BACTERIAL INFECTIONS AND TREATMENT WITH ANTIBIOTICS IN SNAKES, a recent vision. Part 1.

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INTRODUCTION

Bacterial pathogens may play an important role in disease in reptiles, as a primary as well as secondary causitive agent (Cooper, 1981; Ippen and Schroder, 1977; Ross and Marzec,1984). In contrast with the diagnosis of protozoal infections in case of diarrhea e.g. caused by flagellates or coccidia, the diagnosis in bacteriological examninations is not always that clear. Sometimes it is hard to conclusively demonstrate a causal relationship between the bacteria cultured and the disease.
Bacteriological examinations of samples taken from reptiles often reveal a mixture of micro-organisms. Further more comparative research for normal and pathogenic bacterial flora is limited. With some researchers there are contradictions concerning the pathogenic character of certain bacteria, the Salmonella species being the most illustrative example. On the one hand the Salmonella bacteria are considered to belong to the normal flora of reptiles, being non-pathogenic (Cooper, 1981) or only pathogenic under certain conditions (Chiodine, 1983). On the other hand however, others consider them the pathogenic agent of various diseases (Ippen and Schroder,1977; Frye, 1981).
During the last two years, about a hundred bacteriological examinations of samples from

reptiles were carried out. The investigated samples originated from a collection of snakes which were clinically healthy as well as from animals showing clinical signs of disease.
The purpose of this study is to gain insight in the composition of the normal as well as pathogenic flora of reptiles and above all to conclusively demonstrate a causal relationship between clinical infection and cultured micro-organisms.

MATERIALS AND METHODS

The result of bacteriological examination mainly depends on correct sampling and fast transport of the samples to a laboratory. It is of great importance to take the sample from the middle of the focus of infection, thus avoiding already destroyed micro-organisms from the periphery of the spot (Needham, 1983).
For the collection and transport of faecal samples plastic, sterile containers were used. All other samples were taken with sterile swabs and transported in transport medium (Microdiagnostics).
For primary culture the following media were used (depending on the nature of the sample to be investigated: SALMONELLA-SHIGELLA AGER (Oxoid), MAC CONKEY AGAR (Oxoid) no. 3, MANNITOL SALT AGAR (Oxoid), COLUMBIA AGAR NSC (Merck) + 5% horse blood, YERSINIA AGAR 5 (Bio-Merieux), C.L.E.D. MEDIUM (Oxoid), AEROMONAS AGAR (Gibco), CAMPYLOBACTER AGAR (BLASER) (bio-Merieux), BLOOD AGAR BASE (Oxoid) + 5% horse blood, CHOCOLADE-POLYVITEX-BACITRACINE AGAR (Bio-Merieux), SABOURAUD AGAR (gluc. 2%) + ACTIDIONE + CHLORAMHENICOL (Pasteur), DERMATOPHYTEN AGAR to TAPLIN (Merck).
Incubation was at 36 and 28°C under normal, CO2-rich, anaerobic or microaerophyl conditions (BBL Gaspak systems), depending on the sample to be examined. Culturing for anaerobic bacteria was

only carried out when the sample was foul, rotten smelling (e.g. scale rot) and that there was little time between sampling and culturing. Secondary isolation was done on KLIGLER IRON AGAR (Oxoid) and of BASAL MEDIUM (HUGH AND LEIFSON) + 1% glucose (Eco-Bio).
Determination of the bacteria was done with classic determination media:
MOBILITEIT-INDOL-UREASE MEDIUM, SIMMON'S CITRAAT AGAR, FENYLALANINE MEDIUM, DECARBOXYLASE MEDIUM (LYSINE, ARGININE, ORNITHINE) MUCAAT AGAR, MALONATE BROTH, M.R.-V.P. BOUILLON, CYSTINE TRYPTI-CASE MEDIUM (Eco-Bio) + GLUCI-DISKS (B.D.), through enzymatic reactions with Rosco-diagnostic-tablets and commercially obtained systems API.

COMPOSITION OF THE SAMPLES AND POPULATION RESEARCH

One hundred and twenty four samples were taken, more than half being faecal samples. They were collected from a sample of 92 animals, mainly snakes from a zoological collection and from private collections. In table 2 the species sampled are summarized. In this population of snakes clinical symptoms of disease were seen in 46 animals. Pneumonia (14), gastro-intestinal disorders (non-protozoal, 13), stomatitis ulcerosa (2), oropharyngeal cellulitis (1), rhinitis (2), abcesses (2), skin furuncle (1), necrotic dermatitis (3), fungal dermatitis (2), infected burns (1), sepsis (2), death through unknown cause (3).
In additon samples were taken, for the greater part faecal samples, and from the oropharyngeal cavity from clinically healthy animals.

