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AEROBIC ORAL BACTERIA IN HEALTHY CAPTIVE SNAKES

By

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SOVERI, T. and E.-R. SEUNA: *Aerobic oral bacteria in healthy captive snakes.* Acta vet. scand. 1986, 27, 172—181. — Cotton swab samples were taken from the ventral surface of the mouth and from the proximal esophagus from 23 captive nonpoisonous snakes. The samples were cultured and investigated for aerobic bacteria. Both the mouth and the esophagus samples of 6 snakes were negative. When the bacterial isolates of the mouth and the esophagus of the whole snake population were compared, it was found that the flora isolated from both locations were similar. However, when the samples of individual snakes were compared it was found that the same isolates were seldom found in both the mouth and the esophagus. The most common bacteria found were Pseudomonas sp., Alcaligenes-like organisms, Gram-positive rods and Gram-positive cocci belonging to the family Micrococcaceae. Important pathogens were seldom isolated. Salmonella virchow could be found from 2 snakes. The presence of bacteriologically negative samples, great variations in the composition of the flora between individual snakes, and the occurrence of typical environmental bacteria in the oral cavity all suggest that snakes lack a specific autochtonous flora and the bacteria isolated from the oral cavity may be occasional environmental bacteria. The source of pathogens may be the environment, too.

reptile; oral flora; mouth; esophagus; salmonella.

Bacterial stomatitis is considered to be one of the most common diseases of captive snakes (*Page* 1966, *Marcus* 1971, *Cooper* 1981). In a quantitatively small material concerning bacterial diseases of captive snakes in Finland 6 out of the 18 snakes studied suffered from stomatitis (*Soveri* 1984). Many different bacteria have been isolated from clinical cases, mainly belonging to the following genera: Aeromonas, Pseudomonas, Proteus, Staphylococcus and Streptococcus (*Frye* 1981). From these bacteria Gram-negative organisms, especially Pseudomonas aerugi-

nosa and Aeromonas spp., are considered to be very important reptile pathogens (*Cooper & Nares* 1971, *Cooper* 1981, *Selbitz & Elze* 1982). Various predisposing factors which lower the resistance of a snake are important in the pathogenesis of stomatitis (*Cowan* 1968, *Wallach* 1969, *Cooper* 1981).

The origin of reptile pathogenic bacteria has not yet been confirmed. It can be assumed that the pathogenic species are present in the normal flora, but in much lower numbers than in diseased animals. There is not much information available in the literature on the composition of oral flora in healthy snakes. Some studies have been carried out both on captive snakes (*Burke et al.* 1978, *Goldstein et al.* 1981, *Draper et al.* 1981) and snakes which had just been caught (*Williams et al.* 1934, *Ledbetter & Kutscher* 1969). There are no studies published in the Nordic countries, where snakes have been kept in captivity far away from their natural habitats.

The purpose of this study was to investigate the normal aerobic bacterial flora in the mouth and in the esophagus of captive snakes in Finland with reference to the feeding practice of the snakes.

MATERIALS AND METHODS

The snakes were exhibits in a travelling reptile show. Twenty three healthy snakes out of 80 were sampled in February 1983: seven of them were boas; 6 pythons; 4 ratsnakes; 2 anakondas, and 4 were different non-poisonous snakes mainly from North America. Concerning their diet 12 snakes were given chicken, 5 were given mice, 4 were given rats, and 2 were given fish. The frequency of feeding varied from once a week to once a month. Six snakes had been fed during the last 24 h and 17 snakes had not been fed during the last 9 days before sampling. The ages of the snakes varied from 3 months to over 10 years. All of them had either been in captivity for over 2 years or been born in captivity. Terrariums were cleansed at least once a week and the snakes were then usually handled. Fresh water was given to them every second day.

The transport medium for samples was made as follows: the salts solution was prepared according to *Holdeman & Moore* (1977). This solution was diluted 1:1 with distilled water. Gelatin (0.2 %) was added and then melted in a boiling water bath.

The solution was put into anaerobic test tubes under CO_2 flow. The medium was sterilized at 121°C for 15 min. Sterile cotton swabs were moistened with transport medium and parallel samples were taken from the ventral surface of the mouth and from the proximal esophagus. The swabs were transported in tubes filled with CO_2 gas.

The swabs were streaked on the following isolation media within 2 h: blood agar (Orion Diagnostica, Espoo, Finland), Colombia CNA-agar (BBL) and MacConkey agar (Difco). Parallel plates were inoculated from each sample, one being incubated at 20°C for 5 days and the other at 37°C overnight.

The proportion of different colony types was roughly estimated from the primary plates. The dominant colony types from each medium were subcultured on blood agar and nutrient agar for further examination. All isolates were Gram stained and tested for catalase activity. Gram-positive rods were examined for spores. Commercial test systems (Oxi Ferm Tube, F. Hoffmann La Roche, Basel, Switzerland; API 20 E, API-systems, La-Balme Les Grottes, France and Pathotec cytochrome oxidase test strips, General Diagnostics, Warner Lambert Company, New Jersey, U.S.A.) were used for differentiating between the Gram-negative rods. The samples were divided into 2 categories: 1. no growth; 2. one or more isolates.

