Ambulatory electrocardiography and serum cardiac troponin I measurement in 21 dogs envenomated by the European adder (Vipera berus)

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Abstract

Background: Envenomation by the European adder (Vipera berus) is common in dogs in Europe. Cardiac arrhythmias occur but clinical studies of envenomated dogs are limited.

Objectives: To describe arrhythmias in dogs within 48 hours of envenomation, and investigate associations between arrhythmia grade, serum troponin I (cTnI), and snakebite severity score (SS score).

Animals: Twenty-one client-owned dogs bitten by V berus.

Methods: Prospective cohort study of envenomated dogs. Ambulatory electrocardiograms were recorded from presentation to 48 hours after snakebite, and arrhythmias graded 0 to 3 based on frequency and severity. Serum cTnI was measured at presentation, 12 hours, 24 hours, 36 hours, and 14 days after bite. An SS score of 1 to 3 was recorded at admission and based on clinical examination.

Results: All dogs survived. Twelve dogs (57%) developed arrhythmias, all of which were ventricular in origin. Severe complex ventricular arrhythmias (VAs) were observed in 6 dogs (29%). Eighty-one percent of dogs (n = 17) had increased cTnI concentrations at 1 or more time points. Dogs that developed arrhythmias had significantly higher concentrations of cTnI at 12 hours (1.67 [0.04-32.68] versus 0.03 [0.01-0.052]; P = .002), 24 hours (1.88 [0.2-14.23] versus 0.06 [0.01-2.06]; P = .009), and 36 hours (3.7 [0.02-16.62] versus 0.06 [0.01-1.33]; P = .006) after bite compared to those that did not. Contingency table analysis showed that SS score was not significantly associated with arrhythmia grade (P = .9).

Abbreviations: AECG, ambulatory electrocardiography; AVB, atrioventricular block; cTnI, cardiac-specific troponin I; DAP, diastolic arterial pressure; HR, heart rate; MAP, mean arterial pressure; NMBU, Norwegian University of Life Sciences; SAP, systolic arterial pressure; SE, snake envenomation; VA, ventricular arrhythmia; VEC, ventricular ectopic complex; VT, ventricular tachycardia.
1 | INTRODUCTION

Envenomation by the European adder (Vipera berus) is a common seasonal presentation in small animal practice in Europe. During 2014 to 2018, a yearly average of 38 dogs were diagnosed with snake envenomation (SE) at the Faculty of Veterinary Medicine at the Norwegian University of Life Sciences (NMBU), and over 200 SE-related claims were reported yearly by the 2 largest pet insurance companies in Norway (Agria Dyreforsikring, Mo i Rana, Norway; Gjensidige Forsikring ASA, Oslo, Norway). Vipera berus is the only venomous snake in Norway. Signs of SE can therefore be attributed to this species alone.

Clinical signs of envenomation include edema, lethargy, pain, collapse, tachycardia, and cardiac arrhythmias. Electrocardiographic findings, including sinus bradycardia, sinus arrest, supraventricular and ventricular premature complexes, accelerated idioventricular rhythm, and ventricular tachycardia (VT), occur in dogs bitten by V berus and Vipera palaestinae. However, studies describing the time course and severity of these arrhythmias are limited.

Clinical grading systems are used to describe severity of snake bites in both humans and dogs. In dogs envenomated by V berus, severity score correlates with renal injury. However, a scoring system has not yet been assessed in relation to cardiac disease after SE.

Vipera berus venom contains several hemotoxic and cytotoxic proteases including phospholipase A2, serine proteases, and metalloproteinases. Myocardial cell injury might occur secondary to systemic inflammation induced by these toxins. Ammydlytin L, a direct cardiotoxin, is present in V berus venom. Myocardial cell injury could manifest as an arrhythmia.

Commonly used cardiac monitoring methods, including auscultation and short resting ECGs, are insensitive for the detection of arrhythmias when compared to continuous ambulatory ECGs (AECGs). As such, the incidence of arrhythmias after SE might be underestimated.

Cardiac-specific troponin I (cTnl) is a sensitive and specific marker of myocardial cell injury and necrosis in dogs. Troponin I increases both in primary cardiac disease and in myocardial injury secondary to systemic inflammation.

