

## Hemorrhagic stomatitis in a natural hybrid of *Vipera ammodytes* × *Vipera berus* due to inappropriate substrate in terrarium

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**ABSTRACT.** A natural hybrid of *Vipera ammodytes* × *Vipera berus* was presented having low body weight, seizures and generalized swelling of the cephalic region. Based on the history of the case and clinical examination, hemorrhagic stomatitis of traumatic origin was diagnosed. The snake was kept in a terrarium with wood chips as a substrate, and the material had induced trauma in the oral mucosa which was further complicated with *Salmonella* Arizonae and *Morganella morganii* co-infection, abscessation and osteomyelitis. To the best of the authors' knowledge, this is the first reported case of bacterial infection in European snake hybrids and one of a few case reports in European snakes. Although wood chips are an inexpensive substrate, based on our findings, they should be avoided when keeping and breeding European vipers.

**KEY WORDS:** inappropriate substrate, *Morganella morganii*, natural *Vipera* hybrid, *Salmonella* Arizonae, traumatic stomatitis

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Hobby herpetology became very popular in the last few decades; however, snakes are also kept and bred in captivity for venom production, for research and educational purposes or as part of their conservation programs. In all these situations, key factors determining the success or the failure of keeping and breeding snakes are proper hygienic and species-specific husbandry conditions [12, 15]. Several categorizations and prioritizations appeared on the husbandry conditions; however, all of the previous reviews agree that the substrate used in the terrarium is one of the most important factors [12, 15]. The role of the substrate, either natural or artificial, is to not only achieve an adequate physical (e.g., humidity) and physiological security for the reptiles, but also to provide an aesthetic appearance for the terrarium [12].

Several pathological findings were connected to inappropriate substrate usage in reptiles, including snakes [15]. Substrates that are too moist can accumulate different pathogens and invasive forms of parasites (oocysts, eggs, or larvae), while those containing aromatic compounds or that contribute to a dry or moist environment can lead to dermatological and respiratory problems [12, 15]. Several types of substrates can be ingested causing intestinal obstruction, while more “rough” substrates can traumatize the

oral mucosa or the skin, leading to local infections, such as stomatitis or dermatitis [4, 15]. If not treated in time, these local infections can result in more generalized processes, such as septicemia [5, 7].

Herein, we report a case of hemorrhagic stomatitis in a natural hybrid of a nose-horned viper (*Vipera ammodytes*) × European adder (*V. berus*) due to an inappropriate substrate used in the terrarium. Although there is an increasing knowledge about the bacterial flora of the native European venomous snakes [6, 8], we have only limited information available on the bacterial infections of these species, especially in their natural hybrids.

A natural hybrid of *V. ammodytes* × *V. berus* was referred to the Department of Infectious Disease of the Faculty of Veterinary Medicine Cluj-Napoca, Romania. The animal was caught during the summer of 2005 in the Metalliferous Mountains, Transylvania, Romania. This is probably the only known region in Romania, where the two parent species co-occur and based on morphology [14] both *ammodytes*-like and *berus*-like hybrid specimens have been identified (Ghira I., *personal communication*). The present snake was identified as a *berus*-like hybrid and was kept at the Vivarium of the University Babeş-Bolyai for research purposes. The snake was housed in a terrarium that was 100 × 100 × 50 cm (length × width × height) in size with large of 0.5–1 cm diameter, pine wood chips as the substrate (Fig. 1A).

After a couple weeks in captivity, a piece of a wood chip got nipped in one of the fangs of the snake, which was subsequently removed and the wound was disinfected. The referring veterinarian reported that shortly after the treatment, the animal became anorexic and lost weight, and a week later presented seizures and generalized swelling affecting most

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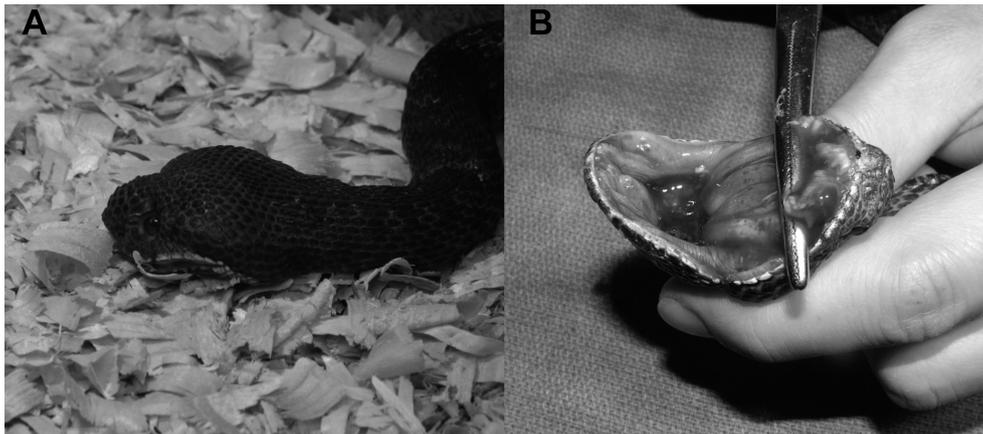


Fig. 1. Clinical aspects of the natural *Vipera ammodytes* × *V. berus* hybrid. A) The animal was kept in a terrarium with wood chips as a substrate and presented large swelling of the cephalic region and seizures. B) Hemorrhagic stomatitis of the oral mucosa resulting in abscessation and osteomyelitis due to *S. Arizonae* and *M. morgani*.

of the cephalic region (Fig. 1A). The animal was anesthetized using an acepromazine (0.02 mg/kg BW, i.m.) and ketamine (5 mg/kg BW, i.m.) combination. At the clinical examination, besides the symptoms mentioned above, the snake presented hemorrhagic stomatitis (Fig. 1B) and osteomyelitis affecting several bones of the skull. In order to release the pressure and to collect microbiological samples, the softest point of the cranial abscess was opened and cleaned as much as possible. The fang regions were also opened and cleaned, and a small piece of a wood chip was removed.

