

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Analysis of the venomous snakebite patients treated in the Užice General Hospital (Western Serbia) between 2006 and 2018

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Introduction/Objective A countrywide survey of venomous snakebites was never conducted in Serbia: the making of a central register was initiated only in 2018. We collected and analyzed the available data regarding venomous snakebites in the Užice region (Western Serbia). The previous analysis from this hospital was published in 1968.

Methods We retrospectively analyzed the data regarding the venomous snakebite patients treated at the Užice General Hospital between 2006 and 2018 and compared these with the data from the 1960s, from one more hospital in Serbia, and from two ex-Yugoslav countries.

Results In 13 years, 249 persons were treated. Of all cases, 10.4% were with inconspicuous symptoms (mild pain at the place of bite), 68.7% were with mild to moderate symptoms, and 20.9% were more or less severe. No fatalities were recorded.

Conclusion Although usually not a life-threatening issue, venomous snakebites are quite common and can cause serious complications. With proper education, many can be avoided. Also, bearing in mind not only the biodiversity *per se* but also the importance of snakes' venoms for the making of various medically important products, we emphasize the need for proper protection of all three venomous snake species in Serbia, namely *Vipera ammodytes* (nose-horned viper), *V. berus* (European adder), and *V. ursinii* (meadow viper).

Keywords: envenomation by *Vipera ammodytes* and *Vipera berus*; interdisciplinary cooperation and education; paucity of information; protected species conservation

INTRODUCTION

As in other countries of former Yugoslavia (except Slovenia), only three autochthonous venomous snake species exist in Serbia, *Vipera ammodytes* (nose-horned viper), *V. berus* (adder), and *V. ursinii* (meadow viper), and all are protected by law [1]. To the best of our knowledge, in the surroundings of the city of Užice (Western Serbia, 43.859° N, 19.849° E, 10×10 km Universal Transverse Mercator, UTM square DP05), only the nose-horned viper can be found (1 provides occurrence maps and lists of localities). The nearest localities (UTM squares in brackets) in South-western and Western Serbia where the presence of adder was confirmed [1] or where suitable habitats exist (boreal forest or/and alpine pastures), are the mountains Zlatibor (CP74, along with *V. ammodytes*), Zlatar, Golija (DP20), Javor (DP21, with *V. ammodytes*), Kamena Gora (CN89, with *V. ammodytes*), and Jadovnik (DN09, with *V. ammodytes*) [1]. The third viper species was found only in remote places in the southwestern margin of the country, at altitudes over 1600 m [1].

Venoms of *Vipera* species are combinations of proteins, polypeptides and enzymes

with specific chemical and biological activities, primarily used to subdue prey. Oxidases, proteases, esterases, hemolysins, neurotoxins, cardiotoxins, myotoxins and factors that modify the coagulation system act on muscles, cardiovascular and neural systems, blood cells, leading to various damages of organs and organ systems [2–8]. Clinical manifestations of envenomation range from negligible to fatal; however, the latter are very rare and often occur in particular cases of bites, for instance, to the neck or directly to blood vessels, in very young patients or as a complication of some chronic disease in the elderly [6–12]. In high contrast to the popular belief, European vipers “are not considered fatal” because the lethal doses for average humans are higher than the amounts of venom the vipers can produce [12]. According to the national Statistical Office, between 2008 and 2017 only four people in Serbia died after being bitten by venomous snakes.

Although Serbia is nowhere near any of the seriously affected areas [13], bites by venomous snakes do occur in our country – and their occurrences are not being analyzed. We found only two papers from Serbia with multi-year data regarding venomous snakebites: 155 cases

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from Užice (1960–1968) and 264 from Priština (1981–1997) [2, 8]. Three other papers we obtained present nine cases in total [14–16]. Also, a report exists of an anaphylactic reaction following the bite of a non-venomous snake (*Zamenis longissimus*): although “examines excluded toxic effects of snakebite,” in the description the author mentions “two punctiform wounds from bites,” which raises suspicion [17]. Contrary to some of the most severely affected regions of the world [e.g. 13], in Serbia, importantly [18], the antivenom is produced in a national institute [19, 20], it is readily available and adequately used.

