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First clinical experiences about the neurotoxic envenomings inflicted by lowland populations of the Balkan adder, *Vipera berus bosniensis*

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ABSTRACT

The first overall clinical description of envenomings by the lowland populations of the Balkan adder (*Vipera berus bosniensis*) is provided by this study. Fifty-four incidents have been collected retrospectively from the south-western Hungarian and the northern Croatian distribution area of the taxon. There were five (9%) asymptomatic, 24 (44%) mild, 12 (22%) moderate, 12 (22%) severe, and one fatal (2%) case according to the Poisoning Severity Score. The single death is a 60-year-old Hungarian case that was caused by *V.b. bosniensis*. Average hospitalisation was 2.75 days. The most common systemic symptoms were gastrointestinal disorders, ECG changes, persisting hypotension and neurological disorders. The initial phase of neurotoxic manifestations was always expressed in cranial nerve disturbances: ptosis, external ophthalmoplegia, diplopia, reduced focusing capability and blurred vision. Neuromuscular paralysis progressed to dyspnoea and lower limb paralysis in the most severe cases. Unusual symptoms were fluctuating arterial hypertension, drowsiness, and hypokalaemia. Laboratory results reveal leucocytosis, while deviation in the other laboratory values is not common. Envenomings by *V.b. bosniensis* significantly differ from those by the European adder (*Vipera berus berus*) in lower manifestation rate of extensive oedema, anaemia, CNS depression, and haematuria but the development of neuromuscular paralysis is high (20%). Their bites rather resulted in mild and moderate local symptoms in envenomed patients than those inflicted by the nominate form. This study presents the evidence of the frequent neurotoxic manifestations in Balkan adder-bitten patients for the first time, which strongly suggests that the venom of the lowland populations of *V.b. bosniensis* has neurotoxic activity.

1. Introduction

The Common adder (*Vipera berus*) is the most widespread terrestrial venomous snake species, occurring from Great Britain through Europe (except for the Iberian Peninsula, southern France, central and southern Italy and most part of Greece), and towards east in northern Asia up to Sakhalin. Populations in the Balkans (Croatia, Serbia, Bosnia–Herzegovina, Bulgaria, Macedonia, Montenegro, Albania, and northern Greece) are known as Balkan adders (*Vipera berus bosniensis*) (Kalyabina-Hauf et al., 2004; Nilson et al., 2005), taxon, which reaches its northernmost distribution in the lowlands of south-western Hungary (Somogy and Zala Counties) at 173–186 m above the sea level (a.s.l.) (Korsós and Krecsák, 2005). Croatian lowland populations occur at max. 400 m a.s.l. Typical specimens from these lowland populations are shown in Fig. 1, which differ in certain morphological characters and in their ecology from the mountainous populations of *V.b. bosniensis* and from the nominate form (Marián, 1956; Korsós and Krecsák, 2005).

In 1927, Reuss described several adders from the valley of the River Sava (Serbia), which he classified into a new genus of *Vipera*, *Mesocoronis*, Reuss 1927 (Krecsák, 2007). Reuss suggested that most of the adder populations in the Balkans (now *V.b. bosniensis*) possess neurotoxic venom (Krecsák, 2007). Otto (1930, 1933) investigated adder venoms from the Sava River valley (Serbia), Igman Mountains (Bosnia and Herzegovina) and southern

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Fig. 1. Specimens from lowland Balkan adder populations. *Legend*: (A) female, (B) male specimen from SW Hungary; (C) female and (D) male specimen from N Croatia. (Photographs: A by I. Sirghel; B by T. Malina; C and D by D. Jelić.)

Hungary, and concluded that the venom of these populations has neurotoxic activity, and it is more potent than that of *Vipera berus berus*, *Vipera ammodytes* and *Vipera aspis* with a similar toxicity to that of *Vipera latastei* (Schöttler, 1938). Reuss (1929a,b, 1930, 1937) characterised the envenomings by Serbian (Valley of the Sava River) *V.b. bosniensis* to result in ptosis and external ophthalmoplegia with complete ocular immobility and reduced visual acuity, dysphagia as a result of palatal weakness, speaking difficulties, the loss of balance with uncoordinated movements, and dyspnoea. The victims' systemic condition resembles to drunken sensation or alcoholic intoxication due to the evolved diplopia, which usually develops within 7 h, even if the fifth of the lethal dose of venom is injected in a previously healthy victim weighing ca. 75 kg (Reuss, 1930). Reuss (1929a,b, 1930) mentioned lower motor neuron-type facial paralysis and tongue paralysis to be a dreadful sign of severe *V.b. bosniensis* bites. Reuss (1937) stated that the specimens of genus *Mesocoronis* are the most dangerous vipers in Europe, as their bite can easily lead to systemic neurotoxicity, if only 2–4 mg (dry weight) venom is injected; thus he named these snakes "European cobras". Other authors (i.e. Schöttler, 1938; Cyrén, 1941; Schiemenz et al., 1987) also mentioned the strong neurotoxic venom of *V.b. bosniensis*. The earliest indication for the neurotoxic envenomings in Somogy County (SW Hungary), documented by Fejérváry (1934), mentioned that the eyes of victim lost their function shortly post-bite, which is very likely the drop of the eyelids. Later, bilateral ptosis was documented in a SW Hungarian case at the end of 1970s (Malina et al., 2008).

