IDENTIFICATION OF SELECTED BACTERIAL PATHOGENS FROM THE VENOM AND ORAL CAVITIES OF AGKISTRODON CONTORTRIX MOKASEN AND AGKISTRODON CONTORTRIX CONTORTRIX, NORTHERN AND SOUTHERN COPPERHEADS

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Don Dailey

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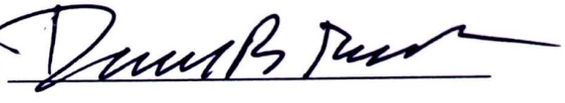
Signature 

Date Dec / 13 / 2001

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A Thesis

Presented for the

Master of Science

Degree

Austin Peay State University

Daniel R. French

December 2001

ACKNOWLEDGMENTS

I would like to thank my major professor, Dr. Don C. Dailey, as well as my other committee members Dr. A. Floyd Scott and Dr. Carol Baskauf, for their guidance and working with me to complete this project over an extended distance. I would also like to thank the Kentucky Reptile Zoo in Slade, Kentucky for allowing me to sample their snakes, and the Freed-Hardeman University Biology Department (Henderson, Tennessee), specifically Dr. Paul Fader for allowing me to use their microbiology laboratory facilities to complete my research while not in residence at Austin Peay State University.

ABSTRACT

The bacterial diversity in the venom and oral cavities of two subspecies of Copperheads, the Northern and Southern Copperheads was investigated. Seventeen Northern and Southern Copperheads in the Kentucky Reptile Zoo in Slade, Kentucky and two Northern Copperheads at Austin Peay State University in Clarksville, Tennessee were surveyed. Eight selective media were used to select for specific groups or genera of bacteria. Eighty-eight isolates were obtained from the oral cavity of the 36 Copperheads. The majority of the isolates were species of *Pseudomonas*; the second- and third-most-common genera were *Staphylococcus* and *Streptococcus*, respectively. Only one bacterium (*Streptococcus* sp.) was isolated from the 36 venom samples. The venom was also used in a disk diffusion assay to test for antibacterial activity. All venom samples demonstrated inhibitory effects on all four test bacteria - *Escherichia coli*, *Aeromonas hydrophilia*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.

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CHAPTER I

INTRODUCTION

Incidence of Snake Bites

Worldwide there are 2.5 million people bitten by snakes each year. In North America 45,000 people receive snakebites annually; 10,000 of these bites are from venomous snakes. As a result of these bites an average of 15 deaths occur each year in the United States (Chippaux, 1998). Death from a snakebite may occur as a result of improper medical treatment, allergies to the venom or antivenin, from refusal to receive medical care, or from complications associated with the bite.

Snake Bite Infection and Pathogens

Infections are complications that can result from snakebites; however, the actual incidence of infection from snakebites is unknown (Rest and Goldstein, 1985). The source of the infecting bacteria may be the snakes oral cavity. These infections may not result in death, but the morbidity can be quite severe, as in the case of a young boy that was described by Hofer *et al.* (1993). After being bitten, the site of the bite became infected and rapidly developed into osteomyelitis and gangrene. Unfortunately, even-though he was treated with antibiotics he developed bone lesions in his arms and legs and skin lesions on his chest, arms, and legs. Two months following the bite a portion of his right leg was amputated. The infectious agent was *Mycobacterium ulcerans*. There is no direct evidence that the offending bacterium originated from the mouth of the snake that bit the

child; however, the genus *Mycobacterium* has been identified in the oral cavity of snakes (Draper *et al.* 1981)

Mycobacterium is one of many human pathogens identified from the oral cavity of snakes. Pathogenic species of *Aeromonas*, *Pseudomonas*, *Staphylococcus*, *Streptococcus*, *Clostridium*, *Corynebacterium*, *Haemophilus*, *Alcaligenes*, *Bacteriodes,* and 11 representatives of the family Enterbacteriaceae, also have been identified (Arroyo *et al.*, 1980; Draper *et al.*, 1981; Goldstein *et al.*, 1981; Ledbetter and Kutscher, 1969; Theakston *et al.* 1990; Soveri and Seuna, 1986). These bacteria may not be restricted to the oral cavity, but also may inhabit the venom of the snakes and be transferred to the bite when the venom is injected.

Role of Venom in Infection

Venom may promote the spread of infection by breaking down the tissue and allowing the bacteria to invade the affected area. This spread is accomplished by the digestive enzymes in the venom: enzymes such as collagenase, which digest the intracellular matrix, hyaluronidase, which breaks down the hyaluronic acid barrier and decreases the viscosity of connective tissue, and phospholipase A, which alters membrane permeability (Grenard, 1994). The overall action of these enzymes is to promote tissue digestion, so that food is digested before it can decay in the snake's stomach.

