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A STUDY OF BACTERIAL CONTAMINATION OF RATTLESNAKE VENOM

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The authors studied the bacterial contamination of rattlesnake venom isolated from snakes in captivity and wild snakes caught recently. The captive snakes showed a relatively high incidence of bacterial contamination of their venom.

Key words: Rattlesnake venom. Bacterial contamination.

Several investigators have studied cold-blooded animals in terms of contamination by different types of organisms. Hinshaw and McNeil[4] isolated *Salmonella rubislaw* from 3 out of 12 *Sceloporus occidentalis* specimens. Zwart[24] reported the isolation of Salmonellae from 37.5% of lizards. Le-Noc and Brygoo[9] isolated 58 different types of salmonellae from 48 out of 127 chameleons examined.

In studies on snakes captured in Pará and in lower Amazonia (Brazil), Lins[10 11] showed that 11.31% of the animals examined were infected with salmonellae. Kourany et al.[8] detected salmonellae in 27.3% of lizards and 6.5% of toads examined. Moreno et al.[14], in a study of 613 cold-blooded animals raised in captivity, most of which were snakes, detected in 271 animals several bacterial species, such as *Citrobacter, Proteus vulgaris, Proteus morganii* and *Proteus rettgeri* as well as several types of salmonellae. This led the authors to emphasize the high incidence of contamination with salmonellae among snakes.

Despite good physiological conditions of the snakes several investigators have found high indices of contamination. Thus, Furlanetto et al [3] detected high levels of contamination with enterobacteriaceae and other bacteria in an epizooty caused by a diphtheroid bacterium among snakes raised in an animal house. This bacillus caused a disease among these snakes that had not been reported before. The disease affected 70.0% of snakes of the genera *Bothrops* and *Crotalus* whose venom was going to be used for the preparation of antibothrops and anticrotalus sera. The snakes were vaccinated with a Wright-type vaccine prepared from the diphtheroid bacillus detected, which was highly effective in lowering the level of infection from 70% to 0.4%.

Several investigators have attempted to establish a bacterial standard for the mouth flora of snakes and have reported the presence of several bacterial species[1 5 6 18 19 21 23]. Soerensen et al[20] described "single visceral drop" disease affecting snakes in captivity. Furlanetto et al.[3] reported a type of infection of the venom glands of *Bothrops linneaeus.* 1758, and *Crotalus wagler,* 1824, raised in the animal house.

Marcus[13] detected "mouth rot" in the upper and lower gums of snakes, and Wallach[22] had also found inflammation of the gums and dental arches of snakes. This type of inflammation caused large amounts of yellowish exudate which later induced secondary osteomyelitis. Klingelhoeffer[7] had also reported two acute forms of "mouth rot" that led to the death of affected snakes. Several other agents such as *Aeromonas*[17] and *P. liquefaciens, Pasteurella* and *Proteus* sp.[19] were reported to be responsible for this type of disease.

In view of the wide interest shown by many researchers in studying cold-blooded animals for infections caused by bacteria and by other microorganisms, we decided to determine the bacterial contamination present in the venom of rattlesnakes, using both wild animals and animals raised in captivity.

MATERIALS AND METHODS

The materials used were rattlesnake (*Crotalus durissus terrificus*) venom and blood-agar bacterial growth medium. The venom was obtained from recently captured rattlesnakes (1 day) and from snakes held in captivity. Venom was obtained from young and adult male and female snakes restrained in the usual manner, by applying pressure to the venom glands. To avoid bacterial contamination of the sterilised venom flask, they were covered with plastic film during collection. Only the snake's fangs were allowed to perforate the plastic film, with the venom being deposited into the flask free from environmental contamination.

Immediately after collection, the venom samples were placed on a blood-agar plate with the aid of a platinum wire. The plates were incubated for 24 hours at 37° C and examined. The colonies detected on the

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plates were examined and the microorganisms identified.

RESULTS

A total of 480 rattlesnakes were examined; 450 of them belong to the animal house of a private laboratory in the State of São Paulo, and the remaining 30 to the animal house of the Faculty of Medicine of Ribeirão Preto, University of São Paulo. The first group is maintained under controlled temperature and humidity conditions, and the second in an open animal pit. The snakes from the private laboratory were divided into two groups: 360 snakes held in captivity and 90 being held in quarantine after being captured (1 day earlier). The snakes belonging to the animal house of the Faculty of Medicine have been captive for a long time. The animals belonging to the private laboratory originated from Bauru, State of São Paulo.

Venom contamination was detected in 42 snakes, as shown in Table 1. The incidence of the microorganisms involved is shown in Table 2.

Table 1 – *Distribution and origin of contaminated snakes.*

Origin	*Captivity*		*Quarantine*		*Total*
	Infected snakes/Total number in the group	*%*	*Infected snakes/Total number in the group*	*%*	
Bauru	35/360	9.7	1/90	1.1	36
Faculty of Medicine of Ribeirão Preto	6/30	20.0	—		6
Total	41/390	10.5	1/90	1.1	42/480

Table 2 – *Incidence of microorganisms among the snakes studied.*

Microorganism	*Captivity*	*Quarantine*	*Total*
Bacillus subtilis	7	–	7
Escherichia coli	2	–	2
Monilia sp	3	–	3
Paracolobactrum aeroginoide	1	–	1
Proteus vulgaris	7	1	8
Pseudomonas aeruginosa	2	–	2
Shigella sp	2	–	2
Staphylococcus epidermides	22	–	22
Streptococcus haemolyticus	4	–	4
Streptococcus beta haemolyticus	2	–	2
Total	52	1	53

DISCUSSION

Several investigators have studied the contamination of cold-blooded animals, specially snakes, with particular emphasis on mouth contamination. Infections caused by salmonellae were detected by Hinshow and McNeil,[4] Zwart,[24] Le-Noc and Brygoo[9], Lins[10 11] Kourany et al [8] and Moreno et al[14]. Moreno et al[14] also detected other bacterial genera such as *Citrobacter* and *Proteus*. The high incidence of contamination with salmonella among snakes may be due to their crawling and to their feeding habits. However, the high incidence of contamination of low-body temperature animals with enterobacteriaceae is more difficult to explain, since these bacteria require higher temperatures to develop.

Conflicting results have been reported by the investigators who have studied the mouth flora of snakes [1 5 6 18 19 21 23]. All the mouth infections of snakes lead to the death of the animal.

In the present study, whose objetive was simply to study the contamination of venom, 8.75% of the captive snakes studied showed contamination as opposed to 1.11% of so-called wild snakes captured less than one day earlier. The wild snake was contaminated with a single bacterial genus, whereas several snakes held in captivity were contaminated with more than one genus of bacteria. These results suggest that contamination is due to handling which involves human contact and the consumption of contamined food and water[2 5 12 15 16]. The recently captured wild snake which was found to be contaminated may have been infected through handling, although it had been captive for only 24 hours. In view of the above considerations it is recommended that individuals bitten by snakes, in addition to being submitted to serum therapy, be also observed clinically for possible signs of bacteremia.

RESUMO

Os autores estudaram a contaminação bacteriana do veneno de cascavéis mantidas em cativeiro e das recentemente capturadas. Verificaram que os venenos dos animais cativos apresentaram alta incidência de contaminação e os tidos como recentemente capturados estavam com baixa contaminação aparente.

Palavras chaves: Veneno de cascavel. Contaminação bacteriana.

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