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REVIEW



Antivenom for European *Vipera* species envenoming

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ABSTRACT

Background: European viper bite is relatively uncommon but can cause serious envenoming, particularly swelling and hemorrhage spreading from limb to trunk that can cause long term disability. Systemic features are relatively mild compared to many other venomous species. Moderate-to-severe envenoming requires antivenom, which is given many hundreds of times each year across the continent. Several *Vipera* spp antivenoms are produced in Europe, but there is little comparative information available for the antivenoms and none is licensed with the European Medicines Agency. We aimed to collect descriptive data on European viper antivenoms and assess their relative effectiveness.

Methods: A systematic review of articles relating to antivenom in Europe was performed using the Medline medical database. The following keywords "Europ*" or the individual names of each European country and "antiven*" or "immun*" or "envenom*" and "snake" or "viper*" or "adder" were used. Articles published between 1 January 1996 and 11 March 2016 pertaining to clinical outcome, including case reports, were selected. Referenced articles in the indexed articles were explored for suitability and included if they met any of the criteria: specific antivenom used, route of antivenom administration, adverse reactions to antivenom therapy and length of hospital admission. All accepted abstracts from EAPCCT conferences since 2000 were searched and abstracts relating to *Vipera* spp envenoming were assessed for suitability. We extracted data on study type, safety and effectiveness. We sought information on antivenoms from manufacturers and individual patient data from authors of publications. Since individual patient data were only rarely available, we compared median length of stay between case series reporting each antivenom. We identified 40 papers and six published abstracts, and one unpublished paper that reported clinical cases and case series of envenomed patients treated with antivenom. No publication reported randomized controlled trials comparing any European *Vipera* antivenom with either placebo or another antivenom. 25 reports were of retrospective hospital- (n = 13) or poison center-based (n = 12) case series including five or more patients; a further 12 reports were either case reports or case series with less than five patients and one paper was a limited literature review. An additional nine papers reported prospective data; seven collected data remotely through poison service telephone communication with the attending physicians.

Antivenoms available in Europe: Eight antivenoms are available for European *Vipera* spp envenoming; a material safety data sheet providing information on manufacture was available for seven. Six are raised against *V. berus* or *V. ammodytes* venom; the seventh is raised against a mixture of *V. ammodytes*, *V. aspis* and *V. berus* venom and the eighth is raised against *V. ammodytes*, *Macrovipera lebetina* and *Montivipera xanthina* venom. Six manufacturers recommended intramuscular administration while two recommended intravenous administration. No randomized control trials comparing the effectiveness of antivenoms were identified.

Pre-clinical data: We found two papers presenting comparative preclinical data.

Clinical data: Clinical studies were predominantly retrospective and contained clinical data on antivenom used in 2602 patients; where the antivenom was identified (n = 2174), 2061 (94.8%) received Zagreb, ViperFAV or ViperTAB antivenoms. There were few published data on the other antivenoms.

Repeated use of antivenom: Repeat doses were reported in 230/1491 of cases (15.4%) where this information was recorded.

Outcome and length of hospital stay: Intravenous administration of antivenom was associated with shorter length of hospital stay (median length of hospital stay in studies of intravenous ViperFAV or ViperTAB ranged from 1 to 4.8 days versus 2 to 18 days for intramuscular Bulbio or Zagreb antivenoms).

Antivenom versus no antivenom: Some small studies demonstrated no difference in the length of hospital stay in patients with equivalent envenomation grading who either did or did not receive antivenom.

Adverse events: Adverse reactions were reported in 37 of 2408 cases (1.5%) including seven cases of anaphylaxis.

ARTICLE HISTORY

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KEYWORDS

Antivenom; *Vipera* snakes; effectiveness

Conclusions: There are very limited pre-clinical comparative data and no randomised controlled trials assessing effectiveness of the antivenoms against different *Vipera* species. Most descriptive data suggest the efficacy of Zagreb, ViperFAV and ViperATab antivenoms by the intravenous route but not intramuscular route, although this is level D evidence. Reported adverse reactions were rare, suggesting that the modern intravenous antivenoms are of good quality. Better and more systematic data, including perhaps randomized controlled trials comparing different antivenoms, are required for the many hundreds of antivenom administrations that occur annually across Europe.

Background

Snake envenoming is an uncommon but potentially severe injury in Europe, estimated to affect 0.4 to 1.1 people per 100,000 population per year and to cause about four deaths annually [1]. Three snake species of the *Vipera* genus (*Vipera berus*, *V. aspis*, and *V. ammodytes*) are responsible for most significant envenoming in Europe (Figure 1) [2]. *Macrovipera lebetina* and *Montivipera xanthina* are clinical problems in the very south-east of Europe and *V. latastei* is clinically relevant in the Iberian peninsula [1,3]. *V. ursinii* and *V. seoanei* appear to cause mostly mild envenoming while some species, e.g., *V. walser*, occur in very restricted localities without reports of bites.

European viper envenoming can cause severe complications including massive local swelling that may spread to the torso, particularly in children, as well as anaphylaxis, cardiovascular shock, neurotoxicity, and coagulopathy (Box 1) [4,5]. Although systemic features do occur, they are typically much less severe than the neurotoxicity or coagulopathies that occur with for example elapids, Russell's viper or American rattlesnakes [5]. Local oedema and bruising of the affected limb (Figure 2) can result in prolonged functional morbidity and lengthy hospital admissions, particularly in children and in cases where there is delayed or inadequate treatment [6,7]. The aim of antivenom treatment is to prevent severe local effects and to reduce hospital stay. Although length of hospital stay is affected by local culture and health care resources, within Europe it is perhaps a reasonable outcome measure since it is an indicator of the degree of local tissue effects.

