Running title: Treatment of viper envenomation in dogs

Abstract

Objective – **To test an equine-derived polyvalent viperid antivenom (EPVA) in the treatment of dogs with evidence of viper envenomation**.

Design – Prospective, **multicenter observational study**.

Setting – Veterinary emergency and critical care facilities.

Animals – **A total of 82 client-owned dogs with progressive manifestations after minimal to severe viperid snakebite were enrolled in the study**.

Interventions – **EPVA was administered at a dosage of 1 ml/kg body weight. Decisions regarding the administration route, possibility of a lower dosage or additional doses were left to each site investigator**.

Measurements and Main Results – **A standardized snakebite severity score (SSS) was used to quantify the severity of envenomation and the clinical course after EPVA treatment. Five dogs died during the observation study as a consequence of the snake bite. A significant proportion of dogs had an improvement in SSS both at 4 (65.8%) and 8 hours (81.7%) following EPVA administration. All surviving dogs showed no abnormalities at the 4-week assessment after discharge, except for 3 dogs that still presented slightly abnormal hematological and/or coagulation parameters.** Antivenom-related acute or intermediate reactions occurred in 12 dogs (14.6%), and no serum sickness was recorded.

Conclusions – **In the first study on antivenom in dogs in Italy, EPVA was shown to stabilize or reverse the effects of progressive viper envenomation, as confirmed by the SSS analyses.**

Keywords: postexposure therapy, snakebite, dogs

Abbreviations

ALP: alkaline phosphatase

aPTT: partial thromboplastin time

AST: aspartate transaminase

CK: creatine kinase

Fab: fragment antigen-binding

IgG: immunoglobulin G

IV: intravenous

PT: prothrombin time

INTRODUCTION

Viper snakebites are a problem in small animals in Italy, where four poisonous species of the *Vipera* genus are commonly found: asp viper (*Vipera aspis*), with the subspecies *V. aspis francisciredi* being the only one found in central Italy,[1] the European adder (*Vipera berus*), sand viper (*Vipera ammodytes*) and meadow viper (*Vipera ursini*), whose bite is not medically significant. Vipers are characterized by a pair of long, hollow, venom-injecting fangs. Their venom is a complex cytotoxic mixture of high molecular weight proteins, the most important including proteases, metalloproteinases, amino acid esterases, phospholipase-A2, phospholipase-B, kininogenases, prothrombin-activating factors, hyaluronidases and neurotoxins.[2]

The clinical features of viper envenomation in dogs are characterized mostly by local tissue injury (i.e. swelling, edema and hematoma), often becoming obvious after some hours.[3,4] Systemic signs include increased vascular permeability, hypotension, hemolysis, anemia, thrombocytopenia, coagulopathy, respiratory depression, myonecrosis, nervous system dysfunction, and acute renal

failure.[3] Gastrointestinal dysfunctions are commonly observed in humans but are infrequent in dogs. The severity of envenomation is influenced by several factors, the most important being the amount of injected poison, dog body mass, dog age, bite location, time spent from bite to medical treatment, fatigue and concurrent diseases at the time of bite.[5] For the aforementioned reasons, a great variability in clinical presentation exists and the definitive diagnosis, when the owner has not observed the snake, may be initially difficult, thus delaying the correct treatment.

Specific antivenoms are considered the only effective treatment of viper snakebite envenoming, although knowledge of their efficacy in dogs is limited. Antivenoms may be constituted by whole IgG, or fragments (Fab, $F(ab')_2$) obtained by pepsin digestion of concentrated IgG. **Fab fragments are rapidly distributed in the tissues and rapidly eliminated**, thus showing a **shorter** half-life (4-24 hours) than whole IgG and $F(ab')_2$ fragments (2-4 days).**[6]** Fab fragments also have only one antigen binding site forming small and reversible immunocomplexes, whereas both whole IgG and $F(ab')_2$ have two antigen binding **sites**, and are thus able to form large and stable complexes. Due to their pharmacokinetic profile, antivenoms constituted by whole IgG or $F(ab')_2$ fragments are therefore more suitable in viper poisoning. This is confirmed by the results of a study conducted in rabbits inoculated with the poison of *Vipera aspis*, in which $F(ab')_2$ fragments showed a greater efficacy than Fab fragments in terms of the ability to neutralize the venom components.[7] Despite current knowledge regarding the safety profiles of antivenoms being limited to humans, recent studies have shown that whole IgG antivenoms may cause both acute and delayed hypersensitivity reactions in dogs,[8,9] whereas $F(ab')_2$ appear to be better tolerated.[10]

