Project title: Globale koagulasjonsanalyser som diagnostiske markører for huggormbitt hos hund

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Evaluation of thrombin generation measurements and global clotting times as diagnostic markers for European adder (*Vipera berus*) envenomation in dogs

Popular science summary

Summer months in Europe bring sunshine and snakes! The European adder, Vipera berus (*V. berus*) is a venomous snake that commonly bites dogs, but surprisingly, there's no test that veterinarians can use to confirm whether a dog has been bitten and injected with venom.

Blood clotting tests could help solve this diagnostic problem. This research looked at how *V. berus* venom affects dogs' ability to clot blood, analysing samples from dogs bitten by *V. berus*, as well as healthy and sick dogs that hadn't been bitten.

Results showed that bitten dogs experienced increased blood clotting (a state called hypercoagulability). This was clear from elevated levels of certain blood markers, especially thrombin and fibrin—proteins involved in forming clots. One simple test, known as the "manual tilt fibrin generation test," stood out as especially promising for use in first-line veterinary clinics.

In short, this study opens the door to a practical way for veterinarians to diagnose adder bites more accurately, which could lead to faster, more targeted treatment for affected dogs.

Introduction

Accidental envenomation of dogs by the European adder, *Vipera berus* (*V. berus*) is a common veterinary emergency during the spring and summer months in Europe, with clinical effects including local swelling, bruising, pain, lethargy, vomiting, cardiac arrythmia, acute kidney injury, coagulopathy, and occasionally, death [1-5].

Despite the medical importance of this snake, definitively diagnosing envenomation in dogs is challenging since bite marks and local reaction are unreliable markers of envenomation and 30% of bites are estimated to be void of venom (dry bites) [1, 6, 7].

There is currently no commercially available test to clinically diagnose *V. berus* envenomation in dogs and treatment is therefore initiated based on a diagnosis of suspicion. Management of this type of snakebite in dogs often involves treatment with antivenom which is both costly and associated with adverse events [1, 8-11]. A definitive diagnosis of *V. berus* envenomation would allow antivenom treatment to be used only when strictly necessary, thereby minimising the risk of adverse events and avoiding unnecessary treatment costs.

A potential diagnostic focus for *V. berus* envenomation is venom-induced coagulopathy. We previously reported an early and persistent procoagulant state in dogs bitten by *V. berus*, measured

as increased thrombin generation (measured by calibrated automated thrombography, CAT) in envenomated dogs compared to healthy controls [4]. However, CAT is not practical for use in the emergency setting, thus simpler manual clotting tests that may be applied in-house, warrant assessment for this diagnostic purpose.

The aim of this study is to further map the coagulation status of dogs bitten by *V. berus* and to assess the suitability of coagulation parameters as clinical tools for the diagnosis of *V. berus* envenomation in dogs, using TAT, thrombin generation, fibrin generation, procoagulant phospholipids and more widely available tests such as PT Quick, aPTT, D-dimer and thrombophilia markers.

Materials and methods

Study design

Dogs presenting with a *V. berus* bite to the first opinion emergency service at the Norwegian University of Life Sciences (NMBU) small animal hospital between April and October in 2022 and 2023 (two snake seasons) were assessed for enrolment in this prospective cohort study. Inclusion in the study required a diagnosis of snakebite based on history, presence of consistent clinical signs at presentation (fang marks, local signs of envenomation such as swelling and pain around the bite site, or systemic signs of envenomation such as lethargy, vomiting, collapse, and cardiac arrhythmias), and a minimum body weight of 6 kg. Exclusion criteria were presentation more than six hours after bite and treatment with antivenom prior to sampling.

Two control groups of privately-owned dogs, not previously bitten by *V. berus*, were recruited during August 2023. The first group consisted of 25 healthy control dogs for which inclusion required a minimum bodyweight of 6 kg, lack of significant acute or chronic disease, and no medication in the preceding two weeks.Healthy status was determined based on information obtained from the owner and a lack of clinically significant abnormalities on physical examination.

The second group consisted of 15 sick control dogs, with clinical signs in common with a *V. berus* bite (lethargy, acute lameness, acute swelling, or combinations thereof), but where snakebite was ruled out through the clinical history or another diagnosis. Dogs were excluded from the sick control group if they had undergone surgery in the preceding 14 days.

