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ENTEROBACTER AGGLOMERANS – A CAUSE OF STOMATITIS IN A SNAKE

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ABSTRACT

Microbiological investigations were carried out of material from the mouth of a common boa (Boa constrictor imperator) with signs of stomatitis, general weakening of the body, difficulty eating and breathing. In the result of the microbiological examinations of the sample from the lesions in the oral cavity were isolated Enterobacter agglomerans 5 and Aspergillus sp. Successful therapy was carried out with enrofloxacin, selected according to the results of the antibiotic gram. The dosage administered was 5 mg/kg Baytril 5 % daily by intramuscularly injection for 10 days. The local lesions were treated with a silver sulfadiazine – cream 1 % and clotrimazole – cream 1 % once a day for 3 weeks up to the recovery.

Key words: stomatitis, Boa constrictor imperator, Enterobacter agglomerans, Aspergillus sp., therapy

Introduction

Cold-blooded animals and particularly reptiles are increasingly grown as pets in many countries around the world. Diseases in these animals, especially infections, are not well studied. Due to the variable body temperature, as well as because of differences in the immune system and the immune response, susceptibility to infections and particularities in their progress in these animals are different from those in warm-blooded animals. Therefore, diseases in these animals are of interest not only in theory but also in practical terms.

In our country in the available literature there is little evidence of infectious pathology of reptiles. Orozova et al. (2010) reported the isolation of poly resistant β-hemolytic strains of Aeromonas hydrophila of dead snakes with haemorrhagic septicemia. Popova et al. (2014) found Clostridium botulinum in peat litter and stomach contents of dead snakes with neurological symptoms.

One of the diseases detected in reptiles is stomatitis. It is an inflammatory-dystrophic disease affecting the oral mucosa. Often the agents are part of the normal microflora of the oral cavity or other parts of the digestive tract, which exhibit its pathogenic effect in periods of immunodeficiency. Accompanying symptoms vary and depend on the primary infectious agent. The most commonly occurs as a result of respiratory disorders, due to incorrect temperature control of raising (Rossi et al., 2006). Temperatures below optimum minimum for the type lead to lowering the body's defenses. When affecting the respiratory system due to lack of diaphragm and cough reflex, snakes are forced to stand with open mouth, trying to compensate for breathing difficulties. This in turn is a prerequisite for entry of a large number of pathogenic microorganisms. Coupled with poor animal hygiene conditions in such a situation the infection develops very quickly. Because of yellowish plaques that are deposited in soft tissues and petechiae in the mucous membrane, the disease is known among herpetologists as Mouth Rot (Girling, 2003).

The purpose of this work is the etiological diagnosis of chronic stomatitis in boa, laboratory setting and conducting optimal etiotropic therapy.

Materials and Methods

Material. Microbiological tests were carried out of material from the mouth of common boa (Boa constrictor imperator), male, aged 3 years. At the review were established pale yellow plaques and deposits in the mouth, bite distorted due to lack of proper occlusion of the jaws, gingival erythema, increased salivation, often opening the mouth with difficulty breathing (Fig. 1). According to the medical history, the snake has refused food for a long time due to painful lesions and general weakening of the body.

Figure 1: Clinical signs of stomatitis in Boa constrictor imperator.

Microbiological tests. From the taken material were made microscopic preparations stained by Gram and native and observed under immersion at a magnification x 1200. For isolation of microorganisms were made cultures in elective and selective growth media for bacteria of different groups, as well as for fungi. They were incubated at 37 °C and 28°C for 24–72 h under aerobic conditions.

Nutrient media. Blood agar (BULBIO – NCIPD Ltd. – Sofia), agar and broth of Mueller Hinton, Eosin Methylene Blue agar for Gram-negative aerobic and facultative anaerobic bacteria, differentiating liquid media (to determine indole, degradation of nitrates, reaction with methyl red and Voges-Proskauer) and Sabouraud agar for fungi (Antisel – Sharlau Chemie S. A., Spain) were used.

