Summer 2023

Characterization of Antimicrobial Properties of Excrement and Functional Microbiome of Black Vultures (Coragyps atratus)

Bridgette Gray
bgray3@stu.jsu.edu

Jacksonville State University
Graduate Studies
700 Pelham Rd N., 320 Bibb Graves Hall, Jacksonville, AL 36265
Phone: (256) 782-5348
http://www.jsu.edu/graduate/index.html

Thesis Approval Form

First Name: * Bridgette Last Name: * Gray

Date: * 07/12/2023

Please indicate the program you are completing:

* (•) Biology
( ) Criminal Justice
( ) English
( ) Geographic Information Science and Technology
( ) History
( ) Mathematics
( ) Music
( ) Psychology

Thesis Title: Characterization of Antimicr

Will you be using additional committee members for your thesis? * ( ) Yes (•) No

Student Signature	Electronically signed by Bridgette Gray on 07/12/2023 9:09:56 PM
Chair Signature	Electronically signed by Ashley Turner on 07/12/2023 9:23:08 PM
Member1 Signature	Electronically signed by Jocelyn Moore on 07/13/2023 11:20:48 AM
Member2 Signature	Electronically signed by Grover Brown on 07/13/2023 1:22:16 PM
Department Head Signature	Electronically signed by Lori Hensley on 07/13/2023 2:12:49 PM
Dean of Graduate Studies Signature	Electronically signed by Channing Ford on 07/31/2023 11:45:32 AM

THESIS APPROVAL

Candidate:	Bridgette Cheree Gray
Major:	Biology
Thesis Title:	Characterization of Antimicrobial Properties of Excrement and Functional Microbiome of Black Vultures (*Coragyps atratus*)

Approval:

Dr. Ashley N. Turner — Date
Assistant Professor of Biology
Major Professor

Dr. Grover J. Brown — Date
Assistant Professor of Biology

Dr. Jocelyn A. Moore — Date
Associate Professor of Biology

Dr. Channing R. Ford — Date
Dean, Graduate Studies

CHARACTERIZATION OF ANTIMICROBIAL PROPERTIES OF EXCREMENT AND FUNCTIONAL MICROBIOME OF BLACK VULTURES (*CORAGYPS ATRATUS*)

A Thesis Submitted to the
Graduate Faculty
of Jacksonville State University
in Partial Fulfillment of the
Requirements for the Degree of
Master of Science
with a Major in
Biology

By

BRIDGETTE CHEREE GRAY

Jacksonville, Alabama

August 4, 2023

Bridgette Cheree Gray August 4, 2023

ABSTRACT

Black vultures, *Coragyps atratus*, are obligate scavenging birds that consume and dispose of decaying carcasses and carrion. They fulfill a key ecological niche in the environments in which they live. It has been observed that these vultures sometimes excrete bodily waste onto their legs. This adaptive behavior could help aid them in controlling bacteria and other microbes they encounter while stepping into a carcass to eat. This study directly examined the antimicrobial properties of the excrement of black vultures across various bacterial species utilizing a zone of inhibition test and a nematode species utilizing a survival assay. The black vulture microbiome was also examined by characterizing the bacterial species present in the vulture excrement utilizing 16S ribosomal RNA sequencing. Antimicrobial experiments revealed the whole vulture excrement yielded positive zones of inhibition for *Bacillus coagulans* and *Staphlococcus aureus* and no impact of development and early survival for *Caenorhabditis elegans*. The microbiome of the black vulture's excrement had 31 bacterial species identified across 9 classes. Overall, there are several current threats to black vultures, including environmental contamination and poisoning. It is important to understand more about the complex biology, health, and status of black vultures to ensure they do not become threatened in Alabama and the United States.

viii, 45 pages

ACKNOWLEDGEMENTS

I would like to thank Dr. Ashley Turner for being a wonderful mentor and for all of her help and support. She was my superstar during this project. Additionally, I would like to thank my committee members Drs. Grover Brown and Jocelyn Moore for their contributions to this project. I would also like to thank Dr. Lindblom for the use of his property for wild vulture sample collection. Thank you to Dr. Rachel Turner and the Birmingham Zoo and Dr. Casey Morrow and the Microbiome Institutional Research Core at University of Alabama at Birmingham for their collaborations on this project. Thank you to the Worm Hole lab and our members, especially to Tanner Vandever and Elise Patrick. A very special thank you to Muerto, Onyx, and Wakinyan. Thank you to my most wonderful partner Trent Moody for being there for me through this process and always supporting me. Last but not least, thank you to my friends and family who supported me throughout this journey.