Antivenom has been raised against venom of different European vipers at several, often national, antivenom production facilities across Europe. However, relatively little is known about several of these antivenoms or about their relative effectiveness.

There is no standardized protocol for antivenom administration across Europe, with variation from country to country, and no antivenom licensed by the European Medicines Agency. Use of unrefined antivenoms in the UK in the 1950s resulted in at least one death from anaphylaxis after *V. berus* bite [4]. Concern about antivenom safety resulted in a period of antivenom underuse, despite the availability of an effective antivenom [4]. In contrast, severe *V. aspis* envenoming in a Swiss patient who did not receive antivenom has recently resulted in increased use in that country [8].

Box 1. Clinical syndromes from European viper bites have been well described [4,5,12,29]. Although there is variation across the range of some species and between species, there is a typical course.

Local envenoming

The first indication of envenoming typically occurs with pain and tingling of the affected limb. Gross oedema of the bitten digit and limb can occur within minutes and continue to extend over the next 48 h (Figure 2); it may extend to affect all of the envenomed limb and then the torso. Tender regional lymphadenopathy and bruising are common with the bruising occasionally gross.

Systemic envenoming

Common features of systemic envenoming include dizziness, vomiting, and diarrhoea as well as fever, tachycardia and hypotension. Although uncommon, marked anaphylaxis-like histamine reactions including urticaria, angioedema, laryngospasm and cardiovascular shock can occur within minutes of the bite, requiring rapid supportive management. Cardiotoxicity demonstrated by dynamic ECG changes such as ventricular ectopic beats, atrial fibrillation, AV block, and t-wave inversion have also been reported. Impairment of consciousness occurs secondary to cardiovascular toxicity.

Neurotoxicity

Neurotoxicity has been well described in cases of *V. aspis*, *V. ammodytes*, and *V. berus bosniensis* [22,29,45,58,69]. Cranial nerve palsies (such as diplopia, ophthalmoplegia, ptosis, and dysphagia) typically develop within 12 h of the bite.

Additional complications

Rare complications include coagulopathy and thrombocytopenia resulting in bleeding diathesis. Although often suspected, deep vein thrombosis is rare. Rhabdomyolysis, acute kidney injury, localized skin necrosis, pancreatitis, acute paralytic ileus, pulmonary and cerebral oedema, and compartment syndrome are all described and appear significantly more common in bites from *V. ammodytes*, occurring in up to 5% of cases [29,30,35,39].

Laboratory findings

Leucocytosis is common and associated with more severe envenoming [21]. Other common abnormalities include anaemia, thrombocytopenia, metabolic acidosis, and microscopic haematuria. Additional laboratory features include prolonged prothrombin time (PT) and activated partial thromboplastin time (APTT), elevated D-Dimer, low fibrinogen, deranged transaminases, elevated creatinine, and creatinine kinase.

The recent availability of newer antivenoms with improved purification techniques has resulted in increased antivenom use for all moderate-severe envenoming in some countries [6,9–11]. Standardized grading systems have been developed in Sweden and France (Supplementary information, Table 2) that may allow identification of patients most likely to benefit from antivenom [12], although neither have been tested against outcome. In this paper, we aimed to identify all the anti-European *Vipera* spp antivenoms currently in clinical use and to seek data on comparative effectiveness and safety.



Figure 1. Distribution of three most widespread species of European *Vipera* genus. (A) *V. berus* (including *V. walser* in blue), (B) *V. aspis*, (C) *V. ammodytes*. Arrows indicate small non-contiguous areas. Reproduced with permission from Speybroeck et al. [2].



Figure 2. Typical local effects of *Vipera* spp envenoming. Consequences of *V. ammodytes* bites to leg (top, 35-year-old woman, day 5 post-bite) or hand (middle, 60-year-old man, hour 26 post-bite) and of *V. berus* bite to leg (bottom, 54-year-old woman, day 5 post-bite). Patients had received 3, 2 and 1 dose(s) of ViperaTAb, respectively. Written consent was obtained for the use of the photos from each patient.

Methods

Antivenoms used for the treatment of *Vipera* spp envenoming in Europe were identified using antivenom databases produced by the World Health Organization (WHO) [13], Munich Poisons Information Centre [14], and Toxinology Department, Women's & Children's Hospital, Adelaide [15] as well as by hand-searching articles identified by the search described below and by searching Google.

A systematic review of articles relating to antivenom in Europe was performed using the Medline medical database. The following keywords "Europ*" or the individual names of each European country and "antiven*" or "immun*" or "envenom*" and "snake" or "viper*" or "adder" were used. Articles published between 1 January 1996 and 11 March 2016 pertaining to clinical outcome, including case reports, were selected. Referenced articles in the indexed articles were explored for suitability and included if they met the outlined criteria. All accepted abstracts from EAPCCT conferences since 2000 were searched and abstracts relating to *Vipera* spp envenoming were assessed for suitability. Authors of papers were contacted for the relevant data.

Papers were selected if they included any of the following: specific antivenom used, route of antivenom administration, adverse reactions to antivenom therapy, and length of hospital admission. Papers were divided into (i) those in which antivenom was administered to patients with unknown envenoming grading and (ii) those in which antivenom was administered to patients with poison severity scores akin to Swedish or French grade 2–3 severity (Supplementary information, Table 2) could be identified. Where insufficient data were documented, authors were contacted for further information. The species of snake responsible for envenoming was determined in the paper by identifying a sample of snakes brought to hospital or by there being only one viper species native to an area (e.g., UK/Scandinavia). Statistical analysis was performed using GraphPad Prism version 6. Median length of stay was calculated where possible for reports presenting data for five or more patients receiving antivenom.