The work on the epidemiology of Viperidae snakebites in Central and Southeastern Europe started recently. However, at the time of this manuscript preparation, the data regarding Serbia was not available [7, 21]. Therefore, our intention was not only to present the information from a single hospital but also to inspire medical workers in other parts of Serbia to collect, analyze and publicize the data they have an insight into. With a better overview of the outcomes of the snake–human encounters, we can better design the necessary education of laypeople regarding snakes. Also, medical workers could expand their knowledge of the distribution of vipers in Serbia and novel approaches in the treatment of venomous snakebites so they could standardize and improve the procedures not only of therapy but also of data gathering, severity score grading, etc. [6, 7].

METHODS

We inspected medical records of the patients treated in the Department for Infectious and Tropical Diseases of the General Hospital in Užice (UGH) between 2006 and 2018. No personal information was used except for the sex and ages of the patients, therefore, the approval of the ethics committee was not necessary.

The UGH is responsible for a population of approximately 300,000 persons. During the entire period covered in this overview, the hospital and its local ambulances were well supplied with antivenom and medications needed for symptomatic therapy. All physicians are well informed and prepared to properly react in the case of a venomous snakebite.

Snakebites were diagnosed according to anamnesis data, through clinical monitoring, and according to the information provided by the patients regarding the snakes that inflicted the bites [22]. We considered three main types of data:

Epidemiological data: sex, age, month in a year, and activity of patients at the time of the bite, the area/locality where the bite occurred, and the reported snake species. Activities of bitten persons were categorized as follows: people who were performing their usual everyday activities related to agriculture (in crop fields or gardens); local persons who were bitten during walks or picnicking in suburban/rural areas (including the picking of berries, fishing, etc.); and tourists from other parts of Serbia, i.e., foreigners.

Clinical data: localization of the bites (part of the body), the severity of the clinical picture, and the administered

therapy. The grading of the clinical courses of the disease was made according to the Severity score of snakebites [23], with grades from 0 (no envenomation; fang marks and minimal pain) to 4 (very severe envenomation).

Laboratory analyses: We considered blood parameters, coagulation status, urea and creatinine, sodium, potassium, alanine aminotransferase, and aspartate aminotransferase; urine was also analyzed. We performed the standard laboratory tests used in Serbia.

Where appropriate, statistical analyses were performed, in Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA, USA) or using an online calculator (www.quirks.com/tools/calculator). All graphs were made in Excel.

RESULTS

Epidemiology, treatment, and outcomes

The data about 249 snake envenomation cases were collected during the 13 year period, 19.15 on average per year. Of those, 30 patients (12%) were treated in primary health care, and 219 (88%) were hospitalized. Antivenom was given to 234 (94%) bitten persons. In all cases, we used the Viekvin® equine antiserum produced by the Torlak Institute of Virology, Vaccines and Sera (5 mL vials). According to the producer, 1 mL of the preparation can neutralize at least 100 LD₅₀ of *V. ammodytes* venom and 50 LD₅₀ of *V. berus* venom [20]. The serum was administered subcutaneously or intramuscularly to all the patients. In 115 (46.2%) cases, symptomatic therapy was administered (antiedematose and analgesic medications). Antibiotics were given to 132 (53%), and corticosteroids to 54 (21.2%) patients. Anti-tetanus protection was given to all patients who had not previously been vaccinated (204: 81.9%). In five cases (2%), local necroses developed, which were surgically treated. The 21 (8.4%) patients with the most severe symptoms were treated at the Intensive Care Unit for 24–48 hours, and were later transferred to the ward for further observation.

In Table 1, we provided the numbers and percentages of the main manifestations of snake venom poisonings. Almost a quarter of patients experienced nausea, vomiting, and diarrhoea. Thrombocytopenia ($< 150 \times 10^9/L$) was the second most frequent manifestation of envenomation. Acute renal failure was accompanied by elevated urea and creatinine, hypokalemia, and proteinuria. Liver damage manifested as

Table 1. Symptoms and signs in organ systems, and abnormalities in laboratory analyses, in the patients treated for snakebites in the Užice General Hospital

Symptoms	Numbers (percentages)
Gastrointestinal disorders	58 (23.3%)
Thrombocytopenia	43 (17.3%)
Acute renal failure	12 (4.8%)
Coagulation disorder	10 (4%)
Shock	9 (3.6%)
Liver damage	7 (2.8%)
Visible bleeding	7 (2.8%)
Neurotoxic symptoms	4 (1.6%)