Sample collection and processing

Citrated plasma was collected at presentation (T0) and 6 hours later (T6) from 14 dogs bitten by *V. berus*, and from 25 healthy controls and 15 sick controls at a single timepoint. Plasma was aliquoted into cryotubes and stored at -20°C for a maximum of 5 days before storage in -80°C for a maximum of 27 months prior to analysis. For the healthy and sick control dogs citrated plasma was collected, prepared and stored as for the envenomated dogs, and analysed within 13 months.

Coagulation parameters measured included prothrombin time (PT), activated partial thromboplastin time (APTT), D-dimer, protein C, antithrombin, fibrinogen, procoagulant phospholipids, thrombin generation, thrombin-antithrombin (TAT) complexes, and fibrin generation (measured spectrophotometrically and using a manual tilt technique).

Statistical analysis

Data were analysed using JMP Pro 14.3.0 (SAS Institute Inc, Cary, NC) and GraphPad Prism 10.2.0 (GraphPad Software LLC, CA, USA). Kruskal-Wallis test with Dunn's multiple comparisons test was

used for comparisons between groups, and Wilcoxon matched-pairs signed rank test was performed for comparisons between timepoints.

Ethics

This study was approved by the Norwegian Food Safety Authority (22/31007) and written, informed owner-consent was obtained for all dogs prior to inclusion.

Results

Demographic data (Table 1)

Table 1. Demographic data (median and range) for envenomated dogs and the healthy and sick control groups.

	Envenomated dogs	**Healthy control dogs**	**Sick control dogs**
Number (n)	14	25	15
Age (years)	2.4 (1.3-12.2)	4.3 (0.5-13)	5.0 (2.1-9.3)
Weight (kg)	19.3 (9.1-38.2)	21.0 (6.0-48.5)	21.9 (8.8-57.0)
Sex (male:female) (n)	10:4	17:8	11:4
Bite location (head:limb) (n)	10:4		
Time from bite to T0 (hours)	2.5 (1.1-6.2)		
Time from T0 to T6 (hours) *(n=10)*	6.0 (4.8-6.5)		

Key Results

Envenomated dogs were hypercoagulable at both timepoints, with increased TAT complexes, thrombin generation and fibrin generation compared to healthy and sick control dogs. D-dimer concentrations were higher in the envenomated dogs at both timepoints, compared to healthy controls but not sick controls. Protein C activity and fibrinogen concentrations were higher in sick controls compared to snake-bitten dogs and healthy controls.

Fibrin generation (clotting time) was measured using a spectrophotometric method and a manuat tilt technique:

Spectrophotometric method

At presentation (T0), envenomated dogs had a significantly shorter median clotting time than both the healthy and sick control groups (193 seconds [range 29-2255 seconds], 3600 seconds [range 1415-3600 seconds] and 3600 seconds [range1254-3600 seconds], respectively; $P \leq 0.0001$, Figure 1). After six hours (T6), envenomated dogs had significantly longer median clotting times compared to T0 (442 seconds [range 153-3600 seconds]; $p \leq 0.01$), but still significantly shorter times than both control groups ($P \leq 0.05$).

Manual tilt technique

At T0, envenomated dogs had significantly shorter median clotting times than both control groups (2.0 minutes [range 1.0-30.0 minutes] vs ≥ 30 minutes for the two control groups; P ≤0.0001, Figure 1). After 6 hours, clotting times were significantly longer in envenomated dogs compared to T0 (6.5 minutes [range 2.0-30.0 minutes]; P ≤0.05), but still significantly shorter than both control groups (P ≤0.001).