Antivenoms available in Europe

We identified eight antivenoms for the treatment of European *Vipera* spp envenoming that are currently available (Table 1). A further 10 *Vipera* antivenoms were previously available but are no longer produced (Supplementary Box 2) [16].

Safety data sheets (SDS) were obtained for seven of the eight antivenoms (produced in Bulgaria, Croatia, France, Poland, Serbia, Turkey, and UK); no SDS could be obtained for the Russian antivenom. Of the seven antivenoms with an SDS, six are equine antivenom products while one is ovine (Table 1). The equine antivenoms are F(ab')₂ products while the ovine antivenom is a Fab product. Two antivenoms are polyvalent: ViperFAV is raised against venom from *V. aspis*, *V. berus*, and *V. ammodytes* while Vetel is raised against venom from *V. ammodytes*, *Macrovipera lebetina*, and *Montivipera xanthina*. The remaining six antivenoms are raised against either *V. berus* or *V. ammodytes* venoms (Table 1).

Estimated total protein per therapeutic dose is documented in five of the seven SDSs – this is less for ViperTAB (200 mg) than ViperFAV (396–468 mg), Biomed (450–850 mg), Zagreb (1000 mg), and Bulbio (900–1700 mg) antivenoms.

Pre-clinical data

Protein profiles of venoms from different *Vipera* spp suggest broad similarities that might allow antivenom cross-neutralization between species [17]. Data on the neutralizing effect of each antivenom against different venoms – where reported in their SDS – are presented in Table 1. ViperFAV, ViperTAB, and Zagreb antivenoms have been tested against *V. ammodytes*, *V. aspis*, and *V. berus* venoms, the Vietkin antivenom has been tested against *V. ammodytes* and *V. berus* venoms, and the Bulbio, Biomed and Vetel antivenoms have been tested against *V. berus* or *V. ammodytes* venom. No data are presented for the Microgen antivenom. The SDS for Vietkin, ViperTAB, and Zagreb antivenoms state that they meet the minimum standard neutralizing effect against venom from *Vipera* species against which they were not raised, suggesting neutralization across species (Table 1).

Animal studies assessing the monovalent *V. ammodytes* Zagreb antivenom demonstrate cross-species efficacy at treating envenoming from *V. berus* [18]. Two published studies have compared the pre-clinical efficacy of *Vipera* antivenoms. A comparison of ViperTAB and Zagreb antivenom showed that both these monospecific antivenoms bind to venom from all the European vipers (Figure 3) [19]. A reduced quantity of ViperTAB required to neutralize venom indicates greater potency compared to the Zagreb antivenom. Testing using the murine ED₅₀ assay showed that ViperTAB is superior to Zagreb antivenom at neutralizing lethal doses of *V. berus*, *V. aspis*, and *V. latastei* venoms while being equipotent versus *V. ammodytes* venom (Figure 3). The second study compared ViperFAV to IPSEER Europe antivenom, which is now no longer available. The study demonstrated a greater venom immunoreactive fraction in ViperFAV and a consequent 51% improvement in specific

neutralizing activity compared to IPSEER Europe against *V. aspis* venom [20].

Due to geographical variation in antivenom use across Europe, clinical experience with each antivenom is often limited to one or two *Vipera* spp. Pre-clinical testing offers the opportunity to test cross-reactivity of antivenoms between *Vipera* spp. However, pre-clinical testing has major limitations, with little similarity to human envenoming, in its use of mice and IV administration of venom.

Clinical data

We identified 40 papers and six published abstracts [6–12,21–66], and one unpublished paper that reported clinical cases and case series of envenomed patients treated with antivenom. No publication reported randomized controlled trials (RCTs) comparing any European *Vipera* antivenom with either placebo or another antivenom. However, there is currently a lack of equipoise in Europe that would allow an RCT versus placebo.

Twenty-five reports were of retrospective hospital- ($n = 13$) or poison center-based ($n = 12$) cases series including five or more patients; a further 12 reports were either case reports or case series with less than five patients and one paper was a limited literature review. Nine papers reported prospective data; seven collected data remotely through poison service telephone communication with the attending physicians.

These reports included 2602 envenomed patients treated with antivenom. Most publications and cases concerned Zagreb (14 publications, 1306 patients), ViperFAV (11 publications, 558 cases), and ViperTAB (14 publications, 197 cases) antivenoms. We found just three publications on Biomed antivenom (43 patients), two publications on Bulbio antivenom (69 patients), one publication for Viekvín (1 patient) and none on Vetel or Microgen antivenoms. Eight papers did not specify the antivenom used.

Five (0.2%) patients receiving antivenom died [29,31,39,52,58]. All five cases were administered either IV or IM Zagreb antivenom prior to death. A further two cases died prior to antivenom therapy [12,51].

Several of these antivenoms are used only in their country of manufacture and neighboring countries. Only the Zagreb and ViperTAB antivenoms have been used widely outside of their country of manufacture; unfortunately, the Zagreb antivenom is no longer available, leaving the affinity-purified ovine Fab antivenom ViperTAB. The cost of the modern more highly purified antivenoms, ViperFAV and ViperTAB, is substantially higher than the older less purified antivenoms.

There is great need for better collection of routine clinical information on the treatment of different envenomings with the variety of antivenoms available across Europe. Individual patient level data are particularly required for meta-analysis.

