Vipera palaestinae envenomation in 327 dogs: a retrospective cohort study and analysis of risk factors for mortality

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Abstract

Vipera palaestinae (Vp), formerly a subspecies of the near east viper Vipera xanthina, is the most common poisonous snake in Israel and neighbouring countries (Jordan, Lebanon and Syria), and is responsible for most envenomations in humans and domestic animals. Hospital records were retrospectively reviewed for confirmed cases of Vp envenomations in dogs over a 13-year period and 327 cases were included in the study. Most envenomations occurred between May and October, and between 02:00 and 10:00 PM. The most frequent clinical signs included: local swelling and oedema (99.6%), viper teeth penetration marks (51%), tachypnoea (50%), panting (44%), increased body temperature (19.2%), tachycardia (>160/min, 19%), salivation (18%) and lameness (15.6%). Common haematological findings included: increased haematocrit (47%), increased haemoglobin concentration (45%), leucocytosis (39%), and thrombocytopenia (30%). The prothrombin time and activated partial thromboplastin time were prolonged in 68 and 21% of the dogs, respectively. Blood biochemistry abnormalities included increased activities of muscle enzymes, hyperglycaemia, hyperbilirubinaemia, hyperglobulinaemia and hypocholesterolaemia.

The mortality rate was 4% (13 dogs). The following variables were significantly associated with mortality: body weight below 15 kg (p = 0.01), limb envenomation (0.008), envenomation at night (p = 0.025), severe lethargy (p < 0.001), hypothermia (p = 0.04), systemic bleeding (p = 0.001), shock (p = 0.007), dyspnorea (p = 0.002), tachycardia (p = 0.002), thrombocytopenia (p = 0.02) and glucocorticosteroid therapy (p = 0.002). Dogs younger than 4 years had a lower death risk (p = 0.01). The association of steroid therapy with increased mortality suggests that the use of steroids in Vp envenomations may be harmful. Specific antivenom therapy (10 ml/dog) was not associated with a higher survival rate, thus its use, dose and timing of administration should be further investigated.

Keywords: Venom; Oedema; Coagulation; Viper; Snakebite

1. Introduction

Vipera palaestinae (Vp) is the most common venomous snake in Israel, and is responsible for most envenomations in humans and domestic animals in Israel (Mann, 1976; Aroch and Harrus, 1999). The snake is endemic in Israel, north of Bee’r Sheva, and is also present in the neighboring countries, Jordan, Lebanon and Syria (Coppola and Hogan, 1992). It has adapted to life in agricultural and suburban areas (Mendelssohn, 1963). Envenomations were reported in people, dogs, cats, and horses, and in a ram (Efrati, 1969; Hoffman et al., 1993; Aroch and Harrus, 1999; Mazaki-Tovi and Lavy, 1999; Yeruham and Avidar, 2002). The viper’s venom contains about 30 components, 16 of which were identified, and the most important ones include proteases, haemorrhagins (metalloproteinases), amino acid esterases, phospholipase-A2 (PLA2), phospholipase-B and neurotoxins (Mann, 1976; Shiloah et al., 1973). The amount of venom injected in a single bite increases with an increase in ambient
temperatures and may reach as much as 1 g (dry weight), however, bites may contain no venom (Allon and Kochva, 1974).

In dogs, most bites are in the head and neck area and less frequently in the limbs (Aroch and Harrus, 1999). Clinical manifestations of Vp envenomations are mostly local, however, systemic signs are not uncommon. The most common local signs in dogs include swelling, oedema and haematoma, attributed mostly to venom haemorrhagin activity, and acute lameness with pain, when limb envenomations occur (Aroch and Harrus, 1999). Systemic signs in humans include anaphylactic, haemorrhagic or neurogenic shock, while in both humans and dogs tachypnoea, tachycardia, local lymphadenomegaly and cardiac arrhythmias were reported (Efrati, 1969; Aroch and Harrus, 1999). Gastrointestinal signs, commonly observed in humans (Efrati and Reif, 1953), are infrequent in dogs (Aroch and Harrus, 1999). Rare complications reported in people and dogs in viper-snake bites include bacterial infections (clostridial or other), local necrosis, upper respiratory airway obstruction due to laryngeal oedema, disseminated intravascular coagulation (DIC), acute renal failure, severe thrombocytopenia and death (Mann, 1976; Chugh et al., 1984; Hutton and Warrell, 1993; Aroch and Harrus, 1999; Lifshitz et al., 2000; Li et al., 2001). Myocardial necrosis in dogs and horses due to Vp envenomation is a rare complication (Hoffman et al., 1993; Mann, 1976; Chugh et al., 1984; Hutton and Warrell, 1993; Aroch and Harrus, 1999; Lifshitz et al., 2000; Li et al., 2001). The mortality rate reported in dogs due to Vp envenomations in a previous study was 3.7% (Aroch and Harrus, 1999). In humans, the mortality had decreased sharply from 6–10% to 0.5–2% since the introduction of a specific antivenom (Efrati and Reif, 1977; Winkler et al., 1993).