Antibacterial Effects of Venom

These enzymatic activities not only promote tissue digestion but may also possess antibacterial properties. Antibacterial effects of snake venom have been described by Stiles *et al.* (1991) and by Talan *et al.* (1991). Venom collected from African and Asian

cobras (*Naja*) had strong activity against *Aeromonas hydrophilia*, but Southern Copperhead (the only member of the *Agkistrodon* complex tested) venom had no antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, or *Aeromonas hydrophilia* (Stiles *et al.*, 1991). Talan *et al.* (1991) demonstrated antibacterial activities against *Staphylococcus*, *Pseudomonas*, *Enterobacter*, *Citrobacter*, *Proteus*, and *Morganella* in the venom of rattlesnakes.

It is not known why snake venoms have antibacterial activities. These antibacterial substances may help control bacterial populations within the venom glands. They may slow bacterial decay of ingested food, or as Thomas and Pough (1979) suggest, they may serve to protect the snake from any pathogens ingested with the food. Possibly the antibacterial effects of the venom are merely alternative actions of the digestive enzymes in the venom. Whatever the reason for the antibacterial effects in the venom, it is evident that snake venoms vary in their effectiveness against different bacterial species. As a result of this narrow spectrum of activity, the venom may select for specific pathogens within the wound. Alternatively, the selective nature of the antibacterial activity may be beneficial to the snake. The venom may act as a biocide or biostatic agent against bacteria that could infect the snake as a result of self-inflicted bites.

Recent research has yielded pharmaceutical compounds derived from snake venoms for the treatment of hypertension, treatment and prevention of thromboemboli, relief from pain, control of vasomotor rhinitis (Grenard 1994), and to inhibit the growth and attachment of various forms of tumor cells, such as Kaposi's sarcoma (Fry *et al.*, 1996;

Senior, 1999). The next chemotheraputeutic derived from snake venoms could possibly be an antimicrobial agent.

As described above, a key complication of snakebite is bacterial infection. Prevention of these infections has been attempted through the prophylactic use of antibiotics. The over use of broad-spectrum antibiotics, has resulted in the selection of bacteria resistant to these commonly prescribed antibiotics. It would be more efficient to use a narrow spectrum antibiotic specific for the bacterium responsible for the infection. Therefore, for proper selection of antibiotic treatment it is imperative to know what bacteria are responsible for infection following the snakebite. The bacteria that inhabit the mouths and venom of Copperheads are not known.

This research surveyed the oral cavity and venom of Northern and Southern Copperheads (*Agkistrodon contortrix mokasen, A. c. contortrix,* respectively) for selected pathogenic bacteria. In addition, the venom was screened for inhibitory activities against bacterial species associated with wound infections. It is hoped that this research will identify venom components for future therapeutic development.

Copperheads

There are two subspecies of Copperheads (Figures 1 and 2) east of the Mississippi River: the Northern and the Southern Copperhead (*Agkistrodon contortrix mokasen,* and *A. c. contortrix,* respectively). The two subspecies intergrade over a large area that runs through West Tennessee, Northeast Mississippi, Central Alabama, Central Georgia, and a large portion of both Carolinas and East Virginia (Figure 3) (Conant and Collins, 1998).

Figure 1. Photographs of *Agkistrodon contortrix mokasen* (Northern Copperhead) taken from Snakes of the *Agkistrodon* Complex: A Monographic Review (Gloyd and Conant, 1990).