There are few clinical (or pre-clinical) data available for the Biomed, Bulbio and Viekvín antivenoms. The SDS for each of these antivenoms recommends IM administration; there is a lack of convincing evidence of envenomation reversal for Bulbio and Viekvín. Whilst there are just two documented cases of adverse reactions in the limited case series

Table 1. Characteristics of the eight antivenoms, using information provided in the SDS.

Antivenom name	Biomed viper venom antitoxin	Bulbio snake venom antiserum	Microgen anti-viper venom serum	Vetel polysera	Viekvin	ViperaTab	ViperFAV	Zagreb, European viper venom antiserum
SDS url	http://biomed.com.pl/pilk/4b702c-5896772-antytoksyna_jadu_zmij_karta_charakterystyki_en.pdf	Available upon request from: http://www.bulbio.com/en.html	Not found	http://www.vetalse-rum.com.tr/panel/images/dosyalar/poliserakullanimtalimati.pdf	http://www.torlakinstitut.com/pdf/Viekvin-en.pdf	http://www.toxinfo.org/antivenoms/resources/anti-venom_uk-micropharm-viperatab_2011-03-23.pdf	http://www.toxinfo.org/antivenoms/resources/anti-venom_france-sanofi-pasteur-viper-fav_2011-11-02.pdf	Available upon request from: http://www.imz.hr
MAH	Biomed Sera and Vaccines Manufacturer, Warsaw, Poland	Bulbio, NCIPD Ltd, Sofia, Bulgaria	Federal State Company for Immunobiological Medicines (Microgen), Moscow, Russia	Vetal Serum and Biological Products Manufacturing Industry and Commerce.	Institute for Virology, Vaccines and Sera, "Torlak", Serbia	Micropharm, UK	Sanofi-Pasteur, France	Institute for Immunology, Zagreb, Croatia
Source Type	Equine F(ab') ₂	Equine F(ab') ₂	NK	Equine F(ab') ₂	Equine F(ab') ₂	Ovine Fab	Equine F(ab') ₂	Equine F(ab') ₂
Vipera spp raised against	<i>V. berus</i>	<i>V. ammodytes</i>	NK	<i>V. ammodytes</i> , <i>Macrovipera lebetina</i> and <i>Montivipera xanthina</i>	<i>V. ammodytes</i>	<i>V. berus</i>	<i>V. berus</i> , <i>aspis</i> & <i>ammodytes</i>	<i>V. ammodytes</i>
Recognised by WHO [13]	Yes	No	Yes	No	Yes	No	Yes	Yes
Antibody concentration	NR	NR	NK	NR	NR	100 mg/mL	99–116 mg/mL	25 mg/mL
Affinity purified	No	No	NK	No	No	Yes	No	No
Recommended dose and route of administration by MAH	5mL IM	10mL SC and 10mL IM	NK	10–50mL IM or IV in severe envenoming	5–10mL IM	8mL IV	4mL IV	10mL IM
Total amount of protein per dose	450–850 mg	900–1700mg	NK	NR	NR	200 mg	396–468 mg	1000 mg
Neutralizing units per ml of antivenom against different venoms	>150	NR	NK	NR	>50	597	125	>50
<i>V. berus</i> venom	NR	NR	NK	NR	NR	690	250	>100
<i>V. aspis</i> venom	NR	>100/ampule	NK	>100	>100	222	250	>100
<i>V. ammodytes</i> venom	NR	NR	NK	NR	NR	176	NR	NR
<i>V. latrostei</i> venom	NR	NR	NK	NR	NR	NR	NR	NR

IM: intramuscular; IV: intravenous; MAH: marketing authorisation holder; MSDS: material safety data sheet; NK: not known; NR: not reported; SC: sub-cutaneous; WHO: World Health Organization.