BACTERIAL FLORA IN OPHIDIA

THE INTESTINAL FLORA

The intestinal flora of snake consists mainly of gram-negative rod-shaped bacteria. In table 3 the proportionally ratio of the bacteria cultured are summarized and expressed as part of the total number of isolations. Enterobacterteriaceae (72,6%) and Pseudomonadaceae (19.1%) were the most frequently cultured micro-organisms.
Gram-positive bacteria were seldom isolated (1.3%). Sometimes yeasts and fungi were isolated from the faecal samples, mostly after prolonged treatment with antibiotics causing dysbacteriosis.
In agreement with others (Mayer and Frank, 1974), a high percentage of Salmonella species was cultured, which is even clearer when the numbers of bacteria cultured are compared with the total number of faecal samples examined (table 4). Salmonella species were isolated from no less than half of the faecal samples cultured. These samples were taken from a population of animals consisting mostly (75%) of clinically asymptomatic animals (proportionally ratio calculated from representative samples: 56 samples were considered representative; not-representative were samples from animals with diarrhea caused by protozoal infection and samples without identification).

THE OROPHARYNGEAL FLORA

The bacterial flora from the oral cavity mainly consists of gram-negative rod-shaped bacteria (table 5). However, a slight proportional shift towards the non-enterobacteriaceae (Pseudomonas, Aeromonas, Acinetobacter) has taken place. When percentages from isolated bacteria spp are expressed as part of the total amount of samples examined (table 6) Escherichia coli and Pseudomonas aeruginosa prove to be the most frequently occuring species, followed by Ps. maltophilia, Salmonella spp, Aeromonas hydrophila and Proteus mirabilis.

Remarkable is the isolation of Salmonella spp from oropharyngeal samples. The Salmonella were cultured from samples taken from snakes with signs of pneumonia. In oropharyngeal samples cultured from freshly captured snakes and captive snakes from Papua-New Guinea no Salmonella spp were isolated (Ross and Marzec, 1984). Unfortunately there was no mention if the specimens examined showed clinical signs of disease.

NUMBER OF THE SAMPLES	124
NATURE OF THE SAMPLES	
FEACAL	67
OROPHARYNGEAL	27
GINGIVAL SECRETUM	6
EPIDERMAL LESIONS	5
CLOACAL SECRETUM	3
NASOPHARYNGEAL MUCUS (PURULENT)	3
TRACHEAL MUCUS (PURULENT)	2
CLOACAL ULCUS	2
LUNG (POST MORTEM)	2
LIVER (POST MORTEM)	2
PIOSON	2
ABDOMINAL CYST (POST MORTEM)	1
FURUNCLE	1
NECROTIC TISSUE	1

Tabel 1: Review of the samples examined.

SAURIA

EUBLEPHARIS MACULARIUS
LACERTA LEPIDA LEPIDA
LACERTA DUGESII

OPHIDIA

PYTHON RETICULATUS
PYTHON MOLURUS
PYTHON CURTUS
PYTHON REGIUS
MORELIA ARGUS VARIEGATA
LIASIS CHILDRENI
CHONDROPYTHON VIRIDIS
EPICRATES ANGULIFER
EPICRATES CENCHRIA CENCHRIA
EPICRATES CENCHRIA MAURA
LICHANURA TRIVIRGATA
CORALLUS CANINUS
CORALLUS ENYDRIS ENYDRIS
BOA CONSTRICTOR
LAMPROPELTIS TRIANGULUM SINALOAE
LAMPROPELTIS MEXICANA ALTERNA
LAMPROPELTIS ZONATA PULCHRA
LAMPROPELTIS GETULUS FLORIDANA
ELAPHE sp.
ELAPHE GUTTATA ssp.
ELAPHE GUTTATA GUTTATA
ELAPHE OBSOLETA LINDHEIMERI
ELAPHE OBSOLETA ROSALLENI
ELAPHE OBSOLETA QUADRIVITTATA
BOIGA DENDROPHILA
NAJA NAJA NAJA
NAJA NAJA KAOUTHIA
NAJA HAJE HAJE
NAJA MOSSAMBICA
NAJA PALLIDA
HEMACHATUS HAEMACHATUS
BUNGARUS MULTICINCTUS

