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ANAEROBIC AND AEROBIC BACTERIOLOGY OF THE SALIVA AND GINGIVA FROM 16 CAPTIVE KOMODO DRAGONS (*VARANUS KOMODOENSIS*): NEW IMPLICATIONS FOR THE "BACTERIA AS VENOM" MODEL

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Abstract: It has been speculated that the oral flora of the Komodo dragon (*Varanus komodoensis*) exerts a lethal effect on its prey; yet, scant information about their specific oral flora bacteriology, especially anaerobes, exists. Consequently, the aerobic and anaerobic oral bacteriology of 16 captive Komodo dragons (10 adults and six neonates), aged 2–17 yr for adults and 7–10 days for neonates, from three U.S. zoos were studied. Saliva and gingival samples were collected by zoo personnel, inoculated into anaerobic transport media, and delivered by courier to a reference laboratory. Samples were cultured for aerobes and anaerobes. Strains were identified by standard methods and 16S rRNA gene sequencing when required. The oral flora consisted of 39 aerobic and 21 anaerobic species, with some variation by zoo. Adult dragons grew 128 isolates, including 37 aerobic gram-negative rods (one to eight per specimen), especially *Enterobacteriaceae*; 50 aerobic gram-positive bacteria (two to nine per specimen), especially *Staphylococcus sciuri* and *Enterococcus faecalis*, present in eight of 10 and nine of 10 dragons, respectively; and 41 anaerobes (one to six per specimen), especially clostridia. All hatchlings grew aerobes but none grew anaerobes. No virulent species were isolated. As with other carnivores, captive Komodo oral flora is simply reflective of the gut and skin flora of their recent meals and environment and is unlikely to cause rapid fatal infection.

Key words: anaerobe, bacteriology, Komodo dragon, oral, venom, *Staphylococcus sciuri*, clostridia.

INTRODUCTION

Komodo dragons are native to only a few of the Indonesian Islands that include Komodo, Rinca, Flores, Gili Motang, and Gili Dasami, but many Komodo dragons are held in captivity in zoos worldwide. They are the largest living lizards, reaching a length of approximately 10 ft and weighing 150 lb. Komodos are carnivores and in the wild they prey on wild pigs, deer, and water buffalo, and they will occasionally eat carrion. In captivity, they are fed a diet that varies by zoo and typically consists of a combination of whole poultry, rodents, fish, and cut meats.

How Komodo dragons kill their prey is a controversial topic.[11] Until recently, the "bacteria as venom" dogma prevailed, based largely on a report by Auffenberg[6] and later echoed in a study by Montgomery et al.[33] After spending a year observing Komodos in the wild, Auffenberg reported that when large Komodos attacked larger prey such as deer or water buffalo, those animals that were only injured would be overcome by infection and vulnerable to future predation. It was therefore postulated that "induction of wound sepsis and bacteremia through the bite of the Komodo dragon may be a mechanism for prey debilitation and mortality." Montgomery et al.[33] later concurred with this model, citing the results in their study of aerobic oral flora; however, this study was before the discovery of venom glands in Komodo dragons.[20,21]

Fry et al.[20,21] published the first in-depth, multidisciplinary analysis of Komodo bite mechanics and venom biochemistry by using magnetic resonance imaging of the skull and showed compound mandibular venom glands with ducts opening between successive serrated pleurodont teeth. Biochemical analysis of the venom revealed components similar to those of snake venoms that cause coagulopathy, hypotension, hemorrhage, and shock. They further proposed that the Komodo dragons actually kill their prey either by tear injury from large serrated teeth acting as the primary weapon, causing severe mechanical

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damage and a very rapid mortality from blood loss (e.g., slicing the femoral artery); or, in instances where injury is not immediately life-threatening, venom exaggerates blood loss, induces shock, and thus facilitates death. Furthermore, it was postulated that Komodo oral flora is obtained by "passive acquisition" and reflective of the bacteria of their diet and that any infection caused by these bacteria is incidental rather than a primary means of killing.[20,21]

Komodo dragons have been reported to bite zoo visitors, park rangers, tour guides, and zoo keepers and even killed a 9-yr-old boy.[2,4,5,15,18,36,37,40] Thus far, the proper antimicrobial therapy for Komodo dragon bite infections has not been defined as there have been no bacteriologic reports about these wounds published; therefore, this study also assists clinicians in the appropriate selection of empirical antimicrobial therapy.

