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Case Report

EFFECTS OF MALNUTRITION AND IMPROPER CAPTIVE MAINTENANCE ON EUROPEAN POND TURTLE (*EMYS ORBICULARIS*): A CASE REPORT

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ABSTRACT

The European pond turtle (*Emys orbicularis*), the most widely distributed European aquatic turtle species, although is protected in most countries under national and international laws, it is also kept in captivity as pet or as part of different conservation programs. Despite these circumstances, there is little to no information on the diseases affecting this species, especially on how certain captivity conditions contribute to these pathologies. Here we describe the effects of improper husbandry conditions on a European pond turtle (*E. orbicularis*) male. The animal was kept exclusively on solid ground and fed with vegetables as would be a terrestrial species, which led to chronic malnutrition, dehydration and stress, and subsequently to the overgrowth of the beak and nails, lameness and secondary stomatitis associated with *Candida* spp. and *Aeromonas hydrophila*. The corrections of husbandry conditions combined with an adequate antimicrobial therapy resulted in full recovery and subsequent release of the turtle. Besides reporting a new case in a European reptile species, we would like to emphasize the role of veterinarians in notifying the owners about correct husbandry conditions for different reptile species and about the conservation and legal aspects of keeping protected indigenous species.

Key words: *Aeromonas hydrophila*, beak overgrowth, *Candida* spp., captivity, *Emys orbicularis*, infectious stomatitis, improper husbandry, lameness.

INTRODUCTION

The European pond turtle (*Emys orbicularis*) is the most widely distributed autochthonous aquatic turtle species of the European fauna. Despite its Eurasian and North African distribution, the population numbers are declining, the species being listed as Near Threatened by the IUCN (2014) and protected by law in most European countries. Habitat destruction and pollution are considered as the main causes of the population decline, however in the recent years invasive species also exerted a significant negative effect (Fritz and Chiari, 2013). Invasive mammals such as raccoons and raccoon dogs are predating the turtles, while the released exotic turtle species (mainly red-eared slider turtle (*Trachemys scripta elegans*) and painted turtle (*Chrysemys picta*)) can directly outcompete the native species or could introduce alien pathogens and parasites with lethal consequences for the naïve host populations (Soccini and Ferri, 2004; Hidalgo-Vila *et al*., 2009; Fritz and Chiari, 2013). Under these circumstances, several *in situ* (e.g. habitat protection) and *ex situ* (e.g. captive breeding programs) measures have been taken in many European countries in order to protect the species (see special issue of Herpetology Notes entitled "*A summary of conservation actions for European pond turtles*"; Fritz and Chiari, 2013).

Since both pollution and diseases (infectious, parasitic and non-infectious) are considered as threatening factors for the European pond turtle, veterinarian involvement in the different field- and conservation programs is warranted and mandatory. Information is available on haematology (reviewed in Arikan and Çiçek, 2014), parasites (Mihalca *et al*., 2007; Hidalgo-Vila *et al*., 2009) and bacterial flora (Soccini and Ferri, 2004; Hacioglu *et al*., 2012; Aleksić-Kovačević *et al*., 2014; Nowakiewicz *et al.,* 2015) of the species, references which are crucial in evaluating the health status of the free-living and captive populations and individuals. The relevance for public health of the species was also studied, European pond turtles being carriers for *Salmonella* (Soccini and Ferri, 2004; Hidalgo-Vila *et al.*, 2007; Marin *et al.*, 2013; Aleksić-Kovačević *et al.*, 2014; Nowakiewicz *et al.*, 2015), *Yersinia* (Nowakiewicz *et al.*, 2015), *Leptospira* (Lindtner-Knific *et al.,* 2013) and *Vibrio* species (Hacioglu *et al.*, 2012; Nowakiewicz *et al.*, 2015). However, due to the relatively low prevalence of these zoonotic pathogens in the European pond turtle and its potentially low contact rate with humans, this species represents a minor public health threat compared to other, especially exotic, reptiles (Nowakiewicz *et al.*, 2015).

