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CLOSTRIDIUM BOTULINUM IN PEAT LITTER – CAUSE OF DEADLY DISSEASE IN REPTILES IN PRIVATE TERRARIUM

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ABSTRACT

Materials from 6 months old snakes of the species shooter snake (*Coluber caspius*), died with neurological symptoms and uncharacteristic eating of peat litter, were tested. At the microscopic examination of samples of liver and peat litter were found bacteria with morphology, typical of *Clostridium botulinum*, then this species was isolated from studied materials. The clinical, pathological and microbiological results show that the most likely cause of these symptoms and mortality was botulinum intoxication. To prevent the disease, the peat and other bedding for reptiles should not be kept in wet and anaerobic conditions.

Key words: reptiles, peat, *Clostridium botulinum*

INTRODUCTION

Some reptiles like snakes, turtles, iguanas and lizards are popular pets. They are beautiful, colorful, quiet, calm and the care for them is relatively easy. However, all reptiles are carriers of various microbes, including bacteria, viruses and parasites, which are not yet well researched. Many of them can be passed on to their owners, especially in different stress situations. One of the dangerous microorganisms found in reptiles is *Clostridium botulinum* (HPSC, 2013). The neurotoxins emitted by it cause botulism - a serious life-threatening illness, characterized by paralysis and death. The bacterium develops in a humid environment devoid of oxygen (anaerobic) and rich in decomposing organic matter - hay, straw, etc., in which small amounts of soil with spores are penetrated (HPSC, 2013; KPP, 2015). As early as 1924, Bachmann and Haynes established strains of *C. botulinum* in samples of clay, sand and peat soil. The growth of this bacterium is favored by presence of organic substances in plant and other substrates such as peat, chopped leaves, dry cut grass or compost at optimal temperature, lack of oxygen and low acidity conditions (Hodges and Schoneweis, 2001).

C. botulinum is a Gram-positive rod-shaped bacterium that can cause lethal form of food poisoning. It forms very persistent endospores, distributed everywhere, as well as extremely strong exotoxin. It is released only from vegetative (actively breeding) bacteria and is the most powerful known natural poisonous substance. One gram of aerosolized botulinum toxin is enough to kill about one million people. Toxic doses are measured in billions parts of a gram. It is a powerful neurotoxin that blocks the release of acetylcholine from the nerves and causes muscle paralysis (Hartsock, 2015).

The spores of clostridia, including C. botulinum, are widespread in the environment, in the soil, on plants, in the intestinal tract of animals, and others. Reptiles, especially aquatic, are often carriers of such spores. Cold-blooded animals are susceptible to the disease. Several outbreaks of

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botulism in fish, as well as in fishery birds, have been registered since 1999 in the eastern part of Lake Erie in the New York area of the United States. They were caused by *C. botulinum* type E. Samples of benthic invertebrates also were gave positive results for spores of this bacterium in tissues. The reduction of dissolved oxygen, pH and redox potential near the sediment was resulted in the bacteria and toxin in the sediments being multiplied. They were taken up through the food chain (Pérez-Fuentetaja et al., 2006; Hartsock, 2015).

The diseases in reptiles are not as well researched as in warm-blooded animals and veterinarian medicians sometimes find it difficult to diagnose, treat and prevent some of these. Therefore, the aim of this study is to perform microbiological studies of materials from snakes in a private terrarium and samples of their peat litter in cases of mortality without characteristic signs.

MATERIALS AND METHODS

Snakes. Carcasses of 2 snakes at 6 months of age of the species *Coluber caspius*, died with nerve signs and allotriophagy (uncharacteristic swallowing of the peat litter) were investigated in a terrarium with 12 snakes imported from abroad. Liver samples for microbiological studies were taken. The animals were grown in a common room but in individual terrariums under optimal conditions.

Peat litter. Three samples of the reptile peat litter taken from different locations just prior to the research were investigated.

Microbiological studies. For isolation of microorganisms, cultures were made from all samples in elective and selective culture media for bacteria of different groups as well as for fungi. These were cultured at 37° C and 28° C for 24 to 72 hours under aerobic and anaerobic conditions (with Anaerocult® A mini – Merck-Bio Lab, Bulgaria).

Nutrient media. Blood agar and Zeisser agar, dextrose broth, folate azide enterococcus medium (BUL BIO - NCIPD LTD - Sofia), agar and broth of Mueller-Hinton were used, as well as Eosin Methylene Blue agar for Gram-negative aerobic and facultative anaerobic bacteria, Cetrimide agar for isolating species of the genus *Pseudomonas*; Chapman Stone agar for staphylococci, Sabouraud agar for fungi (Antisel - Sharlau Chemie S. A., Spain) and selective agar for *Clostridium perfringens* (Merck-Bio Lab, Bulgaria).