An equine-derived polyvalent viperid antivenom (EPVA)[a] for the specific neutralization of the venom of European snakes from the family of *Viperidae* has been developed. The active substances are **$F(ab')_2$** fragments of equine immunoglobulin molecules for the specific neutralization not less than 100 LD_{50} of *Vipera ammodytes* and *V. aspis* venom and 50 LD_{50} of *V.*

berus, *V. xanthina* and *V. lebetina* venom.[b] The other ingredients are: m-cresol (3 mg/ml), sodium chloride (0.9 mg/ml), and water for injection. EPVA is currently approved for human use in several **European Union** countries, including Italy, for the treatment of European viper envenoming in patients with blood coagulation abnormalities or disseminated intravascular coagulation, severe hypotension, **dyspnea** or cardiac arrhythmias. The posology involves the intramuscular injection of 10 ml immediately after the bite or 20-40 ml if at least four hours have elapsed from the bite or if the bite has affected one of the major blood vessels, the head or largely vascularized disctricts (e.g. fingertips). Adverse events in humans include anaphylactic reactions (urticaria, **dyspnea**, vascular collapse due to vascular failure and fall in blood pressure, paleness, cyanosis, increased heart rate) and serum sickness.

This prospective observational study was designed to **evaluate** the **effects** of EPVA for progressive viperid envenomation in dogs. We hypothesized that the administration of EPVA to naturally envenomated dogs would result in stabilization or improvement in snakebite severity scores (SSSs).

MATERIALS AND METHODS

Study design and selection criteria

This prospective multicenter observational study was performed between September 2012 and November 2014 in central Italy. The study protocol was approved by the Ministry of Health, Department of Veterinary Public Health, Food Security and Bodies for Health Protection (DGSAF 001453-P-01/08/2012). The study was carried out at 13 veterinary facilities selected in the provinces of Pisa (San Piero a Grado, San Miniato, Ponte a Egola, Pontedera), Livorno (Livorno), Firenze (Empoli, Certaldo, Montespertoli), Siena (Siena, Poggibonsi, San Gimignano, Casole d'Elsa), and Grosseto (Massa Marittima).

The study included previously healthy dogs of both sexes, which were at least 4 months of age and a minimum body weight of 3 kg. The dogs were conducted to a veterinary facility at one of the study sites with evidence of progressing viper poisoning syndrome, as diagnosed by the site investigator. Before enrollment, owners were briefed of the study risks and informed signed consent was obtained. The only exclusion criterium was the infusion of antivenom before enrollment in the study.

EPVA administration

After the lead investigator had confirmed the eligibility of each dog, EPVA was administered by intravenous or subcutaneous injection. Since no absolute criteria regarding the EPVA dosage have been established, and since the scientific literature indicates that small breeds may be a risk factor in snake poisoning,[11,12] EPVA was administered at a dosage of 1 ml/kg (100 mg/kg) body weight. This dosage was considered suitable to ensure uniformity in the study and predictable **effects** in dogs of different sizes. Decision regarding the administration route and the possibility of a lower dosage, as well as additional doses of EPVA, were left to the site investigator, according to the clinical status of the dog. **In particular, EPVA was administered by intravenous infusion in dogs which presented severe clinical condition and by subcutaneous injection in dogs which presented mild clinical condition. Considering that all dogs were client-owned and would have been at risk without antivenom, no control group was included.**

All dogs included in the study received fluid therapy, **whereas additional therapies (e.g., corticosteroids, analgesics, antibiotics)** were left to the site investigator's discretion.

Adverse events

Dogs were observed for acute and intermediate reactions by the site investigator following EPVA administration until discharge from the facility. Acute and intermediate reactions were defined as any apparent adverse event that occurred within 1 and 24 hours after EPVA administration, respectively. These events included suspected allergic reactions (eg, agitation, pruritus), vomiting, sialorrhea, vestibular signs, and atrioventricular block-related bradycardia. Evaluations of delayed reactions occurring more than 24 hours after EPVA administration were performed by the dog's owner after discharge from the facility. Delayed reactions were defined as any adverse event involving one of the following: swelling, **skin or subcutaneous changes**, arthralgia, fever or chemosis.

