflammation, and no significant effect of arrhythmia was found on the degree of myocardial injury. Systemic inflammation was, therefore, assumed to play a role in the pathogenesis of myocardial injury in these dogs as hypothesized. The same conclusion could not be drawn in the case of dogs envenomed by \textit{N. annulifera} or \textit{B. arietans}. This could possibly be due to differences in the toxic substances of the snake venoms or to differences in the cytokines induced by the venom toxins.

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Footnotes

\(^a\)ADVIA Centaur CP TnI-ultra, Siemens Healthcare Diagnostics Inc, Tarrytown, NY

\(^b\)Canine C-reactive Protein, LifeDiagnostics, West Chester, PA

\(^c\)GraphPad Prism 5.02 for Windows, GraphPad Software, San Diego, CA

References


Figure 1. Cardiac troponin I (cTnI) concentrations in 24 dogs envenomed by the European viper (V. berus) at admission (0h), 12-24 hours post-admission (12-24h) and 5-10 days later (follow-up) as well as 5 dogs envenomed by the African puff adder (B. arietans), and 9 dogs envenomed by the snouted cobra (N. annulifera) at admission (0h) and 12, 24, and 36 hours (h) post-admission. Groups assigned with an asterisk have median (V. berus and B. arietans) or mean (N. annulifera) concentrations significantly different from the control group (clinically healthy dogs).

Figure 2. Relation between presence of systemic inflammation (SI = serum C-reactive protein concentration > 35 mg/L) and myocardial injury assessed by serum concentration of cardiac troponin I in 24 dogs envenomed by the European viper (V. berus) at admission (0h) and 12-24 hours post-admission (12-24h). Groups assigned with an asterisk have median concentrations significantly different from the control group (clinically healthy dogs).