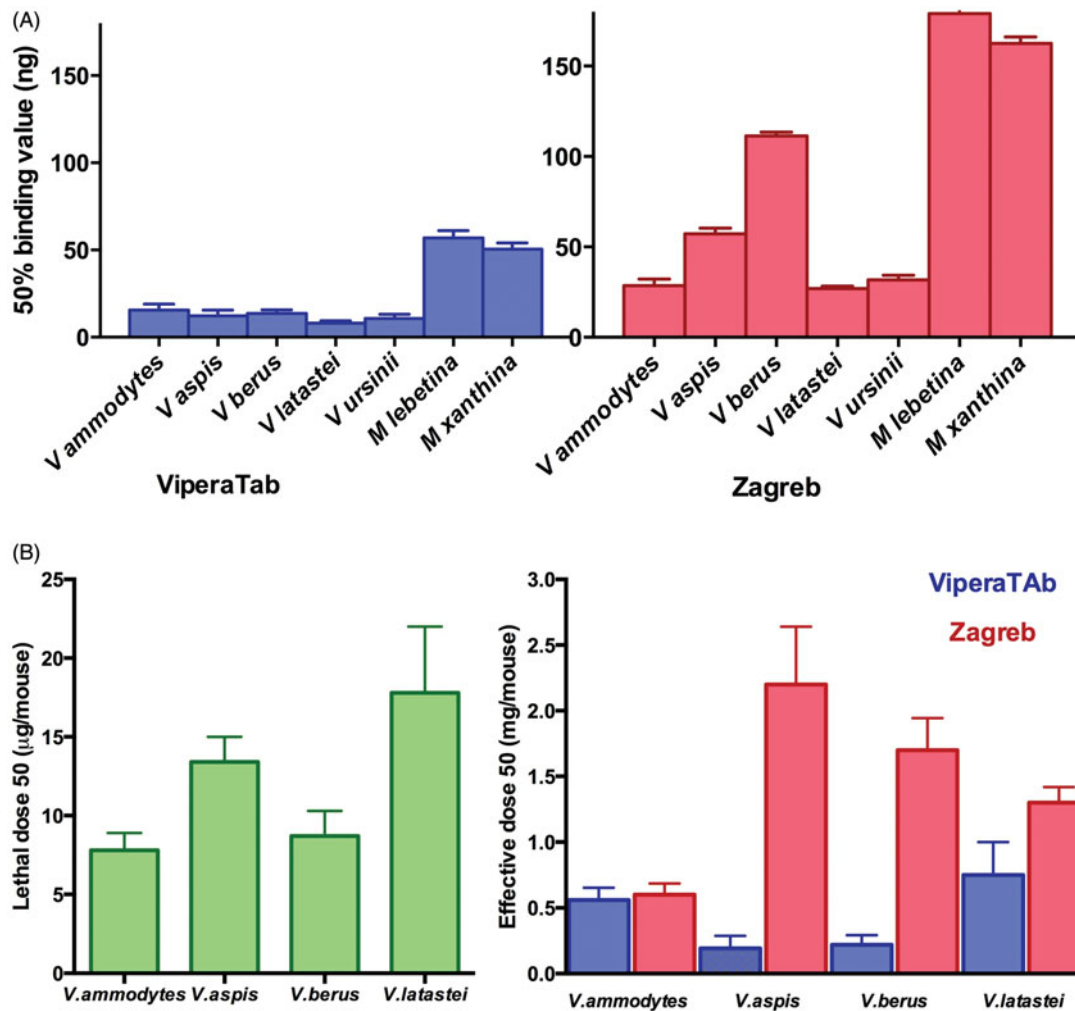


Figure 3. Preclinical data comparing antivenom potency. (A) The quantity of antivenom (ng) required to bind 50% of test venom for ViperaTab and Zagreb antivenoms. (B) The lethal dose for each of four viper venoms (left). The effective dose (ED50) of ViperaTab and Zagreb antivenom required to protect 50% of mice from the lethal doses of each venom (right). Reproduced with permission from Casewell et al. [19].

published, the presence of preservatives such as phenol in Biomed, Bulbio and Viekvin antivenoms might increase the risk of adverse reactions. Further research is required for each of these antivenoms after IV administration, reporting clinical outcome, complications and adverse reactions before recommendations for wider use within Europe can be made.

Outcome and length of hospital stay

We sought individual patient data from publications and authors but these were available for few studies. In the absence of individual patient data that would have allowed calculation of median duration of hospital stay for each antivenom from all patients receiving the antivenom, we compared the median lengths of stay reported in each publication presenting five or more cases for each antivenom, for cases of all severity (Figure 4) and for cases of grade 2 or 3 severity (Figure 5).

The median length of hospital stay for patients in studies of patients of any severity administered Zagreb, ViperFAV, or ViperaTab antivenoms ranged from 3 to 18 days ($n = 734$), 1 to 4.8 days ($n = 558$), and 1 to 8 days ($n = 197$), respectively

(Figures 4 and 5). The median length of stay in studies of patients with grade 2–3 severity ranged from 3 to 14 days ($n = 256$), 1 to 4.8 days ($n = 551$), and 1 to 8 days ($n = 197$), respectively (Figure 5). The viper species responsible for the envenoming could be determined for 1241 cases. However, we found insufficient data to compare the apparent effectiveness of these three antivenoms for the different species of snake.

Both IM and IV routes have been used for the Zagreb antivenom (Figures 4 and 5). The median length of hospital stay in five studies following IM use was 5–18 days [28–30,52,58] compared to 3–6 days in three studies after IV use [7,45,59]. The IV group contained patients envenomed with *V. aspis*, *V. berus*, and *V. ammodytes* whereas the IM group contained patients envenomed with *V. berus*, *V. ammodytes*, and unknown *Vipera* spp.

There are fewer data on the other antivenoms (Figures 4 and 5). The median length of hospital stay in a study conducted in Italy for patients treated with Biomed antivenom IV ($n = 16$) was 5 (IQR 4.0–6.3) days where the viper species was unknown (*V. aspis* or *V. berus*) [7]. In contrast, patients administered Biomed antivenom IM after *V. berus* envenoming