BITIS GABONICA RHINOCEROS
BITIS NASICORNIS
BITIS ARIETANS
CERASTES CERASTES CERASTES
ERISTOCOPHIS NACMAHONII
VIPERA ASPIS ASPIS
VIPERA AMMODYTES TRANSCAUCASIANA
VIPERA KAZNAKOVI
VIPERA URSINII URSINII
VIPERA LEBETINA SCHWEIZERI
VIPERA RADDEI RADDEI
VIPERA RUSSELLI ssp.
CROTALUS LEPIDUS ssp.
CROTALUS CERASTES ssp.
CROTALUS DURISSUS ssp.
CROTALUS DURISSUS TERRIFICUS
CROTALUS ENYO ssp.
CROTALUS WILLARDI
CROTALUS RUBER ssp.
TRIMERESURUS FLAVOVIRIDIS
TRIMERESURUS KANBURIENSIS
TRIMERESURUS OKINAVENSIS

Tabel 2: Review of the species of snakes examined.

Gr - STAPHYLOCOCCUS (93,83%)

ENTEROBACTERIACEAE 72,6%

	N (=146)	%	
SALMONELLA subgroup III	28	19,18	
SALMONELLA subgroup I	5	3,42	23,28
SALMONELLA subgroup II	1	0,68	
ESCHERICHIA COLI	28	19,18	
PROVIDENCIA RETTGERI	11	7,53	
PROTEUS MIRABILIS	11	7,53	
CITROBACTER FREUNDII	9	6,16	
MORGANELLA MORGANII	6	4,11	
PROTEUS VULGARIS	4	2,74	
PROVIDENCIA ALCALIFACIENS	2	1,37	
PROVIDENCIA STUARTII	1	0,68	

PSEUDOMONADACEAE 19,18%

PSEUDOMONAS AERUGINOSA	19	13,01
PSEUDOMONAS PUTIDA	4	2,74
PSEUDOMONAS FLUORESCENS	2	1,37
PSEUDOMONAS STUTZERI	1	0,68
PSEUDOMONAS CEPACIA	1	0,68
PSEUDOMONAS PUTREFACIENS	1	0,68
AREOMONAS HYDROPHILA	1	0,68
ACINETOBACTER CALCOACETICUS (biovar LWOFFI)	1	0,68
PASTEURELLA MULTOCIDA	1	0,68

Gr + COCC. 1,37%

STAPHYLOCOCCUS EPIDERMIDIS	1	0,68
STREPTOCOCCUS FAECALIS	1	0,68

YEAST + FUNGI 4,79%

RHODOTORULA GLUTINIS	1	0,68
CANDIDA GUILLERMONDI	1	0,68

CANDIDA KRUSEI	1	0,68
PHYCOMYCETES sp.	2	1,37
MUCOR sp.	1	0,68
FUNGUS undeterminated	1	0,68

Tabel 3: Proportional ratio of the isolated bacteria spp from faecal samples as part of the total number of bacterial isolations.

Gr - STAPHYLOCOCCUS

ENTEROBACTERIACEAE

	N (=67)	%	
SALMONELLA subgroup III	28	41,79	
SALMONELLA subgroup I	5	7,46	50,74
SALMONELLA subgroup II	1	1,49	
ESCHERICHIA COLI	28	41,79	
PROVIDENCIA RETTGERI	11	16,42	
PROTEUS MIRABILIS	11	16,42	
CITROBACTER FREUNDII	9	13,43	
MORGANELLA MORGANII	6	8,95	
PROTEUS VULGARIS	4	5,97	
PROVIDENCIA ALCALIFACIENS	2	2,98	
PROVIDENCIA STUARTII	1	1,49	

PSEUDOMONADACEAE

PSEUDOMONAS AERUGINOSA	19	28,36	
PSEUDOMONAS PUTIDA	4	5,97	
PSEUDOMONAS FLUORESCENS	2	2,98	
PSEUDOMONAS STUTZERI	1	1,49	
PSEUDOMONAS CEPACIA	1	1,49	
PSEUDOMONAS PUTREFACIENS	1	1,49	
AEROMONAS HYDROPHILA	1	1,49	
ACINETOBACTER CALCOACETICUS (biovar LWOFFI)	1	1,49	
PASTEURELLA MULTOCIDA	1	1,49	

Gr + COCC.