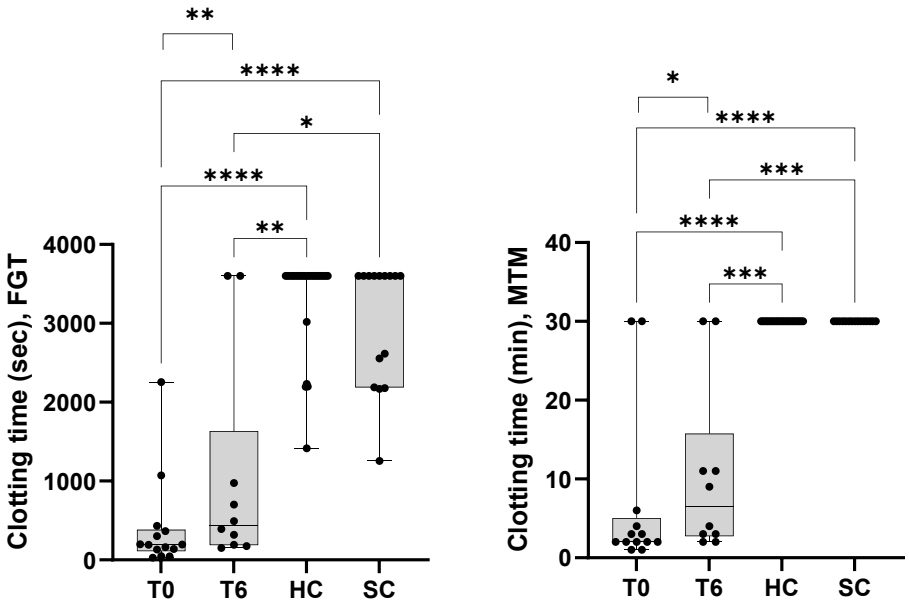

Figure 1. Fibrin generation (clotting time) in envenomated dogs, healthy controls and sick controls. Fibrin generation (clotting time) measured with the fibrin generation test (FGT) and the manual tilt technique (MTT) in envenomated dogs at presentation (T0, n=14/13) and 6 hours after (T6, n=10), and in healthy (HC, n=25) and sick (SC, n=15) controls. * = P ≤0.05, ** = P ≤0.01, *** = P ≤0.001 and **** = P ≤0.0001.

Discussion

We measured the overall coagulation status of dogs bitten by *V. berus* and evaluated the use of coagulation parameters in diagnosing this type of snakebite in dogs. In accordance with our previous findings [4], dogs bitten by *V. berus* were hypercoagulable compared to healthy controls, already upon presentation to the veterinary clinic, and six hours later, as demonstrated by increased thrombin generation, fibrin generation, and TAT complexes. Additionally, envenomated dogs were hypercoagulable compared to sick control dogs, a novel and diagnostically important finding.

Of the parameters we evaluated, fibrin generation (clotting time) tests show the most promise as diagnostic tools, due to minimal overlap between envenomated dogs and sick controls. The manual tilt technique is particularly interesting as it allowed a clear distinction between cases and both healthy and sick control dogs, at presentation, and still six hours later, in all but two case dogs that had longer clotting times. This test is easy to perform in-house, making it a potential rapid clinical diagnostic aid for this type of snakebite in dogs.

In contrast to reports of other types of snakebite in dogs, decreased fibrinogen, AT and protein C activity associated with VICC [12, 13], did not feature in the envenomated dogs in our study, indicating that our dogs did not develop a consumptive coagulopathy. Furthermore, these parameters

did not allow distinction between envenomated dogs and controls, suggesting they have limited use in a diagnostic context.

Conclusions

In conclusion, we demonstrated a potential use for spectrophotometric and manual fibrin generation tests as diagnostic tools for V. berus envenomation in dogs, warranting confirmation in further studies of larger numbers of envenomated dogs. Furthermore, this study confirms the presence of hypercoagulability in dogs bitten by V. berus and may aid hypothesis generation for further studies of the effects of V. berus venom on the haemostatic pathways in vivo.

Such findings positively contribute to the management of suspected *V. berus* bites in dogs, through the potential development of an in-house test for actual envenomation. Such a test would allow targeted treatment with antivenom and enable prioritisation of emergency resources.

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Publications

Results from this study were presented at BSAVA Congress 2025 and form the basis of a manuscript in the submission stages to PLOS One.

HARJEN, H. 2025. Clotting canines: can fibrin generation tests help diagnose adder bites in dogs? BSAVA Congress Proceedings 2025. British Small Animal Veterinary Association. https://doi.org/10.22233/9781913859510.37.59