Different treatment regimens are used for Vp envenomations in animals and human patients, and include antibiotics, antihistamines, steroids and specific antivenom (Ben Abrahaham et al., 2001). Dosing and timing are controversial, and may vary in different medical institutions.

The objectives of this study were to describe the clinical, biochemical and haematological features of Vp dog envenomations in a large patient population, to compare different treatments used, with emphasis on specific antivenom effectiveness and glucocorticosteroid effects, and to evaluate potential risk factors (RF) for association with envenomation related mortality.

2. Materials and methods

2.1. Dogs and general data

All Hebrew University Veterinary Teaching Hospital (HUVTH) records of dogs presented between 1989 and 2002 were retrospectively reviewed for diagnoses of Vp envenomation. Three hundred and twenty-seven dogs were included in this study. All were observed to be bitten by a snake identified as Vp, by the owners, or by HUVTH clinicians, or had typical Vp bite penetration marks. The signalment and case history, physical examination and laboratory data were obtained from the medical records. In addition, data included envenomation date and time, time lag from envenomation to presentation at the HUVTH, geographical location (urban or rural) and the clinical signs with their progression during hospitalisation. Details of hospitalisation, period, treatments, outcomes and complications were also recorded.

2.2. Laboratory data

Blood samples for haematological tests were collected in EDTA tubes, and analysed within 30 min from collection. Complete blood count (CBC) was performed for 281 dogs upon presentation, using an automatic blood analyser calibrated for canine blood (Minos ST-VET, France or Abacus Diatron, Austria). Blood samples from 33 additional dogs were obtained 24 h post presentation. CBC included white blood cell count (WBC), red blood cell count (RBC), haemoglobin, haematocrit, mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC) and platelet count. Packed cell volume (PCV) was performed by centrifugation of heparinised capillaries (228 dogs), and total plasma proteins (TPP) were determined using a standard refractometer (259 dogs). Differential WBCs (68 dogs) were performed manually by counting 100 leucocytes in Giemsa stained blood smears. Blood for biochemical analyses (34 dogs) was collected in plain tubes, centrifuged within 30 min from collection, and sera were refrigerated (4 °C) pending analysis. Biochemistry analyses were performed within 24 h from collection, using an autoanalyser (Kone Progress Selective Chemistry Analyser, Kone Corporation Instrument Group, Finland). Blood samples for individual specific dry chemistry analyses (30 dogs) were collected in heparin tubes, and analysed within 30 min from collection using a dry chemistry analyser (Reflotron, Boehringer Mannheim, Germany or Reflovet Plus, Roche, Germany). Blood samples for coagulation tests (32 dogs) were collected in trisodium-citrate tubes, and analysed within 30 min from collection. Prothrombin (PT) and activated partial thromboplastin times (APTT) were performed using an analyser calibrated for canine blood (KC 1A micro, Amelung, Germany).

2.3. Medications

Antivenom (Vp antivenom Rogof institute, Israel; 10/500 ml Saline, Slow IV) was administered following a hypersensitivity skin test (0.5 ml injected intradermally in a shaved area on the trunk). Diphenhydramine (Phendramine, Vitamed, Israel 2 mg/kg q8h IM) was administered only after the completion of the skin test and before antivenom administration. Other medications included ampicillin.
(Penibrin, Teva, Israel, 25 mg/kg IV q8h), and different glucocorticoid preparations.