Arrhythmias occur in 11% to 47% of dogs bitten by V berus. Increased cTnl concentrations are reported in 33% to 58% of dogs and do not always correlate with the presence of arrhythmias. However, the number and timing of sample collections vary between these studies and only 1 used AECG. Thus, the association between arrhythmias and cTnl concentrations is not fully established in these dogs.

There is no study combining AECG monitoring for longer than 24 hours and serial cTnl measurements in dogs bitten by V berus.

The primary aim of our study was to describe the incidence, nature, and duration of arrhythmias in dogs during the first 48 hours after envenomation and investigate associations with serum cTnl concentrations. A secondary aim was to investigate any association between a snakebite severity score (SS score) assigned at presentation and cTnl or arrhythmia grade. Such information could help to optimize treatment protocols and management of this group of dogs.

2 | MATERIALS AND METHODS

This prospective cohort study was approved by the ethical committee at NMBU. Written owner consent was obtained for all dogs before inclusion in the study.

2.1 Animals

Twenty-six dogs presenting with a V berus bite to the small animal hospital at the Faculty of Veterinary Medicine at NMBU and Anicura Dyresykehus Oslo between April and October 2018 were evaluated for enrollment to the study. Diagnosis of snakebite and thus inclusion in the study was based on history and presence of consistent clinical signs at presentation (fang marks, local swelling, or systemic signs of envenomation). Five dogs were excluded from analyses for the following reasons: a previous history of cardiac disease (n = 1), a murmur detected at presentation (n = 1), treatment with antiarrhythmic medication before recruitment (n = 1), lack of clinical signs within 12 hours of the bite (n = 1), and presentation more than 24 hours after a snakebite (n = 1). Additional exclusion criteria included any preexisting disease and medications (other than levothyroxine [n = 1] and nonsteroidal anti-inflammatory drugs [n = 1]). Cases presented to the first opinion emergency service either directly (n = 16) or were transferred from clinics without an out-of-hours service (n = 5).

2.2 Physical examination and blood sampling

All dogs underwent physical examination including demeanor assessment (normal, lethargic, or markedly lethargic) and blood sampling for cTnl analysis at the following time points after bite: T1: presentation (2-7.5 hours), T2: 10 to 14 hours, T3: 22 to 24 hours, T4: 34 to 38 hours,
and T5: 10 to 21 days. All examinations and blood sampling for project purposes were conducted by a single veterinarian (H.J. Harjen) except for 2 dogs at T5. Treatment decisions were made by the attending clinician.

Whole blood was collected through a venous catheter in the cephalic (n = 16) or saphenous vein (n = 5), into serum tubes and centrifuged at 2700 \( g \) for 10 minutes, 30 to 60 minutes after sampling. Serum was pipetted into cryotubes and frozen within 15 minutes. Samples were stored in \(-80^\circ\)C for a maximum of 200 days before transportation on dry ice to a reference laboratory (Idexx BioAnalytics, Vet Med Labor GmbH, Ludwigsburg, Germany).

Serum cardiac troponin I (cTnI) was measured using an ultrasensitive chemiluminescence assay (Idexx BioAnalytics, Vet Med Labor GmbH, Ludwigsburg, Germany), validated for use in dogs.\(^{19}\) A serum cTnI concentration of up to 0.06 ng/mL was considered normal.

### 2.3 Snakebite severity score

Each dog was assigned an SS score at presentation, using an adaptation of a previously described grading system.\(^{7}\) All scores were assigned by the same veterinarian (H.J. Harjen). Grading criteria are described in Table 1.

### 2.4 Ambulatory electrocardiography

An ambulatory electrocardiogram (Lifecard CF Holter recording system, Spacelabs Healthcare, Snoqualmie, Washington) was placed on each dog at presentation, before blood sampling, and removed after a minimum of 40 hours of hospitalization. A modified bipolar orthogonal lead system (X, Y, Z) was used (see Supporting Information). Electrodes were placed after shaving and skin cleaning with alcohol. A bespoke Holter vest (HeartVets, Exeter, UK) was used to minimize movement artifact.