However, neurotoxicity following Balkan adder bites has been known since the early 1930s, there are no detailed case descriptions on humans envenomed by *V.b. bosniensis*, nor any clinical experiences and/or epidemiological figures. Therefore, our aim was to investigate the pertinence of the statements from the literature, and to examine whether these bites rather cause systemic neurotoxicity than cardiovascular disorders and circulatory instability. We have tried to define the general symptomatic profile of *V.b. bosniensis* envenomings from SW Hungary, N Croatia, and study the risks, the severity, and the prognosis of these bites. Additionally, we have compared the clinical picture of these bites with those caused by *V.b. berus* in C and N Europe.

2. Materials and methods

Incidents were collected by surveying the paper based case reports and/or electric patient databases of hospitals. Only those cases were processed, which were certainly caused by adders and not a harmless, non-venomous species i.e. *Dolichophis*, *Zamenis*, and *Coronella* spp. The latter cases were identified based on the fang marks and/or eye-witness accounts, or voucher specimens brought by the victims on admission. Venom detection by enzyme-linked immunosorbent assay (ELISA) is not applied in Hungary and Croatia, thus the detection of viper venom in the patients' blood could not be possible. Since *V.b. bosniensis* is the single venomous snake species in the surveyed areas misidentification could not occur.

The cases of all but one (Hospital of Nagykanizsa) SW Hungarian hospitals were surveyed where possible adder bitten patients could be admitted and treated (i.e. Kaposvár, Nagyatád, Marcali and Zalaegerszeg). The cases reviewed from the N parts of Croatia derive from the University Hospital of Infectious Diseases "Dr Fran Mihaljević", Zagreb (three victims being pre-admitted in the hospital at Varaždin, Dubrava, and Pakrac); and the General Hospital "Dr Josip Benčević", Slavonski Brod. The following data were collected for each case: the geographical location and the date of accident, the anatomical location of the bite, the age and sex of the patient, local and systemic symptoms, laboratory findings, medical treatment and time to recovery. The Poisoning Severity Score (Karlson-Stiber et al., 2006) was applied to clinically grade the bites, although we have modified the 'moderate' category and amended it with the manifestation of neurological signs and symptoms. The envenomings were divided into five groups: (i) none (dry bites), (ii) mild, (iii) moderate, (iv) severe, and (v) fatal outcomes. We have analyzed the envenomings from the two countries separately as the political border corresponds with a

natural border (i.e. the River Drava) that creates a gap in the distribution of the taxon in the surveyed area. The signs and symptoms observed in *V.b. bosniensis* bites were compared with those that follow *V.b. berus* in N Europe as reported by Karlson-Stiber et al. (1997, 2006), whereas the neurotoxic symptoms with those reported by Reuss (1929a,b, 1930, 1937).

The Mann–Whitney *U* test, Fisher's exact test and exact Chi-squared test were applied in the analysis of the data using the SPSS 17.0 for Windows (SPSS Inc.).

3. Results

3.1. Demographic and epidemiological features of the incidents

Altogether 54 *V.b. bosniensis* envenomings have been collected retrospectively: 21 that occurred between 1979 and 2009 and one fatality between 1945 and 1955 in SW Hungary (Zala and Somogy Counties), while 32 incidents between 1998 and 2009 from Croatia (Zagreb County, Zagreb City County, Krapinsko-zagorska County, Varaždin County, Brod-Posavina County, and Bjelovar-Bilogora County), and one case from 2000 that occurred south of the Sava River in N Bosnia near the Croatian border (Fig. 2). The mean annual incidence in the two Hungarian counties was 0.70 or 0.11/100,000 inhabitants, whereas in the six Croatian counties 2.46 or 0.14/100,000 inhabitants. The earliest bite befell on 11 March, the latest on 29 September. Envenomings accumulated in June and July ($x = 26$, 49%) and in April and May ($x = 16$, 30%). The seasonal patterns of envenomings are very similar in the reviewed areas, the two groups being not significantly different (Mann–Whitney *U* test; $z = -0.135$, $p = 0.89$). All admitted victims were inpatient: 40 (74%) were male and 14 (26%) female. Eight boys and two girls (aged under 17) were among the victims. The youngest victim was a 2-year-old (boy), the oldest 73 (male). All but one patient were bitten for the first time; the single victim had a previous dry bite (asymptomatic). The average hospitalisation time was 2.75 days (min. 24 h, max. 11 days). Twenty-nine bites affected the lower extremity (ankle, dorsum of foot), 24 the upper extremity (finger, hand, rarely the forearm), and one on the waist. All but two patients (bitten twice, consecutively) suffered a single bite.