Despite all these studies, we have no information on diseases affecting this species in captivity or in the wild, situation which is comparable with other European autochthonous reptiles (Köbölkuti *et al.*, 2013a, 2013b; Czirják *et al*., 2015). The only comprehensive study about the diseases and the health status of three free-living European pond turtle populations was done in Serbia, showing that both infectious and non-infectious diseases have an important role and indeed, pollution might be a factor that has a key role in these alterations (Aleksić-Kovačević *et al.*, 2014). Many of the inorganic- (e.g. Hg) and organic (e.g. hexachlorobenzene) contaminants have genotoxic effects (Swartz *et al*., 2003; Matson *et al*., 2005), while cyanobacterial microcystins cause mortalities both in European- and Spanish (*Mauremys leprosa*) pond turtles (Nasri *et al*., 2008). As for the infections of the captive individuals, to our knowledge there are only few reports relating superficial and systemic mycoses (Schildger *et al*., 1991; Mutschmann and Schneeweiss, 2007) and bacterioses (Keymer, 1978b; Martínez-Silvestre and Soler Massana, 2008). In order to increase the medical care and also to significantly improve the conservation of this charismatic species, further studies and case reports are required.

CASE REPORT

A male European pond turtle (*E. orbicularis*) was referred to the Department of Infectious Diseases of the Faculty of Veterinary Medicine Cluj-Napoca, Romania. The owner received the animal few days before the examination and came to learn more about the species, the husbandry conditions and the health status of the turtle. He was puzzled by the information received from the previous owner that the turtle was not eating for several weeks despite the *ad libitum* access to vegetables. Besides the vegetables, the animal also had access to drinking water. It was not possible to find out more information about the origin or the clinical history of the animal, about the captive maintenance, except that the turtle was kept indoors without having access to any water body.

During the clinical examination, besides anorexia, the animal showed overgrowth of the rhinotheca. Thus, the animal could not open its mouth and the nasal cavity was also partly obliterated (Fig. 1). Moreover, the animal was dehydrated, presented lameness, excessive and deforming overgrowth of the nails, especially on the front limbs (Fig. 2). When the owner was informed about the condition of the individual and about the conservation status of the species, he donated the animal to the clinic in order to provide the best care possible, aiming to finally releasing the turtle into the wild.

After anaesthesia with acepromazine (0.2 mg/kg b.w.), the nails and the beak were trimmed and it was possible to open the mouth of the animal in order to examine the oral cavity. The turtle presented stomatitis and white, caseous material was found on the tongue, which was mechanically removed later. Microbiological samples were collected to establish the microbial agents involved and to identify the optimal antimicrobial treatment (Ebani *et al*., 2008).

The swabs were streaked for isolation onto Columbia agar supplemented with 5% sheep blood, MacConkey agar and Sabouraud dextrose agar. After 24 hours of incubation at 37°C, β-haemolytic, lactose negative colonies were isolated on the bacteriological media, which stained Gram negative, were oxidase positive and were identified as *Aeromonas hydrophila* using Analytical Profile Index (API) 20NE biochemical test strips (bioMérieux, France). The sensitivity of the isolate to various antimicrobials commonly used in exotic practices was evaluated using Kirby-Bauer disk diffusion susceptibility test (Ebani *et al*., 2008). The isolate was sensitive to enrofloxacin, showed intermediate sensitivity against cefotaxime and gentamycin and was resistant to ampicillin, amoxicillin, tetracycline, trimethoprim and penicillin.

After 48 hours of incubation at room temperature white, shiny colonies appeared on the Sabouraud dextrose agar, which showed oval, budding yeast-like morphology under the microscope with methylene blue staining. Based on these aspects we identified the microorganism as *Candida* spp..

Based on the laboratory results and textbook recommendations, the following treatment was initiated: 7.5 mg/kg b.w. enrofloxacin intramuscularly every second day for two weeks, 75000 IU/kg b.w. nystatin orally twice in ten days time and 10 ml duphalyte and 2.5 ml glucose 5%, both administered as intraosseous infusion every fifth day for 3 weeks. During the medical treatment, the animal was kept in a large aquaterrarium with access both to water body and land area. The water temperature was ~25°C and a 40W light provided the heat over the land area.

