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Aerobic bacteria in oral cavity of Lancehead snakes (*Bothrops atrox*) with stomatitis

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ABSTRACT. Stomatitis is a common disease found on snake farms, and Gram-negative bacilli are the main etiological agents that play an important role as secondary sources of viral or parasitic infections. The purpose of this work was to identify the aerobic bacteria in the oral cavity of *Bothrops atrox* with stomatitis. Samples for microbiological examination were collected from 12 snakes bred on a commercial snake farm for venom extraction. Samples of the secretion in the oral cavity of each serpent presenting stomatitis were collected from fang sheath, using a cotton swab with sterile alginate. The samples were incubated and cultured on Petri dishes containing blood agar and XLD agar using the agar depletion technique. Bacterial growth occurred in all analyzed samples collected from the oral cavity of *Bothrops atrox* with stomatitis, and some of the samples contained more than one microorganism. The following Gram-negative bacteria were isolated: *Escherichia coli* (26.31%), *Citrobacter* spp. (21.05%), *Proteus* spp. (15.78%) and *Salmonella* spp. (10.52%). The only Gram-positive bacterium that was isolated was *Staphylococcus* spp., which was present in 26.31% of the analyzed samples.

Keywords: bacteriology, infectious diseases, Reptilia, Squamata, Ophidia.

Bactérias aeróbicas da cavidade oral de jararaca-do-norte (*Bothrops atrox*) com estomatite

RESUMO. A estomatite é uma das doenças mais frequentes em criatórios comerciais de serpentes, sendo os bacilos Gram-negativos os principais agentes etiológicos com importante papel, como fontes secundárias, nas infecções virais ou parasitárias. O objetivo do presente trabalho foi analisar as bactérias aeróbicas presentes na cavidade oral em serpentes da espécie *Bothrops atrox*. Utilizaram-se 12 amostras colhidas com auxílio de *swab* estéril na região da bainha da presa, em serpentes que apresentaram estomatite, em um criatório comercial. As amostras foram cultivadas em Ágar-sangue e Ágar XLD. Em todas as amostras analisadas, houve crescimento de, pelo menos, um microrganismo. As bactérias Gram-negativas isoladas foram *Escherichia coli* (26,31%), *Citrobacter* spp. (21,05%), *Proteus* spp. (15,78%) e *Salmonella* spp. (10,52%). A única bactéria Gram-positiva isolada foi a *Staphylococcus* spp., presente em 26,31% das amostras analisadas.

Palavras-chave: bacteriologia, doenças infecciosas, Reptilia, Squamata, Ophidia.

Introduction

The gastrointestinal microbiota of reptiles generally comprises Gram-positive and Gram-negative, aerobic and anaerobic yeasts and protozoa. Gram-negative bacilli are the main etiologic agents of diseases in snakes and play an important role as secondary sources of infections of viral or parasitic etiology. The prevalence of these bacteria in infectious processes is directly related to the opportunistic nature of the normal microbiota of reptiles (Cubas & Baptistotte, 2007).

The stress that these reptiles normally undergoin the process of venom extraction and adaptation to an artificial environment has a direct impact on their health. This dynamic interferes with oral microbiota, which may, in turn, alter the amount and potency of venom (Soveri & Seuna, 1986).

Bacterial diseases in snakes, which are characterized by different clinical manifestations, include stomatitis, ophthalmic and subcutaneous abscesses, glossitis, gastroenteritis, pneumonia, oophoritis and septicemia (Ferreira et al., 2012). Stomatitis is an important disease for animals used in venom obtention because of oral tissue injury and it was detected in 59% of snakes belonging to Boidae and Pythonidae families (Schmidt et al., 2013).

Different authors have reported that the microorganisms commonly found as components of

the microbiota of the digestive system of reptiles can act as etiological agents of gastrointestinal diseases, but few researchers have defined these bacteria in Brazilian species of reptiles. Moreover, the reports are scanty and many of them are outdated (Diaz-Figueroa & Mitchell, 2006).

Captive breeding of lancehead snakes has been extensive because the venom of these reptiles can be used in the production of drugs, which predisposes them to stomatitis. The objective of this study was to identify the aerobic bacteria in the oral cavity of captive *Bothrops atrox* suffering from stomatitis.

Material and methods

All protocols envolving animals have been reviewed and approved by SISBIO, Brazil's Authorization and Information System on Biodiversity, under permit no. 41060-1, and the *Universidade Federal de Uberlândia* Ethics Committee (Animal use permite: 142/13).