To more comprehensively characterize Komodo dragon oral flora and the potential role of oral bacteria in prey mortality, the aerobic and anaerobic flora of 16 captive dragons were studied, including six hatchlings, of which three were cultured before their first meal. No previous study has reported on the anaerobic bacteriology of Komodo dragon flora.

MATERIALS AND METHODS

After a web-based search to identify zoos with resident Komodo dragons (http://app.isis.org/abstracts/Abs50024.asp, accessed: 4 February 2010), participation of these zoos in this project was solicited. Animal Welfare and Research Committee approval was obtained from the participating zoos (Honolulu, Hawaii, USA; Houston, Texas, USA; and Los Angeles, California, USA).

Demographic data, including age, sex, weight, birthplace, diet, meal frequency, and date of last feeding, were collected for each Komodo dragon (Table 1). Samples were collected by zoo personnel from five male and five female adult dragons by pipette for saliva or by swabbing the gingiva at least 1 day after the last feeding. Samples were inoculated into anaerobic transport media (Anaerobe Systems, Morgan Hill, California 95037, USA) that were shipped within 1–2 days via courier to the R. M. Alden Research Laboratory in Culver City, California. Gingival samples from six hatchling dragons, three that had not yet eaten and three that had one meal, were obtained in the same manner. Upon receipt in the laboratory, the samples were placed into the anaerobic chamber for inoculation of anaerobic culture media and then removed from the chamber to inoculate media for aerobic culture. Current bacterial culture techniques were used but did not explore the presence of uncultivable bacteria using molecular methods.

Aerobic media included sheep blood, chocolate, and Rose and MacConkey agars (Hardy Diagnostics, Santa Maria, California 93455, USA) that were incubated aerobically for 24–48 hr at 37°C and then examined. Anaerobic media included supplemented Brucella blood, phenylethyl alcohol blood, laked blood with kanamycin and vancomycin, Bacteroides bile esculin, and egg yolk agars. The plates were incubated in the anaerobic incubator at 37°C and examined after 3–5 days. All plates were held for up to 7 days. The various colony types were subcultured for purity and frozen at −70°C in 20% skim milk.

Strains were identified using standard methods, including gram stain, catalase, oxidase, indole, special potency antibiotic disks, and the identification kits API 20 E, API 20 A (bioMérieux, Inc., Hazelwood, Missouri 63042, USA) and RapID ANA II (Remel, Inc., Lenexa, Kansas 66215, USA).[28,34] Strains with presumptive or no identification achieved by using standard methods were further identified using 16S rRNA gene sequencing.

16S rRNA gene sequencing was performed similarly to the procedure described previously.[46] Cellular DNA was extracted using the DNeasy tissue kit (QIAGEN, Valencia, California 91355, USA). Amplification of 16S rDNA used two universal primers, 8UA and 907B (positions 8 and 907, *Escherichia coli* numbering).[10] Polymerase chain reaction (PCR) products were purified using the QIAquick PCR purification kit (QIAGEN). Purified DNA was sequenced directly (Laguna Scientific Laboratory, Laguna Beach, California 92677, USA) with a Z-BigDye version 3 sequencing kit (Applied Biosystems, Foster City, California 94404, USA) on an ABI 3730XL sequencer (Applied Biosystems). The resulting sequences, approximately 850 base pairs (bp), were compared with sequences in the National Center for Biotechnology Information (Bethesda, Maryland 20894, USA) GenBank database using BLAST software, and the closest similarity to type sequence deposits or validly published type strains was determined.[3,7] Similarity >99% was considered species identity, 97–99% genus identity, and <97% was described as having no genus-level identification. Organisms without a species match and that were <2% dissimilar were grouped together.

Table 1. Captive Komodo dragon demographic, diet, and microbiologic data summary.

