

AEROBIC SALIVARY BACTERIA IN WILD AND CAPTIVE KOMODO DRAGONS

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ABSTRACT: During the months of November 1996, August 1997, and March 1998, saliva and plasma samples were collected for isolation of aerobic bacteria from 26 wild and 13 captive Komodo dragons (*Varanus komodoensis*). Twenty-eight Gram-negative and 29 Gram-positive species of bacteria were isolated from the saliva of the 39 Komodo dragons. A greater number of wild than captive dragons were positive for both Gram-negative and Gram-positive bacteria. The average number of bacterial species within the saliva of wild dragons was 46% greater than for captive dragons. While *Escherichia coli* was the most common bacterium isolated from the saliva of wild dragons, this species was not present in captive dragons. The most common bacteria isolated from the saliva of captive dragons were *Staphylococcus capitis* and *Staphylococcus caseolyticus*, neither of which were found in wild dragons. High mortality was seen among mice injected with saliva from wild dragons and the only bacterium isolated from the blood of dying mice was *Pasteurella multocida*. A competitive inhibition enzyme-linked immunosorbent assay revealed the presence of anti-*Pasteurella* antibody in the plasma of Komodo dragons. Four species of bacteria isolated from dragon saliva showed resistance to one or more of 16 antimicrobics tested. The wide variety of bacteria demonstrated in the saliva of the Komodo dragon in this study, at least one species of which was highly lethal in mice and 54 species of which are known pathogens, support the observation that wounds inflicted by this animal are often associated with sepsis and subsequent bacteremia in prey animals.

Key words: Aerobic bacteria, bacteremia, Komodo dragon, salivary bacteria, survey, *Varanus komodoensis*, wound sepsis.

INTRODUCTION

The Komodo dragon (*Varanus komodoensis*) is the largest living lizard (Auffenberg, 1981). While considered a predator/scavenger, there is some evidence that wounds inflicted by the dragon during encounters with prey species are frequently associated with bacterial infection in the prey animal (Auffenberg, 1981). Moreover, wounds inflicted on domestic animals by the dragon commonly become infected and lead to septicemia (Auffenberg, 1981). Although bacterial infection of bite wounds and septicemia in humans and prey animals have been documented for a variety of predators (Frances et al., 1975; Flandry et al., 1989; Weber and Hansen, 1991), the Komodo dragon is the only major predator/scavenger on the islands it inhabits (Auffenberg, 1981) and would be

the primary beneficiary of prey species killed or debilitated by bacterial infection.

This study was undertaken to identify and compare the aerobic bacteria found in the saliva of wild and captive Komodo dragons, to assess the dragon's saliva as a potential source of pathogenic bacteria, and to evaluate the ability of dragon saliva to induce bacteremia and death in laboratory rodents. In addition, we examined the sensitivity of some of the bacteria isolated from the saliva of dragons to select antimicrobics.

MATERIALS AND METHODS

Saliva, oral swabs, and blood were collected from 23 Komodo dragons in Komodo National Park (Indonesia; 8°24'–8°50'S, 119°21'–119°49'E) during expeditions in November 1996, August 1997, and March 1998. Samples were also obtained August 1997 from 13 captive dragons, 1 to 2.0 m overall length, at the

Gembira Loka Zoo (Yogyakarta, Java, Indonesia; 8°25'S, 112°15'E). Wild dragons were trapped, noosed, or caught manually. A special crocodile noose (Tri-noose®, Fuhmann Diversified, Seabrook, Texas, USA) was used for specimens over 10 kg for capture in the open or fixated on bait. Manual collection of animals involved casting a small fish on a rope near a dragon and attempting capture after the animal fixated on the bait. Trapping utilized wood/wire traps which were triggered by a dragon pulling at the bait or triggered manually using a 10 to 15 m monofilament line. Regardless of how a dragon was captured, a rope was placed around the head for counter tension. Tail, legs, and head were taped or tied with nylon rope. Once under firm manual restraint, the wild dragons became remarkably passive, facilitating sample collection and obviating the need for sedation. All restraint was performed by Komodo National Park rangers, and no serious injuries occurred to human or dragon.