Differences between time of feeding before sampling, different diets and between the 2 sampling locations were compared. The snakes were divided into two classes and into two subclasses each: (a) according to the time after their last feeding: 1. 24 h or less; 2. more than 9 days, and (b) according to diet: 1. chicken; 2. rats or mice. Chi-square tests were used in the statistical analysis. The number of snakes given fish was too low to allow statistical analysis.

RESULTS

Tentative idenfication of the bacteria isolated and the number of isolations are presented in Table 1, and the number of different bacteria isolated from individual snakes is presented in Table 2. Altogether 103 strains were picked from the primary cultures. Both the mouth and the esophagus samples of six out of 23 snakes were negative. Bacterial isolations were made from the esophagus only on 3 snakes, from the mouth only on 4 snakes and from both locations on 10 snakes.

Table 1. Bacteria isolated from healthy snakes.

Bacterium	No. of isolates	
	mouth	esophagus
Pseudomonas sp.	7	9
P. maltophila	2	1
P. aeruginosa	1	0
Alcaligenes-like organism	5	1
Enterobacteriaceae (unidentified)	1	0
Escherichia coli	1	2
Salmonella virchow	1	1
Klebsiella oxytoca	0	1
Proteus vulgaris	0	1
P. morganii	1	1
Serratia marcescens	1	0
S. liquefaciens	1	1
Micrococcaceae	6	5
Gram-positive rods		
— Group 1	7	3
— Group 2	3	6
— Group 3	0	1
Total	37	33

When the bacterial isolates of the mouth and the esophagus of the whole snake population were compared, it was found that the flora isolated from both locations was similiar (Table 1). However, when the parallel samples of individual snakes were compared, it was found that the same isolates were seldom found in both the mouth and the esophagus. There was only 1 snake from which we isolated 4 bacteria in common to both the mouth and the esophagus. From 7 snakes 1 strain was isolated in common to both locations.

Table 2. Number of bacterial strains isolated from 23 snakes.

No. of isolates	Mouth	Esophagus
0	9	10
1	2	5
2	7	1
3	2	3
4	1	2
5	1	1
6	1	1

Eight different oxidase positive Gram-negative rods belonged to the genus Pseudomonas according to the OxiFerm test. Biochemically inactive oxidase positive bacteria were tentatively identified as Alcaligenes-like organisms. Gram-negative bacteria only were isolated from 3 snakes.

Gram-positive rods were isolated from 13 snakes. These bacteria were not further identified. All the strains were catalase positive and did not form spores. Three morphologically different types were found. Group 1 consisted of small coccoid rods. The colonies on blood agar were thin, white and rough with a dry surface and they appeared after 2 days of incubation. Group 2 consisted of small short rods. These organisms formed smooth, grey, convex colonies and they also appeared after a prolonged incubation. Group 3 was isolated just once at 20°C. The colonies were tiny, translucent and smooth. The bacteria in group 3 were large rods.

Gram-positive cocci belonged to the family Micrococcaceae. All the strains formed a yellow pigment and were non-haemolytic. Six snakes had an abundant Gram-positive flora, and in 4 samples of these the Gram-positive flora exceeded the Gram-negative flora.

No association was found between the presence of bacterial flora and recent feeding in the statistical analysis of the data. Moreover the type of the feed was not in association with the presence of the flora.

DISCUSSION

Considerable variation was shown in the composition of the oral bacterial flora of the individual snakes. Because all the snakes were sampled at the same time and in the same manner, we assume that methodological errors cannot fully account for the fact that both the number of different strains and also the amount of the growth on the primary plates varied considerably. Negative findings may be false negatives and may indicate the insensitivity of the method to detect low numbers of bacteria.

There is no general agreement about the typical oral bacterial flora of snakes. *Burke et al.* (1978) and *Goldstein et al.* (1981) reported a predominantly Gram-negative flora, whereas Corynebacterium sp. was the organism most frequently isolated by *Draper et al.* (1981). *Goldstein et al.* and *Ledbetter & Kutscher* (1969) did not isolate corynebacteria. Corynebacteria were also

rare in the study of *Burke et al.* We isolated Gram-positive rods that may be corynebacteria, Kurthia and related species from 13 out of 23 snakes.

Pseudomonas sp. were isolated more frequently in the present study than previously reported in *Burke et al., Goldstein et al.,* and *Draper et al.* Mima, which is now included in the genera Acinetobacter and Moraxella (*Carter* 1984) was a common isolation in the study of *Burke et al. Goldstein et al.* isolated Acinetobacter frequently. We did not isolate any organisms of that group. However, our group Alcaligenes-like organisms may be related to Mima organisms. The present confusion in the taxonomy as well as the differences in methods used for identification makes it difficult to compare the results of different studies.