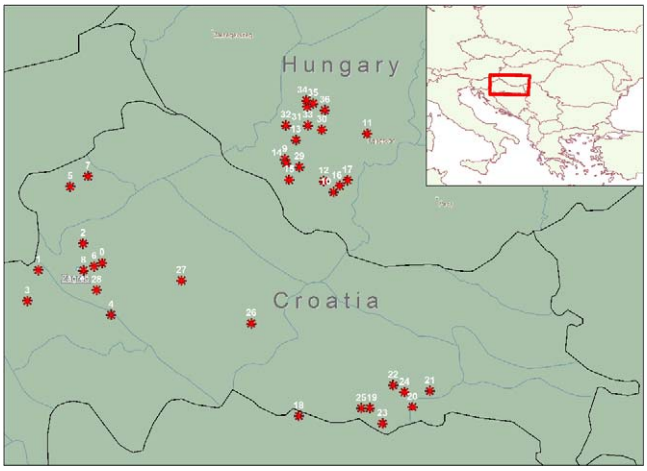

Fig. 2. Localities of incidents caused by *Vipera berus bosniensis* in SW Hungary and N Croatia. 0, Sesvete; 1, Samobor; 2, Kašina; 3, Jastrebarsko; 4, Peščenica, Zagreb; 5, Radoboj; 6, Vukomerec; 7, Lepoglava; 8, Zagreb; 9, Bolhás; 10, Homokszentgyörgy; 11, Kaposvár; 12, Rinyabesenyő; 13, Segesd; 14, Szenta; 15, Tarany; 16, Tiltványpuszta; 17, Visnye; 18, Bosanska Posavina; 19, Oriovac; 20, Slavonski Brod; 21, Bukovlje; 22, Čelikoviči; 23, Bebrina; 24, Glogovica; 25, Lužani; 26, Blagorodovac; 27, Čazma; 28, Velika Gorica (Turopolje); 29, Nagyatád; 30, Nagybajom; 31, Mesztegnyő; 32, Vése; 33, Homokpuszta; 34, Kelevíz; 35, Hosszúvíz; 36, Felsőkakpuszta.

3.2. General aspects of the envenomings

Five victims (9%) were completely asymptomatic (i.e. neither local nor systemic signs or symptoms appeared, only fang marks were visible) and 49 were symptomatic. Fifteen cases (28%) resulted in local reactions only, and eight (15%) in local symptoms with mild and transient systemic symptoms that resolved spontaneously (mild severity). The maximum severity grade was: none ("dry-bites") 5 (9%), mild 24 (44%), moderate 12 (22%), severe 12 (22%), and fatal one (2%). However, we did not document fatalities during the surveyed period (1979–2009), neither in Hungary, nor in Croatia, one fatality that occurred in SW Hungary between 1945 and 1955, was also considered in the reviewed series. The cases from the two countries did not differ significantly in respect of severity, is shown in Fig. 3A (Mann–Whitney *U* test; $z = -0.881$, $p = 0.743$).