Quantitative AECG analysis was performed by a blinded, single operator with experience in canine AECG analysis (J. Harris) using commercially available computer software (Pathfinder Digital V9.019, Spacelabs Healthcare Ltd, Hertford, UK). The analysis system was programmed using agreed measurement criteria adapted for dogs in the absence of published criteria, as previously described.\(^{20}\) Beats were categorized as normal or aberrant morphologies. Ventricular tachycardia was defined as a minimum of 4 consecutive ventricular ectopic complexes (VECs) at \(\geq 200\) beats/min (bpm).

Arrhythmias were graded based on type, frequency, and severity, using previously described grading systems, modified to reflect clinical significance of previously reported arrhythmias in canine SE (Table 2).\(^{2,3,6,21,22}\) Grades 1 to 3 were considered increasingly abnormal. Definitions of AECG arrhythmia criteria are presented in the Supporting Information.

Ambulatory ECG recordings were tabulated, analyzed, and graded according to time after SE as this was considered most clinically useful and allowed comparisons to be made between individuals. Thus, day 1 and 2 correspond to the first and second 24-hour periods after SE.

### 2.5 Electrocardiography follow-up

A 5-minute, 6 lead ECG was performed on each dog upon reexamination (T5). A total of 5 dogs were available for 24-hour home Holter analysis 1 year after SE. Owners were instructed to carry out normal activities with the dog, except swimming.

### 2.6 Blood pressure measurement

Indirect blood pressure (Cardell; Midmark, Versailles, Ohio) measurements were recorded at T1 to T5. A cuff size of approximately 40% TABLE 1 Snakebite Severity Score definitions

| Snakebite Severity Score | Clinical features |
|-------------------------|------------------|
| 1                       | Mild             |
|                         | Local swelling around the bite, no systemic signs. |
| 2                       | Moderate         |
|                         | Extensive swelling extending beyond the immediate bite site or mild systemic signs (lethargy, isolated episode of vomiting). |
| 3                       | Severe           |
|                         | Pain and extensive swelling progressing beyond the limb or head, with marked systemic signs (collapse, cardiac arrhythmia, repeated vomiting, diarrhea, bleeding). |

Abbreviations: 2DAVB, 2nd degree atrioventricular block; 3DAVB, 3rd degree atrioventricular block; AVBR, accelerated idioventricular rhythm; AVB, atrioventricular block; ECG, electrocardiography; SVPCs, supraventricular premature complexes; VECs, ventricular ectopic complexes; VT, ventricular tachycardia.

### Table 2 Arrhythmia grading criteria

| Arrhythmia grade | ECG criteria per 24-h period |
|------------------|-------------------------------|
| 0                | Normal                        |
|                  | • `<50 VECs`                   |
|                  | • `<50 SVPCs`                  |
|                  | • No complex arrhythmia (couplets, triplets, AIVR/VT, bigeminy, or trigeminy) |
|                  | • No high grade AVB           |
| 1a               | Mild                          |
|                  | • 50-1000 VECs                |
|                  | • No couplets, triplets or AIVR/VT, bigeminy, or trigeminy |
|                  | • No high grade AVB           |
| 1b               | 50–1000 VECs                  |
|                  | • Any couplets, triplets, bigeminy/ trigeminy, or AIVR |
|                  | • No VT                       |
|                  | • No high grade AVB           |
| 2a               | Moderate                      |
|                  | • Grade 1a + >1000 VECs       |
| 2b               | Grade 1b + >1000 VECs         |
| 3                | Severe                        |
|                  | • Presence of any VT (>200 bpm) irrespective of VEC number, and/or high grade 2DAVB/3DAVB |

Abbreviations: 2DAVB, 2nd degree atrioventricular block; 3DAVB, 3rd degree atrioventricular block; AIVR, accelerated idioventricular rhythm; AVB, atrioventricular block; ECG, electrocardiography; SVPCs, supraventricular premature complexes; VECs, ventricular ectopic complexes; VT, ventricular tachycardia.
limb circumference was placed on either the distal radius or metatarsus with the dog in lateral recumbency. Twelve serial measurements of systolic arterial pressure (SAP), diastolic arterial pressure (DAP), and mean arterial pressure (MAP) were recorded per time point. The first 2 measurements and any obvious outlying values were discarded. The mean of the remaining measurements was used.