STAPHYLOCOCCUS EPIDERMIDIS	1	1,49	
STREPTOCOCCUS FAECALIS	1	1,49	

YEAST + FUNGI

RHODOTORULA GLUTINIS	1	1,49	

CANDIDA GUILLERMONDI	1	1,49
CANDIDA KRUSEI	1	1,49
PHYCOMYCETES sp.	2	2,98
MUCOR sp.	1	1,49
FUNGUS undeterm.	1	1,49

Tabel 4: Proportional ratio of the isolated bacterria spp in faecal samples as part of the total number of faecal samples examined.

Gr - STAPHYLOCOCCUS (82%)

ENTEROBACTERIACEAE (50%)

	N (=50)	%	
ESCHERICHIA COLI	7	14,00	
SALMONELLA subgroup III	3	6,00	8,00
SALMONELLA subgroup I	1	2,00	
PROTEUS MIRABILIS	3	6,00	
MORGANELLA MORGANII	2	4,00	
CITROBACTER FREUNDII	2	4,00	
PROVIDENCIA RETTGERI	1	2,00	
PROVIDENCIA ALCALIFACIENS	1	2,00	
ENTEROBACTER CLOACEAE	1	2,00	
KLEBSIELLA PNEUMONIAE	1	2,00	
KLEBSIELLA OXYTOCA	1	2,00	

PSEUDOMONADACEAE (26%)

PSEUDOMONAS AERUGINOSA	7	14,00	
PSEUDOMONAS MALTOPHILIA	4	8,00	
PSEUDOMONAS STUTZERI	1	2,00	
PSEUDOMONAS ALCALIGENES	1	2,00	
AEREOMONAS HYDROPHILA	3	6,00	
ACINETOBACTER CALCOACETICUS (biovar LWOFFI)	2	4,00	

Gr + COCC. (8%)

STAPHYLOCOCCUS EPIDERMIDIS	2	4,00	
MICROCOCCUS sp.	2	4,00	

Gr + staphylococcus (2%)

CORYNEBACTERIUM sp.	1	2,00	

YEAST + FUNGI (8%)

RHODOTORULA RUBRA	1	2,00
RHODOTORULA GLUTINIS	1	2,00
CANDIDA PARAPSILOSIS	1	2,00
MUCOR sp.	1	2,00

Tabel 5: Proportional ratio of the isolated bacteria spp in oropharyngeal samples as part of the total number of bacterial isolations.

Gr - STAPHYLOCOCCUS

ENTEROBACTERIACEAE

	N (=27)	%
ESCHERICHIA COLI	7	25,92
SALMONELLA subgroup III	3	11,11
SALMONELLA subgroup I	1	3,70
PROTEUS MIRABILIS	3	11,11
MORGANELLA MORGANII	2	7,41
CITROBACTER FREUNDII	2	7,41
PROVIDENCIA RETTGERI	1	3,70
PROVIDENCIA ALCALIFACIENS	1	3,70
ENTEROBACTER CLOACEAE	1	3,70
KLEBSIELLA PNEUMONIAE	1	3,70
KLEBSIELLA OXYTOCA	1	3,70
PSEUDOMONADACEAE		
PSEUDOMONAS AERUGINOSA	7	25,92
PSEUDOMONAS MALTOPHILIA	4	14,80
PSEUDOMONAS STUTZERI	1	3,70
PSEUDOMONAS ALCALIGENES	1	3,70
AEREOMONAS HYDROPHILA	3	11,11
ACINETOBACTER CALCOACETICUS (biovar LWOFFI)	2	7,41
Gr + COCC.		
STAPHYLOCOCCUS EPIDERMIDIS	2	7,41
MICROCOCCUS sp.	2	7,41
Gr + staphylococcus		
CORYNEBACTERIUM sp.	1	3,70
YEAST + FUNGI		

RHODOTORULA RUBRA	1	3,70
RHODOTORULA GLUTINIS	1	3,70
CANDIDA PARAPSILOSIS	1	3,70
MUCOR sp.	1	3,70

Tabel 6: Proportional ratio of the isolated bacteria spp from oropharyngeal samples as part of the total number of samples examined.