2.4. Statistical analysis

The data were analysed by SPSS software for Windows 10.0 (SPSS Inc.). As this was a cohort study, risk ratios (RR) were calculated. However, since exact analysis software for epidemiologic pure count data are available only for the calculation of odds ratios (OR; Greenland and Rothman, 1998), 95% confidence intervals (CI) based on small sample methods could be calculated only for OR, thus the ORs and their 95% CI are also depicted. Fisher’s exact tests were used to evaluate the crude associations between potential RF and mortality. In order to control for potential confounders, association of steroids therapy and mortality was further analysed by controlling separately for variables that were significantly associated with mortality in the univariate analysis (p < 0.1). Conditional maximum likelihood estimate of the OR with 95% CI were calculated after stratifying for each of these variables (one at a time), using PEPI 4.0 statistical software (Abramson and Gahlinger, 2001). Unless stated otherwise, p values of < 0.05 were considered statistically significant in all tests applied.

3. Results

Of all dog envenomations presented to the HUVTH between 1989 and 2002, 327 of 475 dogs were included in the study, and 148 cases were excluded, because these could not be confirmed as Vp envenomations. There were 152 males (46.5%) and 175 females (53.5%), with no significant difference between genders. The most common breeds were crossbreeds (29%), Rottweilers (6%), Boxers (6%), Doberman Pinschers (4%), Weimaraners and Golden retrievers (3% each), Labrador retrievers, miniature Pinschers and Cocker Spaniels (2% each). Mean age was 43 months (standard deviation (SD) 33; median 36; range 1.5 months–14 years). Mean body weight was 27.1 kg (SD 11.9; median 27; range 3–77).

Most envenomations occurred during the hot season (May–October, 86%) and between 14:00 and 22:00 (61%). Mean lag time from envenomation to presentation at the HUVTH was 256 min (SD 537, median 120, range 15–5760). A hundred and fifty-seven dogs (48%) were bitten in urban districts and the rest (52%) in suburban and agricultural districts.

3.1. Clinical signs

Most dogs were bitten once (96.3%), while some were bitten twice in one time (3.7%). Envenomations occurred in the head and submandibular area (78%), limbs (21.4%), and other body areas (0.6%). Clinical signs were mostly local, and included swelling and oedema (99.6%), viper’s teeth penetration marks (51%), salivation (18%), lameness (15.6%), bleeding tendencies (local and from body orifices; 5.8%) and local petechiae (3.2%). Systemic signs included tachypnoea (>40/min, 50%), panting (44%), increased body temperature (>39.5 °C, 19.2%), tachycardia (>160/min, 19%), lymphadenomegaly (15.3%), mental status abnormalities (varying degrees of depression up to coma, 13%) and dyspnoea (7%).

Viper’s teeth penetration marks were observed in 44% of the dogs when envenomated in the head and submandibular area, and in 75% of the dogs envenomated in the limbs. This difference was found to be statistically significant (p < 0.01). There was also a difference in the presence of bite marks between fatal (77%) and non-fatal cases (51%; p = 0.056).

3.2. Haematological and coagulation results

Haematological and coagulation profile data upon presentation are summarised in Table 1. The most common findings included haemoconcentration (47%), increased haemoglobin concentrations (45%) and MCHC (34%), leucocytosis (39%) and thrombocytopenia (30%). The most common abnormalities, observed one day after admission, were thrombocytopenia (52%), increased MCHC (47%) and anaemia (36%). Mean TPP on arrival was 66 g/l (SD 11) and dropped to 55 g/l (SD 10) a day later. Upon presentation the PT was mildly prolonged in most dogs (68.4%), while the APTT was prolonged in 21.1% of the dogs. One day later, the APTT was mildly prolonged in 75.8% of the dogs, while no significant change in the frequency of prolonged PT was noted.

3.3. Blood biochemistry results

Biochemistry analyses results are summarised in Table 2. The most common abnormalities included increased activities of muscle enzymes: lactate dehydrogenase (LDH, 67%), creatine kinase (CK, 64%) and aspartate aminotransferase (AST, 40%). Additional abnormalities included hypertriglyceridaemia (62.2%), mild hyperglycaemia (38%), hyperbilirubinaemia (37%), hyperglobulinaemia (35%) and hypocholesterolaemia (28%).