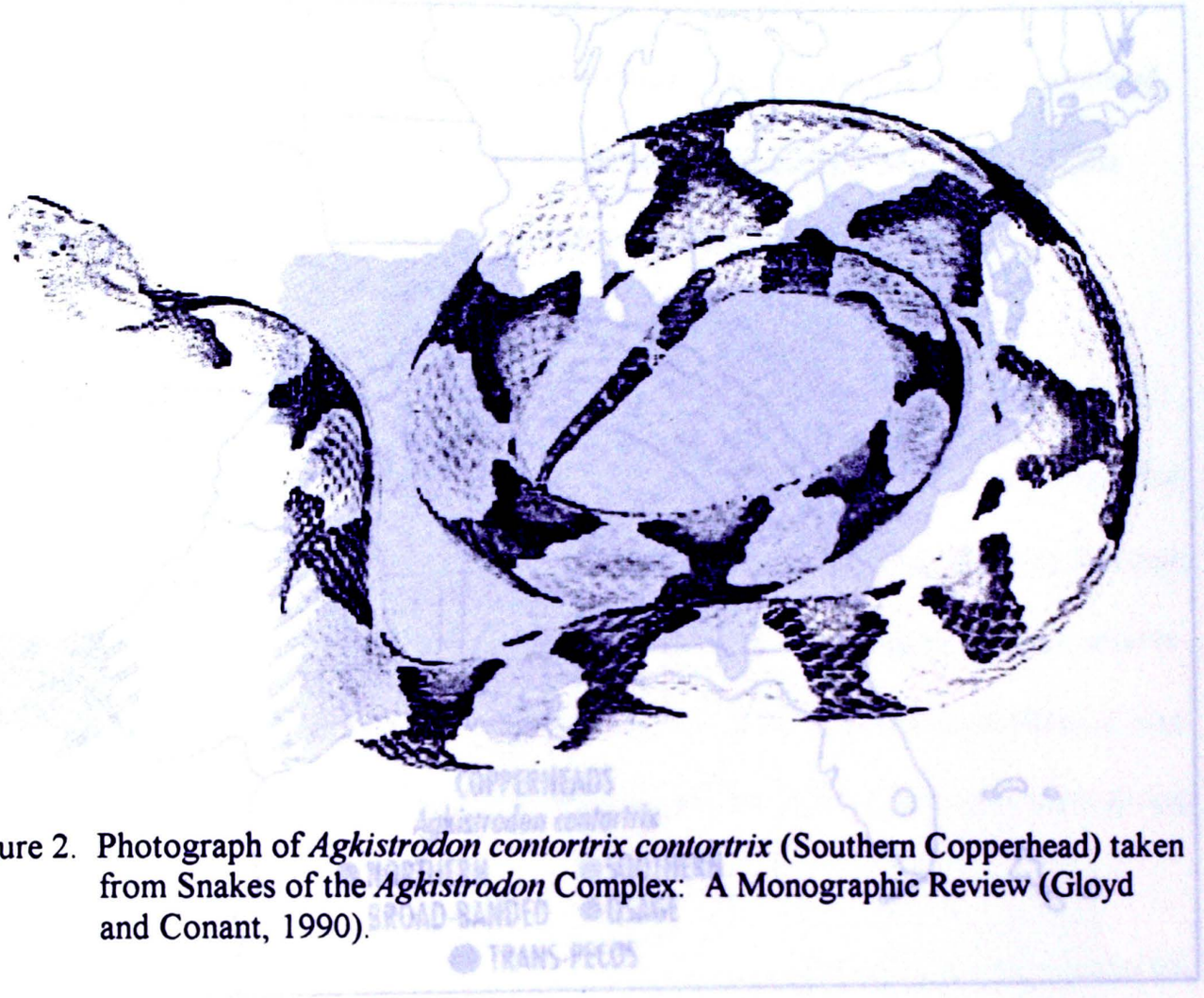

Figure 2. Photograph of *Agkistrodon contortrix contortrix* (Southern Copperhead) taken from Snakes of the *Agkistrodon* Complex: A Monographic Review (Gloyd and Conant, 1990).