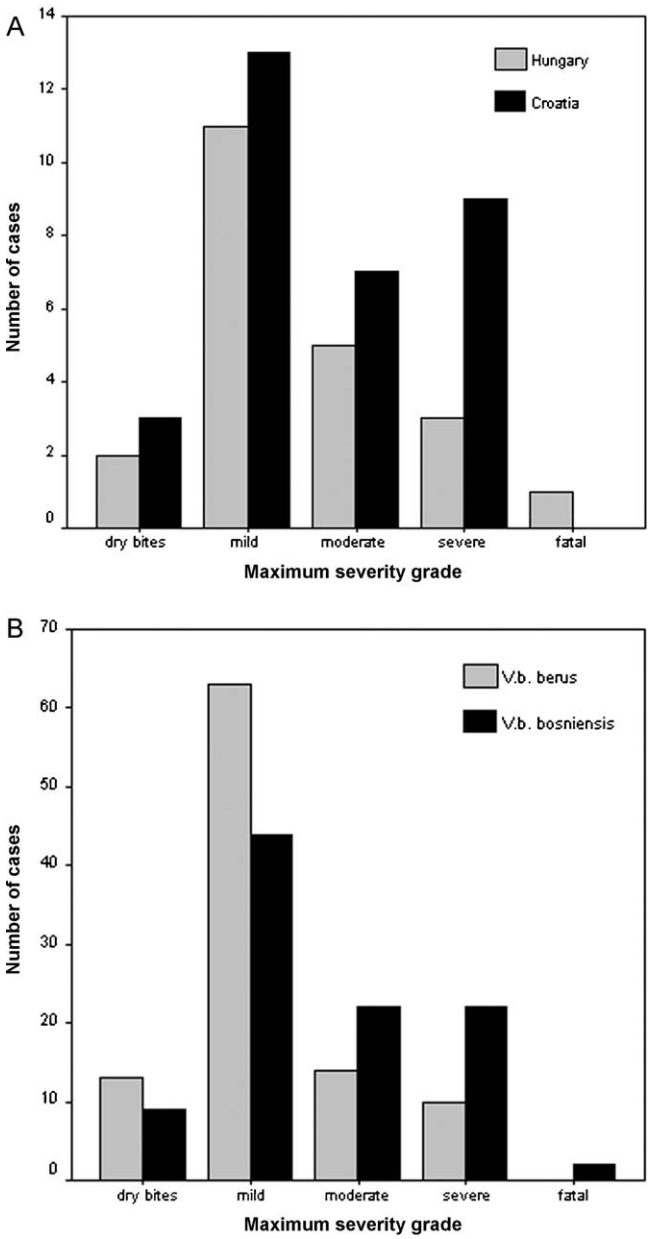

Fig. 3. Maximum severity grade of envenomings. (A) Maximum severity grade of *V.b. bosniensis* envenomings in Hungary and Croatia. (B) Maximum severity grade of envenomings by *V.b. bosniensis* and *V.b. berus* (Karlson-Stiber et al., 2006).

Table 1
Manifestations of the various symptoms on the bitten patients with different stadiums of envenomings were inflicted by *V.b. bosniensis* in SW Hungary and N Croatia.

Clinical aspects of envenomings	Maximum grade of severity[a]			
	Mild (%) $n=24$	Moderate (%) $n=12$	Severe (%) $n=12$	Fatal (%) $n=1$[d]
Local symptoms				
Local swelling only	24 (100)	3 (25)		
Moderate oedema		9 (75)	10 (83.3)	
Extensive oedaema			2 (16.7)	
Erythema	10 (41.6)	1 (8.3)	1 (8.3)	
Petechiae	1 (4.2)			
Local haematoma	1 (4.2)	6 (50)	3 (25)	
Moderate haemorrhage	2 (8.3)		2 (16.7)	
Extensive haemorrhage			1 (8.3)	
Cellulitis, phlegmon			1 (8.3)	
Bulla			1 (8.3)	
Local necrosis			1 (8.3)	
Systemic symptoms				
Gastrointestinal symptoms	3 (12.5)	9 (75)	12 (100)	1 (100)
Cardiovascular symptoms				
Hypotension	1 (4.2)	8 (66.7)	8 (66.7)	1 (100)
Hypertension	0	2 (16.7)		
Dizziness	1 (4.2)	7 (58.3)	10 (83.3)	1 (100)
ECG changes	1 (4.2)	9 (75)	9 (75)	1 (100)
CNS depression				
Faint		1 (8.3)	3 (25)	1 (100)
Shock			1 (8.3)	1 (100)
Neurological symptoms				
Cranial nerve disturbances[b]		5 (41.7)	6 (50)	
Dyspnoea			2 (16.7)	
Limb paralysis			1 (8.3)	
Other acute symptoms				
Drowsiness		2 (16.7)		
Autonomic disorders[c]	1 (4.2)	1 (8.3)		
Numbness of the bitten extremity	1 (4.2)			
Bleeding from gums			1 (8.3)	
Generally muscle weakness		2 (16.7)	3 (25)	
Breathing difficulties	3 (12.5)	1 (8.3)	2 (16.7)	1 (100)
Paraesthesia	1 (4.2)	1 (8.3)	2 (16.7)	
Dysthesia		1 (8.3)		
Tinnitus		1 (8.3)		
Vertigo		2 (16.7)	2 (16.7)	
Laboratory findings				
Leucocytosis ($>11\times10^9$/L)	3 (12.5)	7 (58.3)	9 (75)	1 (100)
Thrombocytopenia (below 150×10^9/L)			7 (58.3)	
Anaemia (Hb $>10\%$)		1 (8.3)	2 (16.7)	
Haematuria			1 (8.3)	
Prolonged PT		1 (8.3)	4 (33.3)	
Raised fibrin degradation		1 (8.3)	1 (8.3)	
Hypokalaemia ($K^+>3$ mmol/L)		1 (8.3)	3 (25)	
Increased creatine kinase		1 (8.3)	1 (8.3)	
Increased aspartate aminotransferase (AST)		1 (8.3)	2 (16.7)	
Increased alanine aminotransferase (ALT)			1 (8.3)	