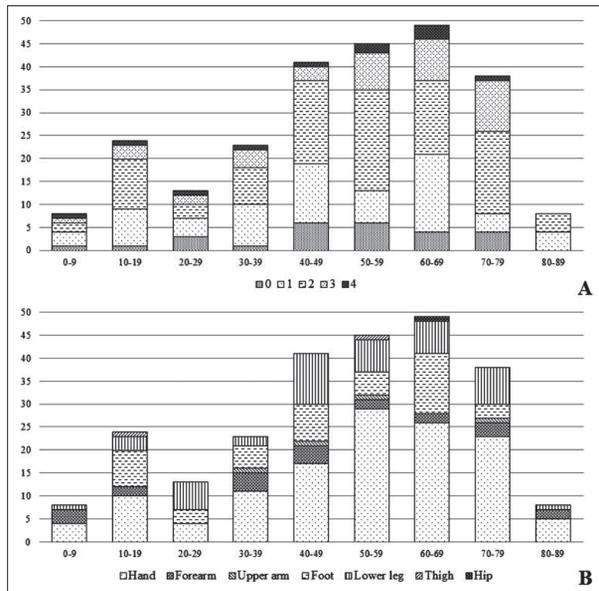


Figure 1. (A) Numbers of bites and 0–4 severity grades in 10-year age groups; (B) Bite sites (bitten body parts) in different age categories

elevated levels of liver enzymes alanine aminotransferase and aspartate aminotransferase. Visible bleeding expressed as petechiae and hematomas, rarely as hematuria; in only one patient bleeding from the digestive tract was recorded (hematemesis and melena). Neurotoxic symptoms expressed as transient limb pareses at the bites sites.

More male (59.04%) than female (40.96%) patients were bitten (one-sample t-test between percents): $t_{248} = 2.901$, $p = 0.004$.

The average time elapsed between the bite and antivenom administration was 53.15 minutes (range 15–300 minutes). Milder clinical pictures, e.g. grade 1, developed after shorter times to antivenom administration (37.61 minutes on average) compared to grade 4, which developed in patients treated on average 99.54 minutes after the bite. The Pearson's coefficient R was 0.5365, with a high statistical significance of correlation ($p < 0.000$) between severity grades and minutes to antivenom.

On average, the patients were hospitalized for 2.5 days (range 0–9). All patients with the symptoms grade 4 were held for six or more days.

Calculated against the 300,000 population, the annual incidence of venomous snakebites per 100,000 persons ranged from 4.3 to 8.7, with an average of 6.4.

Severity grades and ages of the patients

The percentages of 0–4 severity grades were 10.4, 27.7, 41.0, 16.5, and 4.4. Not a single death was recorded as a consequence of a venomous snakebite. The youngest patient was a baby boy less than a year old (grade 4), and the oldest a man of 88 (grade 2). The correlation between ages and severity grades was not significant (Pearson's $R = 0.0628$, $p = 0.324$). Both the bites inflicted certainly by the nose-horned vipers and those possibly made by adders caused the symptoms of all grades.

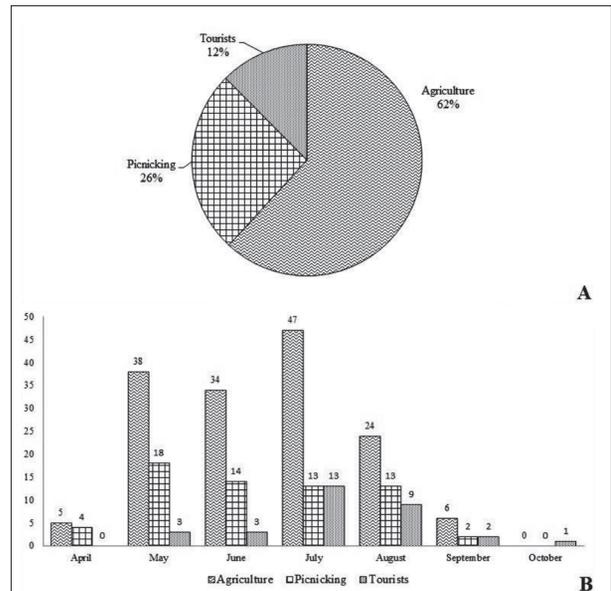


Figure 2. (A) Percentages of patients according to their activity/occupation; (B) Seasonal variations in numbers of bites according to the patients' occupation/activity

In Figure 1A, we graphed the numbers of bitten individuals in 10-year age groups, with their symptoms severity grades. The highest number of patients were the people in their 60s. Among those younger than 40, the most bites were recorded in the 10–19 years age group. In all age categories, more bites were inflicted on the upper extremities (Figure 1B). Significantly more people had bites on hands and arms (62.2% of the whole sample) compared to feet and legs (37.80%): $t_{248} = 3.97$, $p < 0.001$ (one-sample t-test between percents).