Smaller captive specimens under 1.2 m total length were restrained manually wearing heavy gloves for protection. Larger captive dragons were moved into a wooden or metal crate by feeding or operant conditioning. Openings in the walls of the crate were located to allow the taking of samples from the restrained animal.

Saliva samples were obtained using sterile cotton swabs which were used to obtain material from the gum line and dorsal palette. Using 10 ml catheter-tipped syringes, larger saliva samples were collected from some dragons which were actively salivating (only small amounts of saliva were obtainable from most dragons using this method). Swabs were placed in sterile vials containing aerobic transport media (Fisher Scientific, Dallas, Texas, USA) and immersed in liquid nitrogen. Saliva samples were placed in sterile cryopreservation vials (Fisher Scientific) for transport to the University of Texas (Arlington, Texas). The methods of sample collection and storage were not designed to support recovery of anaerobic bacteria. Blood was collected by a lateral approach to the ventral tail vein and placed in lithium heparin tubes (Scientific Products, Grand Prairie, Texas, USA). Blood samples were stored in a dark, cool (5–10 C) container until returned to base camp each day where they were treated by conventional methods for isolation of plasma. Plasma samples were transferred to sterile cryotubes (Nalgen Company, Rochester, New York, USA) and stored in liquid nitrogen for transport to the University of Texas. Blood was not obtained from all dragons from which saliva was collected.

During bacterial identification at the University of Texas, swab samples were removed from liquid nitrogen and thawed at room tempera-

ture. Trypticase soy broth (Fisher Scientific) was inoculated with the swabs and incubated for 24 hr at 37 C. Following incubation, broth cultures were streaked onto petri plates containing trypticase soy agar (TSA), MacConkey agar, and TSA enriched with 5% defibrinated sheep blood agar (Fisher Scientific) and incubated at 37 C for 24 hr to produce colony growth. Initially isolates were separated based on Gram stain reaction and colony morphology. The Vitek Gram-positive and Gram-negative identification system (Vitek Systems, Inc., Hazelton, Missouri, USA), BBL Enterotube II (Fisher Scientific), BBL Oxi/Ferm II (Fisher Scientific), and standard biochemical assays were used for identification of bacterial species.

Mortality assays were conducted on a limited number of saliva samples from Komodo dragons. Briefly, saliva samples were removed from liquid nitrogen and allowed to thaw at room temperature, diluted 1:10 with sterile phosphate-buffered saline (sPBS; pH 7.4) under aseptic conditions, and 100 µl of diluted saliva passed through a sterile 0.22 µ filter (Fisher Scientific), or sPBS alone were injected intraperitoneally into each of 10 6–8-wk old female, ICR Swiss albino mice (Harlan Sprague-Dawley, Houston, Texas, USA). The number of mice dying within 96 hr following injection of dragon saliva was recorded. Blood was collected aseptically via cardiac puncture immediately following death or just prior to death of mice. The presence of bacteria in the blood of mice injected with dragon saliva was determined by culture of blood samples on TSA enriched with 5% defibrinated sheep blood agar, incubated for 24 hr at 37 C, and analyzed as above for bacterial species. Samples of blood from each of five mice not injected with dragon saliva were analyzed as above.

Plasma samples from three dragons were analyzed for antibodies to the bacterial species that killed the mice in the above described experiment. Enzyme-linked immunosorbent assay (ELISA) was used to detect the presence of anti-*Pasteurella multocida* antibody in dragon plasma. Bacterial antigen was prepared by overnight incubation of *P. multocida* in Luria-Bertani (LB) culture media (Fisher Scientific). The *P. multocida* isolate obtained from the oral cavity of the Komodo dragon in this current study was the source of bacterial antigen employed in these assays. Following incubation, bacteria were pelleted by centrifugation at 1,200×G for 15 min at 5 C; the bacterial pellet was washed five times in sPBS by centrifugation, sonicated at 100 W for 5–10 sec bursts on a sonicator (Vibra-Cell, Sonics and Metrials, Inc., Newtown, Connecticut, USA) and centrifuged as above to remove unlysed bacteria.