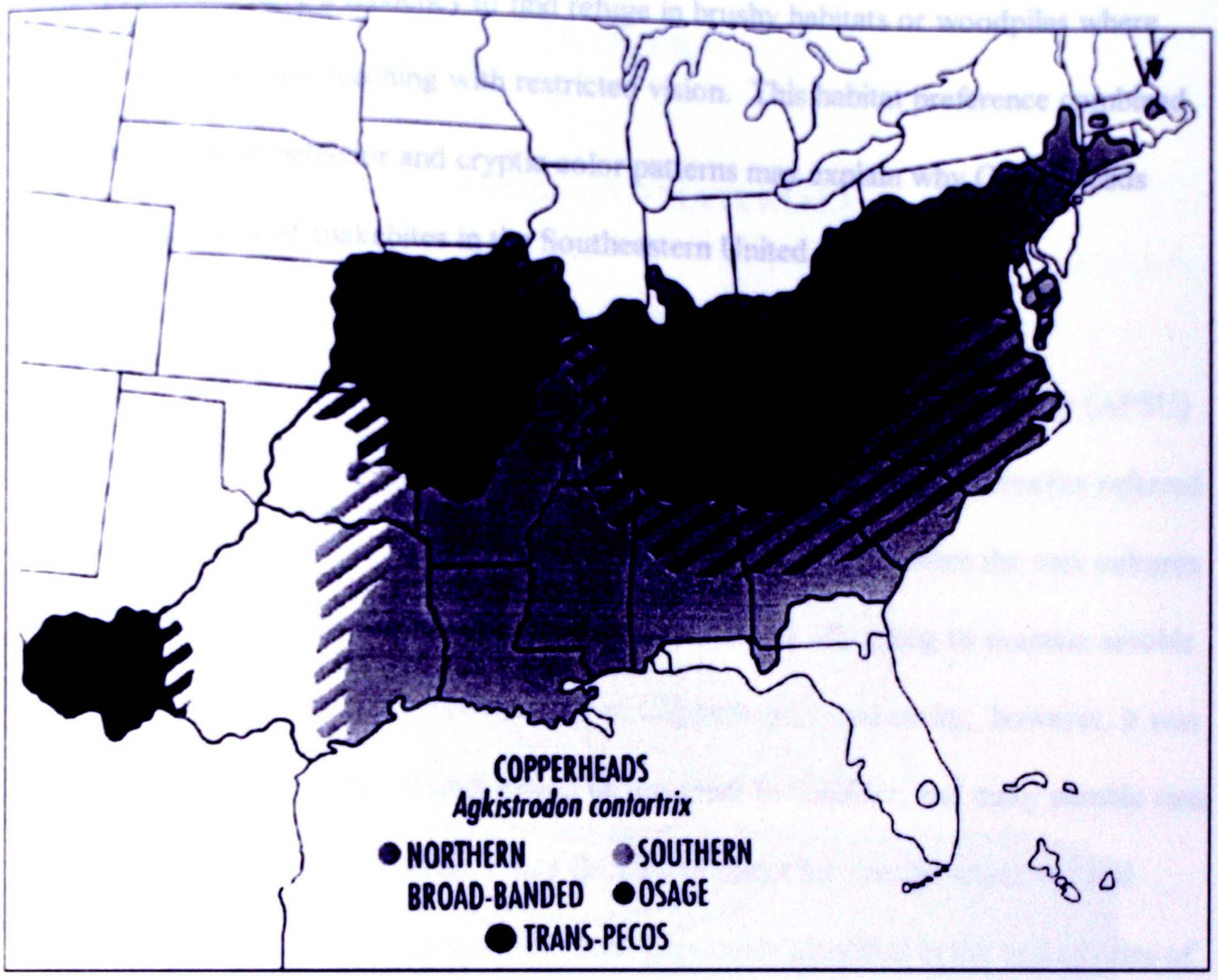

Figure 3. Range map of Copperheads in the United States. The map is from Conant and Collins (1991).

Copperheads have a tendency to find refuge in brushy habitats or woodpiles where humans frequently are reaching with restricted vision. This habitat preference combined with their sluggish behavior and cryptic color patterns may explain why Copperheads inflict the majority of snakebites in the Southeastern United States.

CHAPTER II

METHODS AND MATERIALS

Study Specimens

Two wild-caught Northern Copperheads in the Austin Peay State University (APSU) living amphibian and reptile collection were sampled. These snakes are hereafter referred to as APSU1 and APSU2. The cultures from APSU1 and APSU2 were the only cultures that were incubated aerobically at 25^{0}C on TSA. I was initially going to examine aerobic and facultative anaerobic bacterial flora of the Copperhead's oral cavity; however, it was determined that the number of taxa would be too great to consider, and many aerobic taxa would be nonpathogenic bacteria. I then decided to select for specific anaerobic and facultative anaerobic pathogens that have been previously identified in the oral cavities of other snakes.

Seventeen each of Northern and Southern Copperheads in the reptile collection of the Kentucky Reptile Zoo (Figure 4) were sampled. Included in the sample were both captive-bred and wild-caught snakes. Because of the manner in which the snakes were kept it was not possible to determine which snakes were captive-bred or wild-caught, nor was it possible to determine how long each snake had been in captivity.

Bacteria and venom were obtained from the sample specimens using the following routine procedures. Snakes were restrained and grasped by the back of the neck with the thumb and second finger, being sure to avoid placing fingers under the head, because the

a.

b.

Figure 4. Photographs taken at the Kentucky Reptile Zoo. A) Storages containers for snakes kept in the main building. B) Storage containers of snakes similar to those of the Copperheads that were sampled.

fangs can pierce the lower jaw. By gently squeezing inward on the jaws the mouth opened slightly allowing for insertion of a sterile swab.

Venom was collected by placing the lip of a sterile 10-ml snap-cap centrifuge tube under the upper mandible with the fangs pointing into the tube. The index finger was then pressed down on the head to express the venom.

Cultures

Bacteria were collected from the oral cavities of snakes using calcium alginate swabs. Once a swab was used to collect the sample it was dropped into AMIES transport media (Atlas, 1993) for transport back to the lab. In the lab, samples were streaked onto selective media (Table 1) which were used to detect selected specific pathogens in the samples. The cultures were incubated anaerobically for twenty-four hours at 37 ^{0}C. Anaerobic conditions were established using BBL® mot anaerobic gas pouches ™. The anaerobic conditions were used to help select for specific pathogens.