Despite the initial poor prognosis, the animal responded well to the new husbandry (e.g. access to water) and to the treatment, starting to eat after three weeks of medication. The condition of the limbs and the nails were not perfectly improved after the first three weeks, thus we decided to keep the animal in captivity for a longer period in the Vivarium of the Babeş-Bolyai University, Cluj-Napoca, Romania. Initially, the turtle was housed in an individual aquarium, while later kept together with other exotic and native aquatic turtle species (other European pond turtles and red-eared sliders). The animal was fed with bovine and chicken meat supplemented with vitamins and minerals, but occasionally also received frogs and fishes. After three months in our custody, the turtle was released in good body condition in the Danube Delta Biosphere Reserve.

Fig. 1: Overgrown beak in European pond turtle (*Emys orbicularis*) – due to this condition the animal could not open his mouth, was anorexic and the respiration was also affected.

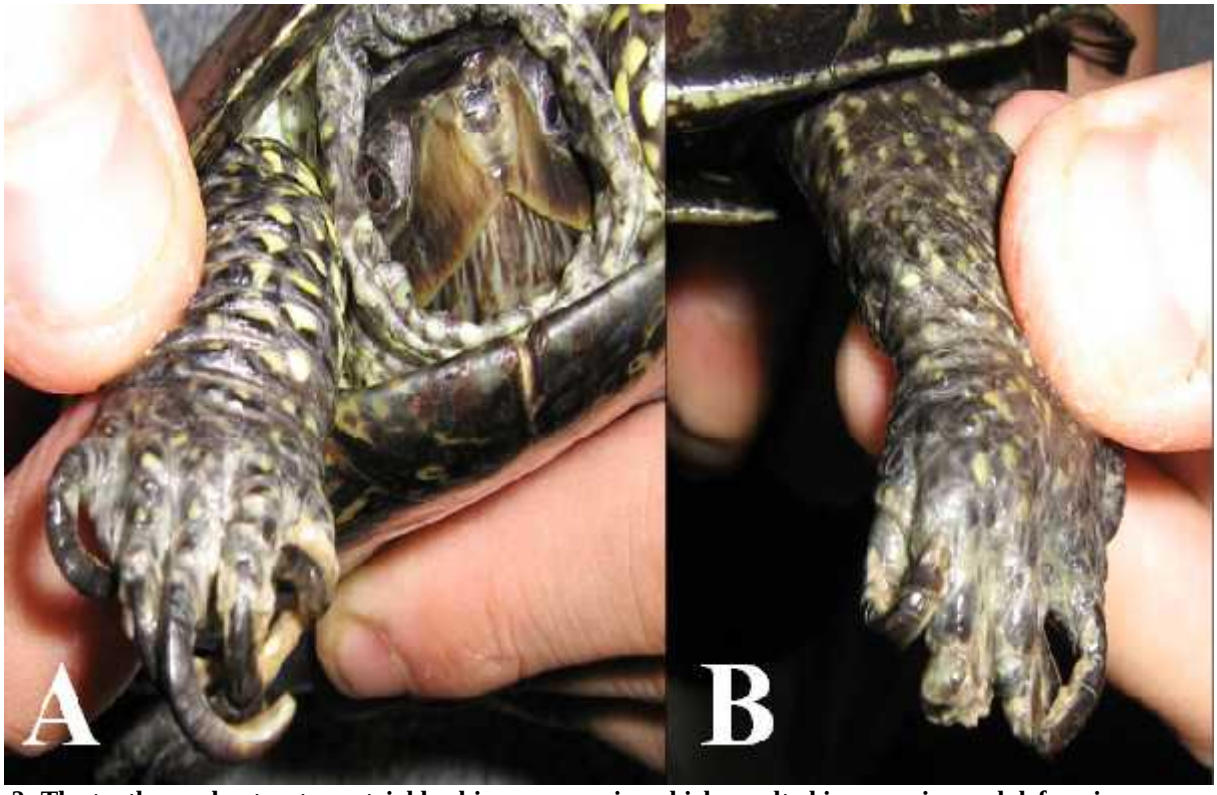

Fig. 2: The turtle was kept as terrestrial herbivorous species which resulted in excessive and deforming overgrowth of the nails both on the front (A) and hind (B) limbs.

DISCUSSION

Despite their conservation status, many European reptiles, including pond turtles, are kept in captivity either as pets or as part of conservation or scientific programs. Similarly to the exotic species, numerous health problems can appear if the proper, species-specific nutritional and husbandry conditions are lacking (Czirják *et al*., 2015).