Ninety-six-well microtiter plates (Dyna Tech Laboratories, Inc., Chantilly, Virginia, USA) were coated with *P. multocida* antigen (5 µg/well) and incubated overnight at 5 C. Antigen coated plates were washed and blocked with a solution of 2.5% bovine serum albumin (Sigma, St. Louis, Missouri, USA) for 1 hr at 37 C. Blocked plates were washed once with washing buffer (WB), and either mouse or human (controls; 12 wells each for undiluted control plasma) or dragon plasma either undiluted or diluted 1:10, 1:25, or 1:50 were added to wells with a 1 hr incubation at 37 C. Following incubation, dragon plasma-coated wells were washed once with WB and mouse anti-*Pasteurella* serum (diluted 1:50) was added to each well and incubated for 1 hr at 37 C. Anti-*Pasteurella* IgG was collected via cardiac puncture from female ICR Swiss albino mice following five weekly subcutaneous injections (100 µl) of a 1:1 mixture of bacterial antigen (100 µg) and Freund's complete adjuvant. Following incubation with anti-*Pasteurella* antibody, peroxidase-labeled sheep anti-mouse IgG (Jackson Immuno Research Laboratories, Inc., West Grove, Pennsylvania, USA) was added to each well and incubated for 1 hr at 37 C. Finally, plates were washed once with WB, substrate (o-phenylenediamine/peroxidase, 750 µg/ml; Sigma) was added to each well, and optical density was determined at 490 nm on a microplate reader (Model MR 600, Dynatech Labs, Inc., Burlington, Massachusetts, USA).

The National Committee for Clinical Laboratory Standards (NCCLS; Wayne, Pennsylvania, USA) diffusion method was used to determine the sensitivity/resistance to 12 different antimicrobics (amikacin, chloramphenicol, ciprofloxacin, doxycycline, erythromycin, gentamicin, penicillin G, piperacillin, streptomycin, tetracycline, tobramycin, and vancomycin) of 16 bacterial isolates from saliva of Komodo dragons. Briefly, LB broths (Fisher Scientific) were inoculated with a purified bacterial isolate (see above) and incubated at 37 C for 24 hr. Following incubation, 100 µl of each bacterial culture was aseptically pipetted and spread onto the surface of each of five Mueller Hinton II agar (MH II) plates (Fisher Scientific). Disks impregnated with the appropriate antimicrobial (Fisher Scientific; concentration of the antimicrobial was according to NCCL Standards—amikacin, chloramphenicol, doxycycline, tetracycline, and vancomycin at 30 µg/disk; ciprofloxacin at 5 µg/disk; erythromycin at 15 µg/disk; gentamicin, streptomycin, and tobramycin at 10 µg/disk; penicillin G at 10 units/disk; and piperacillin at 100 µg/disk) were applied to the surface of the MH II plates onto which the bacteria had been spread. Plates treated in this

manner were incubated at 37 C for 24 hr. Resistance or sensitivity of each bacterial species to each of the antibiotics tested was determined by measuring the diameter of the zone of inhibition using the standards of the NCCLS (amikacin, sensitive (S) >17 mm or resistant (R) <14 mm; chloramphenicol, S>21, R<20; ciprofloxacin, S>21, R<15; doxycycline, S>16, R<12; erythromycin, S>23, R<13; gentamicin, S>15, R<12; penicillin G, S>29, R<28; piperacillin, S>18, R<17; streptomycin, S>15, R<11; tetracycline, S>19, R<14; tobramycin, S>15, R<12; and vancomycin, S>17, R<14).

One-way analysis of variance (Zar, 1974) was used to determine the significance of optical density reduction compared to controls and percent inhibition among all tested groups in the competitive inhibition ELISA. Significant differences between controls and all dragon serum samples were determined using Tukey's honest significant difference for all pair-wise comparisons. Student's *t*-test was used to assess the significance of differences between the mean number of species of salivary bacteria in wild versus captive dragons. All analyses were performed using Statistica (Statsoft, Inc., Tulsa, Oklahoma, USA) or StatMost (DataMost Corp., Salt Lake City, Utah, USA) statistical software.