Thanks so much everyone, I could not have done it without you!

Bee Gray

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

I. INTRODUCTION

Vultures are large, obligate scavenging birds that fulfill a key ecological niche in the environments in which they live. There are two distinct groups of vultures, New World vultures and Old World vultures. These groups of vultures are not genetically close to each other and have similarities with their carrion eating behaviors and morphology from convergent evolution (Wink, 1995). New World vultures are in the family *Cathartidae* which includes 7 extant species of vultures which reside in North, South, and Central America (Carlson, 2021; *Vulture | San Diego Zoo Animals & Plants*, n.d.). While Old World vultures are in the family *Accipitridae* which also includes eagles, kites, and hawks and these vultures reside in Africa, Asia, and Europe (*Vulture | San Diego Zoo Animals & Plants*, n.d.; Wünschmann et al., 2018).

Two distinct species of New World vultures are native to North America and inhabit Alabama, black vultures (*Coragyps atratus*) and turkey vultures (*Cathartes aura*). Black vultures have distinctive bald heads, black faces, and black bodies (Figure 1). They consume and dispose of substantial amounts of decaying carcasses or carrion to help keep ecosystems healthy and balanced (Buechley & Şekercioğlu, 2016b). In doing this, they help reduce the spread of disease (Buechley & Şekercioğlu, 2016b). Importantly, vultures are federally protected by the Migratory Bird Treaty Act of 1918 administered by the U.S. Fish and Wildlife Services (Migratory Bird Treaty Act, 1918; *USDA APHIS | Species*, 2020).

The U.S. North American Bird Conservation Initiative Committee released the 2019 State of the Birds: America's Birds are in Crisis Report shares that 30% of all North

American birds have disappeared in the last 50 years and there are massive reported losses among U.S. bird populations (*State of the Birds 2019*, 2019). Vultures have experienced the most rapid decline in conservation status of any group of birds over the past decade and comprise the most threatened avian functional guild in the world, largely due to poisoning (Buechley & Şekercioğlu, 2016a). Globally, nine of 23 vulture species are classified as critically endangered and on the brink of extinction, three are endangered, four are near threatened, and seven with least concern (Buechley & Şekercioğlu, 2016b).

Both black vultures and turkey vultures faced decline from 1900-1970s due to the misconception they spread disease that led to trapping and killing along with the use of the pesticide Dichlorodiphenyltrichloroethane (DDT) that led to bioaccumulation and eggshell thinning and reproductive decline (Kiff et al., 1983; *The Black Vulture (Coragyps Atratus) | MSEM in the Field*, 2017). However, with better environmental awareness and actions on our part, today these two vultures are among the most common large carnivorous birds in North America (*The Black Vulture (Coragyps Atratus) | MSEM in the Field*, 2017). According to the North American Breeding Bird Survey, both black and turkey vultures have increased in number across North America from 1966 to 2014 (Sauer et al., 2014). Both the Alabama Department of Conservation and Natural Resources and United States Department of Agriculture's Animal and Plant Health Inspection Service report these vultures have a low conservation concern status in Alabama and the United States at large (Bird Life International, 2016, 2018; Moore, n.d.; Pugh, n.d.; *USDA APHIS | Species*, 2020). Overall, they are currently one of the seven

vulture species with least concern by the International Union for Conservation of Nature (IUCN) and stable in the world mentioned previously (Buechley & Şekercioğlu, 2016b).