Samples for microbiological examination were collected from 12 snakes of the species *Bothrops atrox* which presented stomatitis, four males and eight females. The samples were taken from animals bred on a commercial snake farm for venom extraction belonging to the company *Pentapharm do Brasil Comércio e Exportação LTDA*, in Uberlândia, Minas Gerais, registered with the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA) under no. 11904.

To avoid adding to the animals' stress, the samples were collected along with the routine procedures of the breeding farm, which follows all the animal welfare and biosafety standards. The samples were collected from snakes presenting stomatitis before they underwent any treatment.

The snakes were physically restrained by placing a snake hook near the final third of the head, then positioning a hand in the region of the animal's temporomandibular joints, thereby keeping its mouth open (Wilkinson, 2014). Samples of the secretion in the oral cavity of each serpent presenting stomatitis were then collected from its fang sheath, using a cotton swab with sterile alginate (Figure 1) (Jorge et al., 1990).

The samples were stored in tubes containing Stuart transport medium and sent to the Laboratory of Infectious Diseases of the *Universidade Federal de Uberlândia*. At the laboratory the samples were transferred to test tubes containing thioglycolate broth, a highly nutritious medium for culturing a variety of microorganisms, and were incubated in a bacteriological incubator at 37º C for 24 hours (Oplustil, 2004).

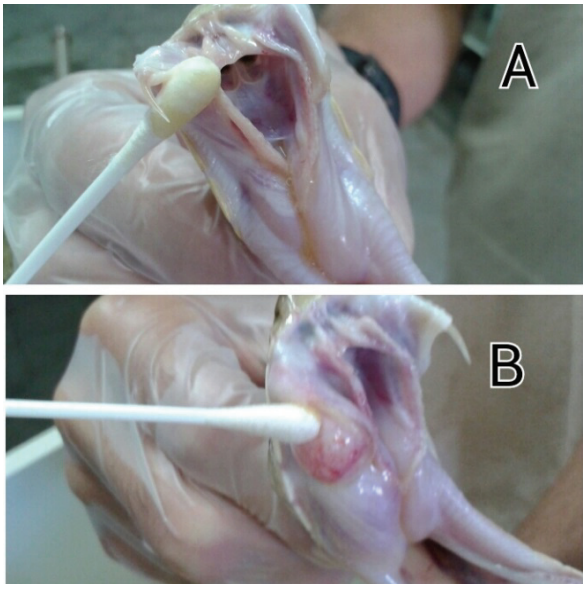

Figure 1. Collecting material with a sterile swab in the region of the fang sheath. A. Healthy oral cavity of *Bothrops atrox*; B. Snake with stomatitis in the region of the right fang sheath.

To isolate the bacterial colonies, the samples were cultured with the aid of a platinum wire loop on Petri dishes containing blood agar and XLDagar (Xylose Lysine Deoxycholate agar), using the agar depletion technique. The seeded dishes were then incubated again in a bacteriological incubator at 37º C for 24 hours (Quinn et al., 2004).

Colonies cultivated on blood agar were Gram stained to identify Gram-positive and Gram-negative bacteria. Catalase and mannitol tests were used for the identification of Gram-positive bacteria (Oplustil, 2004).

The colonies cultivated on XLDagar were identified using commercial Mini Kits containing Rugai medium with lysine, which is used mainly in biochemical screening of colonies grown on media selective for Gram-negative bacteria of the family Enterobacteriaceae. A fresh Rugai medium with lysine was employed separately for each different colony grown on XLDagar in order to identify each bacterial genus or species, as recommended by the manufacturer (Oplustil, 2004).

Results

Bacterial growth occurred in the all twelve analyzed samples collected from the oral cavity of *Bothrops atrox* with stomatitis, and some of the samples contained more than one microorganism. The following Gram-negative bacteria were isolated: *Escherichia coli* (26.31%), *Citrobacter* spp. (21.05%), *Proteus* spp. (15.78%) and *Salmonella* spp. (10.52%). The only Gram-positive bacterium that was isolated

was *Staphylococcus* spp., which was present in 26.31% of the analyzed samples (Table 1).

Table 1. Frequency of bacteria found in the oral cavity of *Bothrops atrox* with stomatitis

Microorganisms	Number of positive samples	Frequency (%)
Escherichia coli	5	26.31
Staphylococcus spp.	5	26.31
Citrobacter spp.	4	21.05
Proteus spp.	3	15.78
Salmonella spp.	2	10.52

Discussion

A study evaluated the aerobic microbiota in the oral cavity, cloaca and venom of specimens of the species *Crotalus durissus terrificus* newly captured in the wild and quarantined, snakes kept in collective captivity, and snakes bred in individual captivity. The most common bacteria they found were *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Morganella morganii*; however, *Pseudomonas aeruginosa* and *Morganella morganii* have not been found in *Bothrops atrox* (Ferreria Jr. et al., 2009).