RESULTS

Twenty-eight Gram-negative and 29 Gram-positive species of aerobic bacteria were identified from saliva collected from 39 Komodo dragons (Table 1). Saliva samples from wild dragons were positive for 93% of the Gram-negative bacteria isolated, only 36% of which were observed in the saliva of captive dragons. Among the Gram-positive bacteria identified, 83% were seen in the saliva of wild dragons and only 31% were isolated from the saliva of captive dragons. Captive dragons had a significantly lower average number of species of salivary bacteria (\pm SE = 2.8 ± 0.3 ; $n=13$) than did wild dragons (5.2 ± 0.4 ; $n=26$; $P<0.03$; data not shown). The most common bacterium identified to species isolated from wild dragons was *Escherichia coli* (nine dragons), but it was not found in the saliva of any of the captive animals. Several species of *Staphylococcus* and *Streptococcus* also were commonly encountered among bacteria identified from wild dragon saliva. The most frequently

TABLE 1. Gram-positive and Gram-negative bacteria isolated from saliva samples from wild ($n=26$) and captive ($n=13$) Komodo dragons.

Bacterial species	Number of dragons Gram-negative culture positive		Bacterial species	Number of dragons Gram-positive culture positive	
	Wild	Captive		Wild	Captive
<i>Acinetobacter calcoaceticus</i>	2	0	<i>Aerococcus</i> sp.	2	0
<i>Aeromonas hydrophila</i>	3	1	<i>Bacillus cereus</i>	3	0
<i>Alcaligenes faecalis</i>	2	0	<i>Bacillus coagulans</i>	1	0
<i>Burkholderia cepacia</i>	3	0	<i>Bacillus stearothermophilus</i>	1	0
<i>Citrobacter koseri</i>	2	1	<i>Bacillus subtilis</i>	4	0
<i>Chryseobacterium indologes</i>	2	0	<i>Bacillus</i> sp.	4	0
<i>Enterobacter aerogenes</i>	2	1	<i>Brevundimonas diminuta</i>	2	0
<i>Enterobacter agglomerans</i>	1	2	<i>Corynebacterium</i> sp.	7	0
<i>Enterobacter cloacae</i>	1	0	<i>Enterococcus faecalis</i>	1	0
<i>Enterobacter sakazakii</i>	2	0	<i>Enterococcus casseliflavus</i>	1	0
<i>Escherichia coli</i>	9	0	<i>Kurthia</i> sp.	0	2
<i>Flavomonas oryzae</i>	2	0	<i>Micrococcus</i> sp.	5	0
<i>Klebsiella pneumoniae</i>	0	1	<i>Staphylococcus aureus</i>	8	3
<i>Klebsiella</i> sp.	8	0	<i>Staphylococcus auricularis</i>	1	0
<i>Moranella morgani</i>	1	0	<i>Staphylococcus capitis</i>	0	5
<i>Moraxella</i> sp.	2	0	<i>Staphylococcus caseolyticus</i>	0	5
<i>Pasteurella multocida</i>	2	0	<i>Staphylococcus cohnii</i>	0	1
<i>Pasteurella pneumotropica</i>	1	0	<i>Staphylococcus gallinarum</i>	1	0
<i>Proteus mirabilis</i>	5	2	<i>Staphylococcus haemolyticus</i>	2	1
<i>Providencia rettgeri</i>	1	0	<i>Staphylococcus hominis</i>	1	0
<i>Pseudomonas aeruginosa</i>	1	0	<i>Staphylococcus kloasii</i>	1	0
<i>Pseudomonas mendocina</i>	1	0	<i>Staphylococcus saprophyticus</i>	1	0
<i>Pseudomonas</i> sp.	3	1	<i>Staphylococcus</i> sp.	10	0
<i>Serratia marcescens</i>	2	1	<i>Staphylococcus sciuri</i>	2	0
<i>Serratia</i> sp.	2	0	<i>Staphylococcus warneri</i>	1	2
<i>Shigella</i> sp.	1	0	<i>Staphylococcus xylosus</i>	1	1
<i>Sphingobacterium multivorum</i>	2	0	<i>Streptococcus bovis</i>	1	0
<i>Stenotrophomonas maltophilia</i>	0	1	<i>Streptococcus agalactiae</i>	1	0
			<i>Streptococcus</i> sp.	14	0

isolated bacteria from the saliva of captive dragons were *Staphylococcus capitis* (five dragons) and *Staphylococcus caseolyticus* (five dragons). Neither of these bacteria were found in the saliva of wild dragons.