2.7 | Statistical analysis

Statistical analysis was conducted using commercially available statistical software packages (JMP Pro 14.3.0, SAS Institute, Inc, Cary, North Carolina and Stata/SE 15.1, StataCorp, College Station, Texas). Data were tested for normality using the Shapiro-Wilk test. Fisher’s exact test was used to compare categorical variables. For nonparametric data, Wilcoxon exact test and Steel-Dwass test for multiple comparisons were used to analyze cTnI concentrations between arrhythmia grade and SS scores. Nonparametric receiver operating characteristic curves were used to analyze potential cTnI cut-off values for diagnosis of arrhythmia grades at T1 and T2. For all analyses, a P value of <.05 was considered significant. Where relevant, 95% confidence intervals (CIs) are presented. For parametric data, mean values ± SD are reported and a 2-tailed t test was used for between group comparisons.

3 | RESULTS

3.1 | Animals

Twenty-one dogs were included in the final study group. The snake, snake-bite, or fang marks were observed in 17 dogs. In the remaining 4, the diagnosis was made based on the presence of clinical signs and history.
consistent with Vb e r u s envenomation. Fifteen dogs were female and 6 were male. Median age was 3 years (range 7 months to 18.5 years). Median weight was 19 kg (range 5.5-43 kg). Breeds included 6 crossbreeds and 1 each of Border Collie, English Setter, Miniature Schnauzer, Samoyed, Nova Scotia Duck Tolling Retriever, Boston terrier, Cavalier King Charles Spaniel, Australian Kelpie, Standard Poodle, Flat Coated Retriever, Shetland Sheepdog, Staffordshire Bull Terrier, Akita, Kleiner Münsterländer, and Toy Poodle.

Median time from estimated snakebite to presentation was 1.5 hours (range 0.5-0.9 hours). Missing data included 1 T1 sample and 1 T4 sample, in separate dogs. Two dogs were lost to follow up at T5. All dogs were examined, and blood sampled, at a minimum of 4 time points.

3.2 | Treatment

All dogs received treatment of crystalloid fluid IV (Ringer-acetate, \( n = 21 \), NaCl \( n = 1 \), day 1) for the entire hospitalization period. Median fluid rate during the sampling period was 4 mL/(kg hr) (range 2.7-6.3 mL/(kg hr)).

Analgesics used included buprenorphine (Vetergesic vet, Ceva Santé Animale, France) at a dose of 0.01 to 0.02 mg/kg IV or IM q8h (\( n = 3 \)) and methadone (Metadon, Norges Apotek, Norway) at a dose of 0.1 to 0.2 mg/kg IV q4h (\( n = 13 \)). Five dogs received methadone on day 1 and subsequently buprenorphine on day 2.

Lidocaine (Xylocain, Aspen Pharma trading Ltd, Ireland) was administered at dose of 2 mg/(kg hr) (continuous rate infusion) to 1 dog 39 hours after envenomation because of a ventricular arrhythmia (VA) observed on resting ECG by the attending clinician.

Sixteen dogs received intravenous equine F(ab’)2 antivenom (Viper Venom Antitoxin, SIS Biomed, Warsaw, Poland) IV, 7 of which received it before recruitment to the study. Median time from snakebite to antivenom treatment was 3.8 hours (range 0.75-0.24 hours).

Other treatments before presentation included penicillin and streptomycin (\( n = 1 \)), prednisolone (\( n = 1 \)), and dexamethasone (\( n = 1 \)).

3.3 | Ambulatory electrocardiography

Median time from estimated SE to the start of AECG recording was 3.5 hours (range 2 hours 45 minutes to 9 hours) and median total AECG recording duration was 41 hours 43 minutes (range 24 hours 15 minutes to 50 hours 58 minutes). Recordings were standardized for analysis according to time after SE. Median duration of analyzed recordings for days 1 and 2 were thus 20 hours (range 14-21) and 23.5 hours (19–24), respectively. Day 2 AECG data were excluded for 3 dogs in which only 5 hours of recordings were available.