After the initial inoculation to selective media, each morphologically different culture was streaked for isolation onto separate tryptic soy agar (TSA) plates; the original plates were stored at 4 °C. All cultures were incubated anaerobically at 37 °C for 24 hours. Once isolation had been confirmed, stock cultures were prepared in tryptic soy broth (TSB), and adjusted to 50 % glycerol and stored at –80 °C. Bacterial isolates were identified using standard biochemical reactions as described in Bergy's Manual of Determinative Bacteriology (Holt *et al.* 1994).

Table 1. Listing of the selective and differential media used to isolate specific pathogens from the venom and oral cavity of Copperheads. The table also identifies the selective agents and the organisms for which the media selects.

Media[1]	Selective Property	Organism Selected
Bismuth Sulfite Agar (Difco)	Bismuth Sulfite	*Salmonella* sp.
Clostridium perfringens Agar (Atlas, 1993)	Oleandomycin and Polymyxin B	*Clostridium* sp. particularly *C. perfringens*
MacConkey Agar (Difco)	Bile Salts. No. # 3 and Crystal Violet	Gram negative enteric bacilli
Mannitol Salt Agar (Difco)	7.5% NaCl	*Staphylococci* sp.
Pasteurella Selective Agar (Moore *et al*, 1994)	Gentamicin, Potassium Tellurite and Amphotericin	*Pasteurella* sp.
Phenylethanol Agar (Difco)	Phenylethyl Alcohol	Gram positive bacteria
Cetrimide Agar (Difco)	Cetrimide	*Pseudomonas* sp.
Ryan's Media[2] (Atlas, 1993)	Ampicillin	*Aeromonas* sp.

[1]Difco media are available as commercial mixes, the remaining media were prepared as described in the citations.
[2]Modified original recipe to double strength of ampicillin.

Venom and Antibacterial Susceptibility Testing

Twenty microliters of collected venom were dropped onto a TSA plate and incubated anaerobically for 24 hours at 37 °C using BBL® mot anaerobic gas pouches ™. The remainder of the venom was transferred to a small culture tube and stored at –80 °C. There was no need to sterilize the venom for later use in the susceptibility assays, because only one sample had bacterial growth. This sample did not show growth again. Lyophilized Northern Copperhead venom, obtained from Sigma Chemical Company, was used in the susceptibility assays described below after it had been rehydrated with sterile saline (20mg/ml, 10mg/ml).

Venom collected from Copperheads and lyophilized Northern Copperhead venom were used in standard antibacterial susceptibility assays. The assays were adapted from Stiles *et al.* (1991), who tested different snake venoms for activity against *Pseudomonas aeruginosa* (ATCC no. 27853), *Staphylococcus aureus* (ATCC no. 29213), *Escherichia coli* (ATCC no. 25922), *Aeromonas hydrophilia* (ATCC no. 7965) and *Bacillus subtillus* (ATCC no. 6051). These same bacteria, with the exception of *Bacillus subtillus* (a non-pathogenic species), were used in this study to test for any antibacterial activities associated with the venom of the Copperheads.

The test bacteria were grown in Mueller-Hinton broth to an OD_{600} of 0.3; sterile cotton swabs were then moistened with the culture and spread across the surface of Mueller-Hinton agar plates. Sterile 5-mm Whatman 3M filter paper disks were saturated with the venom sample and placed on the surface of the inoculated Mueller-Hinton agar plates. The lyophilized Sigma venom was reconstituted by suspending 10 mg of venom in

500 µl of sterile saline yielding a final concentration of 20mg/ml. This concentration was referred to as Sigma. The venom was then diluted to a half strength solution (10mg/ml) which was labeled ½ Sigma.

The plates were incubated at 37^{0}C for 24 hours. After the 24-hour period the zones of inhibition were measured and recorded in millimeters.

Differences in the resulting zones of inhibition were evaluated using a two-factor nested ANOVA, where the factors were bacteria species and Copperheads subspecies (venom source) with the individual snake nested within Copperhead subspecies. ANOVA was followed by Tukey HSD pairwise comparisons.

Zone width data was transformed (1/zone^2) before analysis to normalize the data. All statistical tests were carried out using JMP IN Statistical Software (SAS, 2001).

CHAPTER III

RESULTS

Bacteriology

Bacteriology of the Oral Cavity

A total of eighty-eight non-replicated bacterial isolates were obtained from the 36 Copperheads sampled. This number is composed of the isolates listed in Tables 2 and 3, as well as 18 *Pseudomonas* isolates, and 15 Gram positive, non-sporing, irregular rods, not listed in the tables. Table 2 lists the bacterial taxa identified, the source (venom or oral cavity) and the percentage of sample specimens each taxon was found in.