Pasteurella multocida was the only aerobic bacterium recovered from the blood of dying mice injected with saliva samples from three (KD 4, 6, and 8) of the five wild dragons tested in this fashion. None of the mice injected with samples of filtered saliva from these same dragons died. Saliva from KDS elicited 100% mortality among mice injected, while saliva from KD 4 and 6 elicited only 60% mortality. Mice receiving saliva from the other two dragons (KD 5 and 7) tested showed mor-

bidity (ruffled fur, hunched appearance, decreased mobility) but no mortality (Table 2). Culture of the saliva from the five dragons tested in this experiment detected *P. multocida* in only one of the dragons (KD 6). No changes were observed in morbidity and mortality after 96 hr among mice injected with dragon saliva.

In identifying *P. multocida* the following biochemical test results were obtained: the Gram-negative organism was positive for glucose and mannitol fermentation and negative for adonitol, arabinose, dulcitol, lactose, maltose, and sorbitol fermentation; glucose fermentation did not lead to the formation of acetylmethylcarbinol or Voges-Proskauer; and the organism was

TABLE 2. Mortality in mice injected intraperitoneally with 100 μ l of dragon saliva diluted 1:10 in sterile phosphate-buffered saline (PBS; pH 7.4). Each of five groups of mice ($n=10$) received saliva from one of five Komodo dragons designated KD4, KD5, KD6, KD7, and KD8. No mortality was seen among un-injected mice and mice injected with sterile PBS alone (controls).

Dragon #	Hour postinjection	Percent of mice dead
KD4	0	0
	24	0
	48	0
	72	60
	96	60
KD5	Morbidity but no mortality	
KD6	0	0
	24	0
	48	0
	72	40
	96	60
KD7	Morbidity but no mortality	
KD8	0	0
	24	60
	48	80
	72	80
	96	100
Mice injected with PBS only	0	0
	24	0
	48	0
	72	0
	96	0

negative for lysine decarboxylase, phenylalanine deaminase, urease, β -hemolysis (sheep red blood cells), and citrate utilization but positive for nitritase, ornithine decarboxylase, oxidase, hydrogen sulfide production, and indole formation.

Plasma from three wild Komodo dragons was tested for anti-*Pasteurella* antibody in a competitive inhibition ELISA. *Pasteurella multocida* was not cultured from the saliva of any of these three dragons. All three of the dragons tested showed the presence of anti-*Pasteurella* antibody in their plasma (Table 3). A dose response was evident with decreasing dilution of plasma, and while insufficient amounts of plasma were available from the Komodo dragon designated KD 7 to run an undiluted plasma sample, strong inhi-

TABLE 3. Competitive inhibition by Komodo dragon plasma of mouse anti-*Pasteurella* antibody in an ELISA. Plasma from three dragons designated KD3, KD5, and KD7 were examined. Dragon plasma was tested undiluted (except for KD7) or diluted 1:10, 1:25, or 1:50.

Dragon	Dilution dragon plasma	Percent inhibition	n	SD of detection of mouse anti- <i>Pasteurella</i> antibody
KD3	Undiluted	50.8 ^a	4	0.79
	1:10	26.1 ^a	11	1.59
	1:25	17.3	12	0.81
	1:50	13.7	7	1.43
KD5	Undiluted	44.8 ^a	3	1.58
	1:10	32.1 ^a	11	0.78
	1:25	27.8 ^a	11	0.88
KD7	1:10	4.8	5	0.6
	1:25	25.5 ^a	4	0.79
	1:50	15.7	4	1.69
	1:50	25.9 ^a	3	1.52

^a Statistical analyses run on optical densities showed that these points differed significantly from controls $P < 0.05$.

bitation was evident for plasma samples from all three of the dragons examined. Neither normal human nor normal mouse plasma inhibited the detection of anti-*Pasteurella* antibody by ELISA.

Four of 16 species of bacteria tested showed resistance to one or more of the 12 different antimicrobics examined (Table 4). *Enterobacter cloacae* was resistant to gentamicin and piperacillin, two drugs to which they are normally susceptible; *Moraxella* sp. was resistant to penicillin, piperacillin, and erythromycin, all drugs to which this genus is normally susceptible; *Bacillus* sp. was resistant to penicillin, to which it is thought to be susceptible, and *Enterococcus faecalis* showed resistance to gentamicin and streptomycin, both drugs usually showing strong activity against this organism. All of the other drugs tested that had proven effectiveness against these 16 bacteria strongly inhibited growth of bacteria.