Nine dogs (42.9%, CI 24.5%-63.5%) were classified as not having an arrhythmia (grade 0). Arrhythmias were detected in 12 dogs (57%, CI 34%-76%), of which 5 were classified as grade 1b, 1 as grade 2b, and 6 as grade 3. All arrhythmias were ventricular in origin. Extracts from AECG recordings are presented in Figure 1. Overall grade was consistent across day 1 and day 2 in 13 dogs. Two dogs progressed...
from grade 0 on day 1 to grade 1b on day 2. Three dogs showed an improvement on day 2 compared to day 1, from grade 1b to 0 (n = 1) and 3 to 2b (n = 2). Continuous percentage VECs and cTnI concentrations are presented for 6 dogs with grade 3 arrhythmias, in Figure 2. Arrhythmia grade was not significantly different between dogs with and without antivenom treatment (P = .6).

No arrhythmias were detected on resting ECG at T5. Five dogs with arrhythmias (≥grade 1) were available for 1-year follow-up AECG. Mean AECG recording duration at that time was 19.3 hours in these dogs, and no VECs were detected.

### 3.4 | Cardiac troponin I

Sixty-seven percent (CI 45%-83%) of dogs (n = 14) had increased serum cTnI concentrations at 3 or more time points, and 81% of dogs (n = 17) had increased cTnI at 1 time point or more. Five dogs (24%, CI 1.6%-45.1%) had cTnI concentrations above the reference cut-off value at all time points from T1 to T4. Three dogs had T5 cTnI concentrations of 0.06 ng/mL, 0.07 ng/mL, and 0.11 ng/mL, respectively. In 2 of these dogs, cTnI had decreased compared to all other time points.

The highest cTnI concentration of 32.68 ng/mL was observed in the oldest dog (18.5 years) at T2. This individual also had the highest T5 cTnI concentration (0.11 ng/mL) and was available for 1-year follow-up at which point cTnI was 0.12 ng/mL. Individual peak cTnI concentrations were observed across T2 to T4 and were significantly higher in dogs with arrhythmias (≥grade 1) compared to those without (Table 3). Cardiac troponin I concentrations were also significantly higher in dogs with arrhythmias (≥grade 1) than dogs without arrhythmias, at T2, T3, and T4 (Figure 3, Table 3). Peak cTnI concentration was not significantly different between dogs with and without antivenom treatment (P = .7).

Five dogs had increased cTnI concentrations at a minimum of 2 time points, in the absence of an arrhythmia. Troponin I concentrations of 0.52 to 2.06 ng/mL were detected in 1 of these dogs, whereas concentrations did not exceed 0.09 ng/mL in the other 4. One dog with a grade 2b arrhythmia had normal cTnI concentrations at all time points except T5 where it was marginally increased at 0.07 ng/mL.

### 3.5 | Receiver operating characteristics curve analysis

Receiver operating characteristics curve analysis was used to investigate cTnI concentrations at T1 and T2 as an indicator of the presence of an arrhythmia ≥grade 1 at any time during hospitalization. Troponin I cut-off concentrations of 0.04 ng/mL (sensitivity: 75% [CI 46.8%-100%], specificity: 79% [CI 55.1%-95.5%]) were used to assess arrhythmia presence. Receiver operating characteristics curve analysis was calculated for each time point individually (Table 3). Ratios of arrhythmia presence were calculated for dogs with and without antivenom treatment (P = .7).

### 3.4 | Cardiac troponin I

#### Table 3  Summary of characteristics by arrhythmia group with relevant P values for comparisons between dogs with and without an arrhythmia ≥ grade 1