Percentages of bites related to activity/occupation

Almost 62% of our patients were people engaged in some agricultural activity; a quarter were local people picnicking, and the lowest number of snakebites was recorded in tourists (Figure 2A).

In the "agriculture" group, significantly more people were bitten in the upper compared to lower extremities ($t_{153} = 7.041$, $p < 0.001$). Of those who spent time outdoors picnicking in a suburban/rural environment (25.7%) similar numbers of patients were bitten in upper and lower extremities ($t_{63} = 0.256$, $p = 0.803$), and among tourists, presumably hikers (12.4%), the majority were bitten in lower extremities ($t_{30} = 2.572$, $p = 0.015$). One-sample t-tests between percents were used.

The numbers of bites among seasons varied differently in the three occupations/activity categories (Figure 2B). Agricultural activities and hiking were the riskiest in July while picnicking appeared hazardous in May.

Annual and seasonal distribution of venomous snakebites

In the analyzed 13-year-long period, up to two-fold oscillations in the annual numbers of bites occurred, in intervals

of approximately four-five years (Figure 3A). The first bites were recorded in April, and their numbers were the highest in July; October was the last month to have records of snakebites (Figure 3B).

Snake identification

In 38.96% of cases, the snakes were not identified by the bitten persons. Of those who were sure that some venomous snake bit them, 44.58% claimed it was an adder. An exceptionally low number of patients reported nose-horned vipers (16.47%). Of the four patients with neurological symptoms, only one claimed that an adder bit him; the rest reported *V. ammodytes*.

DISCUSSION

In the past several years, venomous snakebites and their treatment, as well as the respective education/training of both the general population and medical workers, are gaining global attention, and actions have been announced/undertaken to reduce their impact [6, 7, 18, 24]. Although mortality due to European *Vipera* spp. bites is generally low, the fear and disgust regarding snakes is widespread and deeply rooted [7–12, 25].

We compared our data with those previously published from the UGH and with four other publications depicting high numbers of venomous snakebites: 264 from Serbia (hospital in Priština), 542 and 93 from Croatia, and 341 from and Bosnia and Herzegovina, during 17, 21, 11, and 24 years, respectively [2, 8, 9, 10, 26].

All aspects of envenomations treated in the UGH correspond to those from the comparable studies, from the duration of hospitalization through severity grades and average incidences per 100,000 inhabitants, to the most severely affected groups of people and the antivenom application frequencies [2, 8, 9, 10, 26]. A recent overview for Europe showed similar trends [7]. Annually, we treated more patients on average (19.15) than reported in the 1960s (17.22) and in Priština (15.53) [2, 8]. In contrast to the 1960s (and Priština), in our study more people were bitten to the hands: $t_{402} = 4.359$, $p < 0.001$, while previously significantly more bites to the feet were recorded: $t_{402} = 5.733$, $p < 0.001$ (t-tests between percents). Previously “children and pupils” dominated [2], while nowadays the most bites were inflicted on people over 40 (Figure 1A). In Priština, 0–20-year-old patients were the dominant age group [8]. Such differences were already observed, and they depend on the prevalent activities in the target groups [8, 9, 10]. Contrary to two neighboring countries [9, 10], where the gender ratios among the bitten persons were equal, in Serbia, more males suffered the bites – in our study almost every year (Figure 3A) – similarly to the data obtained for Europe [7, 8]. This can be related either to their occupation or to the lack of fear.