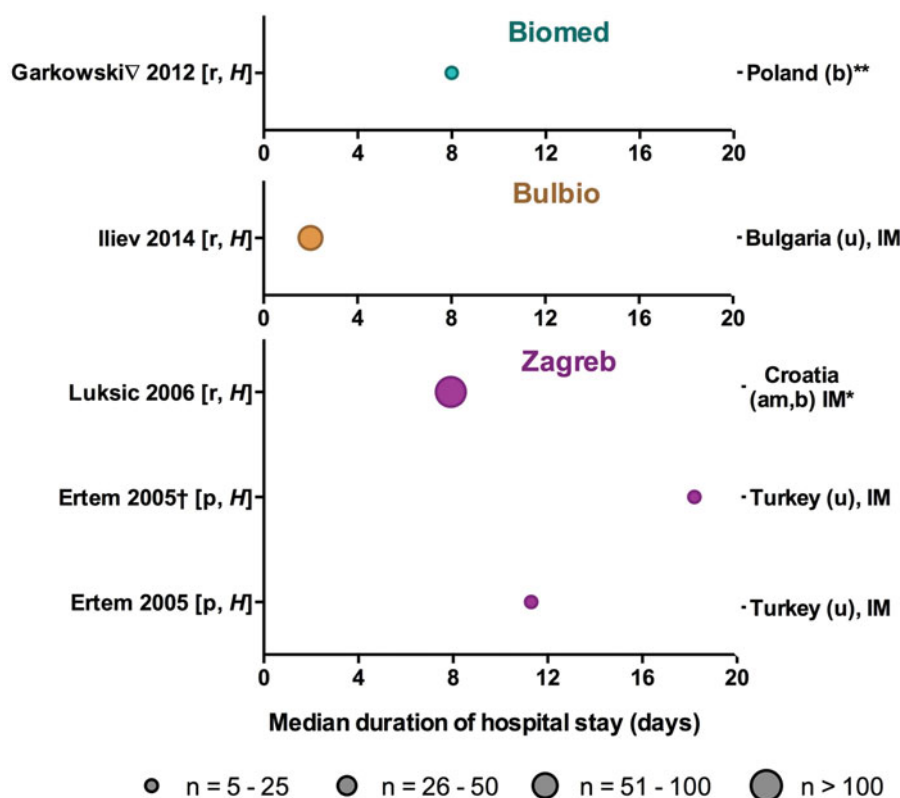


Figure 4. Median length of hospital stay (and IQR where known) for envenomed patients of unspecified poison severity score treated with antivenom and without antivenom in studies with five or more patients. Left y-axis shows first author, year of publication, in square brackets retrospective [r] or prospective [p] and italics hospital [H] or Poison information centre [PIC]. Right y-axis shows location of study, predominant *Vipera* spp responsible (am, *V. ammodytes*; b, *V. berus*; u, *Vipera* spp unspecified), and route of administration (IM, IV). Garkowski 2012: the mean length of hospital stay is displayed since neither the median nor individual patient data were provided. Ertem 2005†: these patients required surgical care in addition to antivenom, hence the longer hospital stay. *Antivenom predominantly administered IM but a few patients administered IV, **Route of administration unspecified.

($n=13$) remained in hospital for a mean duration of eight days (median/IQR not known), despite five of these patients having their envenoming graded as minor [40].

Patients treated with Bulbio antivenom IM after *V. ammodytes* or *V. berus bosniensis* envenoming ($n=68$) had a median duration of 2 days in hospital. Although not graded in the paper, envenoming appeared mild with 67% displaying local swelling of <1% body surface area and rare mild systemic signs [48]. There is one published case of Viekvin antivenom administered IV. The responsible viper spp was unknown; the length of hospital stay was 7 days [7].

Overall, IV administration of antivenom was associated with shorter length of hospital stay (median length of hospital stay in studies of IV ViperFAV or ViperATAB ranged from 1 to 4.8 days versus 2 to 18 days for IM Bulbio or Zagreb antivenoms).

Of note, there were eight reports of patients with grade 2–3 envenoming who did not receive antivenom, who were discharged after a median duration of 1–8 days (Figure 5). The rapid discharge of some of these patients does draw into question the natural history of *Vipera* envenoming of this grade.

Introduction of purified antivenoms for the management of European *Vipera* envenoming has been reported to cause a significant reduction in complications and hospital length of stay, although this is low-level grade D evidence [6,9,12,21,31,60]. This is consistent with data reported by Reid

[4] who outlined a series of fatal outcomes before the widespread application of antivenom in the UK. In patients who received antivenom, fatal outcome was rare, occurring in just 0.2% of reported cases.

Length of hospital stay is likely to be a reasonable marker of treatment effectiveness due to the major local effects of European *Vipera* venoms and resulting morbidity if therapy is ineffective. However, variations in care across the continent complicate the picture, as does the variability both within and between species, and the poor quality of many of the studies.

The long length of hospital stay after antivenom administration for moderate-to-severe (grades 2 or 3) envenoming suggests that antivenom may be poorly effective if given late, once venom has spread into the lymphatics and caused local tissue damage, or with some particular species.

Individual patient data were not available to allow calculation of median lengths of stay for each antivenom against each *Vipera* species. However, patients treated IV with ViperFAV and ViperATAB appeared to have a reduced length of hospital stay when compared to Zagreb antivenom (Figures 4 and 5). An RCT comparing these antivenoms would allow a more accurate comparison.

Several explanations exist for the increased length of hospital stay and high incidence of complications in patients treated with Zagreb antivenom. There is geographical variation in the use of each antivenom with ViperFAV