|                      | Arrhythmia grade |
|----------------------|------------------|
|                      | Grade 0 | ≥Grade 1 | P value   |
| Number               | 9       | 12      |           |
| Age (y)              | 2 (0.58-11) | 5 (0.58-18.5) | .25<sup>a</sup> |
| Weight (mean) (kg)   | 17.7 (±9.6) | 25.2 (±9.8) | .36<sup>b</sup> |
| Sex (male/female)    | 2/7     | 4/8     | .66<sup>c</sup> |
| Hours from bite to presentation | 1.25 (0.75-6.5) | 1.625 (0.5-5) | .89<sup>c</sup> |
| Bite location (head/limb) | 6/3     | 10/2    | .61<sup>c</sup> |
| Antivenom treatment (yes/no) | 6/3     | 10/2    | .61<sup>c</sup> |
| Treatment with antivenom before recruitment (yes/no) | 4/5     | 3/9     | .39<sup>c</sup> |
| Arrhythmia detected on initial examination (yes/no) | 0/9     | 2/12    | .49<sup>c</sup> |
| Buprenorphine (yes/no) | 5/6     | 4/8     | .67<sup>c</sup> |
| Methadone (yes/no)   | 9/0     | 9/3     | .22<sup>c</sup> |
| SS score (n) (1/2/3) | 2/6/1   | 2/8/2   | .82<sup>c</sup> |
| Cardiac troponin I (ng/mL) |          |          |           |
| T1                   | 0.02<sup>b</sup> (0.01-0.08) | 0.055 (0.01-2.27) | .086<sup>a</sup> |
| T2                   | 0.03 (0.01-0.52) | 1.665 (0.04-32.68) | .002<sup>a</sup> |
| T3                   | 0.06 (0.01-2.06) | 1.875 (0.02-14.23) | .009<sup>a</sup> |
| T4                   | 0.06 (0.01-1.33) | 3.7<sup>a</sup> (0.02-16.62) | .005<sup>a</sup> |
| T5                   | 0.02<sup>b</sup> (0.01-0.06) | 0.03 (0.01-0.11) | .28<sup>a</sup> |
| Peak troponin I (ng/mL) | 0.07 (0.03-2.06) | 4.7 (0.07-32.68) | .001<sup>a</sup> |

Note: Median and range values are presented for group data.
Abbreviation: SS score, snakebite severity score.
<sup>a</sup>Wilcoxon test (= Mann-Whitney test).
<sup>b</sup>t test.
<sup>c</sup>Fisher’s exact test.
91.1%] and specificity: 75% [CI 4.9%-92.9%]) and 0.1 ng/mL (sensitivity: 83% [CI 55.2%-95.3%], specificity: 89% [CI 56.5%-98%]), for diagnosis of arrhythmias ≥ grade 1, we found for T1 (Area under the curve (AUC) 0.73, CI 0.49-0.96) and T2 (AUC 0.9, CI 0.77-1.0), respectively. For indication of a grade 3 arrhythmia, cTnI cut-off concentrations of 0.15 ng/mL (sensitivity: 66.6% [CI 30%-9.3%], specificity: 100% [CI 78.5%-100%]) and 1.89 ng/mL (sensitivity: 100% [CI 61%-100%], specificity: 100% [CI 79.6%-100%]), were found for T1 (AUC 0.9, CI 0.9-1.0) and T2 (AUC 1.0, CI 1.0-1.0), respectively.

3.6 | Snakebite severity score

Nineteen percent (CI 7%-40%) of dogs (n = 4) had an SS score of 1, 67% (CI 45.4%-82.9%) (n = 14) had a score of 2, and 14% (CI 4.9%-34.6%) (n = 3) had a score of 3. No significant associations were found between SS score and cTnI concentrations at presentation (P = .5) or SS score and arrhythmia grade (P = .9).

3.7 | Blood pressure

Blood pressure was measured in 20 dogs (metatarsus n = 18, forelimb n = 2) at T1 to T5. A mean of 10 measurements was used in all but 8 cases (single time point) in which 5 to 8 measurements were used.

Overall mean SAP, DAP, and MAP during hospitalization (T1-T4) were 131.7 ± 14.6 mm Hg (range 104-169), 73.4 ± 13.5 mm Hg (range 52-119), and 95.6 ± 13.2 mm Hg (range 70-134), respectively. At T5, these values were 131.7 ± 12.8 mm Hg (117-154), 77.3 ± 13.9 mm Hg (55-103), and 98.4 ± 11.5 mm Hg (81-117), respectively. No significant difference in SAP, DAP, or MAP was found between dogs with or without an arrhythmia ≥ grade 1 at each given time point other than at T2 where SAP and MAP were significantly higher in dogs with an arrhythmia ≥ grade 1 (SAP 137 ± 14.8 mm Hg versus 123.9 ± 11.8 mm Hg, P = .04; MAP 99.6 ± 13.3 mm Hg versus 87.6 ± 1.6 mm Hg, P = .04).