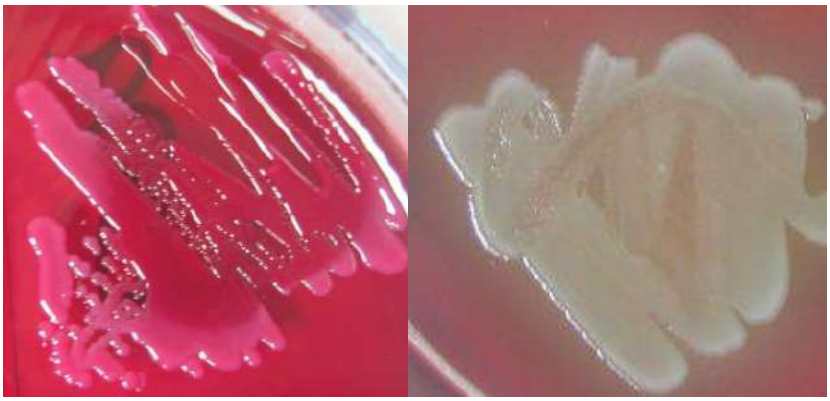
Taxonomic identification of the isolated microorganisms was made by microscopic examination of Gram stained preparations, as well as by reading of cultural characteristics on solid and in liquid media and of biochemical properties using differentiating liquid media and additional tests for H2S, oxidase and catalase with reagents of Antisel - Sharlau Chemie S. A., Spain. Isolation and identification of bacteria was conducted in accordance with the international identifier of Bergey (Holt et al., 1994) and using an identification table of Biomerieux-France, and of fungi - according Murray et al. (2003).

Determination of the sensitivity of isolated bacteria to antimicrobial means was done through the classic agar-gel diffusion method of Bauer et al. (1966). Standard antibiotic discs (BULBIO – NCIPD Ltd. – Sofia) and such prepared by us were applied after inoculation of bacterial suspension in exponential growth phase with a concentration of 2.106 cells/ml on blood agar. Incubation was performed at 37 °C for 24 hours. The results were interpreted in a three-tier system of Bauer et al. (1966) after measuring the diameters of inhibitory zones in mm.

Results

At microscopic examination of preparations of the secretion were established polymorphic medium sized Gram-negative rods. As a result of the cultural studies were isolated Enterobacter agglomerans 5 and Aspergillus sp. in less quantity.

On Figure 2 are presents the results from the cultural studies.



а **в**

Figure 2: Colonies of Enterobacter agglomerans on Endo agar (а) and on blood agar (в) – *ά*-hemolytic zone is visible.

From Table 1 and Figure 3 can be seen a part of the results of the biochemical tests conducted to identify the isolated bacterial strain.

Table 1: Biochemical characterization of the isolated bacteria

Test (enzyme, end product, substrate)	Reaction of the strain
Hemolysis	ά
Motility	+
Nitrate reductase	+
Catalase	+
Oxidase	-
Indole	-
Hydrogen sulfide	-
Reaction with methyl red	+
Voges-Proskauer reaction	-
D-glucose	+
Lactose	+
ONPG	+
Urease	-

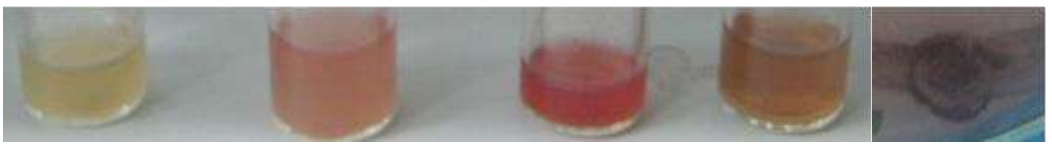


Figure 3: Results of some stages of the biochemical identification of the isolated strain (a – for nitrate reductase; b – for indole; c – with methyl red; d – reaction of Voges-Proskauer; e – for oxidase).

The results of the study to determine the sensitivity of the isolated bacteria to antimicrobial agents are presented in Fig. 4 and in Table 2.