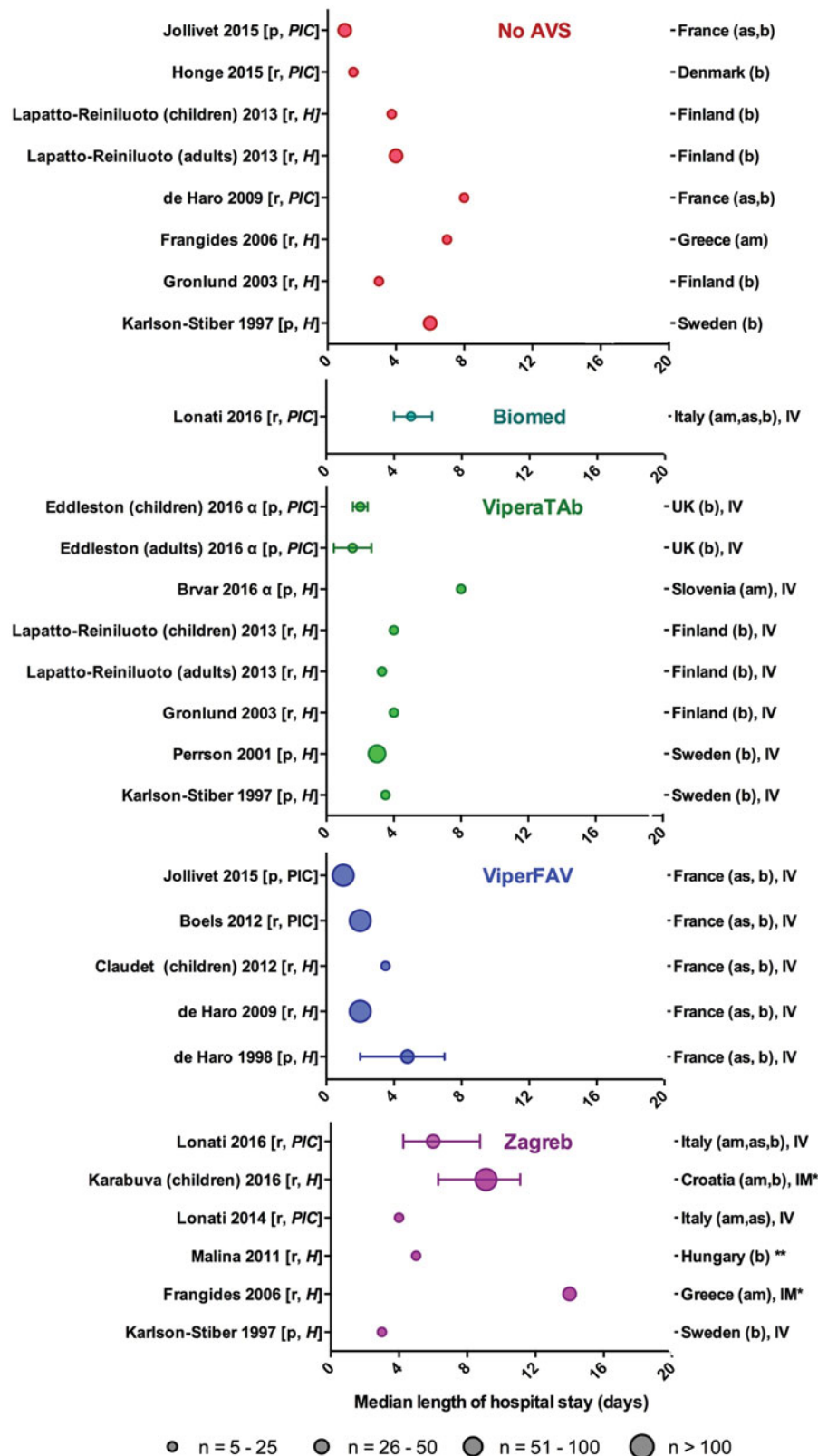


Figure 5. Median length of hospital stay (and IQR where known) for envenomed patients of poison severity score 2–3 treated with antivenom, or without antivenom, in studies with five or more patients. Left y-axis shows first author, year of publication, in square brackets retrospective [r] or prospective [p] and italics hospital [H] or Poison information centre [PIC]. Right y-axis shows location of study, predominant *Vipera* spp responsible (am, *V. ammodytes*; as, *V. aspis*; b, *V. berus*; u, *Vipera* spp unspecified), and route of administration (IM, IV). Eddleston 2016 α: unpublished series of 31 patients with *V. berus* envenoming.

predominately used in France, ViperaTab used in Scandinavia, and Zagreb antivenom used in Southern Europe with healthcare systems varying across the European continent. In the latter region, in contrast to the other regions, *V. ammodytes* is responsible for most envenoming and is considered by some to be the most venomous European snake [29,61]. Recent data suggest that ViperTab (raised against *V. berus*) may not counter the neurotoxins found in *V. ammodytes* venom [57].

The route of administration may also be important. The manufacturers of six of the antivenoms recommend IM use [Table 2 in Supplementary material], which results in reduced antivenom bioavailability, slow absorption into the blood stream, pain at injection site, and risk of haematoma (particularly in the presence of abnormal clotting) [62]. The WHO recommends that antivenoms should no longer be given by the IM route [63].

The limited clinical evidence for the Biomed, Bulbio, and Viekvin antivenoms does not demonstrate good effectiveness after IM administration [37,40,48]. A comparison with ViperaTAb, ViperFAV, and Zagreb antivenoms given IV is hindered by the lack of case series for Biomed, Bulbio, and Viekvin antivenoms containing only patients with moderate-to-severe envenoming.

Repeated use of antivenom

Repeated administration of antivenom due to poor effect or recurrence of clinical features was reported in 230/1490 cases (15.4%). Repeated doses were more common in the use of Zagreb antivenom 101/564 (17.9%) and ViperaTAb 77/488 (15.8%) than ViperFAV 52/438 (11.8%) (Table 3 in Supplementary information). One case report described a case of severe systemic *V. ammodytes* envenoming in which multiple doses of Bulbio antivenom were administered IM without apparent beneficial effect [37].

Antivenom versus no antivenom

Some studies demonstrated no difference in the length of hospital stay in patients with equivalent envenomation grading who either did or did not receive antivenom (Figure 5) [25,44,49,51]. The numbers of patients with envenoming of sufficient severity to warrant antivenom who do not get it are small. There is large spectrum of severity that exists within Audebert's classification grade 2 [6]. It is possible that a selection bias results in less severe envenoming grading (e.g., grade 2a) being managed conservatively without antivenom.