The taxonomic identification of the isolated bacteria was performed by microscopic examination under imersion at magnification of 1200 x of statained by Gram and Pfiffer preparations, reading the cultural features and biochemical properties using Polymicrotest (BUL BIO - NCIPD LTD - Sofia) and additional tests for oxidase, catalase and others with reagents from Antisel (Sharlau Chemie S. A., Spain). The isolation and identification of the bacteria has been carried out in accordance with the Bergey International Identifier (Holt et al., 1994).

Quantification of microorganisms was performed by the classical method in serial 10-fold increasing dilutions of the test materials in sterile saline solution. Cultures on the selected media were prepared from these dilutions, by three for each medium and dilution. After incubation at 37^{o}C for 24–72 h under aerobic and anaerobic conditions (with Anaerocult® A mini – Merck-Bio Lab, Bulgaria), the mean arithmetical number of the developed colonies was calculated and the colony forming units (CFU) in 1 g of the initial material were determined.

Statistical analysis of the results of triplicate made quantification of microorganisms in the peat litter was carried out using the classic method of Student-Fisher.

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Results

Changes in tissues and organs were not detected at autopsies except presence of peat in the oral cavity and the digestive tract (Figure 1).

Figure 1. Stage of the autopsy of one of the snakes. The presence of peat in the digestive tract is visible

In the initial microscopic examinations of the materials from liver and peat litter, bacteria and spores with morphology characteristic of *Clostridium botulinum* were found therein. As a result of the performed culture studies *C. botulinum* was isolated from the liver of the examined carcasses. The same species was also isolated from the peat litter of the reptiles. The results of some of the performed cultural and microscopic studies are presented in Fig. 2 and 3. The typical growth of *C. botulinum* in liquid culture with turbidity, sludge formation and gas emission with specific odor is seen in Figure 2 a. The characteristic whitish-gray grained asbestos-like colonies with β-hemolytic zone were also established (Figure 2 b), as well as the typical cell and spore morphology of this species (Figure 3).

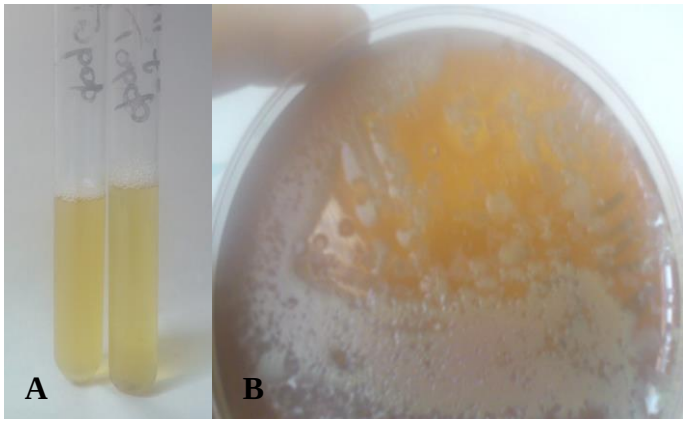

Figure 2. Cultures of Clostridium botulinum in glucose broth (A) and on Tsaysler agar (B) isolated from peat litter of tested snakes

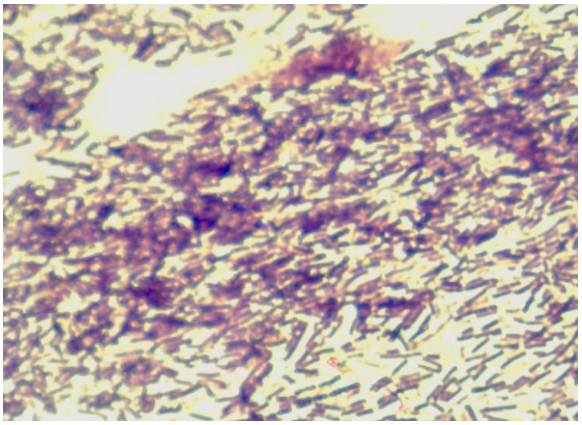

Figure 3. Cells and spores of *Clostridium botulinum*, isolated in pure culture from peat, x 1200, stained by Gram.

From the peat we also isolated other microorganisms (Table 1), as part of these microorganisms are also isolated from goat milk samples (Hristov et al., 2016). The summary data in the table shows that the total number of micro-organisms was not high. Gram-negative bacteria prevaled, predominantly Proteus vulgaris, as well as spore-forming species. Pathogenic microorganisms such as Clostridium perfringens, Escherichia coli, Salmonella sp., Pseudomonas aeruginosa or other species of genus Pseudomonas were not isolated.