The families Micrococcaceae and Enterobacteriaceae were the most prevalent among the isolates with the genera *Staphylococcus* and *Streptococcus* predominating. Only two representatives of the Bacteroidaceae family were obtained from these isolates and both were identified on *Pasteurella*-selective agar. Table 3 lists the bacteria that were isolated from the wild-caught Copperheads housed at APSU. The most commonly isolated bacterium was *Pseudomonas*.

I isolated 15 Gram positive, non-sporing, irregular rods that could not be identified. This number was the same as the *Staphylococcus* species. I was only able to group the Gram positive , non-sporing, irregular rods into one of three categories. There were eight catalase positive/fermentors, five catalase negative/fermentors, and only two catalase

Table 2. Bacteria identified from 46 non-replicated isolates from the venom and oral cavity of 34 Copperheads housed at the Kentucky Reptile Zoo.

Origin	Family[1]	Lowest taxon identified	% of Sample in which isolates were found: Individuals	Total Family
Venom				
	Micrococcaceae	*Streptococcus* sp.	3 %	
Oral Cavity				
	Enterobacteriaceae	only to family	6 %	38 %
		Salmonella sp.	6 %	
		Salmonella typhi	3 %	
		Enterobacter intermedium	3 %	
		Serratia rubidaea	6 %	
		Proteus vulgaris	6 %	
		Proteus mirabilis	3 %	
		Providencia rettegeri	3 %	
		Cedecea sp.	3 %	
	Pasturellaceae	only to family	15 %	18 %
		Pasterulla haemolytica	3 %	
	Bacteroidaceae	only to family		6 %
	Micrococcaceae	*Staphylococcus* sp.	44 %	74 %
		Streptococcus sp.	29 %	

[1] Family arrangement based on Bergy's Manual of Systematic Bacteriology (Krieg *et al.*, 1984)

Table 3. Bacteria isolated from the oral cavity of 2 wild-caught Copperheads housed at Austin Peay State University. Cultures were incubated aerobically at 25^{0}C.

Origin	Genus species
Oral Cavity of APSU1	*Micrococcus sedentarius*
	Bacillus sp.
	Staphylococcus sp.
	Pseudomonas sp.
Oral Cavity of APSU2	*Acetobacteraceae* sp.
	Brevundimonas vesicularis
	Acinetobacter calcoaceticus/Genospecies 1
	Micrococcus sedentarius
	Moraxella (Branhamella) ovis
	Arthrobacter histidinolovrans

positive/non-fermentors. *Staphylococcus* species and the Gram positive , non-sporing, irregular rods were the second-most common bacteria with *Streptococcus* being the third-most common bacteria overall.

Bacteriology of the Venom

Only one venom sample yielded bacteria, which were identified as *Streptococcus*. All other samples failed to produce any growth, including the lyophilized venom sample purchased from Sigma Chemical Company.

Antibacterial activity of venom

A disk-diffusion assay was used to screen for the presence antibacterial activity in venom samples of the two subspecies of Copperheads tested. The data in Table 4 shows that all venom samples had antibacterial activity against the four selected pathogens. ANOVA indicates that different bacteria species were inhibited by venom to differing degrees ($P < 0.0001$), but that the subspecies of Copperheads from which the venom was obtained is not a significant factor ($P = 0.7707$) (Table 5). Tukey HSD tests indicate that the mean zone widths of *Staphylococcus aureus* (X=16.97) and *Aeromonas hydrophilia* (X=12.14) differed significantly from the other bacteria tested. However there was no significant difference between the mean zone widths of *Escherichia coli* (X=9.17) and *Pseudomonas aeruginosa* (X=9.20).

Thus, although venom inhibited the growth of all four bacteria, *Staphylococcus aureus* was most inhibited, followed by *Aeromonas hydrophilia*, with *Escherichia coli* and *Pseudomonas aeruginosa* least inhibited. In addition, venom from both Copperhead subspecies inhibits the bacteria to a similar extent.

Table 4. Disk-diffusion assay of 36 venom samples from the Northern Copperhead (Acm), and the Southern Copperhead (Acc) for antimicrobial activity.

Venom Sample	*E. coli*		*A. hydrophilia*		*S. aureus*		*P. aeruginosa*	
	Acm	Acc	Acm	Acc	Acm	Acc	Acm	Acc
1	9	9	11	13	21	19	9	9
2	9	9	11	13	17	11	9	9
3	7	9	9	13	15	19	9	9
4	9	9	11	13	17	11	11	9
5	9	11	13	0	19	11	11	9
6	7	9	13	13	17	13	9	9
7	9	7	13	11	17	17	11	9
8	9	9	15	11	19	15	9	9
9	9	9	25	15	19	17	9	9
10	7	7	11	13	17	17	7	9
11	7	9	11	11	17	17	9	9
12	9	9	13	11	22	17	9	11
13	9	9	11	9	17	17	11	11
14	11	9	15	11	19	17	9	9
15	9	9	17	11	21	19	8	9
16	7	9	11	11	13	19	7	9
17	25	9	13	11	17	17	9	9
Means*	9.17[a]		12.14[b]		16.97[c]		9.20[a]	
Sigma[1]	2	nt	5	nt	6	nt	2	nt
½ Sigma[2]	1	nt	3	nt	5	nt	2	nt

nt: not tested, freeze-dried venom from *Agkistrodon contortrix contortrix* was tested by Stiles *et al.* (1991)