Clinical observation

In order to quantify the severity of the poisoning at baseline, as well as its evolution, a snakebite severity score (SSS) was used which has been validated in human crotalid poisoned patients (Table 1).[13] **Envenomation was considered minimal (SSS = 0-2), moderate (SSS = 3-5), or severe (SSS >5).** In view of the possible limited **effects** of EPVA in the case of delayed administration, the time elapsed between the viper bite and inclusion was also considered. The SSSs were assessed at baseline and at 4 and 8 hours after inclusion.

Blood collection and analyses

Blood samples for hematological and coagulation tests were collected and analysed upon inclusion, at 4 and 8 hours after inclusion and whenever an assay was deemed clinically necessary by the site investigator. Complete blood count, including WBC, RBC, HCT, and platelet count, as well as coagulation parameters (prothrombin time and partial thromboplastin time) were performed by each veterinary facility laboratory within 30 min after blood collection.

144

145 ***Follow-up***

146 Each dog was discharged 8 hours after inclusion, except for those whose clinical conditions required
147 hospitalization. In these cases, the dog remained under observation until an improvement in general
148 conditions and laboratory data was registered. Following discharge, each dog was monitored by the
149 owner for the next 4 weeks. The owners were thus instructed to notify the site investigator of any
150 problems by phone, email or direct visit. A further SSS assessment was performed 4 weeks after
151 discharge from the veterinary facilities.

152

153 ***Statistical methods***

154 Statistical analyses were performed using a commercial statistics program.[c] The primary endpoint
155 for assessment the **effect** of treatment was a change in SSS. **An improvement** was defined as a
156 decreasing SSS during the study period. For each dog, for each assessment time, and for each
157 component of the SSS, a change in score or score component from the baseline values was
158 calculated. Subsequently, the proportion of dogs with a lower score (improvement), the same score,
159 or a higher score (worsening) than that recorded at baseline was determined. All data were tested for
160 normality by the Kolmogorov-Smirnov test. One-way ANOVA analysis of variance among mean
161 score values, as well as the single score components, for each assessment time was performed by a
162 Friedman test followed by a Dunn multiple comparison test. **Spearman test was performed to**
163 **evaluate correlation between SSS at baseline and the weight of the dogs.** A value of $P<0.05$ was
164 considered significant.

165

166 **RESULTS**

A total of 82 dogs, all of which met the inclusion criteria, were enrolled from the 13 study sites. **No dogs which met the exclusion criteria (infusion of antivenom before enrollment in the study) were reported by the site investigators.** Thirty-nine dogs (47.5%) were referred to the hospital within 1 hour of the snake bite, 21 dogs (25.6%) within 2 hours, 11 dogs (13.4%) within 6 hours and 2 dogs (2.4%) within 12 hours of the bite. The remaining 9 dogs (11%) were referred to the hospital >13 hours after the bite. The dogs consisted of 48 females (58.5%) and 34 males (41.5%). The most common breeds were English Setter (19.5%), Brittany dog (18.3%), cross breed (15.9%), and English Springer Spaniel (11%). The ages of the dogs ranged from 6 months to 14 years, with a mean ± standard deviation (SD) age of 63.8 ± 39.9 months. The mean ± SD body weight was 18.1 ± 7.0 kg (range 5 / 50 kg).

Most dogs were bitten on the head/muzzle (62.2%). Other locations were front limbs (25.6%), rear limbs (11%), and **teat** (1.2%). For 29 of the 82 dogs (35.4%) the owner had observed the dog being bitten or a viper near the dog and in all dogs the clinical signs were strongly indicative of a viper bite, including decreased sensory response (72%), hematuria (47.6%), tachypnea and/or tachycardia (41.5%), hypotension (30.5%), petechiae and/or hemorrhage (19.5%). The most common clinical pathology abnormalities were increased CK, ALP and AST (39%), prolonged PT and aPTT (34.1%), proteinuria and increased urine protein-creatinine ratio (31.7%), thrombocytopenia, anemia and hemolysis (28%), hypofibrinogenemia (15.9%), and increased urea and creatinine levels (4.9%).