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Table 1. Results of microbiological investigations of peat litter of snakes of the species *Coluber caspius*, kept in a terrarium

Isolated microorganisms – CFU/ g		
Total number		$1,52.10^4 \pm 0,12$
Gram-positive	**Staphylococci**	$3,38.10^2 \pm 0,68$
	Enterococci	$1,08.10^2 \pm 0,18$
	Spore forming	$5,49.10^3 \pm 1,37$
Gram-negative		$5,85.10^3 \pm 1,98$
Fungi	**Oval**	$2,98.10^3 \pm 1,49$
	Filamentous	$4,37.10^2 \pm 2,45$

As a result of the present study, measures had been taken to change the contaminated peat litter of the reptiles and there were no new cases of disease.

DISCUSSION

As can be seen from the presented results, pathogenic microorganisms other than C. botulinum were not isolated from the samples we studied. This is the first case of isolation of this species from snakes and peat in Bulgaria. Obviously, the nerve signs and the lethal outcome of the disease of the reptiles examined are due to its toxins, which have permeated orally from the peat litter, or due to the penetration and multiplication of this microorganism in the digestive tract and the liver of the reptiles. Botulism most often develops as a result of food intoxication but in some cases also after bacterial colonization of the intestinal tract (Pérez-Fuentetaja et al., 2006). The disease is characterized by neuropathic signs and mortality (Pérez-Fuentetaja et al., 2006). Regardless of the presence of different serovariants of the bacterium, the mechanism of action of their neurotoxins is the same. They do not lose their toxicity under the influence of the proteases and bile juice in the small intestine. The swallowed toxin quickly passes from the digestive tract into the bloodstream and reaches the nerve cells. Once it penetrates into them, the toxin stops the transmission of the impulses stimulating the movement of the muscles. It causes the neuromuscular blockade, which manifests itself with general weakness and eventual paralysis. Because of central nervous system affecting, the symptoms can mimic those of other diseases such as rabies, and in horses - viral encephalitis or the nervous form of rhinopneumonitis. The prognosis of the disease is directly related to the amount of toxin ingested (KPP, 2015). The established signs and mortality in the animals we investigatea were obviously attributable to this toxin action. Because of the absence of pathological changes in their tissues and organs except the presence of peat in the digestive tract, our studies were conducted only on liver and peat bedding materials. The other snakes in the investigated terrarium may have taken less toxins and due to the zoohygienic measures taken, the outcome was favorable for them.

The HPSC (2013) emphasizes that reptiles (especially turtles) are not suitable for pet-friendly for small children and should not be grown in households with children under five. It is recommended that their owners wash their hands immediately after contact with the reptiles or terrariums. It has recently been reported that contact with turtles or food for them is the most likely

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cause of two cases of childhood botulism in Ireland (HPSC, 2013; KPP, 2015). Case of childhood botulism caused by *C. botulinum* type A toxin is also established. Source is poorly treated water from a well with a peat layer (Kobayashi et al., 2014).

Contamination with *C. botulinum* of plant substrates such as hay, peat and others occurs mainly in two circumstances: when improperly dried feed materials are baled, stored and reserved while are still wet or when an animal carcase (mouse, rabbit or snake) is accidentally intercepted by the baler (KPP, 2015). Other authors such as Zied et al. (2014) highlight the risk of contamination of preserved mushrooms grown and stored in peat with pathogenic microorganisms including *C. botulinum*. They recommend processing in an autoclave as the best form of sterilization of preserved mushrooms. Plant materials such as ground peat and others should be pre-sterilized prior to storage. This is not easy, because in complex carbon-based materials such as peat some resident microbes can survive even after gamma-irradiation at doses exceeding 50 Kgy. Such are actinomycetes or spore-forming clostridia, species of genus *Bacillus* (Yardin et al., 2000) or *Paenibacillus*, isolated by Ming et al. (2012) from a swamp sample of peat in northern China. Likewise, Leclair et al. (2012) isolated 53 strains of *C. botulinum* from two peat bogs, soil samples, as well as from the skin and the content of the intestines of seals.

In substrates that are treated with high temperature or acids, C. botulinum is killed. Oxygen access prevents the development of spores and the formation of toxins. Heating of the materials to 80-90° C for 10 minutes is sufficient for their disposal due to the denaturing of the toxins that are proteins. Because the disease is caused by them and not by the bacteria themselves, when C. botulinum is unable to produce exotoxins, it is harmless, but even if the cells are not actively growing, the residual toxin can cause disease (Hartsock, 2015).

CONCLUSIONS:

Home-grown reptiles may suffer from botulism. The litter used for reptiles in terrariums, especially if it is from peat, may be the source of the disease. It should be pre-sterilized or not allowed to be used humid or anaerobically stored material.

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