Table 2: Sensitivity of the isolated bacteria to antimicrobial means *in vitro.*

Antimicrobial mean	Disc contents (μg)	Sensitivity of the strains
Thiamphenicol	30	R
Tetracycline	30	S
Doxycycline	30	S
Lincomycin	15	R
Penicillin	15	R
Oxacillin	1	R
Amoxycillin	10	R
Ampicillin	10	R
Cefuroxime	30	R
Cefotaxime	30	I
Novobiocin	30	R
Gentamicin	10	S
Amikacin	10	S
Enrofloxacin	5	S
Ciprofloxacin	5	S
Sulfamethoxazole+Trimethoprim	23.75/1.25	S

S – sensitive; I – intermediate; R – resistant

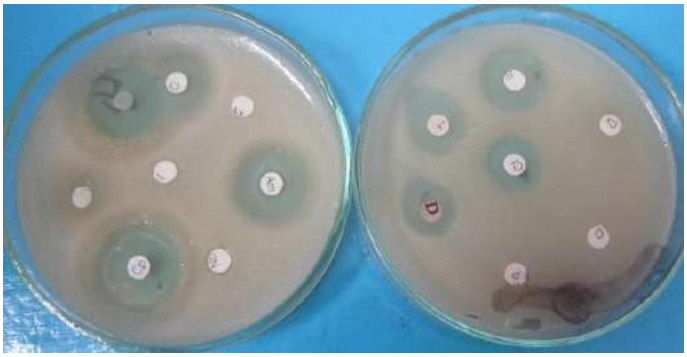


Figure 4. Antibiotic grams of the isolated *Enterobacter agglomerans*.

As it is seen from the data presented, the isolated Enterobacter agglomerans 5 show sensitivity to tetracycline antibiotics, aminoglycosides, quinolones and potentiated sulfonamides. This show resistance to penicillin antibiotics and to novobiocin.

Therapy. The therapeutic regimen constructed and implemented by us is presented in Table 3.

Table 3: Regimen for the treatment of stomatitis at a serpent with the participation of *Enterobacter agglomerans 5* and *Aspergillus sp.*

Active substance	Medicinal form	Single dose	Route of administration	Duration of administration
Enrofloxacin	Injection solution 5 %	0.4 ml	intramuscularly	10 days
Silver sulfadiazin	Cream 1 %	-	local, external	3 weeks
Clotrimazole	Cream 1 %	-	local, external	3 weeks

Figure 5: Stage of the control inspection. It is seen that the lesions are disappeared, the oral mucosa is restored and the animal is clinically healthy.

Successfully treatment was conducted with enrofloxacin, selected according to the results of the antibiotic grams. The dosage administered was by the scheme: 5 mg/kg Baytril 5 % injection, in our case about 4 kg body weight – 0,4 ml daily, intramuscularly for 10 days, according to Kaplan et al. (1995). It is desirable its dilution to 1 ml with physiological saline, as it irritates the inoculation site and may lead to tissue necrosis.

Locally the lesions were treated with a silver sulfadiazine – cream 1 % and clotrimazole – cream 1 % (Girling, 2003) once a day until restoration.

As a result of the conducted complex therapy by created by us scheme has achieved successful recovery of the patient within three weeks. On Figure 5 are visible the result of the successful therapy.

Discussion

Isolated in these studies Gram-negative bacterium Enterobacter agglomerans is currently relocated to another family and is known as Pantoea agglomerans, belonging to the family Enetrobacteriaceae. It is identified in the 60s among hospital-acquired infections and post-operative complications. Accepted for conditionally pathogenic, not very high virulence and invasive in animals and humans. However, lowering the immune reactivity creates favorable conditions for the development of bacterial infections in the mucous membranes and urinary tract (Public Health Agency of Canada, 2010).

Infection in terrarium conditions can be done by direct or indirect contact of mucous membranes with infectious agent - contaminated hands or contaminated accessories and tools such as tweezers to clean water containers, etc. The most common case of contamination in snakes is by fecal-oral route, as members of Enterobacteriaceae normally occur in the lower part of the digestive tract. Poor hygiene in the cage and untimely cleaning prerequisite for contamination of the environment with these bacteria, such as research and our case. Existing respiratory infection and subsequent frequent opening of the mouth, combined with biodynamics of snakes are certain prerequisites for entry and multiplication of bacteria in the oral cavity (De Vasjoli et al., 2004).