In this report we presented a case where a male European pond turtle was exclusively kept on solid ground and fed with vegetables as a terrestrial species (*Testudo* sp.) would have been . The pond turtle being a predominantly carnivorous species, these conditions lead to chronic malnutrition (Mehler and Bennett, 2005),, dehydration and stress (Dupoué *et al*., 2014). Although it is rare in carnivorous turtle species (Chitty and Raftery, 2013), our patient presented beak overgrowth, most probably due to the malnutrition, which further aggravated the condition of the animal. Chronic anorexia and stress compromised the immune system of the turtle which facilitated the secondary invasion by the opportunistic pathogens *Candida* spp. and *Aeromonas hydrophila*, resulting in stomatitis. The corrections of husbandry conditions combined with an adequate antimicrobial therapy lead to the full recovery and release of the turtle.

Stomatitis in reptiles is usually a secondary complication on the background of improper environmental (e.g. temperature, humidity) and/or nutritional conditions, stress, lesions of the mucosa of various origins (Mehler and Bennett, 2006; Jacobson, 2007), similar to our case. Most of the stomatitis-associated bacteria and fungi, including those presented in this report, are part of the normal bacterial flora of the host's digestive tract, however under chronic stress and/or immunosuppression these microorganisms become clinically impacting. If the local lesions are not treated properly, their progress can affect other tissues of the region, e.g. central nervous system (Czirják *et al*., 2015) or can result in more generalized processes such as septicaemia (Köbölkuti *et al.*, 2013b).

Aeromonas hydrophila is considered an important pathogen for poikiloterm animals, especially for reptiles (Jacobson, 2007; Kim and Kwak, 2013). While causing mainly septicaemia in different chelonian and crocodilian species, it is usually associated with stomatitis in snakes (Jacobson 2007). When comparing different chelonian species, it has been shown that aquatic turtles are more often affected by bacterial pathogens as opposed to terrestrial species, although the prevalence of *A. hydrophila* infection is similar between the two groups (Keymer, 1978a, 1978b). In European chelonians, this bacteria was found to be a part of the oral and cloacal flora in free-living pond turtles (European- (*E. orbicularis*) and Balkan pond turtle (*Mauremys rivulata*)) (Soccini & Ferri, 2004; Hacioglu *et al*., 2012; Aleksić-Kovačević *et al.*, 2014), which could cause septicaemia in captivity (in case of *E. orbicularis* and *M. leprosa*; Keymer, 1978b).

Yeasts from the genus *Candida* are common isolates in reptiles, being more prevalent in herbivore species when compared to the carnivore ones (Kostka *et al*., 1997). The number of reported reptile candidiasis cases however was lower when compared to mammalian and avian studies. Nevertheless, it is still unknown whether this is a true difference or the prevalence is underdiagnosed and underreported in this vertebrate group (Jacobson, 2007).

Several forms of candidiasis have been described in reptiles, *C. tropicalis* and *C. albicans* being the dominant species involved. Many of these reports refer to species of the European fauna. *C. albicans* and *C. tropicalis* caused respiratory infection in terrestrial turtles species (*Testudo greaca* and *T. hermanni* respectively, Schildger *et al.*, 1991; Hernandez-Divers, 2001); *C. albicans* was associated with stomatitis and hepatitis in a smooth snake (*Coronella austriaca*) (Austwick and Keymer, 1981) and enteritis in loggerhead sea turtle (*Caretta caretta*) (Orós *et al*., 2004). We are adding to this list our case, reporting *Candida* spp. associated stomatitis in a male European pond turtle.

While seemingly European marine- and terrestrial turtles received enhanced veterinary medical attention mainly due to their conservation status (all marine species being listed as endangered or critically endangered) and popularity as pet species (*Testudo* sp.), with the decline in the populations sizes of the native aquatic species (two *Emys* and three *Mauremys* species) we hope this trend will change in the future. With our case report we would like to support these efforts and also, by reviewing the information available on the medical aspects of the European pond turtle, we would request the reptile and wildlife veterinarian and herpetologist community for further studies on this matter.

Moreover, owners and veterinarians dealing with protected species have to be familiar not only with the biology and husbandry of the animals in order to avoid husbandry related pathologies, but also with the conservation and legal aspects of such acts.

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