Taking into account the animal's condition, the severity and advancement of the inflammation affecting the bones and the central nervous system, the snake was euthanized with ketamine (20 mg/kg BW i.m.), and its dead body was stored frozen until examination [9].

In order to establish the potential microbial agents involved, the swabs collected were streaked onto Columbia agar supplemented with 5% sheep blood. After 24 hr of incubation at 37°C, two kinds of mid-sized colonies appeared, one of which showed β-hemolysis. Both of these isolates stained Gram negative and were oxidase negative. The isolates were further characterized with API 20E biochemical test strips (bioMérieux, Marcy l'Étoile, France) in accordance with the manufacturer's directions. The sensitivity of the isolates to various antimicrobials commonly used in exotic practices was evaluated using a disk diffusion susceptibility test.

After morphological and biochemical analyses, the isolates were identified as *Salmonella Arizonae* and *Morganella morgani*. Both species showed resistance to most of the antibiotics tested, but were sensitive to enrofloxacin.

Based on the history, clinical examination, surgical treatment and microbiological findings, the snake was diagnosed with traumatic stomatitis caused by the inappropriate substrate used in the terrarium, which later was complicated by the secondary infection with the two opportunistic pathogens. With the progression of the infection, the inflammation affected both the soft and hard tissues of the regions,

including the central nervous system.

The European fauna contains ten venomous Viperidae snakes with several subspecies [10], many of them having important conservation values, e.g., the Hungarian meadow viper (*Vipera ursinii rakosiensis*) and the Milos viper (*Macrovipera schweizeri*). In the recent years, several studies have examined the *Salmonella*-carrier state or the bacterial flora (cloaca, oral or venom) of different European vipers [6, 8]; however, there is virtually no information on the diseases affecting these snakes. Most of the information available on this topic has come from the different conservation programs, both husbandry problems (e.g., low humidity) [2] and parasites/pathogens [13] playing an important role. The present case adds to this knowledge; we diagnosed a traumatic stomatitis in a *Vipera* hybrid caused by the wood chips used as a substrate in its terrarium, and this was further complicated by secondary bacterial infection with *S. Arizonae* and *M. morgani*.

Stomatitis is one of the most common health problems in captive snakes [4]. Traumatic injuries caused by different objects in the cage, bites from prey and poor husbandry (improper nutrition or environmental temperature) are the predisposing and primary causes of infection [4]. The initial lesions are colonized by the opportunistic bacterial species, causing local infection [15]. Many bacterial species have been isolated from oral lesions of snakes, especially Gram-negative bacteria, including both *Salmonella* spp. and *M. morgani* [4], which are part of the resident digestive flora of the European vipers [8]. Facilitated by captivity-associated stress and/or immunosuppression, the initial damage of the mucosa may progress to osteomyelitis or abscessation [4], which might have been also the case in our hybrid snake. Due to the advanced status of the infection, we could not treat the animal, although antibiotic treatment based on the susceptibility test proved to be a good option in the initial phases of the stomatitis in captive *Vipera* species (*V. ammodytes* and *V. berus*; Köbölkuti *et al.*, *in preparation*). In

addition to these, European adders (*V. berus*) are thought to be more sensitive to captivity and associated stressors compared to other native venomous snakes, resulting frequently in clinical illnesses and fatalities (Kelemen A., *personal observation*), which might have further facilitated the outcome of this case.

To our knowledge, this report is not only one of a few case reports in European autochthonous snakes but is also the first bacterial case on a European viper hybrid. Although the information available on the snake hybrids, especially on their immune responses, is scarce, based on knowledge available from other vertebrate hybrids (e.g. fishes, amphibians or birds), we hypothesized that hybrids would be more resistant against pathogens and parasites compared to their parent species due to the heterogeneity in immunity. For example, it has been shown that the hybrid *Pelophylax esculentus* has a more effective and more diverse antimicrobial peptide defence against the chytrid fungus *Batrachochytrium dendrobatidis* than its parent species, *P. lessonae* and *P. ridibundus* [1]. Moreover, F1 hybrids of *Xenopus laevis* × *X. muelleri* are resistant to the specific parasites of the parent taxons [3]. Despite these advantages, under certain conditions (e.g., captivity, inappropriate husbandry) the effect of common pathogens and parasites can be significant even in hybrids, as it is demonstrated by the case reported here and by a previously documented case of infection with *Caryospora simplex* in the same hybrid [11]. Although wood chips are a commonly used substrate for many reptile species, in the case of European vipers, large pine wood chips should be avoided as a substrate, especially for longer time periods, as they might cause injuries and could facilitate the invasion with opportunistic bacterial species.

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