Low incidence of local necroses (2%) can be attributed to the fact that all emergency stations in the entire Užice region, including local ambulances in villages, possess and

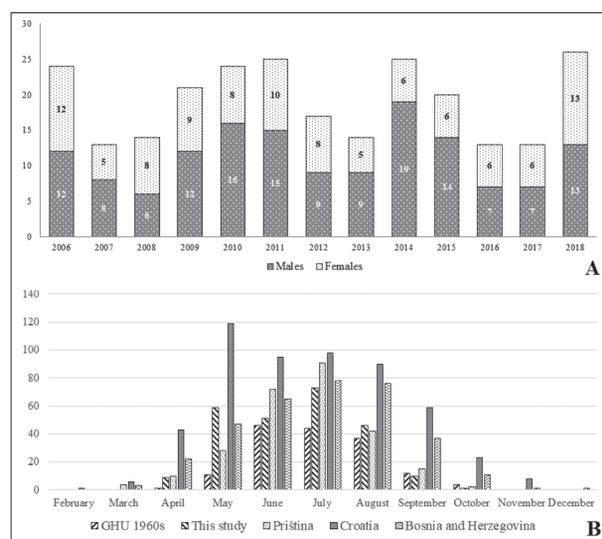


Figure 3. (A) Annual numbers of bites in the present study: male and female patients; (B) Seasonal distribution of bites reported in this study compared to previously published data from the same hospital, and the data from Priština, Croatia, and Bosnia and Herzegovina



Figure 4. (A) and (B): Typically colored female and male *Vipera ammodytes*; (C) and (D): typically colored female and male *V. berus*; (E): atypically colored female *V. ammodytes*, and (F) unusually colored male *V. berus* (Photos: Aleksandar Simović)

correctly utilize the anti-viperine serum. For certain snake species, or in certain cases of *Vipera* sp. bites, intravenous antivenom application was recommended [6]. To all our patients the serum was administered subcutaneously or intramuscularly: that was probably the reason no adverse reactions developed. Antibiotics therapy was given to one-half of our patients (the cases of complicated infections). This is in accordance with the existing recommendations and usual practice in the adjacent countries [6, 8, 9, 10].

According to the physicians’ notes, all analyzed bites were legitimate (hazardous), often occupational, contrary to some previous reports [15, 16] and our suspicions. Illegitimate bites (by the snakes which are observed and provoked or irritated intentionally), although highly probable in natural surroundings, often are inflicted by captive,

sometimes exotic species and can result in more severe envenomation compared to bites on laymen by autochthonous snakes [27, 28]. Judging solely by the frequencies of bitten persons and locations of the bites in various environments/activities, we suppose that those engaged in some agricultural work either did not see the snakes or tried to remove them by hand hence significantly more bites to the upper extremities. For the local people on a picnic, no conclusion could be made regarding their behaviour in contact with snakes. We presume that most of the tourists did not see the snakes and provoked them unintentionally.

Figure 3B presents seasonal variations in the numbers of bites in our study and four other analyses [2, 8, 9, 10]. In the 1960s in Užice, the most bites were recorded in June, but in our study as well as the one made in Priština, this occurred in July. All studies showed variations in months and years with the highest numbers of bites: this can probably be attributed to differences in climate, but also in human activities. This is also consistent with the findings for Europe [7].

As noted already in 1968, the prevailing weather conditions influence the activity of snakes i.e., the numbers of bites to people. People reported that in rainy years the snakes were more abundant [2]. Comparatively humid conditions favour the growth of vegetation, which can lead to increased numbers of small mammals, the snakes' main prey.

We sought some regularity regarding the severity of symptoms due to snakebite and the time of the year when the bite was inflicted. In May, the most bites of grades 3 and 4 happened. However, for sound conclusions, we need more information (besides the snake identity), including the data regarding the snakes' ecology and behavior [29]. There were findings that during winter, *V. ammodytes* venom contains less of the lethal components [30] and that in spring and summer viper venom is more potent [31]. It was speculated [8] that during the snakes' winter inactivity, the venom accumulates in their venomous glands hence its amount at the first bite is large, which results in severe clinical pictures in persons bitten in spring. To the best of our knowledge, there is no proof for such an assumption.

Many of our patients had no or only mild symptoms. This can, *inter alia*, be due to bites of non-venomous snakes (in almost 40% of cases the snakes were not identified) or to 'dry' bites by venomous species.

In other analyses, only a few fatal cases were reported and very small numbers of serum sickness and anaphylactic reactions developed [8, 9, 10]; we had none. In all three countries, *Vipera ammodytes* inflicted the most bites. Like in our case, in the cited studies often the snakes could not be identified, and most probably many cases remained unreported. The latter is a global problem though [7, 32].