n = number of patients
[a] 5 asymptomatic cases are not listed.
[b] Paralysis of the cranial nerves resulted in ptosis, ophthalmoplegia, diplopia, and reduced visual acuity.
[c] Bladder tension and urine incontinence.
[d] Only partial data are available.

Table 1 shows the local consequences of the bites. The most common and prominent systemic symptoms were: gastrointestinal disorders – nausea, vomiting, abdominal pain and/or cramp, and diarrhea – ($x=25$, 46%), ECG changes ($x=20$, 37%) and persisting hypotension ($x=18$, 33%) (Tables 1 and 2). In some ($x=3$, 5%) mild cases gastrointestinal symptoms (mainly nausea) and slight dizziness spontaneously resolved, while transient hypotension normalised without any medical treatment in one case. Continuous ECG monitoring showed mainly sinus tachycardia ($x=18$, 33%), but paroxysmal supraventricular tachycardia with 200/min frequency was also detected. Sinus tachycardia was detected with normal blood pressure twice. In one of the most severe cases, the patient vomited several times previously, then haematemesis appeared with two episodes. CNS depression (faint and/or shock) was recorded in seven (13%) victims (Table 1). All of them were strongly hypotensive and tachycardic, suffered from intense dizziness and general weakness before they lost their consciousness. Breathing difficulties, from mild suffocation feeling through superficial respiration to severe respiratory distress developed occasionally ($x=7$, 13%). High blood pressure could be documented twice on adult, previously healthy males: one of them was a 41-year-old (bitten near Segesd, SW Hungary) with hypertension peaking at 200/120 mmHg 32 h post-bite, while the other was a 42 year-old victim (bitten near Lepoglava, Croatia), who was slightly hypertensive (140/80 mmHg). Drowsiness as an early systemic symptom evolved also in two patients only (one Hungarian, one Croatian) and in one case it was accompanied with an unambiguous neurological sign (i.e. palpebral ptosis). Other acute symptoms and relevant laboratory findings are also summarized in Table 1. Nor angioneurotic oedema, nor bronhospasm was recorded in this series.

Table 2
Symptoms and sings observed on patients bitten by *V.b. berus* in N Europe, and *V.b. bosniensis* lowland populations in Hungary and Croatia. Comparison made with exact Chi-squared test, two-sided, with 1 degrees of freedom.

Symptoms and laboratory findings	*V.b. berus* envenomings $n = 204$[a] and 30[b] (%)	*V.b. bosniensis* envenomings $n = 54$ (%)	χ^2	p
Local swelling only	40.2[a]	50	1.682	0.195
Moderate oedema (extended to the half extremity)	25[a]	35.2	2.240	0.134
Extensive oedema				
Involving the whole extremity	27.9[a]	3.7	14.220	[d]<0.001***
Involving the trunk as well	4.9[a]	0	1.595	[d]0.207
Gastrointestinal disorders	83.3[b]	46.2	10.980	0.001**
Hypotension	83.3[b]	33.3	18.702	<0.001***
Hypertension	0[b]	3.7	0.102	[d]0.748
CNS depression	53.3[b]	13	15.807	<0.001***
Neurological disorders	0[b]	20.4	7.032	[d]0.008**
ECG changes	16.7[b]	37	3.828	0.05*
Breathing difficulties[c]	20[b]	13	0.730	0.393
Angioedema	23.3[b]	0	10.861	[d]0.001**
Anaemia	23.3[b]	5.5	4.240	[d]<0.039*
Haematuria	36.7[b]	1.9	16.353	[d]<0.001***
Leucocytosis	60[b]	37	4.105	0.043*

[a] Karlson-Stiber et al. (2006).
[b] Karlson-Stiber et al. (1997).
[c] Involving mild suffocation feeling through superficial respiration to severe respiratory distress.
[d] Yates continuity correction applied.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