3.4. Treatments and outcomes

Specific antivenom therapy was administrated to 222 dogs (62%), while 105 dogs received no antivenom therapy, in most cases, due to financial considerations. No hypersensitivity reactions to antivenom were observed. Diphenhydramine was administered to 288 dogs (88%), and glucocorticoids were used in 68 dogs (21%).

Mean hospitalisation time in the general population of envenomated dogs was 1.3 days (SD 1.7, range 0–19) while in fatal cases 2.2 days (SD 2.5, range 0–7). The mortality
Table 1
Complete blood count and coagulation profile on arrival

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Mean ± SD</th>
<th>Median</th>
<th>% Below RI</th>
<th>% Above RI</th>
<th>Range</th>
<th>RI</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (10^9/l)</td>
<td>16.1 ± 9.0</td>
<td>14.5</td>
<td>1.75</td>
<td>39.0</td>
<td>0.9–104.0</td>
<td>5.0–16.0</td>
</tr>
<tr>
<td>RBC (10^12/l)</td>
<td>7.08 ± 1.22</td>
<td>7.14</td>
<td>9.4</td>
<td>21.32</td>
<td>3.35–10.37</td>
<td>5.50–8.00</td>
</tr>
<tr>
<td>HB (g/dl)</td>
<td>17.6 ± 5.8</td>
<td>17.4</td>
<td>6.96</td>
<td>45.26</td>
<td>7.8–25.0</td>
<td>12.0–17.5</td>
</tr>
<tr>
<td>HT (l/l)</td>
<td>49.1 ± 8.3</td>
<td>49.3</td>
<td>4.54</td>
<td>46.69</td>
<td>23.5–69.7</td>
<td>35.0–50.0</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>24.5 ± 2.1</td>
<td>24.3</td>
<td>0.00</td>
<td>43.30</td>
<td>19.0–30.4</td>
<td>19.5–24.5</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>35.2 ± 2.6</td>
<td>35.3</td>
<td>9.60</td>
<td>33.80</td>
<td>25.70–42.80</td>
<td>32.0–36.0</td>
</tr>
<tr>
<td>PLT (10^9/l)</td>
<td>240 ± 152</td>
<td>227</td>
<td>29.94</td>
<td>5.08</td>
<td>10–1262</td>
<td>150–500</td>
</tr>
<tr>
<td>PT (s)</td>
<td>9.87 ± 2.88</td>
<td>9.80</td>
<td>2.63</td>
<td>68.42</td>
<td>5.10–22.00</td>
<td>6.00–8.40</td>
</tr>
<tr>
<td>PTT (s)</td>
<td>15.24 ± 3.85</td>
<td>14.70</td>
<td>7.89</td>
<td>21.05</td>
<td>7.90–27.80</td>
<td>11.00–17.40</td>
</tr>
</tbody>
</table>

rate was 4% (13/327 dogs). Eight of the 13 (62%) fatal cases were treated with glucocorticosteroids, and eight (62%) received specific antivenom.

3.5. Risk factors for mortality

RF for mortality were divided into four categories: envenomation characteristics, clinical signs, clinical-pathological abnormalities, and type of treatment, and are summarised in Table 3. Limb envenomation, bites during night hours (22:00–06:00) and low body weight (<15 kg) were significantly associated (p = 0.004) with mortality (Table 3). Young age (<4 years) was significantly associated with higher survival (Table 3).

The following clinical signs were found to be significantly associated with mortality (Table 3): mental status abnormalities (i.e. severe depression or coma) upon arrival (p < 0.001), haematuria (p = 0.001), hypothermia (p = 0.004), bleeding tendencies (p = 0.001), shock (p = 0.007), dyspnoea (p = 0.002) and tachycardia (>160/min, p = 0.002). Dehydration on arrival was associated with mortality, (p = 0.057).