In our study, many people reported being bitten by adders (111 of 249, 44.58%), which could result from the common misidentification stemming from great variability within and similarities between adders and nose-horned vipers (Figure 4). Another source of confusion may be the differences in local names of snake species. In as many as 97 cases (38.96%), the snake was not identified, and in the remaining 41 (16.47%) people reported nose-horned

vipers. According to expert opinion (given before the publication of exact localities where the adders were recorded), bites by adders were possible in only 40 (16.1%) cases. However, when we compared the reported localities of bites by "adders" with the published [1] UTM squares where adders were recorded (eight in total, in four together with *V. ammodytes*), no overlap was found. Nevertheless, we cannot exclude the possibility that adders are present in certain places that are still formally unknown.

In all localities noted in medical records available for our study, nose-horned vipers were previously recorded, and in some places (Zlatibor, Zlatar, Uvac – Nova Varoš) they are abundant in the places frequented by tourists, mountaineers, recreationists, or farmers. Encounters with adders are possible in some parts of the Zlatar mountain, and in the surroundings of Sjenica, where the terrain and climate suit them. Even in places where they are present, adders are comparatively scarce and spend most of the daytime hiding in low vegetation. The periods when they can be seen are early morning and/or late afternoon. They can be more easily encountered at the end of summer (second half of August) when people collect forest fruits. Also, people working in places 1000 meters above the sea level can come across adders. These snakes often hide in piles of cut trees hence the workers can get bitten while manipulating trunks. Also, adders can be transported with logs/timber to the places they naturally do not occur in. For these reasons, it is important to precisely record the localities where the bites occur.

Importantly, in four places in South-western and Western Serbia, *Vipera ammodytes* and *V. berus* live in sympatry. Nevertheless, as the effects of their venoms differ [3], it is possible to deduce which species inflict bites. However, it would be best if the perpetrator animals could be identified and left alive [24].

CONCLUSION

In certain parts of the world, venomous snakebites present a severe threat to people. Nevertheless, in Serbia (like elsewhere in the Balkans and Europe), venomous snakes are neither as numerous nor as dangerous as for instance, in Asia or Africa. We are deeply convinced that people in Serbia can be properly educated regarding the three venomous snake species (out of the total ten) and that many snakebites can be prevented or avoided.

Variations in seasonal/annual numbers of bites – and the changes thereof – highlight the fact that more investigation at the ecology of venomous snakes has to be undertaken and that information should be exchanged between medical professionals and professional biologists to create adequate education, advice, and preventive measures. Also, the vipers' distribution data could be filled in more detail. A series of lectures should be organized to inform the physicians on the necessity for a more thorough approach to this issue so both the collection of data and medical treatment could be standardized and improved. In this way, both people and snakes would be protected.

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Conflicts of interests: None declared.

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Анализа болесника лечених од уједа змија отровница у Општој болници Ужице (Западна Србија), у периоду од 2006. до 2018. године

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САЖЕТАК

Увод/Циљ До сада није сачињен преглед уједа отровних змија у целој Србији: са прављењем централног регистра започето је тек 2018. године. Прикупили смо и обрадили доступне податке везане за уједе отровних змија на подручју Ужица (Западна Србија). Претходна анализа из ове болнице објављена је 1968. године.

Методe Ретроспективно смо анализирали податке о пацијентима леченим у Општој болници Ужице након уједа отровних змија, у периоду 2006–2018. године, и упоредили их са подацима из 1960-их, са студијом из још једне болнице у Србији и са подацима из две земље бивше Југославије.

Резултати Током 13 година збринато је 249 особа. Од свих случајева, 10,4% је било са неупадљивим симптомима (благ бол на месту уједа), 68,7% је прошло са благим до умере-

ним симптомима, а мање или више озбиљних је било 20,9%. Смртних случајева није било.

Закључак Иако углавном нису животно угрожавајући, уједе отровних змија су релативно чести и могу довести до озбиљних компликација. Уз одговарајућу едукацију многи се могу избећи. Такође, имајући у виду не само биолошку разноврсност као такву него и значај отрова змија за производњу различитих медицински значајних препарата, наглашавамо потребу за одговарајућом заштитом све три врсте отровница у Србији – поскока (*Vipera ammodytes*), шарке (*V. berus*) и шаргана (*V. ursinii*).

Кључне речи: међудисциплинарна сарадња и образовање; недостатак информација; очување заштићених врста; уједи *Vipera ammodytes* и *Vipera berus*.