*Different letters indicate significantly different means ($P < 0.05$), as indicated by Tukey HSD tests

[1]The lyophilized Sigma venom was reconstituted by suspending 10 mg of venom in 500 µl of sterile saline yielding a final concentration of 20 mg/ml (Sigma).

[2]The venom was then diluted to a half strength solution (½ Sigma).

Table 5. Two-factor, nested ANOVA. Effect test from the whole model

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Subspecies	1	1	0.00000061	0.0854	0.7707
Bacterial species	3	3	0.00197906	92.2177	<0.0001
Snake [subspecies]*	37	37	0.00037847	1.4299	0.0873
Snake subspecies X Bacterial species	3	3	0.00002334	1.0873	0.3587

*Indicates individual snakes nested in subspecies

CHAPTER IV

DISCUSSION

Bacteriology

Oral cavity

Several bacteria identified in my study of Copperheads have also been previously identified in the oral cavity of various other species of snakes. Included among these previously identified bacteria were *Pseudomonas*, *Staphylococcus*, *Salmonella*, *Proteus*, and various other representatives of the Enterbacteriaceae and Bacteroidaceae families (Arroyo *et al.*, 1980; Draper *et al.*, 1981; Goldstein *et al.*, 1981; Ledbetter and Kutscher, 1969; Theakston *et al.* 1990; Soveri and Seuna, 1986). However, there is no previous mention of representatives of the Pasteurellaceae family, several of which were isolated in my study.

Another difference between my study and the results of previous work is the abundance of Gram positive, non-spore forming, irregular rods that I found. Other researchers (Arroyo *et al.*, 1980; Draper *et al.*, 1981; Goldstein *et al.*, 1981; Ledbetter and Kutscher, 1969; Theakston *et al.*, 1990; Soveri and Seuna, 1986) have previously found Gram positive rods in the oral cavity of snakes, but none have mentioned finding irregular rods, which were isolated in my study.

Venom

In Goldstein *et al.* (1979) the researchers collected venom from disinfected fangs and non-disinfected fangs. Fifty percent of venom samples taken from the disinfected fangs had no growth; the other 50% had only slight growth. Goldstein *et al.* (1979) concluded that, similar to other body fluids, venom is sterile (it was believed that the growth was a result of oral contamination). Evidence from my Copperhead study supports this conclusion. Of the thirty-seven (36 fresh samples and one from Sigma Chemical Company) venom samples only one showed any evidence of bacterial growth. The bacterium identified from the single venom sample was not a unique isolate; it was a species of *Streptococcus*. There were several isolates of *Streptococcus* identified from the oral cavity of the Copperheads. It is possible that the isolate could have been a contaminant from the oral cavity.

The conclusion that venom is a sterile body fluid is also supported by the results of the venom assay. Each of the venom samples had a negative effect on the growth of the pathogens they were tested against; *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli*, and *Aeromonas hydrophilia.*

This venom assay was a disk diffusion assay developed with slight modifications of the procedure described by Stiles *et al.* (1991), and four of the same bacteria that were used in Stiles' *et al.* (1991) assays were used for my assays. The results listed in Table 4 show that each of the venom samples had an inhibitory effect on each of the bacteria. Stiles *et al.* (1991) failed to demonstrate any effect of Copperhead venom on these bacteria. Stiles *et al.* (1991) used only Southern Copperhead venom; however, my research indicates that

there is no significant difference between the Northern and Southern Copperhead venoms in regards to their antimicrobial activities against the four pathogens used in the venom assay.

As mentioned previously, it is believed that one of the benefits of the antimicrobial activity of snake venom is to protect the snake from pathogens (Thomas and Pough, 1979). *Aeromonas hydrophilia* is widely considered a pathogen of reptiles. These data may help support Thomas and Pough's (1979) hypothesis, *Escherichia coli* is not usually a reptilian pathogen and *Pseudomonas aeruginosa* requires a compromised host for infection. It is possible that the external membrane of the Gram negative bacteria helps to protect them better than the Gram positive *Staphylococcus aureus*, which has no external membrane.