Snakes from different regions of the world present a variety of oral microbiota. Among the factors suggested to explain such differences are the particularities of the specimens evaluated, such as the species of snake, its origin, whether it is captive or free-living, its health status, whether or not it has fed recently, and the condition of the prey it feeds on (Jorge et al., 1990).

The oral cavity of venomous and non-venomous snakes is colonized by a wide variety of anaerobic and aerobic microorganisms, and infections caused by *Aeromonas hydrophila*, *Pseudomonas* spp., *Proteus* spp., *Salmonella* spp., *Citrobacter* spp., *Escherichia coli*, *Providencia* spp., and *Xanthomonas maltophilia* stand out, among others (Blaylock, 2001). Gram-positive agents are also sometimes isolated, particularly *Streptococcus* spp. and *Staphylococcus* spp. (Cubas & Baptistotte, 2006). *Staphylococcus* spp. has been identified frequently in *Bothrops atrox*, appearing in 26% of samples.

Some bacteria with a zoonotic potential have been isolated as part of the normal microbiota of snakes, and the main etiological agents are Gam-negative bacilli such as the bacterium *Morganella morganii*, which is distributed worldwide and is found in the normal microbiota of the oral and cloacal cavities of snakes. This microorganism is one of the bacterial agents involved in local complications by secondary necrosis in humans following a snake bite (Mader, 1998). In the analysis of a subcutaneous abscess in a *Boa constrictor*, *Morganella morganii* was the etiologic agent (Ferreira et al., 2012).

Studies of *Salmonella* spp. in serpents suggest that the entry of infectious organisms in a snake collection is related not only to the addition of new reptiles but possibly also to the food that reptiles in captivity are fed, such as rodents (Williams, 2008).

The prevalence of enterobacteria in *Bothrops jararaca* in the state of São Paulo, based on a microbiological survey using samples taken directly from the colon of healthy adult snakes was evaluated. Several genera of the family Enterobacteriaceae were obtained (*Citrobacter, Enterobacter, Escherichia, Klebsiella, Kluyvera, Morganella, Proteus, Providencia* and *Salmonella*) and a morphologically similar genus of Gram-negative bacterium (*Aeromonas*). *Salmonella, Citrobacter* and *Escherichia* were the most frequent isolates. *Escherichia, Proteus, Salmonella* and *Citrobacter* were also identified in the *Bothrops atrox* specimens analyzed (Bastos, Lopes, Gattamorta, & Matushima, 2008). A study of the microbiota in the fangs, fangs heath and venom of *Bothrops jararaca*, reported that the bacterial species most frequently found were: group D streptococci, *Enterobacter* sp., *Providencia rettgeri*, *Providencia* spp., *Escherichia coli*, *Morganella morganii* and *Clostridium* sp., but less frequently *Pseudomonas* spp., *Proteus mirabilis*, *Staphylococcus aureus*, *Salmonella typhimurium*, and *Citrobacter* spp. (Jorge et al., 1990).

A microbiological study of the oral cavity often different snake species belonging to the families Boidae, Colubridae, Elapidae and Viperidae, identified the bacteria *Actinomyces* sp., *Burkholderia* spp., *Moraxella* spp., *Proteus* spp., *Sarcina* spp., *Bacillussubtilis*, *Staphylococcus aureus*, coagulase-negative *Staphylococcus* and *Yersinia enterocolitica*, and also isolated *Proteus* spp. and *Staphylococcus* spp. from *Bothrops atrox* (Fonseca, Moreira, Cunha, Ribeiro, & Almeida, 2009).

Macroscopic and microscopic changes in *Micrurus corallinus* snakes kept in captivity, were observed in appearance of vesicles in the animals, and in their microbiological examination, isolated *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Blood cultures of these specimens were also performed using blood collected aseptically by intracardiac blood puncture, in which *Staphylococcus aureus* was also isolated, indicating septicemia caused by this agent. *Staphylococcus* spp. was isolated in 26% of *Bothrops atrox* samples analyzed (Serapicos et al., 2005).

Conclusion

Bacterial growth was frequently present in the oral cavity of *Bothrops atrox* with stomatitis, and usually contain more than one species of microorganism. The most commonly isolated microorganisms were Gram-negative bacterium and the only Gram-positive bacterium that was isolated was *Staphylococcus* spp., which was present in 26.31% of the analyzed samples.

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