The development of Aspergillus sp. in the oral cavity is obviously favored by the lower temperature in the cold-blooded and of course by the moisture on the mucosa and disbacteriosis. Perhaps it cames from the bedding, where can develop in high humidity and occasional cleaning.

As snakes have no lymph nodes unlike the mammals, the pathogens could not be hampered locally. At entering the blood rapidly develops generalized septicemia with lesions in the internal organs. In severe cases, the infection may spread from the oral mucosa in the maxillary or mandibular bones. So rapid identification of the infectious agent and taking promptly measures is important (Jacobson, 2007).

The applied antibacterial therapy based on held antibiotic gram must be accompanied by a maintenance therapy - balanced diet with vitamins, a smaller prey, and in more severe cases – with

parenteral introduction of fluids, as in reptiles' dehydration is much more dangerous than starvation. It is need also raising the temperature in terrarium, which is important for faster recovery of reptiles and for optimal action of the antibacterial means (Kaplan et al., 1995).

Conclusions

Conditionally pathogenic bacteria of the family Enterobacteriaceae such as Enterobacter agglomerans can cause stomatitis in terrarium captive reptiles at violation of the zoo hygienic conditions.

Parenteral antimicrobial therapy based on antibiotic grams must be accompanied by topical treatment of lesions with appropriate means and with supportive therapy –balanced diet with vitamins, smaller food and increased temperature of breeding, conducive to more rapid recovery of reptiles and optimally action of antibacterial means.

References

1. Bauer, A. W., W. M. Kirby, J. C. Cherris, M. Truck. (1966). *Antibiotic susceptibility testing by a standardized single disk method*. The Am. J. of Clin. Pathol., 1966, 45–49, 493–496.
2. De Vosjoli, P., R. Kligenberg, J. Ronne. (2004). *The Boa Constrictor Manual, Advanced Vivarium Systems*. Irvine, California, 76–99.
3. Girling, S. (2003). *Veterinary Nursing of Exotic Pets*. Blackwell Publishing Ltd, 161–174, 175–191.
4. Holt, J. G., N. R. Krieg, P. H. A. Sneart, J. T. Staley, S. T. Williams. (1994). *Bergey's manual of determinative bacteriology*. Ninth edition, Williams & Wilkins.
5. Jacobson, E. R. (2007). *Infectious Diseases and Pathology of Reptiles*. University of Florida, College of Veterinary Medicine, Gainesville, Florida, 131–161.
6. Kaplan, M., R. Jereb. (1995). *Ulcerative Stomatitis (Mouthrot) in Reptiles*. Journal of Wildlife Rehabilitation, 1995, [http://www.anapsid.org/stomatitis.html].
7. Murray, P. R., E. J. Baron, J. H. Jorgensen, M. A. Pfaller, R. H. Yolken. (2003). *Mannual of Clinical Microbiology*. 8th Edition, Vol. 2, ASM Press, Washington, D. C., 1726–1753.
8. Orozova, P., Y. Petkov, M. Bonovska, V. Chikova. (2010). *Isolation and identification of Aeromonas hydrophila of snakes with haemorrhagic septicemia*. Conference of FVM – FTU with international participation "Tradition and Modernity in veterinary medicine", Reports, 216–226, 2010 (in Bulgarian).
9. Popova, T. P., M. D. Kaleva, V. Manov, B. Nikolov. (2014). *Clostridium botulinum in peat litter – cause of deadly disease in reptiles in private terrarium*. Agricultural science, 4, 2014 (in press, in Bulgarian).
10. *Public Health of Agency of Canada*. November, 2010. [https://www.msdsonline.com/resources/msds-resources/free-safety-data-sheet-index/enterobacter-spp.aspx].
11. Rossi, J., M. A., Roxanne Rossi. (2006). *What's Wrong with My Snake?* The Herpetocultural Library, Advanced Vivarium Systems, Irvine, California, 2006, 59–61, 141–149.