Salmonella spp. have been isolated frequently from reptiles, including snakes (*Müller* 1972, *Mayer & Frank* 1974, *Chiodini & Sundberg* 1981). Salmonella spp. are rather common isolates from reptiles also in Finland (Annual Reports of the State Veterinary Medical Institute 1970—1984). These isolations have been made mainly from intestines and faeces. Only a few reports of Salmonella spp. from the oral flora of snakes have been published (*Ledbetter & Kutscher* 1969, *Goldstein et al.* 1981), which suggests that Salmonella spp. are much more common in the intestinal than in the oral flora of snakes. Still, mouths of snakes are potential sources of human salmonellosis. S. virchow could be isolated from 2 snakes in our investigation. This serotype is very rare in animals in Finland. It has been isolated only 4 times during the last 15 years. All the isolations have been done from faeces of captive snakes (Annual Reports of the State Veterinary Medical Institute 1970—1984). According to these reports it seems evident that snakes may have had S. virchow from other snakes or originally from their breeding environments rather than from their prey in Finland. S. virchow isolations from human sources in Finland have been more common during the years 1979—1983 (10—54 cases yearly) than 1971—1978 (0—10 cases yearly). Over 70 % of them were of foreign origin (Annual Reports of the National Public Health Institute 1971—1983). Theoretically these human cases can be sources for snake salmonellosis and vice versa.

In mammals the autochtonous oral flora colonizes the mucous membranes persistently (*Sonnenwirth et al.* 1980). Attach-

ment to the host cells is the first step in colonization. This mechanism was not studied in present work or in former investigations about reptile oral flora. Thus, the bacteria isolated from the oral cavity may be either autochtonous or allochtonous organisms. Snakes are in close contact with their environment, which act as a constant source of bacteria for them. That can give an impression of a persistent flora even when the bacteria do not colonize. It is worth noting that most of the bacteria frequently isolated from the oral cavity of snakes are common inhabitants of soil and fresh water (*Buchanan & Gibbons* 1974). Many of them are also commonly found in the intestines of snakes (*Mayer & Frank* 1974).

The presence of bacteriologically negative samples, great variations in the composition of the flora between individual snakes when housed separately, and the occurrence of typical environmental bacteria in the oral cavity all suggest that snakes lack a specific autochtonous flora and the bacteria isolated from the oral cavity may be occasional environmental bacteria. However, the presence of a rare Salmonella serotype, on the other hand, indicates persistent colonization either in the mouth or intestines, because this organism is not present or is very rare in the environment of the snakes in Finland. The oral flora of mammals needs time for development (*Sonnenwirth et al.* 1980), but the oral flora of snakes seems to be complete soon after birth. *Goldstein et al.* (1981) found that the flora of snakes sampled within 4 h after birth and before the first feeding was comparable with the flora of older snakes; although the number of sterile samples was greater in young snakes. This agrees with our findings that the feed did not have any effect on the flora.

Potential pathogens like Pseudomonas aeruginosa and Proteus sp. were seldom isolated. Aeromonas spp. were not found. The source of these bacteria is probably the environment, too. P. aeruginosa, A. hydrophila and Proteus sp. are not primary pathogens for mammals, but they are important organisms in oral infections of snakes. One reason can be the ability of these organisms to grow at low temperatures and form toxins. Secondly, they may be able to attach themselves to the damaged mucous membranes of snakes more effectively than to mammal cells. A third explanation could be the lack of protective normal flora in the oral cavity of snakes, which would then favour the colonization of pathogens.

The animals in our study generally had a rather simple flora and the number of negative findings was higher than reported in other studies. If the oral flora were dependent on the environment, snakes kept in captivity in clean terrariums and given only tap water should have flora which is less complex and lower in number than either the flora of snakes kept in less hygienic conditions, or the flora of wild snakes living in habitats rich in bacteria. *Williams et al.* (1934) and *Fischer et al.* (1961) have reported that snakes which had just been caught had less bacteria than captive snakes. This may be due to scanty environmental flora, e.g. on hot, dry areas, or to the accumulation of bacteria in terrariums not cleansed frequently. Much more work should be done to find out the effect of the environment on the oral bacterial flora of snakes. Wild snakes living in different habitats could be an interesting study material for this purpose.

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SAMMANDRAG

Aeroba orala bakterier hos friska ormar i fångenskap.

Med hjälp av bomullsstickor togs prov från munhålans ventrala yta och från matstrupens proximala del från 23 ickegiftiga ormar i fångenskap. Proven odlades med avseende på aeroba bakterier. Hos 6 ormar var proven från bode munhålan och matstrupen neagtiva. När bakterieisolaten från munhålan och matstrupen från hela ormpopulationen jämfördes, kunde man konstatera att floran isolerad från boda ställena var likartad. När däremot prov från individuella ormar jämfördes sinsemellan fann man att samma isolat sällan påvisades både i munhålan och matstrupen. De vanligaste bakterierna var Pseudomonas sp., Alcaligenes-liknande organismer, Gram-positiva stavar och Gram-positiva cocci hörande till familjen Micrococcaecae. Viktiga patogener isolerades sällan. Förekomsten av bagteriologiskt

negativa prov, stora variationer i sammansättningen av floran mellan individuella ormar, och förekomst av typiska miljöbakterier i munhålan tyder på att ormarna saknar en specifik autokton flora och att bakterierna isolerade från munhålan kan vara tillfälliga miljöbakterier. De patogena bakterierna kan också härstamma från den omgivande miljön.

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