Adverse events

The incidence of adverse reactions to antivenom was clearly reported in 34 papers or abstracts covering 2408 patients; however, the antivenom administered could only be identified for 2008 (83.3%) patients. Adverse reactions were reported to complicate 37/2400 cases (1.5%) in which antivenom was administered, varying from 0.5 to 2.0% in

patients treated with ViperaTAb, Zagreb, and ViperFAV antivenoms (Supplementary information, Table 3). Adverse reactions were reported in 2/43 (4.7%) of cases involving Biomed antivenom. No adverse reactions were reported to have occurred in the 67 patients who were administered Bulbio antivenom and the single patient administered Viekvin antivenom. Of note, for cases reported by Poison Information Centres, the authors reporting the case had not seen the patient. It is unclear how carefully many of the patients were observed for the adverse reactions, suggesting likely under-reporting.

Of the 37 documented adverse reactions, 21 demonstrated immediate hypersensitivity reactions of which seven were anaphylaxis. Other patients showed signs of mild anaphylaxis with pruritus and rash (6), urticaria (4), nausea and sweating (2), angioedema (1) and hypotension alone (1). There were 13 cases of delayed hypersensitivity consistent with serum sickness. The remaining reactions consisted of a pyogenic reaction (1) or an unspecified reaction (2). There were no reported fatalities related to adverse reactions.

Overall, reported adverse reactions for the antivenoms were rare. Of the three most widely published antivenoms (Zagreb, ViperFAV, and ViperaTAb), the ViperaTAb Fab antivenom recorded the lowest incidence of adverse reactions, perhaps due to the reduced protein load and shorter half-life [19]. However, caution should be used in interpreting the antivenom safety profiles since the studies were predominantly retrospective and, in some cases, performed remotely, this reducing the likelihood of adverse reactions being recognized and ensuring undercounting.

Venom recrudescence

The shorter half-life of Fab compared to $F(ab')_2$ antivenoms is associated with greater incidence of recrudescence and need for additional doses of antivenom, as seen in the USA with CroFab antivenom [64,65]. This effect does not appear important with ViperaTAb – multiple doses were required in about 16% of patients compared to 18% and 12% of patients receiving Zagreb and ViperFAV, respectively. Case reports have described recurrent or ongoing systemic envenoming after late administration of ViperaTAb to severely envenomed children [23,26]. Repeated doses of ViperFAV administered in a French series were reported as being given for ongoing oedema rather than systemic envenoming, and did not appear to alter these local effects [6]. National guidance in France no longer recommends the use of multiple doses of ViperFAV.

Venom variation across Europe

Within each *Vipera* spp, there is variation in the venom composition across their respective geographical distributions [67,68]. Guiavarch and colleagues report that intra-species venom variation results in variable efficacy of ViperFAV antivenom for *V. aspis* neurotoxicity [53]. Further data are required on the variation in antivenom efficacy by region; in general, the data are not currently available.

Vipera latastei and *V. ammodytes*

V. latastei is the only venomous snake reported in central and southern Spain [2], reported to be responsible for around five deaths over a 12-year-period [41]. Gonzalez reported a large cohort of envenoming from Spain and deemed *V. latastei* envenoming the most severe [66]. There are no antivenoms raised against *V. latastei*; however, both Zagreb and ViperaTAB show efficacy in pre-clinical tests. Carvalho and colleagues reported severe envenoming following a *V. latastei* bite that appeared to respond well to prompt ViperFAV antivenom administration [55]. Other data suggest inconsistent management of *Vipera* spp envenoming within Spain with substantial delays in antivenom administration and inappropriate IM administration of antivenom [54].

Similarly, there are substantial data demonstrating prolonged hospital admission and high incidence of complications in patients envenomed by *V. ammodytes* [29,30,39]. There is a need for clinically relevant animal studies and for more clinical studies exploring the efficacy of ViperaTAB and ViperFAV in treating such envenoming. Both antivenoms exceed the WHO required minimum neutralizing capacity for this species of snake.

Limitations

The data reported here are predominantly retrospective in nature, suffering from the inherent weaknesses of such data. There are no RCTs that prove efficacy. Effectiveness is only suggested by reports of swelling and pain being controlled rapidly after IV administration of antivenom.

There is currently no standardized method of reporting snake bite outcome resulting in significant heterogeneity in publication quality. Many papers lacked sufficient information on the antivenom used, the envenomation grade, and the length of hospital stay, making comparisons difficult. The low incidence of snake envenoming, and of antivenom administration in Europe, has resulted in a predominance of retrospective observational publications spanning large study periods, yielding low-quality scientific evidence for the effectiveness of antivenoms.

The use of the length of hospital stay as a proxy for case severity is compromised by variation in provision and culture of healthcare across Europe, with different approaches to managing envenoming. However, it is perhaps a useful marker of treatment outcome due to predominant local effects. The lack of individual patient data from either papers or authors meant that median lengths of stay could not be calculated across different studies; instead, the reported median (or mean if former not available) was compared between antivenoms. There was a lack of published data on effectiveness for different species with the individual antivenoms, meaning it was difficult to tease out the effect of antivenom versus species on the length of stay.

Conclusions

There is a surprising lack of clear data on the effectiveness on the eight antivenoms that are used to treat *Vipera*

envenoming across Europe. The recent stop in the production of Zagreb antivenom means that only two antivenoms – ViperaTAB and ViperFAV – are recommended to be given by the IV route as advised by the WHO. The other antivenoms are usually given by the IM route and lack any evidence of effectiveness. Adverse reactions are reported rarely with these antivenoms and appear to be treatable with standard therapy. Recrudescence is not common. More pre-clinical and clinical data are required to ensure appropriate treatment of envenomed patients with high-quality effective antivenoms.

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