At baseline, 5, 28 and 49 dogs showed minimal (SSS=0-2), moderate (SSS=3-5), and severe (SSS>5) envenomation symptoms, respectively, with a median scores of 2 (range 1 / 2), 4 (range 3 / 5), and 8 (range 6 / 16) points, respectively. No correlation was found between SSS at baseline and the weight of the dogs (P>0.05).

Five animals with **initial median SSS** of 11 (range 5 / 16) died during the study, resulting in a 6.1% **mortality rate**. One of these deaths occurred immediately after the 8-hour evaluation, whereas the remaining four dogs died after an average time of 130 hours (range 29 / 360 hours).

EPVA administration and additional therapy

EPVA was administered in 69 out of 82 dogs (84%) by intravenous infusion **over 30 minutes**, whereas in the remaining 13 dogs (16%) EPVA was administered by subcutaneous injection given the good clinical status of the dogs. Seventy-four dogs (90%) received EPVA at the dosage set in the study protocol (1 ml/kg) after inclusion in the study, whereas 8 dogs (10%) received a lower dosage (range 0.3 / 0.8 ml/kg). This deviation from the protocol was approved by the lead investigator when requested by the site investigator because of the good clinical conditions of the dogs at entry. Seven dogs received additional EPVA after an average time period of 39 hours after the first administration (range 14 / 84 hours). These additional treatments were approved by the lead investigator when requested by the site investigator because of the serious clinical conditions of the dogs and/or a dramatic worsening of the envenomation syndrome as assessed by an increase in SSS.

All dogs received fluid therapy, consisting of isotonic crystalloid solution.[d] Altogether 75 dogs were treated with glucocorticoids. Sixty-nine dogs were treated on arrival at the hospital, whereas 6 dogs had already been treated by the dog's owner. Seven dogs did not receive glucocorticoid treatment. Fourteen dogs were treated with H2 antagonists (ranitidine) for 1-6 days, and 63 dogs were given antibiotics, including β-lactams (alone or in combination with streptomycin) and fluoroquinolones (enrofloxacin). Clinical signs related to infection of the snake bite were not observed in any dog.

Changes in SSS components

A significant proportion of dogs demonstrated an improvement in SSS both at 4 hours (54/82, 65.8%) and 8 hours (67/82, 81.7%) (P<0.0001) compared to the baseline value (Table 2). No significant change in SSS was observed in 19.5% (16/82) and 4.9% (4/82) of dogs at 4 and 8 hours, respectively (P≥0.05), and a not significant worsening of SSS was observed in 14.6% (12/82) and 13.4% (11/82) of dogs at 4 and 8 hours, respectively (P≥0.05). The median change in SSS was -1.7 points (range -7 / +3) at 4 hours and -2.5 points (range -10 / +7) at 8 hours. In terms of the individual score components, a significant improvement (P<0.0001) was observed both at 4 and 8 hours for the central nervous system (38/82, 46.3% and 52/82, 63.4%, respectively), the pulmonary system (35/82, 42.7% and 39/82, 47.6%, respectively) and the cardiovascular system (34/82, 41.5% and 37/82, 45.1%, respectively). On the other hand for the local wound, a significant improvement was observed only at 8 hours (33/82, 40.2%) (Tables 3-4). No significant improvement was observed at any assessment time for the gastrointestinal system and the coagulative pattern, whereas a significant worsening (P<0.0001) was observed for the hematological system at 8 hours (27/82, 32.9%).

Adverse events

A total of five possibly antivenom-related reactions occurred in 12 dogs, signifying a 14.6% incidence rate for adverse reactions (Table 5). Eleven dogs experienced four acute antivenom reactions, occurring within 1 hour following EPVA administration, which included agitation, pruritus, vomiting and sialorrhea. Agitation and pruritus occurred in 3 dogs, vomiting occurred in 4 dogs and sialorrhea and/or tremor occurred in 4 dogs. Vomiting was treated with maropitant (0.2 mg/kg, IV) in three cases and metoclopramide (25 mg/kg, **once** IV) in one case. All other events resolved without treatment in less than 3 hours.

No intermediate antivenom reactions were reported, whereas delayed reactions, occurring more than 24 hours following EPVA administration, were observed in 1 dog which reported arthralgia ten days after EPVA administration. The event resolved itself without treatment prior to the end of the study period.