The only haematological abnormality associated with higher mortality was thrombocytopenia (p = 0.02). Haemconcentration on arrival tended to be associated with mortality (p = 0.09). Prolonged PT and APTT upon arrival and in the second day of hospitalisation were not found to be significant RFs.

Specific antivenom and diphenhydramine administration did not influence mortality. Glucocorticoid therapy, however, was significantly associated with mortality (p = 0.002). Testing this association after controlling for...
other significant RF (one at a time) did not alter this association (Table 4).

4. Discussion

Vipera plalaestinae is the only poisonous snake present in Israel north of Bee’r Sheva and up to the Lebanese and Syrian borders, with the exclusion of the Hermon mountain (>1200 m above sea level) and the Dead Sea areas (Efrati and Ref, 1977). All the dogs included in the present study were envenomated in this geographical zone. This fact, along with the selection criteria described in Section 2 above led to a confirmation of Vp envenomation.

Most of Vp envenomations occurred between May and October between 14:00 and 22:00, and parallel viper’s seasonal and diurnal activity. This pattern, however, may also result from increased seasonal and diurnal dog activity. Similar findings were reported previously in dogs (Aroch and Harrus, 1999; Hackett et al., 2002). Likewise, in humans, most frequently reported Vp envenomations were between 18:00 and 22:00 (Mann, 1976).

A large proportion of fatal envenomation cases (6/13, 46%) in the present study occurred between 22:00 and 06:00, leading to an increased and statistically significant risk for mortality. This could theoretically be explained by prolonged time lag from envenomation to treatment initiation. However, prolonged time lag from envenomation to presentation was not found to be a RF for mortality in the present study, suggesting that other unidentified factors were involved.

In contrast to a previous study (Aroch and Harrus, 1999), in which only 20% of bitten dogs were from urban areas in
Israel, in the present study there was no significant difference in the distribution of cases from rural versus urban districts. This difference might be attributed to an increase in urbanisation at the expense of rural and agricultural areas in Israel during the recent years, leading to pressure on Vp to appear in more populated locations. The previously reported annual number of dog envenomations in the HUVTH was 15 (Aroch and Harrus, 1999) while in the present study, a 67% rise was observed (25 dogs per year). This fact might also result from an increasing overlap between densely populated areas and Vp habitat.

Most envenomated dogs were young (< 4 years, 71%), of large breeds (≥ 25 kg, 66%) with no significant difference between genders, as was previously reported (Aroch and Harrus, 1999).

The mortality rate in the present study (4%) was similar to that observed in the past (3.7%; Aroch and Harrus, 1999), and also similar to that observed in dogs envenomed by Vipera berus in Sweden (3.5%; Kangström, 1989). This rate is higher than that reported in human patients envenomed by Vp in Israel (0.5–2%; Mann, 1976; Ben Abraham et al., 2001).

It has been shown before that the venom quantity injected by Vp in a single envenomation did not correlate with prey size (Allon and Kochva, 1974). This might have led to higher venom to body weight ratio in small breed dogs, and account for the increased RR for mortality observed in small dogs in the present study. In another retrospective study conducted on 100 dogs envenomed by rattlesnakes, small dogs required significantly longer hospitalisation period (Hackett et al., 2002), a finding that further supports this explanation.

Young dogs (< 4 years) had a lower risk for mortality. This finding is in agreement with studies in human patients, where adults were found to be more susceptible to Vp envenomations compared to children (Mann, 1976). Young healthy dogs, with mature and active immune systems, are probably also less likely to develop systemic complications following envenomation compared to old dogs, prone to other concurrent systemic diseases, organ dysfunction and/or immunodeficiency.

The local signs of local tissue swelling, oedema, haematoma, and local bleeding in Vp envenomations are attributed mainly to the venom’s haemorrhagin and PLA₂ actions (Lloret and Moreno, 1993; Moreira et al., 1994; Gutiérrez and Rucavado, 2000). Electron microscopy studies have shown that this activity leads to endothelial cell damage, increased erythrocyte tissue extravagation and platelet destruction and dysfunction (Grotto et al., 1969). Vp venom anticoagulant properties also negatively influence both platelet aggregation, and factor VIII activity (Grotto et al., 1969), leading to further tissue bleeding and swelling.