						Diet					Strains isolated			
											Aerobes		Anaerobes	
Dragon no.	Sex	Weight (kg)	Age	Specimens/ dragon	Feeding frequency	Poultry whole	Poultry parts	Rodents whole	Fish whole	Other	Gram negative	Gram positive	Gram negative	Gram positive
Adults														
HH1[a]	Female	19	9 yr 7 mo	3[b]	One time/wk	Chicks		Rats	Smelt		3	7	2	4
HH2	Female	17	9 yr 7 mo	3[b]	One time/wk	Chicks		Rats	Smelt		7	6	0	6
HH3	Female	15	9 yr 7 mo	1	One time/wk	Chicks		Rats	Smelt		3	4	0	3
HH4	Male	46	17 yr	3[b]	One time/wk	Chicks		Rats	Smelt		8	0	1	4
HT1	Male	84	11 yr 9 mo	3[b]	One time/wk plus snacks	Quail, turkey		"Rodents"		Rabbits, eggs[c]	6	5	0	5
HT2	Male	8	2 yr 10 mo	1	Two to three times/wk	Quail		"Rodents"		Eggs	1	3	0	2
HT3	Male	7	2 yr 10 mo	1	Two to three times/wk	Quail		"Rodents"		Eggs	2	2	2	2
HT4	Female	7	2 yr 10 mo	1	Two to three times/wk	Quail		"Rodents"		Eggs	1	3	0	2
LA1	Female	29	5 yr 10 mo	1	Two times/wk	Quail	Chicken	Rats	Fish	Horse meat	2	6	3	2
LA2	Male	58	11 yr 2 mo	1	Two times/wk	Quail	Chicken	Rats	Fish	Horse meat	4	5	1	2
Hatchlings														
LA4, 5, 6	—	—	9–10 days	1	Once			Baby mice			12	12	0	0
LA3	—	—	7 days	1	Never						0	2	0	0
LA7, 8	—	—	7 days	1	Never						7	7	0	0

[a] HH, Honolulu, Hawaii; HT, Houston, Texas; LA, Los Angeles, California.
[b] Including one saliva and two gingival samples; number of strains isolated were averaged from the three specimens.
[c] Hard-boiled chicken eggs.

There were two instances of cases where there were several strains with genus-level identification but that had no named species (six *Clostridium* and five *Corynebacterium* spp.). A multiple alignment of the strains was performed to determine whether any of the strains within each genus had similarity. The multiple alignment of each group of sequences was created with MEGA4[44] using its native implementation of CLUSTAL W in the Alignment Explorer Tool, followed by further manual sequence correction using MEGA4 and FinchTV (Geospiza, Inc., Seattle, Washington 98119, USA) and trimming to uniform dataset length (804 and 770 bp, respectively).[44] Evolutionary analyses were conducted in MEGA4. Divergence between sequences was determined using a bootstrap procedure (500 repetitions).[17] Analyses were conducted using the Jukes–Cantor model, and all positions containing gaps and missing data were eliminated.[29] Phylogenetically similar organisms were grouped together.

RESULTS

Culture results are summarized in Table 1, along with demographic data and dietary information. The five males and five females ranged in age from 2 yr 9 mo to 17 yr and weighed 7–84 kg. All six hatchlings were born at the Los Angeles Zoo between 14 and 17 August 2010. Three were cultured on day 7 after hatching and before their first feeding, and three were cultured on days 9–10 just after their first meal of baby mice.

The aerobic culture results from this study are given in Table 2 and compared with the findings of a previous study.[33] We isolated 128 strains (87 aerobic and 41 anaerobic), with a median of 10 isolates per culture (range, 6–22) per adult dragon. Most isolates were recovered in moderate-to-heavy growth. All adult dragons grew aerobic gram-negative rods (37 total, one to eight per specimen), 95% of which were *Enterobacteriaceae*; aerobic gram-positive bacteria (50 total, two to nine per specimen), especially *Staphylococcus sciuri* and *Enterococcus faecalis*, were present in eight of 10 and nine of 10 adult dragons, respectively. In total, there were 18 gram-negative and 21 gram-positive aerobic species found.

Table 3 lists the anaerobic isolates recovered in the current study. All adult dragons grew anaerobic gram-negative and gram-positive species (41 total, one to six per specimen), especially clostridia. There were 13 different *Clostridium* spp. isolated, constituting 62% (13/ 21) of the anaerobic species recovered and 87% of the total gram-positive anaerobic species; moreover, clostridia were present in every adult dragon. *Bacteroides fragilis* group was present in some dragons from all zoos. There were few other anaerobic genera recovered. In total, there were six gram-negative and 15 gram-positive anaerobic species found.