DISCUSSION

The Komodo dragon is the only large predator/scavenger within its range and would, therefore, be the primary benefac-

TABLE 4. Sensitivity to select antimicrobics of 10 Gram-negative and six Gram-positive species of bacteria isolated from Komodo dragons. The 12 antimicrobics tested were amikacin (Ami), gentamicin (Gen), ciprofloxacin (Cip), chloramphenicol (Chl), tobramycin (Tob), streptomycin (Str), penicillin (Pen), piperacillin (Pip), doxycycline (Dox), erythromycin (Ery), vancomycin (Van), and tetracycline (Tet). All species of bacteria except *Staphylococcus caseolyticus* were from wild dragons. Antimicrobial inhibited bacterial growth (I) or did not inhibit bacterial growth (N). A zero (0) indicates that the antimicrobial has been determined not to be effective against this species/genus of bacterium.

Bacteria antimicrobial	Ami	Gen	Cip	Chl	Tob	Str	Pen	Pip	Dox	Ery	Van	Tet
Gram-negative species												
<i>Acinetobacter calcoaceticus</i>	0	0	I	0	I	0	0	I	I	0	0	0
<i>Alcaligenes faecalis</i>	I	I	0	0	I	0	0	I	0	0	0	0
<i>Enterobacter aerogenes</i>	I	I	I	0	I	0	0	I	0	0	0	0
<i>Enterobacter cloacae</i>	I	N	I	0	I	0	0	N	0	0	0	0
<i>Enterobacter sakazakii</i>	I	I	I	0	I	0	0	I	0	0	0	0
<i>Escherichia coli</i>	I	I	I	0	I	0	0	I	0	0	0	0
<i>Moraxella</i> sp.	I	I	I	I	I	0	N	N	I	N	0	I
<i>Pasteurella multocida</i>	0	I	I	I	0	0	I	I	I	I	0	I
<i>Providencia rettgeri</i>	I	I	I	0	I	0	0	I	0	0	0	0
<i>Pseudomonas aeruginosa</i>	I	I	I	0	I	0	0	I	0	0	0	0
Gram-positive species												
<i>Bacillus</i> sp.	I	I	I	I	I	0	N	I	0	I	I	I
<i>Enterococcus faecalis</i>	0	N	0	0	0	N	0	I	0	I	I	0
<i>Staphylococcus aureus</i>	I	I	I	0	I	0	0	I	0	I	I	0
<i>Staphylococcus caseolyticus</i>	I	I	I	0	I	0	0	I	0	I	I	0
<i>Streptococcus bovis</i>	0	0	I	I	0	0	I	I	I	I	I	I
<i>Streptococcus</i> sp.	0	0	I	I	0	0	I	I	I	I	I	I

tor from prey animals that were killed or debilitated by bacterial infection from wounds inflicted during encounters (Auffenberg, 1981). In an earlier study, Auffenberg (1981) reported isolation of four species of pathogenic bacteria from the oral cavity of the Komodo dragon (*Staphylococcus* sp., *Providencia* sp., *Proteus mirabilis*, and *Proteus morgani*). The present study has demonstrated that the Komodo dragon is infected with a wide variety of bacterial species in its saliva including *P. mirabilis* and several species of *Staphylococcus* and *Providencia*, organisms reported by Auffenberg (1981). Of the 20 different species of aerobic bacteria isolated from the mouths of wild alligators (*Alligator mississippiensis*) from the southeastern United States (Flandry et al., 1989), six also were isolated in the present study from the oral surfaces of the Komodo dragon. Moreover, the frequency of occurrence of each species of bacterium isolated from the alligator was similar to that seen in the dragon, with 16 of the 20 bacteria observed in less than