Follow-up

Seventy-four dogs (90.2%) were discharged after 8 hours of observation and treatment, whereas for the remaining 8 dogs (9.8%), hospitalization at the veterinary facility was necessary before discharge (range 1 / 6 days). After adjusting for fatalities (5/82), there were 77 cases available for follow-up evaluation. All surviving dogs were cured and no abnormalities were observed in chemical chemistry at the 4-week assessment after discharge (average SSS=0.1, range 0 / 3), except for 3 dogs which still presented slightly abnormal hematological (**hematocrit**) and/or coagulation parameters (**PT, aPTT**).

DISCUSSION

The present study describes the clinical and hematologic parameters in dogs bitten by *V. aspis* and investigates the **effects** of EPVA as reflected in the stabilization or improvement of clinical conditions and laboratory results. To our knowledge, this is the first prospective observational study investigating the **effects** of viperid antivenom in dogs bitten by *V. aspis* in Italy.

The large majority of viper envenomations were observed in hunting dogs, which are the breeds at higher risk of snakebites because they frequent the countryside where reptiles are more abundant. This finding is similar to the only **Italian** data on canine *Vipera aspis* bites.[14] The large majority of dogs (87.8%) were bitten in the front areas of the body (head/nose/neck, front limbs), reflecting their tendency to look for or attack snakes. These data confirm those of previous reports

on dog envenomation by *Vipera xanthina palaestinae* and *Vipera berus*,[9,11,12,15,16,17,18,19] unlike in humans where bites are adventitious and occur mainly in the distal areas of limbs.[e] As a consequence, 77 out of 82 dogs enrolled in the present study (94%) showed moderate to severe envenomation signs at baseline. This is because when snakes feel threatened, **they are apt to** inject a larger dose of poison in defense.[20]

Since no prospective studies on canine envenomation syndrome by Asp viper and the efficacy of EPVA treatment in Italy are available, the dogs included in this survey were considered at risk of severe envenomation on the basis of personal experience. As a consequence, dogs were treated with EPVA administered at a dose of 100 mg/kg, except for 8 dogs, which were administered **a median dose** of 56 mg/kg, given the mild signs of envenomation at baseline. Improvements in symptoms and laboratory data were observed within 8 hours post treatment in the large majority of dogs (81.7%), and 90.2% were discharged because they were considered no longer at risk. Stabilization of envenomation was observed in 19.5% and 4.9% of dogs at 4 and 8 hours, respectively. Combining the dogs that improved or displayed no further progression of envenomation highlights the **effect** of the treatment in the large majority of the dogs already at 4 hours after administration of EPVA (87%).

Because an untreated control group was not included, it is not possible to assert that the resolution of clinical signs was directly related to the EPVA administration, **and that improvement wouldn't have occurred if the dogs received only supportive therapy. The absence of a control group was partially avoided by comparing the SSS of each dog at baseline to its own subsequent severity score.[21]** Experience with human victims of vipers suggests that SSS would be expected to worsen without treatment, rather than improve or remain unchanged.[22] The efficacy of antivenom therapy in the treatment of viper bites is also well known in humans, where symptoms resolve shortly after antivenom infusion, reducing the incidence of hematomas, functional

discomfort and length of hospitalization.[20,23,24] On the other hand, supportive therapy alone may not be able to rapidly reduce signs including mental status and swelling at the site of the bite.[16,25]

Forty-nine dogs showed an initial SSS higher than 5, which would be considered as severe envenomation in human medicine.[13] Among these, 8 dogs weighed less than 11 kg and only 3 dogs required a second dose of antivenom. Unlike previously reported,[11,12] our results did not indicate that small breeds may be a risk factor in snake poisoning, as no correlation between the severity of envenomation and the weight of the dogs was found.

Five animals died during the study period. One death occurred immediately after the protocol evaluation at 8 hours due to the rapid worsening of the dog's clinical conditions. This dog also had a high SSS at entry. One dog was treated with EPVA 1 hour after the bite and antivenom administration was repeated 74 hours after the bite, however despite the moderate SSS at entry, the dog died 15 days later because of renal failure. One dog was treated 18 hours after the bite and died 29 hours from the baseline evaluation, while the remaining two dogs received EPVA 8 to 79 hours after the bites and died 40 and 92 hours later respectively, because of severe hemolysis and coagulopathy. In a retrospective study conducted in the Poison Control Centre of Milan (Italy), a 21% mortality rate by *V. aspis* bite was reported, mainly due to hematologic problems, renal failure and shock.[14] Unfortunately, the epidemiological information did not report whether any antivenom therapy had been administered to the dogs. In a similar study by the Veterinary Poisons Information Service in the UK, fatalities after *V. berus* envenomation occurred in 3% of dogs treated with antivenom and in 4.8% of dogs that did not receive antivenom therapy.[3] Other studies on *V. berus* and *V. palestinae* poisoning reported mortality in 0% to 15% of dogs.[12,15,17,26,27] Although the percentages are relatively similar, the different species of vipers, geographical countries and climates make it difficult to compare the mortality rate of the present study to other studies.