3.3. Neurotoxic signs and symptoms

Systemic neurotoxicity developed in 20% of the cases ($x = 11$) (Table 2). The development rate of the different neurological symptoms and signs varies between the two countries, being 23% in Hungary and 18% in Croatia, although the difference was not significant (Fisher's exact test, two-sided $df = 1$, $p = 0.324$) in the reviewed areas. The most common ($n = 10$) neurotoxic symptoms were cranial nerve disturbances (i.e. ptosis, ophthalmoplegia, diplopia, reduced visual acuity). Of these neurological disturbances, the paresis of the third cranial nerve (i.e. ptosis) was the most common; six patients (11%) showed this sign. Ptosis developed with relatively high frequency (18%) in the Hungarian cases, while this typical paralytic sign was less frequent (only 6%) among the Croatian victims but the symptoms of neurotoxicity were more various at the latter. The shortest time to the development of the clinically detectable ptosis was 1.5–2 h after the accident, while the latest was 6 h (mean 3 h). In a Croatian patient, ptosis lasted for 5 days, while in a Hungarian, it was still present on the second day post-bite. External ophthalmoplegia was confirmed medically in four occasions. Vision troubles included diplopia, reduced focusing capability, and blurred vision. A patient with diplopia complained of pain in the eyeballs on the next two days following the bite. Two patients suffered from dyspnoea: a 34-year-old previously healthy Croatian male was transported in very critical condition to the hospital, he was unable to move because of lower limb paralysis, prior experienced balance disorder and dyspneic episodes; while the other had breathlessness, then later dyspnoea was medically confirmed. Bilateral ptosis with ophthalmoplegia and accommodation trouble has already developed during the transportation to hospital in this person. All patients completely recovered from the neuromuscular paralysis.

3.4. Fatal envenoming by V.b. bosniensis

Fatalities inflicted by *V.b. bosniensis* are almost inaccessible or unrecorded. A single briefly documented fatality is known from SW Hungary from the past 60 years, when a soldier died during military service near Böhönye (central part of Somogy County) between 1945 and 1955 (exact date is unknown), after being bitten on his waist when he sat in the grass (Marián, 1956; Dolecskó, 1964). He was transported in an unconscious and highly critical condition to hospital, where death supervened shortly post-admission due to respiratory and circulatory failure, and there was no time for antivenom therapy (Mihályi, pers. comm.). However, autopsy was performed (Mihályi, pers. comm.), the report could not be accessed as that particular military hospital was liquidated since, and all reports are all lost.

3.5. Prehospital and hospital treatment

First-aid was often self-applied or applied by the person accompanying the victims (Table 3). Majority of the patients treated in hospitals were transported to hospital within 3 h after the incident. Of the 54 patients 27 (50%) received antivenom. All moderate and severe cases ($n = 24$) required medical treatment and hospitalisation, antivenom therapy being applied in 21 cases. Skin test was performed on one victim because of her relevant medical history (involved allergy) and antivenom therapy was contraindicated. In 22 cases, the polyvalent equine derived European Viper Venom Antiserum® (Institute of Immunology, Zagreb, Croatia) was used (18 × 1 ampoule, 4 × 2 ampules). Four patients received the trivalent Viperfav® antivenom (Sanofi-Aventis, Lyon, France), two of them double dose. In 1979, the Pasteur ER antivenin (unknown dose) was given to a Hungarian victim. In mild cases the patients received supportive treatment only. A combination of plasma expanders and crystalloids was used in the severe envenomings (Table 3).

3.6. Comparison of the severity of envenomings by V.b. bosniensis and V.b. berus

Envenomings by the Balkan adder significantly differ (Mann–Whitney U test; $z = -3.049$, $p = 0.02$) in respect of severity from *V.b. berus* envenomings (Fig. 3B). Envenomings with mild course are more common in case of bitten patients by *V.b. berus*, while *V.b. bosniensis* causes more moderate and severe envenomings. A comparison of the symptoms in *V.b. bosniensis* envenomings with those inflicted by *V.b. berus*, based on the literature data (Karlson-Stiber et al., 1997, 2006), is provided in Table 2.

Table 3
Prehospital and hospital treatment of the envenomed patients by the Balkan adder in SW Hungary and N Croatia.