The high percentage of envenomations in the head area (75%) might indicate that dogs initiated the contact with the viper, unlike envenomations in humans, which are almost exclusively accidental and 83% were reported to occur in the distal parts of the limbs (Mann, 1976).

Viper’s teeth penetration marks were evident in 51% of envenomated dogs only, although all cases included in the present study were confirmed envenomations. Thus, lack of penetration marks cannot rule out snakebite. In both fatal and limb envenomations, a higher frequency of these marks (77 and 75%, respectively) was observed. Though not statistically significant, their presence was associated with approximately three times higher risk for mortality. Although, penetration marks were significantly associated with limb envenomations, this association was not altered after statistically controlling for limb envenomations, and thus, these marks can cautiously be considered as independent indicators for poor survival.

The higher mortality rate in limb envenomations could result from a more rapid spread of the venom to the general circulation through large superficial blood vessels present in the limbs, compared with those present in the submandibular, head and neck areas, and a relative lack of soft tissue, capable of absorbing the venom, in the limbs. For this same reason, limb bites were probably more likely to be observed by clinicians.

The systemic clinical signs present in Vp envenomations may vary due to individual patient susceptibility, and changes in the venom amount injected in a single bite. Common systemic signs observed upon arrival, as tachypnoea, tachycardia and depression, could partially be attributed to the presence of pain and excitement following envenomation, but might also result from shock. In this study, tachycardia, dyspnoea, hypothermia, severe lethargy or coma, shock, systemic bleeding or haematuria upon arrival were found to be indicators for poor survival. Systemic bleeding and haematuria upon arrival may have been markers of DIC presence early in the disease course. It has previously been demonstrated in human patients envenomated by Vp that severe shock and/or severe

| Table 4 |
| Odds ratio for the association of glucocorticosteroids therapy with mortality |

<table>
<thead>
<tr>
<th>Adjusted for</th>
<th>Odds ratio (OR)</th>
<th>Confidence interval (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &lt; 4 years</td>
<td>5.58</td>
<td>1.52–22.8</td>
</tr>
<tr>
<td>Night envenomation</td>
<td>7.87</td>
<td>1.97–38.2</td>
</tr>
<tr>
<td>Light weight (&lt; 15 Kg)</td>
<td>5.91</td>
<td>1.57–24.7</td>
</tr>
<tr>
<td>Limb envenomation</td>
<td>5.4</td>
<td>1.39–22.8</td>
</tr>
<tr>
<td>Dehydration</td>
<td>6.19</td>
<td>1.71–25</td>
</tr>
<tr>
<td>Severe lethargy</td>
<td>5.66</td>
<td>1.48–23.8</td>
</tr>
<tr>
<td>Dyspnoea</td>
<td>5.53</td>
<td>1.49–22.7</td>
</tr>
<tr>
<td>Systemic bleeding</td>
<td>5.96</td>
<td>1.61–24.5</td>
</tr>
<tr>
<td>Shock</td>
<td>7.86</td>
<td>2.04–36.7</td>
</tr>
<tr>
<td>Tachycardia</td>
<td>5.27</td>
<td>1.41–21.9</td>
</tr>
<tr>
<td>Hypothermia</td>
<td>4.58</td>
<td>1.12–19.8</td>
</tr>
<tr>
<td>Haemoconcentration</td>
<td>6.55</td>
<td>1.78–26.9</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>4.29</td>
<td>1–19.4</td>
</tr>
</tbody>
</table>
systemic signs were associated with higher mortality (Winkler et al., 1993). Shock may occur as a result of a hypersensitivity reaction (i.e. anaphylaxis) or through direct actions of venom components. The neurotoxins present in Vp venom only rarely lead to neurological signs in dogs (Aroch and Harrus, 1999), but can induce severe vasodilatation through effects on the medullary vasoactive center and peripheral nerves (Krupnick et al., 1968) and exacerbate a state of shock. Venom haemorrhagins also contribute to the progression of shock by causing bleeding and fluid extravasation into inflamed tissues.