A comparison of my research with that of Stiles *et al.* (1991) may provide some explanation for the differences found between our two studies. We both used the same ATCC strains of bacteria, and we both used the same concentration of reconstituted venom from Sigma (10mg/ml) of venom, with the addition of the double concentration in my research. Stiles *et al.* (1991) grew their bacterial cultures to an OD_{600} 0.1, whereas my density was three times greater. I used a lesser amount of fresh venom (~10 µl) whereas Stiles *et al.* (1991) used 15 µl of rehydrated venom, I allowed my plate to incubate longer (24 hrs compared to 18 hrs). It is possible that the only significant differences between these two studies are the differences between the snakes from which the venom was collected.

CHAPTER V

CONCLUSIONS

From a review of the literature and data collected in this study it is obvious that the oral cavity of snakes has a high diversity of bacterial flora. As Goldstein *et al.* (1979) suggests, this flora is probably related to the food that the snakes ingest. A future study might compare the transmittable bacteria of a food animal to that of the oral bacteria of the snakes fed on that animal.

This diverse flora makes finding a narrow-spectrum antibiotic to be used as a propholytic for snake bits very difficult. Ledbetter and Kutscher (1969) indicated that the *Clostridium* species they isolated were susceptible to penicillin, erythromycin, tetracycline, and chloramphenicol. Theakston *et al.* (1990) indicates that gentamicin was most effective against the bacteria that were isolated in their study; however, in my study a *Pasteurella* selective media was used that contains gentamicin. Since this media allowed for the isolation of several organisms, it would not be effective in cases where members of the Pasteurellaceae were present.

Flandry *et al.* (1989) characterized the bacterial flora of the Alligator (*Alligator mississippiensis*) and found it to be similar to that of snakes. In their research of antibiotic therapy for alligator bites, it was determined that 87% of the isolates were susceptible to chloramphenicol, and 77% were effected by gentamicin. Their research

also indicated that the isolates were resistant to trimethoprim-sulfamethoxazole. One group of antibiotics that was not mentioned was the cephalosporins. Several of the bacteria identified in my study are usually susceptible to either a first, third, or fifth generation cephalosporin. Cephalosporins have a low toxicity level to humans (Brock *et al.*, 1994), but they have not been directly tested against bacteria isolated from the oral cavity of snakes. Chloramphenicol seems to have the greatest, although not complete, effect out of those previously mentioned and has been tested against wild-reptile isolated bacteria (Ledbetter and Kutscher, 1969; Flandry *et al.*, 1989); however, its negative side effects usually out weigh its benefits (Totora *et al.*, 1992).

One aspect to consider is that these antibiotics when used were not used in clinical trials. It is possible that an antibiotic that works in laboratory trials, or is effective in treating one type of infection, may not be effective in clinical trials or in wound infections.

Copperhead venom has a significant effect on ***Aeromonas hydrophilia*** and *Staphylococcus aureus*, as well as an effect on ***Pseudomonas aeruginosa*** and ***Escherichia coli***. Further research needs to be preformed to characterize antimicrobial components of the venom and whether these components could be viable chemotherapeutics.

The taxa of bacteria I found in the venom and oral cavities of Copperheads represent an incomplete sample of the total diversity. Some limitations that account for this are the small sample size and a failure to detect certain common snake pathogens (e.g.

Aeromonas and *Clostridium*) that may have been destroyed during the sampling process. Still, the results of my study contribute to a better understanding of what pathogens are associated with the venom and oral cavity of Northern and Southern Copperheads, and help further the knowledge of ophidian biology. It is hoped that data from this study will spawn new research into the field of chemotherapeutics and contribute to a better understanding of one of the animals most feared by man: the Copperhead.

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VITA

Daniel R. French was born in Lexington, Tennessee on February 16, 1975. He graduated from Lexington High School in May 1993. The following semester he enrolled at Freed-Hardeman University. A medium-sized private, church of Christ affiliated university located in Henderson, Tennessee. While there he worked as a microbiology laboratory assistant for Dr. Paul Fader; this stirred his interest in microbiology. Later, he participated in various research projects relating to herpetology under the supervision of Dr. Brian Butterfield. These two men were instrumental in the development of Daniel's particular interest in microbiology and herpetology. In December 1997, he received a Bachelor of Science in Biology, with minors in chemistry and biblical studies. Daniel then entered the Master of Science in Biology program at Austin Peay State University, where he began work with Dr. A. Floyd Scott in herpetology; however, he later decided to develop a research project that centered around microbiology. The result was this thesis supervised by Dr. Don Dailey. After graduation from Austin Peay State University (2001) he plans to pursue a Doctorate of Philosophy in Environmental Biology at Arkansas State University, continuing this theme of microbiology/herpetology by working with pathogens of reptiles and amphibians.

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