All hatchlings grew aerobes, but no anaerobes. The three hatchlings that were fed one meal grew more organisms (average nine) compared with those not fed. Of the hatchlings that had not been fed, one hatchling grew only two strains of *Bacillus*, whereas the other two hatchlings grew seven species of gram-negative and gram-positive aerobes each, similarly to findings for the fed hatchlings.

There was marked interzoo variation in species. Isolates common to all zoos included *Bacillus cereus*, *E. faecalis*, *S. sciuri*, *E. coli*, *Klebsiella oxytoca*, and *Providencia rettgeri*. Twenty-six unique species were isolated from the Honolulu dragons but were not isolated from dragons in the other zoos. Dragons in the Houston and Los Angeles zoos grew 12 and 10 unique species, respectively. The unique species recovered from Honolulu dragons included nine *Enterobacteriaceae*; seven aerobic, nonspore-forming gram-positive rods; six clostridia; and one *Bacteroides thetaiotaomicron*; three of their four dragons grew *Enterobacter aerogenes* and *Clostridium bifermentans* and four of four dragons grew *Actinomyces nasicola*.

Three adult dragons from the Honolulu Zoo that were all fed a similar diet each had one salivary and two gingival samples cultured (front and back) to examine possible differences in floral composition (Fig. 1). Although only three dragons were compared, there was a marked shift in types of recovered organisms: saliva strains were predominantly *Enterobacteriaceae* spp., followed by similar numbers of aerobic gram-positive strains and clostridia, as well as a few *B. fragilis* group isolates. In contrast, gingival samples grew approximately twice as many aerobic gram-positive and clostridia strains compared with saliva, and there were one third fewer *Enterobacteriaceae* spp. and no *B. fragilis* group strains. A single adult Houston Zoo dragon that had a different diet from the Honolulu dragons also had one salivary and two gingival samples taken (not included in Fig. 1). Similar numbers of aerobic gram-positive and gram-negative strains and no anaerobic gram-negative bacilli were recovered from both the saliva and gingiva of this dragon; however, there was a marked increase in clostridia recovered from the gingiva.

Table 2. Comparative aerobic bacteriology of captive and wild Komodo dragon oral flora.

	Current study				
		Hatchlings		Montgomery et al.[33]	
Organism	Adults ($n = 10$)	Fed ($n = 3$)	Not fed ($n = 3$)	Wild ($n = 26$)	Captive ($n = 13$)
Gram-negative					
Acinetobacter calcoaceticus				2	
Aeromonas hydrophila	1			3	1
Alcaligenes faecalis				2	
Brevundimonas (*Pseudomonas*) *diminuta*				2	
Burkholderia cepacia				3	
Chryseobacterium indologenes				2	
Citrobacter braakii	2				
Citrobacter freundii	2				
Citrobacter koseri	2		1	2	1
Enterobacter aerogenes	3			2	1
Enterobacter agglomerans				1	2
Enterobacter cloacae				1	
Enterobacter cloacae, biovar 1		2	2		
Enterobacter cloacae, biovar 2		2			
Enterobacter sakazakii				2	
Escherichia coli	5			9	
Flavimonas oryzihabitans				2	
Klebsiella oxytoca	3				
Klebsiella pneumoniae	2	3	2		1
Klebsiella sp.[a]				8	
Moraxella sp.[a]				2	
Morganella morganii	1			1	
Pantoea ananatus	1				
Pasteurella multocida				2	
Pasteurella pneumotropica				1	
Proteus mirabilis	2			5	2
Proteus vulgaris group	1				
Providencia alcalifaciens	1				
Providencia rettgeri group	5			1	
Pseudomonas aeruginosa	1	3	2	1	
Pseudomonas mendocina				1	
Pseudomonas sp.[a]				3	1
Raoultella ornithinolytica	1				
Salmonella enterica ssp. *arizonae*	3				
Serratia marcescens				2	1
Serratia sp.[a]				2	
Shigella sp.[a]				1	
Sphingobacterium mizutaii	1				
Sphingobacterium multivorum				2	
Stenotrophomonas maltophilia		2			1
Gram-positive					
Actinomyces nasicola	4				
Aerococcus sp.[a]				2	
Bacillus cereus group	5	3	1	3	
Bacillus coagulans				1	
Bacillus megaterium group			1		
Bacillus sp.[a]				4	
Bacillus stearothermophilus				1	
Bacillus subtilis				4	
Chryseobacterium gleum		2	1		
Corynebacterium falsenii	1				

Table 2. Continued.