	No. of the cases in three different grades of the envenomings[a]		
	Mild (%) $n=24$	Moderate (%) $n=12$	Severe (%) $n=12$
Pre-hospital treatment/first-aid			
Pressure dressing/tourniquet	1 (4.1)	2 (16.6)	2 (16.7)
Cold pack on the bitten area	1 (4.1)		
Excision		3 (25)	1 (8.3)
Bleeding out of the bite site		3 (25)	1 (8.3)
Wound suction	1 (4.1)		
Hospital treatment			
Antivenom therapy			
European Viper Venom Antiserum	3 (12.5)	8 (66.6)	11 (91.7)
Viperfav	4 (16.6)	1 (8.3)	
Pasteur ER antivenom[b]		1 (8.3)	
Supportive treatment			
Analgesic	3 (12.5)	10 (83.3)	12 (100)
Antemetic	1 (4.1)	7 (58.3)	8 (66.7)
Antihistamine and/or calcium administration	8 (33.3)	6 (50)	6 (50)
Adrenaline administration			5 (41.7)
Anti-tetanus injection	13 (54.1)	9 (75)	8 (66.7)
Blood transfusion			3 (25)
Heparin administration		1 (8.3)	
Corticoid therapy	2 (8.3)	4 (33.3)	5 (41.7)
Antibiotic therapy	9 (37.5)	8 (66.6)	10 (83.3)
Combination of plasma expanders and crystalloids			4 (33.3)
Surgical intervention	1[c] (4.1)	3[c] (25)	1[d] (8.3)

[a] 5 asymptomatic cases and one fatal case are not listed.
[b] It was administrated in a single case in SW Hungary in 1979.
[c] Exposure of local suppuration.
[d] Removing of the necrotic tissue (4 cm × 4 cm).

4. Discussion

Balkan adder bites are considered to be rare, compared to *V.b. berus* envenomings in N and NW Europe (i.e. 231/year in Sweden (Karlson-Stiber et al., 2006), or in the UK, 100/year (Warrell, 2005)). In Hungary, at present the population density of *V.b. bosniensis* is low (i.e. ca. 4 specimens/1 km^2 – calculation based on the data by Újvári et al. (2001)), which can be the main reason for the infrequent human-adder encounters, though incidents were also rare in the 1940–1950s: 4 cases/12 years in one hospital of Somogy County (Marián, 1956). In Croatia, where the adder population density is higher (ca. 6–8 specimens/1 km^2 around Zagreb; Jelić et al., 2009), these incidents are more common. Our incidence rates are underestimated, as several victims (mainly in Hungary) do not present to the doctor when only mild local symptoms develop (Varga, pers. comm.). Additionally, our review of the six Croatian and the two Hungarian counties did not include all hospitals in the examined area because of the lack of approval to review their cases.

The "classical" systemic envenoming by *V.b. berus* mainly results in gastrointestinal symptoms and circulatory disturbances (Warrell, 2005; Karlson-Stiber et al., 1997, 2006). Neurotoxicity is highly uncommon (reported only twice) and the neurotoxic activity of *V.b. berus* venom was not proved until now (Malina et al., 2008). Envenomings by *V.b. bosniensis* are particularly interesting clinically, as the course of envenoming has been almost unknown up to now, since the published reports (i.e. Bubalo et al., 2004; Lukšic et al., 2006) recorded the envenomings by *V.b. bosniensis* together with those inflicted by *V. ammodytes*. Mirković (1901) mentioned three viper-bites from Fruška Gora (Serbia) without reporting the culprit species; although, only *V.b. bosniensis* occurs there. Our results confirm that the development of neurotoxicity following the bites of these lowland *V.b. bosniensis* populations is significant. Additionally due to the connected antihaemostatic effects, in the moderate and severe cases antivenom therapy is strongly indicated. The high possibility of manifestation of ECG changes following these bites further increases the risk for severe envenomings. Reuss (1930) has already noted that different arrhythmias or heart block can develop in the most severe cases. Certain unusual and rare symptoms such as fluctuating arterial hypertension may also occur in *V.b. bosniensis* envenomings and also in case of *V.b. berus* (Krue and Hansen, 1999; Malina et al., 2008; Hoegberg et al., 2009), but hypokalaemia was also detected in envenoming by the nominate form (Hoegberg et al., 2009). Interestingly, hypertension is an occasionally observable systemic symptom on the victims of *V. latastei* (Gonzales, 1991). Deviation from normal in laboratory values was not as expressed and common in our patients as reported in envenomed humans by *V.b. berus* (Karlson-Stiber et al., 2006; Hoegberg et al., 2009) (Table 2).