The most pronounced haematological abnormalities of haemoconcentration (47%), increased MCHC (34%), leucocytosis (30%) and thrombocytopenia (30%) observed in the present study were previously reported in Vp envenomations of dogs and men (Mann, 1976; Aroch and Harrus, 1999). The presence of haemoconcentration could not be explained by dehydration merely, since only 3% of the dogs exhibited clinical dehydration. The pathogenesis of haemoconcentration might involve splenic contraction due to catecholamine release as a result of severe stress and excitement and actions of venom components. These same mechanisms could explain the presence of leucocytosis upon arrival. The MCHC increase might have been the result of intravascular haemolysis, due to the actions of a haemolysin identified in Vp venom (Gitter et al., 1959; Kochva et al., 1960). Indirect haemolysis could also be caused by the PLA2 action on erythrocytes membrane, degradation of trapped erythrocytes in fibrin clots, and severe vasculitis observed shortly after the envenomation (Grotto et al., 1969). This severe vasculitis can lead to thrombocytopenia with bleeding, platelet sequestration in inflamed tissues and possible formation of DIC. In the present study, thrombocytopenia upon arrival was often severe, and in many cases worsened during the following days. Thrombocytopenia upon arrival was indeed found to be a RF for mortality, and its observed worsening over time may also serve as an indicator of poor survival. No other haematological abnormality was found to be associated with increased mortality. Haemoconcentration was the only one to show a tendency toward statistically significant association.

Mean PT and APTT upon arrival were slightly prolonged, and were further prolonged a day later. Therefore, their clinical significance is hard to assess as a biochemical parameter could be evaluated as a risk factor and may have been elevated along with other muscle enzymes (CK, LDH, AST), probably due to local tissue damage induced by Vp venom actions. The possibility that some of these increased activities were due to myocardial damage cannot be ruled out, however, isoenzyme profiling and cardiac troponin concentration measurement were not performed. Ventricular arrhythmias (ventricular premature complexes, ventricular tachycardia, accelerated (idioventricular) rhythm and paroxysmal ventricular tachycardia) were observed in 37% out of 16 cases in which electrocardiograms were performed. This finding might support presence of myocardial damage (e.g. myocarditis and/or infarction) as has been reported previously in dogs and horses envenomated by Vp (Hoffman et al., 1993; Leisner et al., 1999), however, ventricular arrhythmias in most dogs probably resulted from extracardiac causes (e.g. thrombosis, bleeding and DIC).

Mild hyperglycaemia, observed in 38% of the dogs, was probably the result of envenomation associated stress. Bilirubinaemia, observed in 37% of the dogs probably resulted from combination of haemolysis and ischemic hepatoapthy. The latter mechanism could be supported by presence of increased activity of ALT observed in 25% of the dogs, although ALT activity is present also in muscles, and may have been elevated along with other muscle enzymes due to muscle damage.

Cholesterol concentration was inversely correlated with Vp envenomation severity in human patients, and albumin concentration showed the same tendency (Winkler et al., 1993). The authors hypothesized that hypocholesterolaemia was the result of lipoprotein leakage through capillaries, along with changes in lipoprotein transport and metabolism caused by PLA2 activity (Winkler et al., 1993). In the present study, hypcholesterolaemia could not be included in the risk factor analysis due to the low number of biochemical analyses performed in fatal cases, but, when measured, was noted in 29% of dogs. Cholesterol concentrations in the fatal cases were low (2.02, 3.12, 3.23 and 5.06 mmol/l, reference interval (RI) 3.1–6.7).

Hypoalbuminaemia was observed in 17% of the dogs, while globulin concentration was slightly increased in 35% of the dogs. This suggests that hypoproteinemia was a result of albumin leakage, probably due to vasculitis and capillary damage. The decline of total plasma protein, observed mainly during the second day, probably reflected worsening of hypoalbuminaemia. Both albumin and cholesterol concentrations in Vp envenomations should be further investigated and evaluated as RF and markers of severity in a large dog population. No other biochemical parameter could be evaluated as a risk factor...
due to the small number of biochemical analyses performed in fatal cases.