Organism	Current study			Montgomery et al.[33]	
		Hatchlings			
	Adults ($n = 10$)	Fed ($n = 3$)	Not fed ($n = 3$)	Wild ($n = 26$)	Captive ($n = 13$)
Corynebacterium freneyi	3				
Corynebacterium hansenii	2				
Corynebacterium sp.[a]				7	
Corynebacterium sp. 1[b]	3				
Corynebacterium sp. 2[b]	1				
Corynebacterium sp. 3[b]	1				
Corynebacterium ulceribovis	1				
Corynebacterium xerosis-like	2				
Enterococcus casseliflavus	1	1	1	1	
Enterococcus faecalis	9	2	2	1	
Gram-positive coccus[c]	1				
Kocuria marina	1				
Kurthia sp.[a]					2
Microbacterium sp.[b]			1		
Micrococcus sp.[a]				5	
Rothia sp.[b]	1				
Rummeliibacillus stabekisii			1		
Staphylococcus aureus				8	3
Staphylococcus auricularis				1	
Staphylococcus capitis					5
Staphylococcus caseolyticus					5
Staphylococcus cohnii					1
Staphylococcus gallinarum		2		1	
Staphylococcus haemolyticus				2	1
Staphylococcus hominis				1	
Staphylococcus kloosii				1	
Staphylococcus saprophyticus				1	
Staphylococcus sciuri	8	2	1	2	
Staphylococcus sp.[a]				10	
Staphylococcus warneri				1	2
Staphylococcus xylosus	1			1	1
Streptococcus agalactiae				1	
Streptococcus bovis				1	
Streptococcus dysgalactiae ssp. *dysgalactiae*	1				
Streptococcus dysgalactiae ssp. *equisimilis*	1				
Streptococcus sp.[a]				14	
Vagococcus fluvialis	2				

[a] No further identification reported.
[b] No species-level GenBank match.
[c] No genus-level GenBank match.

DISCUSSION

Previous studies concluded that a Komodo's bite caused fatal infection, resulting from virulent oral bacteria, and that the Komodo simply follows the stricken prey for miles until the creature succumbs.[6,33] More recent and comprehensive physiologic findings describing the role of Komodo venom as a means of facilitating prey mortality have resulted in controversy among biologists.[20,21] The previous "bacteria as venom" dogma is still espoused by some zoos and other authorities, despite the new information on venom and even though no single bacterial species capable of such massive and rapid infection has been reported in either wild or captive dragons. One early and very limited study[6] reported the isolation of *Staphylococcus* sp., *Providencia* sp., *Proteus mirabilis*, and *Morganella morganii* from the oral cavity of three wild Komodo dragons. A subsequent study by Montgomery et al.[33] obtained oral specimens

Table 3. Anaerobic bacteria recovered from oral samples of 10 captive adult Komodo dragons (no anaerobes were present in hatchling dragons).

Organism	No. of dragons
Gram-negative	
Bacteroides fragilis	4
Bacteroides thetaiotaomicron	1
Bacteroides sp.[a]	1
Fusobacterium varium	1
Parabacteroides sp.[a]	1
Gram-negative rod[b]	1
Gram-positive	
Clostridium bifermentans	3
Clostridium butyricum	2
Clostridium difficile	2
Clostridium fallax	1
Clostridium glycolicum	1
Clostridium perfringens	5
Clostridium sardiniense	1
Clostridium sordellii	7
Clostridium subterminale group	2
Clostridium sp. 1[a]	2
Clostridium sp. 2[a]	1
Clostridium sp. 3[a]	2
Clostridium sp. 4[a]	1
Eubacterium moniliforme	1
Gram-positive rod, spore-forming[b]	1