No animal in the present study developed clinical bleeding, purpura, ecchymosis, or any other evidence of recurrence recognized by the owners within the 4 weeks post-hospitalization. Acute adverse reactions were observed in 11 dogs (13.4%) only 4 of whom required medical intervention beyond slowing the time of antivenom infusion **up to 1 hour**, suggesting that these were most likely anaphylactoid reactions.[28,29] **No skin or subcutaneous changes were observed**, whereas one dog with arthralgia was reported ten days after EPVA administration, which may have been due to ongoing venom toxicity rather than reactions to the antivenom.

In conclusion, the administration of EPVA stabilized or reversed the effects of progressive viper envenomation syndrome in the large majority of afflicted dogs included in this study. This was shown by the SSS analysis which was more often lower or unchanged than increased within hours after therapy.

FOOTNOTES

[a]Institute of Immunology Inc., Rockefellerova 2, HR-10 000 Zagreb, Croatia.

[b]Package insert, Viper venom antiserum, European (equine), Institute of Immunology Inc., Rockefellerova 2, HR-10 000 Zagreb, Croatia.

[c]GraphPad Prism 5.0 for Windows, GraphPad Software, San Diego, CA, USA.

[d]Lactate Ringer with Glucose, Electrolytic for Rehydratation III, Galenica Senese, Siena, Italy.

[e]Mann G. 1976. Snake bites in Israel, PhD Thesis, School of Medicine, The Hebrew University of Jerusalem.

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426 treatment. West J Med 1988;148(1):37-44.

Table 1. Snakebite Severity Score*.

Time elapsed between viper bite and inclusion in the study	**Score**
< 59 minutes	0
1-2 hours	1
3-6 hours	2
7-12 hours	3
> 13 hours	4
Local wound	
Signs within normal limits	0
Minimal - pain, swelling, ecchymosis, erythema limited to bite site	1
Moderate - pain, swelling, ecchymosis, erythema slowly spreading	2
Severe - pain, swelling, ecchymosis, erythema rapidly spreading	3
Very severe - pain, swelling, ecchymosis, erythema extends beyond the bite site, or significant tissue slough	4
Central nervous system	
Signs within normal limits	0
Minimal - apprehension	1
Moderate - chills, weakness, faintness, ataxia	2
Severe - lethargy, seizures, coma	3
Pulmonary system	
Signs within normal limits	0
Minimal - slight tachypnea	1
Moderate - respiratory compromise, tachypnea, use of accessory muscles	2
Severe - cyanosis, dyspnea, extreme tachypnea, respiratory insufficiency, or respiratory arrest from any cause	3

Table 1. (*continued*).

Cardiovascular system	**Score**
Signs within normal limits	0
Minimal - tachycardia, general weakness, benign dysrhythmia, hypertension	1
Moderate - tachycardia, hypotension (but tarsal pulse still palpable)	2
Severe - marked tachycardia, hypotension (nonpalpable tarsal pulse or systolic blood pressure < 80mmHg), malignant dysrhythmia, or cardiac arrest	3
Gastrointestinal system	
Signs within normal limits	0
Minimal - abdominal pain, tenesmus	1
Moderate - vomiting, diarrhea	2
Severe - repetitive vomiting, diarrhea, or hematemesis	3
Hematological system	
Hct within normal limits	0
30<Hct<37.4	1
20<Hct<29	2
10<Hct<19	3
Hct < 9	4
Coagulative pattern	
Within normal limits	0
Minimal - coagulation parameters slightly abnormal (PT<20 sec, aPTT<50 sec, platelets: 100,000-150,000/mm^3)	1
Moderate - coagulation parameters abnormal (PT: 20-50 sec, aPTT: 50-75 sec, platelets: 50,000-100,000/mm^3)	2
Severe - coagulation parameters abnormal (PT: 50-100 sec, aPTT: 75-100 sec, platelets: 20,000-50,000/mm^3)	3
Very severe - coagulation parameters markedly abnormal with bleeding present or the threat of spontaneous bleeding, including PT unmeasurable, aPTT unmeasurable, platelets < 20,000/mm3	4

[*] Adapted from Dart *et al.* (1996)[1].