There is no standard treatment protocol for Vp envenomation in dogs or humans. Combination of fluid therapy, steroids, antibiotics and specific antivenom therapy are the treatments most often employed. The current recommended HUVTH protocol for Vp dog envenomations includes IV fluid therapy and ampicillin, diphenhydramine and IV specific antivenom. Envenomated dogs are monitored for haematological, biochemical, coagulation and ECG abnormalities for 24 h. Additional treatments, as colloids or FFP, are added if needed. In this study, specific antivenom treatment efficacy was evaluated based upon mortality rate rather than clinical signs severity and progression. In human patients, mortality rate decreased sharply with the introduction of a specific antivenom, and at present, death occurs mainly due to anaphylactic shock. In the latter cases, both in humans and dogs, antivenom seems to be ineffective (Winkler et al., 1993). No difference in mortality rate was observed in the present study between dogs receiving antivenom compared with untreated dogs. In fact, eight of 13 fatal cases did receive specific antivenom therapy. The possibility of confounding by indication, in which antivenom was selectively administered to dogs, which suffered from worse condition is hardly conceivable since this treatment is a part of the HUVTH protocol for the treatment of snakebites. It was therefore not administered only as a result of economic considerations of the owners. Indeed, controlling separately for the factors that were found to be associated with mortality did not alter the indifference in mortality between the antivenom treated and untreated group (data not shown).

In prairie rattlesnake dog envenomations, antivenom treatment significantly influenced only platelet counts, whereas no influence on other parameters was observed (Hackett et al., 2002). This apparent antivenom treatment ineffectiveness could be due to relatively low doses used. In human medicine, antivenom is used mostly in cases exhibiting systemic signs and in these instances it is usually given in high doses. The initial antivenom dose in human patients is 30–80 ml (Efrati, 1969), and treatment is continued until clinical signs wane. Antivenom doses may reach as much as 200–300 ml in extremely severe cases. In dogs, regardless of bodyweight or envenomation severity, the currently employed antivenom dose is almost always limited to 10 ml, mostly due to financial considerations. Based on the data from human medicine, there is every reason to believe that correct specific antivenom therapy should reduce mortality in dogs as well. Therefore, higher antivenom doses should be considered for the treatment of dogs presenting severe clinical signs or RF for mortality, while dogs with no evidence of systemic signs may do well without any antivenom. Timing of antivenom therapy may also be an important factor to be considered. The time lag from envenomation to treatment initiation in dogs is usually longer compared to that in humans, and antivenom administration is thus delayed and this may lead to less favorable results in dogs. Indeed, some studies of snakebites in humans strongly recommend early antivenom treatment (Ya and Perry, 1960; Mann, 1976). Antivenom effectiveness warrants further studies in larger dog populations due to the low mortality observed in the present study.

The use of glucocorticosteroids in snakebites has always been controversial. They are used in cases of shock and severe oedema, particularly in the larynx area, when they may minimize and/or prevent further endothelial damage. However, steroids may slow and diminish antivenom activity (Mann, 1976) and increase the risk for bacterial infection. Some clinicians believe that the use of steroids is contraindicated in snakebites (Garland, 2000). In the HUVTH, steroids are not included in the currently recommended treatment protocol for Vp envenomations in dogs. Steroid administration was found to be a risk factor for mortality. Controlling for other RF (one by one) did not alter this effect. Therefore, the opinion that corticosteroids administration increases mortality and hence contraindicated is supported. However, due to small number of death cases, a powerful multivariate analysis could not be performed. Thus, confounding by indication in which clinicians administered corticosteroids to dogs that presented combination of clinical signs suggestive of poor condition could not be entirely ruled out.

In conclusion, this study showed an increase in Vp annual envenomations of dogs, and a shift from rural to urban pattern, probably due to increased urbanisation. RF for mortality included late night envenomation, low patient body weight, limb envenomation, severe depression or coma upon arrival, dyspnoea, systemic bleeding, shock, haematuria, tachycardia, hyperthermia and thrombocytopenia. Some of these signs might have been associated with shock and/or DIC. The presence of the above RF for mortality should alarm clinicians, and warrant aggressive treatment. The use of glucocorticosteroids cannot be recommended in Vp envenomation in dogs, and may even be contraindicated, as it was found to be associated with increased mortality. Treatment with a low dose of specific antivenom (10 ml) did not decrease mortality rate and the use of higher doses should be further investigated.

References