[a] No species-level GenBank match.
[b] No genus-level GenBank match.

from 26 wild and 13 captive Komodo dragons, for aerobic culture only, and recovered a much wider variety of aerobic organisms that were identified phenotypically with biochemical methods and without the advantage of molecular methods. They found 28 gram-negative species in total, 27 from wild and nine from captive dragons, of which 46 and 56%, respectively, were enteric species. Two of their isolates were identified as *Pasteurella multocida*; however, *P. multocida* isolates were not found in the dragons in our study. The rest of the gram-negative organisms that they isolated are commonly found in soil and water. They also found 29 gram-positive species, 25 from wild and eight from captive dragons; most were staphylococci and streptococci that are typically found on animal skin, as well as *Bacillus* spp., that are commonly found in soil and on vegetation.[34] They considered 54 of the 57 species to be "potentially pathogenic";[33] however, these members of the normal microbiome are in fact of low virulence and unlikely to be the cause of rapid fatal infection when present in a wound.[1,9] Komodo venom was not considered to contribute to mortality, because the discovery of venom glands was published years later.[20,21]

Although *P. multocida* infections from bite wounds of mammals are pathogenic, they are associated with sepsis infrequently.[1] *Pasteurella multocida* is part of the normal oral flora of dogs, cats, and other animals and is only associated with infection when outside its usual ecologic niche. Montgomery et al.[33] proposed *P. multocida* as the cause of Komodo-associated prey sepsis and mortality, even though it was reported in only 5% (2/39) of their dragons. None of the dragons in this current study grew *P. multocida*, nor has it been isolated in studies of the oral flora of other reptiles.[8,16,19,24–27,39,45] Rather than Komodo dragon oral flora consisting of virulent bacteria that are the likely cause of sepsis and rapid death, this investigation found that captive dragon oral flora is reflective of the skin and gut flora of their recent meals as well as environmental flora, a finding similar to many other venomous and nonvenomous reptiles and other carnivores. In fact, numerous studies detailing the oral aerobic and anaerobic flora of both captive and wild reptiles, including rattlesnakes, gartersnakes, cobras, and vipers, as well as alligators and tortoises, have all isolated varieties of organisms that largely overlap with the findings of this study.[8,16,19,24–27,39,45] No wild dragons were evaluated in this current study, so the presence of *P. multocida* remains unresolved for wild Komodos.

Montgomery et al.[33] noted major differences in the diversity and quantity of bacteria isolated from the saliva samples between wild and captive Komodo dragons. The bacteria recovered from the captive dragons in this study, however, more closely resembled those of the wild dragons in the Montgomery et al.[33] study, perhaps as a function of diet. For example, *E. coli* grew in 31% of the wild but none of the captive Komodos in their study, but it was present in 50% of the captive adults in our study. Although they did not specify whether the diets of the captive dragons sampled included whole prey, the diet of the dragons in the current study included frozen and then thawed whole poultry (chicken, turkey, and quail), rodents, rabbits, and fish, including the gut contents that typically harbor *E. coli*.

Other bacteria found in the captive dragons in this study and only in the wild but not captive Komodo dragons by Montgomery et al.[33] were *B. cereus*, *E. faecalis*, *Enterococcus* casseliflavus, *S. sciuri*, and *P. rettgeri*. *Staphylococcus sciuri* is a veterinary strain commonly isolated from a wide range of pets and farm animals, including dogs, chickens, and pigs,[12,30,41] and it was found in eight of 10 of the captive adults in this study and in two