Hct: hematocrit; PT: prothrombin time; aPTT: partial thromboplastin time.

Table 2. Summary of changes in overall severity score 4 and 8 hours after EPVA administration in 82 dogs with viper envenomation in Italy.

Assessment time (hours)	Change in score from baseline			Median change score (minimum, maximum)*
	Improvement	No change	Worsening	
4	54 (65.8%)§	16 (19.5%)	12 (14.6%)	-1.7 (-7/+3)
8	67 (81.7%)§	4 (4.9%)	11 (13.4%)	-2.5 (-10/+7)

Each cell gives the number of dogs (%).

* A negative change score corresponds to an improvement, a positive change score corresponds to a worsening, and a zero change score corresponds to no change.

§ $P<0.0001$, one-way ANOVA.

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Table 3. Changes in the individual components of the severity score 4 hours after EPVA administration in 82 dogs with viper envenomation in Italy.

Individual SSS component	Change in score from baseline			Median change score (minimum, maximum)*
	Improvement	No change	Worsening	
Local wound	14 (17.1%)	62 (75.6%)	6 (7.3%)	-0.1 (-2/+3)
Central nervous system	38 (46.3%)§	41 (50%)	3 (3.6%)	-0.5 (-3/+2)
Pulmonary system	35 (42.7%)§	42 (51.2%)	5 (6.1%)	-0.4 (-2/+2)
Cardiovascular system	34 (41.5%)§	43 (52.4%)	5 (6.1%)	-0.5 (-2/+3)
Gastrointestinal system	16 (19.5%)	63 (76.8%)	3 (3.6%)	-0.3 (-3/+1)
Hematological system	3 (3.6%)	56 (68.3%)	23 (28%)	+0.3 (-2/+3)
Coagulative pattern	20 (24.4%)	52 (63.4%)	10 (12.2%)	-0.2 (-4/+1)

Each cell gives the number of dogs (%).

* A negative change score corresponds to an improvement, a positive change score corresponds to a worsening, and a zero change score corresponds to no change.

§ $P<0.0001$, one-way ANOVA.

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Table 4. Changes in the individual components of the severity score 8 hours after EPVA administration in 82 dogs with viper envenomation in Italy.

Individual SSS component	Change in score from baseline			Median change score (minimum, maximum)*
	Improvement	No change	Worsening	
Local wound	33 (40.2%)§	44 (53.6%)	5 (6.1%)	-0.4 (-2/+2)
Central nervous system	52 (63.4%)§	27 (32.9%)	3 (3.7%)	-0.7 (-2/+2)
Pulmonary system	39 (47.6%)§	38 (46.3%)	5 (6.1%)	-0.5 (-2/+1)
Cardiovascular system	37 (45.1%)§	40 (48.8%)	5 (6.1%)	-0.5 (-3/+2)
Gastrointestinal system	18 (21.9%)	62 (35.6%)	2 (2.4%)	-0.3 (-2/+1)
Hematological system	6 (7.3%)	49 (59.7%)	27 (32.9%)§	+0.3 (-2/+2)
Coagulative pattern	29 (35.6%)	41 (50%)	12 (14.6%)	-0.4 (-4/+2)

Each cell gives the number of dogs (%).

* A negative change score corresponds to an improvement, a positive change score corresponds to a worsening, and a zero change score corresponds to no change.

§ $P<0.0001$, one-way ANOVA.

Table 5. EPVA-related acute and intermediate adverse events in dogs with viper envenomation in Italy.

Type of reaction	No. of dogs (%)	Treatment	Outcome
Acute*			
vomiting	4/82 (4.9%)	maropitant (3) metoclopramide (1)	survived
sialorrhea/tremor	4/82 (4.9%)	none	survived
agitation, pruritus	3/82 (3.6%)	none	survived
Delayed§			
arthralgia	1/82 (1.2%)	none	survived

* Occurred within 1 hour following EPVA administration.
§ Occurred more than 24 hours following EPVA administration.

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