three alligators each, and nine of these identified from only a single animal. A strong association has been reported between wounds inflicted by dragons on wild and domestic prey species and the occurrence of wound sepsis and septicemia (Auffenberg, 1981). Fifty-four of the 58 bacterial species isolated in the present study are potentially pathogenic (Lennette et al., 1985), and at least one species, tentatively identified as *P. multocida*, caused high mortality among mice injected with dragon saliva. *Pasteurella multocida* has been recovered from the oral surfaces of a wide variety of wild and domestic animals (Yu et al., 1995), and this bacterium is the leading cause of animal bite wound infections in humans (Hombal and Dincsoy, 1992). That we were unable to isolate *Pasteurella* from saliva of the five dragons associated with mouse mortality and morbidity is probably related to the presence of anti-*Pasteurella* antibody in the saliva, as well as to the fact that this group of bacteria normally resides intracellularly (Lennette et al., 1985). Al-

though samples collected from dragons were not treated to preserve anaerobic bacteria, we cannot eliminate the possibility that an unidentified anaerobe could have contributed to mortality and morbidity among mice injected with dragon saliva.

The findings of the present study support the hypothesis (Auffenberg, 1981) that infliction of wounds in prey animals by the Komodo dragon provides a strong opportunity for wound contamination by one or more of a wide variety of potentially pathogenic bacteria found on the oral surfaces of the dragon. Induction of wound sepsis and bacteremia through the bite of the Komodo dragon may be a mechanism for debilitation and death of prey, providing an important food source for all Komodo dragons in a population.

Wild Komodo dragons have both a higher average number of salivary bacteria as well as a much greater variety of salivary bacteria than do captive dragons. The apparently routine feeding by wild dragons on putrefying carcasses (Auffenberg, 1981) would be an important contributor to these differences. Many of the organisms isolated from the wild caught dragons and not seen in the saliva of captive dragons were bacteria normally associated with human and animal feces.

The four species of bacteria resistant to antimicrobics to which they are normally susceptible may have been acquired by the wild Komodo dragons through feeding on domestic animals (Auffenberg, 1981) or animal and human feces. This information on antimicrobial resistance could be important in the treatment of infections in humans and domestic animals developing from bite wounds inflicted by the dragon.

Komodo dragons in general, but large adults in particular, have numerous scars on their bodies inflicted by other dragons during fights over carcasses and territory (Auffenberg, 1981). In addition, routine bleeding of the gums is seen in adult dragons during feeding. Thus, Komodo dragon may be exposed to potential infection by its own salivary bacteria. If this is the case

then one would expect to see immunological indicators of past exposure to the bacteria found in the animal's blood. The high level of competitive inhibition of the ELISA for mouse anti-*Pasteurella* antibody by dragon plasma suggests that dragons have ample exposure to this pathogen for generation of an antibody response to this and perhaps other pathogenic bacteria present in its oral cavity. These and probably other immunologic components may play a key role in protecting the dragon from the pathogenic salivary bacteria that are responsible for inducing wound sepsis and bacteremia in prey animals.

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LITERATURE CITED

- AUFFENBERG, W. 1981. The behavioral ecology of the Komodo monitor. University Press of Florida, Gainesville, Florida, 406 pp.
- FLANDRY, F., E. LISECKI, G. DOMINGUE, R. NICHOLS, D. GREER, AND R. HADDAD. 1989. Initial antibiotic therapy for alligator bites: Characterization of the oral flora of *Alligator mississippiensis*. Southern Medical Journal 82: 262–266.
- FRANCES, D., M. HOLMES, AND G. BRANDON. 1975. *Pasteurella multocida*: Infections after domestic animal bites and scratches. Journal of the American Medical Association 233: 42–45.
- HOMBAL, S., AND H. DINCISOY. 1992. *Pasteurella multocida* endocarditis. American Journal of Clinical Pathology 98: 565–568.
- LENNETTE, E., A. BALOWS, W. HAUSLER, JR., AND H. SHADOMY. 1985. Manual of clinical microbiology, American Society for Microbiology, Washington, D.C., 1149 pp.
- WEBER, D., AND A. HANSEN. 1991. Infections resulting from animal bites. Infectious Disease Clinics of North America 5: 663–680.
- YU, G., A. BOIKE, AND J. HLADIK. 1995. An unusual case of diabetic cellulitis due to *Pasteurella multocida*. Journal of Foot and Ankle Surgery 34: 91–95.
- ZAR, J. 1974. Biostatistical analysis. Prentice-Hall, Inc., Englewood Cliffs, New Jersey, 620 pp.

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