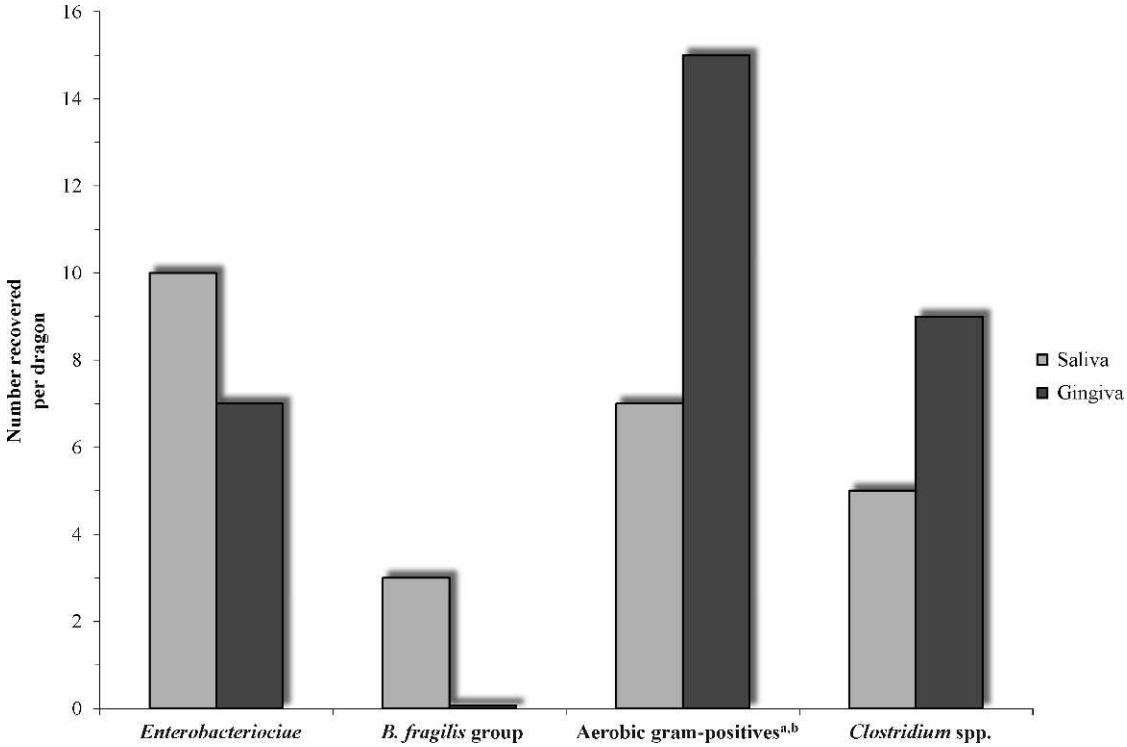

Figure 1. Bacterial species recovered from salivary (one) and gingival (two) samples of three Komodo dragons from the Honolulu Zoo. [a,b]For aerobic gram-positives, saliva samples contained *Actinomyces nasicola* (one), *Bacillus cereus* group (one), *Corynebacterium falsenii* (one), *Enterococcus faecalis* (two), and *Staphylococcus sciuri* (two). Gingiva samples contained *A. nasicola* (two); *B. cereus* group (one); *Corynebacterium freneyi* (two); *Corynebacterium hansenii* (one); *Corynebacterium xerosis* (one); *Corynebacterium* spp. 1, 2, and 3 (one each); *E. faecalis* (one), *Kocuria marina* (one); *S. sciuri* (one); *Vagococcus fluvialis* (one); and aerobic gram-positive coccus (one).

of three fed and one of three unfed hatchlings. Montgomery et al.[33] found it in two of 26 wild but none of the captive dragons.

The most common isolates found by Montgomery et al.[33] in captive Komodo dragon mouths, *S. capitis* and *Staphylococcus caseolyticus*, were not present in their wild dragons or in any of the captive dragons in this study. Interestingly, *S. aureus*, found in 31 and 23% of wild and captive dragons, respectively, in the Montgomery et al.[33] study was not detected in this study, nor was *Streptococcus intermedius*, a species that is also coagulase positive and can have an overlapping identification with *S. aureus* in many commercial identification kits.

Vagococcus fluvialis was among the unusual aerobic strains recovered in this study and has been isolated from variety of animals, including pigs, horses, cattle, and cats.[38] Other unusual bacteria include the marine-associated species *Kocuria marina*[31] and *Aeromonas hydrophila* that were recovered from two different dragons at the Honolulu Zoo where smelts are included in the diet. *Salmonella enterica* ssp. *arizonae* was found in three dragons from two zoos. *Salmonella enterica* ssp. *arizonae* was isolated previously in the oral flora of a rattlesnake and in gartersnake egg yolk sacks, suggesting cloacal transfer as a possible mode of acquisition.[24,25] Others have noted *S. enterica* ssp. *arizonae*, albeit infrequently, in a variety of poultry and meat products; therefore, whole poultry in their diets could have been another source of acquisition[32,35,42] *Clostridium difficile* was recovered from two dragons in the Honolulu Zoo.