3.8 | Other clinical findings

Overall median heart rate (HR) during hospitalization (T1-T4) was 90 bpm (range 40-200). At T5, median HR was 100 bpm (range 72-135). Heart rate was significantly higher in dogs with an arrhythmia ≥ grade 1 than those without at T2 (mean 106 ± 2.9 bpm versus 78.2 ± 22.8 bpm, P = .009) and T3 (mean 100 ± 29.2 bpm versus 77.2 ± 11 bpm, P = .03). Overall, no statistically significant difference in demeanor, mucus membrane color, capillary refill time (CRT), or femoral pulse quality was found between dogs with and without an arrhythmia ≥ grade 1. All dogs survived. All the dogs examined at T5 (n = 19) were assessed as being healthy. Both dogs that were unavailable for examination at T5 were reported as healthy by the owner.
4 | DISCUSSION

Our study shows that cardiotoxicity, evident as an arrhythmia or increased cTnI concentrations, is a common sequel to V berus envenomation in dogs. Previous studies using 2 to 5 minute ECGs reported arrhythmia incidences of 25% to 41.6% compared to 57% (CI 34%-77%) in our study. Cardiac effects were reported in 11% and 14% of dogs with V berus SE in 2 other studies. However, ECGs were not performed in all dogs in these 2 studies, thereby likely underestimating the true arrhythmia incidence compared to the present study. The other AECG study of V berus envenomated dogs reported a similar arrhythmia incidence of 47%.2

Ventricular tachycardia, present in 6 dogs in our study, occurs in SE dogs. However, quantification of VT incidence is lacking from previous work, and differences in VT definition make comparisons between studies challenging. The grading system, and specifically the assignment of a grade 3 arrhythmia, in the current study is conservative in terms of VT definition (4 consecutive VECs at an instantaneous rate of ≥200 bpm) when compared to other SE studies where VT has been defined as a minimum of 3 VECs at ≥100 or ≥160 bpm.2,6

Perhaps surprisingly, no dogs developed atrioventricular block (AVB) in the current study, compared to 21% in a study of dogs with V palestinae SE. The lack of AVB and other previously described ECG findings, such as ST segment depression and sinus arrest, might reflect variations in venom dose and composition, individual response to venom, individual or species variation in response to myocardial injury, or different mechanisms of cardiotoxicity.24

Previous longitudinal studies of V berus SE describe cTnI concentrations suggestive of myocardial cell injury in 33% and 58% of dogs, respectively. The equivalent figure appears higher in the current study (81%, CI 60%-92%) and could reflect higher assay sensitivity and a lower upper reference cut-off concentration than previous studies. The differences observed could also be an effect of small sample size.

Cardiac troponin I findings from the T1 time point in our study are comparable to another study that reported no significant difference in cTnI concentrations between dogs with and without arrhythmias, up to 8 hours after SE.2 Serial cTnIs in our study demonstrate, as previously suggested, that insufficient time between SE and sampling is a likely explanation for the lack of cTnI increase at this time point and that measurement at the time of presentation is of limited value in separating dogs that develop arrhythmias from those that do not.

Troponin I concentrations 12 to 36 hours after bite were higher in dogs with an arrhythmia grade ≥ 1 compared to those without an arrhythmia. However, normal cTnI concentrations did not rule out the presence of an arrhythmia and vice versa. Troponin I concentrations of ≥1.89 ng/mL 12 hours after envenomation, were useful in predicting a grade 3 arrhythmia in our study. Given the small sample size in the current study, further studies of cTnI concentrations 12 hours after envenomation would be of interest to determine whether a true diagnostic cTnI cut-off can be established and to assess risk of sudden cardiac death in this population.

The clinical relevance of marginal increases in cTnI in dogs is not known. cTnI concentrations of up to 0.136 ng/mL have been described in dogs screened and found to be free of cardiac disease.17 Interbreed variation, extreme exercise, and age are documented causes of mild cTnI increase in otherwise apparently healthy dogs.17,25,26 Troponin I kinetics are reflected in the finding of peak cTnI concentrations 12 to 36 hours after SE in our study. Studies in humans describe biphasic cTnI release, with a rapid release of a small unbound cytoplasmic pool within 4 to 6 hours, peaking at 12 to 24 hours after insult, followed by a more gradual release of bound, structural cTnI creating a second peak 2 to 4 days after injury.27 The duration of cardiomyocyte injury after SE is not fully documented. A previous study found that 28.6% of dogs have increased cTnI 5 to 10 days after SE. In the current study, 3 dogs had abnormal cTnI 10 to 14 days after SE. Given the short half-life of cTnI in the dog (1.85 hours), this might indicate on-going cardiac injury, but could also be explained by individual variation in baseline cTnI due to age, physical activity, or breed.25,26,27 Five dogs had abnormal cTnI in the absence of an arrhythmia. Individual variation in baseline cTnI is a possible explanation for this finding. Myocarditis can be present in the absence of ECG changes and could therefore also explain the finding of raised cTnI concentrations in the absence of arrhythmia in envenomated dogs.