These envenomings rather result in mild or moderate local symptoms, than those caused by *V.b. berus*, corresponding to Reuss' statements (1937). Therefore, only some patients required corticosteroid therapy due to the relatively mild local consequences of envenomings, conversely to *V.b. berus* envenomings (Karlson-Stiber et al., 2006). Neurological symptoms usually follow the moderate and severe cases only in our series. Nevertheless, in a case that was graded as mild (Treskavica, Bosnia–Herzegovina, 1979; Peranić, pers. comm.) because of only local swelling appeared without any general systemic symptom but neurotoxicity (diplopia with spontaneous resolution within 1 h) developed. The onset of neurotoxic manifestation is usually rapid and always expresses as vision troubles, and often with ptosis. Regardless of the onset time, paralytic symptoms worsen rapidly if the adequate treatment is delaying and/or a higher dose of venom in injected, as showed by certain cases i.e. patients with neuromuscular weakness, dyspnoetic episodes, and lower limb paralysis. The latter characteristic symptom was also noted by Reuss (1930, 1937) but it has never documented in *V.b. berus*. Bladder tension and urine incontinence are rare, however the inability to control this excretory function could be connected to neurotoxicity indirectly, through autonomic actions that result in autonomic disorders such as involuntary urination. Persson (1995) stated that involuntary urination and urine incontinence might be connected to neurological disorders in case of viper envenomings in Europe. True vertigo was also observed in our patients that can be associated with viper-bite neurotoxicity as well as drowsiness, mentioned by de Haro et al. (2009), although, its pathophysiological cause is unclear. Drowsiness sometimes occurs in *V.b. berus* envenomings too, mainly when children are affected (Cederholm and Lennmarken, 1987; Reading et al., 1995). According to certain authors (i.e. Persson, 1995; Ferquel et al., 2007; de Haro et al., 2009), the real cause of this symptom is connected to systemic neurotoxicity, while others (i.e. Warrell, 1996) suppose a venom-induced endorphin release in its background. In our opinion, drowsiness most probably cannot be connected to neurotoxicity in Balkan adder bites. Only one patient suffered from this symptom who showed unambiguous neurotoxic sign (i.e. bilateral ptosis). The antivenom therapy was effective in all cases, however, in patients with severe clinical picture, neurotoxic symptoms resolved slowly (ptosis lasted 2 and 5 days) despite the repeated doses of antivenom. Their slow response to antivenom can be explained by the prejunctionally acting phospholipase A_2(s) (PLA_2) content of venom, which destroys the nerve endings and delays the nerve regeneration. Therefore, the prompt antivenom therapy after the onset of the first sign of paralysis can be more effective to prevent the progression of neurotoxicity. The prejunctionally acting PLA_2s are more distributed in the other species of European *Vipera* (i.e. certain subspecies of *V. aspis* and *V. ammodytes*), than

the postjunctionally acting ones (Ferquel et al., 2007). Wide range of different antibiotics and anti-tetanus administration were routinely used in both countries, although it is not indicated in *V. berus* bites (Warrell, 2005).

Today, fatality by *V. berus* is rare throughout Europe (Warrell, 2005). We deem this to be the result of the relatively low venom yield (14 mg in dry weight; Bubalo et al., 2004) and the advanced hospital treatment with antivenom therapy, but not the venom's toxicity. Fatalities by *V.b. bosniensis* are similarly rare in the surveyed areas. We believe it can be associated with the lower venom yield of *V.b. bosniensis* (1.7–10.3 mg in adults, mean 5 mg in dry weight, SW Hungarian population; $n = 10$ (Malina et al., unpublished data)), which significantly decreases the risk of a fatal bite. Despite their lower venom yield, envenoming by *V.b. bosniensis* can result in more severe consequences than that of the N European *V.b. berus* that was reported by Karlson-Stiber et al. (2006). This supports that early presumption according to which, venom of *V.b. bosninenis* is more potent than that of *V.b. berus* (Otto, 1933) and very probably because of its neurotoxic activity. The lower occurrence rate of dry-bites can be explained by the more aggressive temperament of these adders.

5. Conclusion

Our results corroborate with the old literature reports indicating that the bites of the lowland Balkan adders rather result in mild and moderate local consequences with relatively high rate of neurotoxic manifestations. Neuromuscular paralysis firstly affects the cranial nerves, mainly the III, IV and VI, and can progress to lower limb paralysis, dyspnoea then respiratory failure, which require emergency medical attention and healthcare. The development of these neurological deficiencies clearly reflects the well-known signs of the acute descending paralysis characterising the neurotoxic viper-envenomings. Haematuria, haematemesis, anaemia and thrombocytopenia are significantly less frequent than following *V.b. berus* bites. The clinical picture of humans envenomed by these adder populations differs significantly in several aspects from the C and N European *V.b. berus* envenomings. In addition, the neurotoxic symptoms that develop in *V.b. bosniensis* envenomings vary in their intensity and development-rate inter populations, probably as a result of the different expression levels of neurotoxins in their venom, which is a known phenomenon in other Palaearctic *Vipera*, such as *V. aspis* (Ferquel et al., 2007).

Conflict of interest

The authors declare that there are no conflicts of interest.

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