There are no previous studies of anaerobic oral flora in the Komodo dragon. In this study, 41 different anaerobes (one to six per specimen) were isolated, with *Clostridium* spp. accounting for 63% (25/41) isolates and *B. fragilis* present in dragons from each zoo. There was a paucity of other anaerobic genera, and none of the hatchlings, fed

or unfed, grew anaerobic bacteria. This difference might be because they had not fed on entire animals that have anaerobes in their gut flora.

The oral flora of animals is complex and there are varieties of different ecologic niches in the oral cavity. Four dragons had both salivary and gingival specimens obtained, showing some differences between the sites. The gingival samples contained more gram-positive species, both aerobes and anaerobes, especially those commonly found as skin flora, whereas the saliva contained more gram-negative enteric species typical of those found in gut contents of their whole animal diet. Previous studies did not differentiate results among oral sites including saliva, gum line, and dorsal palette swabs.[33] Because the diet of the captive dragons in this study included whole prey, and their oral bacteriology included skin and enteric flora common to these prey, it seems reasonable that wild Komodo dragon oral flora also would simply reflect the skin and enteric flora of their local prey.

Very few studies have been systematic and have attempted to define the presentation, epidemiology, bacteriology, and therapy of bite wounds, and they have been generally limited to dog or cat bites,[43] leaving the clinician to extrapolate the best form of antimicrobial therapy for other types of bites from clinical infectious disease references.[1,23] Most of the bacteria recovered from bite wounds are reflective of the oral flora of the biting animal.[22] In a minority of cases, the pathogenic bacteria come from the victim's own skin or from the physical environment at the time of injury, for example, bites by aquatic animals have a bacteriology that is reflective of their water environment. In addition, the oral flora of the biting animal not only contains their usual "normal flora" but also contains members of the microbiomes of their ingested prey and other foods when not fed a processed diet.[25]

The findings in this study of the oral flora of Komodo dragons correlate with studies of other reptiles, including snakes and alligators.[16,19,24,25] Treatment of individual bite cases should be based on specific bacteriology of the cultured wounds; however, when such data are unavailable, physicians must predict the best empirical antimicrobial therapy. Antimicrobial susceptibility testing was not part of this study. Clinicians should be cautious in using the susceptibility data provided by Montgomery et al.,[33] who performed limited antimicrobial susceptibility testing of several aerobic species only and used methodology that did not conform to standard National Committee for Clinical Laboratory Standards (now Clinical and Laboratory Standards Institute) procedures.[13,14] In particular, they used overly heavy inoculum and, for some of the organisms, nonstandard interpretive susceptibility breakpoints. When selecting empiric antimicrobial therapy, clinicians are best advised to assume wound infections are polymicrobic, including aerobic and anaerobic enteric and skin flora of the Komodo dragon prey, and to use therapeutic agents effective against this range of organisms. No single oral antimicrobial agent is likely to cover the full range of the isolated organisms. Moxifloxacin was shown to be active against the spectrum of dog and cat bite pathogens,[43] and it likely has the broadest range for single agents that might be active against Komodo flora. A variety of intravenous agents, such as carbapenems or piperacillin-tazobactam, should cover the spectrum as well. Perhaps in the future, a researcher will culture and report the bacteriology of Komodo bite wound infections in humans to allow more directed therapy.

CONCLUSIONS

The aerobic and anaerobic bacteriology of captive Komodo dragons is diverse and reflects their diet that includes the skin flora and gut contents of their prey or ingested carrion, as well as organisms commonly found in soil and on vegetation. This study did not evaluate any wild dragons, thus, it can only be speculated that their flora would be similar to those of the captive dragons. Although some of these strains are opportunistic pathogens, they are of low virulence and would not be considered to have a primary pathogenic role that would cause rapid death of prey. No single pathogen was found common to all dragons that could be considered part of an evolutionary mechanism on which the Komodo dragon could rely for prey capture.

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