The exact pathophysiology of arrhythmias after SE is unknown. Cardiomyocyte injury might occur secondary to a systemic inflammatory state induced by venom proteases.10-12 Previously described findings of a correlation between concentrations of C-reactive protein and cTnI in V berus envenomated dogs6 and hypersensitivity myocarditis after V berus SE in a human patient support an inflammatory mechanism of myocardial injury. A direct cardiotoxin (Ammodytin L) has also been isolated in V berus venom.11,13 Tachyarrhythmias themselves can also contribute to cTnI increase.

Arrhythmias resolved without anti-arrhythmic treatment in 11 of 12 dogs in our study, similar to another study of 126 dogs envenomated by V berus.23

Snakebite severity score, based on clinical examination at presentation, was investigated as an indicator of arrhythmia development. Scoring was carried out by the same clinician, in order to maximize comparability between individuals. However, the results of our study suggest that it does not provide additional information regarding severity of cardiac effects of SE.

There are limitations to our study. Echocardiography would have been a useful addition before inclusion in the present study to rule out preexisting cardiac disease. However, as cTnI has a high specificity and sensitivity for cardiac injury, reduction in cTnI concentration from the initial 38 hours after SE to 14 days after SE was considered consistent with the absence of underlying heart disease. Antivenom treatment was an unavoidable confounding factor in our study. Arrhythmia occurrence and cTnI concentrations were, however, not significantly different between antivenom and non-antivenom treated dogs. Three dogs received anti-inflammatory medication before recruitment to our study. Given that mechanism of arrhythmia after SE is unknown, we cannot rule out that this might have impacted the findings in these individuals. Another previously mentioned limitation of our study was the sample size; more definitive conclusions could likely be drawn from a larger study population.
While we have demonstrated that continuous ECG is a useful tool in detecting and quantifying arrhythmias in SE dogs, Holter AECG is not ideal for real-time arrhythmia monitoring due to the need for retrospective ECG analysis. Telemetric ECG, which combines continuous monitoring with real-time viewing, would be a more useful monitoring tool in SE dogs in the clinical setting.

Clinical effects of SE vary in severity from benign signs to sudden death,34 to which severe systemic inflammatory response, arrhythmia, and anaphylaxis are potential contributory factors.35-37 Arrhythmias appeared to be well tolerated by dogs in our study, and therefore the clinical importance of the arrhythmias is unclear. The post hoc diagnosis and lack of continuous blood pressure measurement in our study might have resulted in an underestimation of hemodynamic effects. The finding of higher SAP and MAP at T2 in dogs with an arrhythmia ≥ grade 1 is contrary to what might be physiologically expected. However, given the small sample size and the marginal P value (0.4), we suggest that this finding is likely incidental and not arrhythmia-related.

Risk associated with VA has not been assessed in SE in dogs; however, in Doberman pinschers with dilated cardiomyopathy, presence of fast VT (instantaneous rate > 260 bpm) and high cTnI were both predictors of sudden cardiac death.38 Therefore, dogs with similar findings secondary to SE warrant monitoring and might be at similar risk. Given that increased cTnI and presence of VT are risk factors for sudden cardiac death in myocardial disease in dogs,37-39 we suggest that continuous ECG monitoring of SE dogs is advisable.

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CONFLICT OF INTEREST DECLARATION
Joanne Harris is a director of HeartVet Consultants Ltd who provided commercial Holter monitor rental and analysis. All Holter recordings in our study were analyzed on a research platform with J. Harris blinded to all patients details during analysis. The remaining authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION
Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION
The study was approved by the Committee for Ethical Approval of Studies with Animal Patients at the Norwegian University of Life Sciences (NMBU).

HUMAN ETHICS APPROVAL DECLARATION
Authors declare human ethics approval was not needed for this study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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