However, it is important to remember current threats to these vultures include environmental contamination and poisoning, including lead, mercury, pesticides, and insecticides. As scavengers who live on rotting carrion, they can fall victim to poisons or chemicals in dead animals. This was the case with their New World vulture cousin, the California condor, that declined to near extinction due to poaching, habitat destruction, and lead poisoning (Johnson et al., 2013; Rideout et al., 2012). It is extremely important to understand as much as we can about the biology, health, and status of these two New World vultures to ensure they do not become threatened in Alabama and the United States.

It has been observed and noted that New World vultures sometimes excrete waste onto their legs. There are two probable ideas as to why this behavior is carried out and maintained by these birds. The first idea is the birds excrete on their legs for thermoregulation using evaporative cooling in a process called urohidrosis (Cabello-Vergel et al., 2021; "Energy Conserving and Heat Dissipating Mechanisms of the Turkey Vulture," 1970). This thermoregulation benefit has been documented among various species of birds, including storks and New World vultures (Cabello-Vergel et al., 2021; Graves, 2019). The second postulate is they might excrete on themselves to control bacteria and other microbes they encounter while eating and encountering a carcass. The highly acidic uric acid may act as an antiseptic body fluid helping the animal control microbial communities on their legs that they are exposed to while eating carrion.

There have been some interesting studies involving New World vultures over the past ten years that have unintentionally scratched the surface of examining the second proposed idea above, however only indirectly. The first microbiome study with New World vultures highlighted the remarkable tolerance that these vultures have evolved to bacterial toxins in decaying carrion (Roggenbuck et al., 2014). Face and gut samples were examined. It was discovered that *Clostridium* and *Fusobacterium* species dominate both the black vulture's and turkey vulture's gut microbiota, both widely pathogenic to other vertebrates. However, this study did not directly examine the microbiome through vulture excrement, but face and gut samples. Another study was interested in examining whether griffon vultures, an Old World vulture, might be a useful reservoir of bacteriocin-producing lactic acid bacteria (LAB) (Arbulu et al., 2016). Fresh excrement samples were collected, and LAB were isolated from the excrement and evaluated for antimicrobial activity against various bacterial species, including Gram-positive and Gram–negative bacteria. There were several LAB species identified with remarkable antimicrobial activity. However, this study was carried out with Old World vultures and did not assess the whole excrement antimicrobial activity for this vulture species. Overall, reviewing the literature, the second hypothesis appears to not have been directly examined experimentally.

This study proposes to gain a better understanding of how these sophisticated scavengers have evolved to do what they do through two major arms of the project (Figure 2). We proposed and set out to directly examine the antimicrobial properties of vulture excrement of the black vulture, *Coragyps atratus*, native to Alabama against

various bacterial species. We also wanted to identify the bacterial species present in vulture excrement by characterizing the microbiome of the black vulture.

II. MATERIALS AND METHODS

Wild Vulture Excrement Sample Collection

Attempts were made to collect excrement samples from wild black vultures in Jacksonville, Alabama. Permission was obtained to bait in a cotton field on private property (North 33.8330513, West -85.7672834) (Figure 3). To prepare, a large plastic tarp was disinfected with a 10% bleach solution and allowed to dry overnight. The sterilized tarp was stored in a large trash bag. Five deer rib cages were used to bait and were obtained from Trail Ends Deer Processing. The deer carcasses were stored at -20°C in a chest freezer for 2 weeks and thawed the morning of prior to baiting.

In the field, the plastic tarp was placed and staked using standard metal tent stakes. All thawed deer rib cages were placed in the center of the tarp and securely tied to a cement block to prevent mesocarnivores from stealing the bait (Figure 3B). The baited tarp was observed for 4 days at the original location (North 33.8330513, West -85.7672834) (Figure 3C). It was then transported and observed for 3 days at a second location within the same field (North 33.8329101, West -85.7697980) (Figure 3D). Following this wild sample collection, the deer carcasses were disposed, and the plastic tarp disinfected and disposed.

Captive Vulture Excrement Sample Collection

Excrement samples were collected and obtained from three captive black vultures at the Birmingham Zoo in Birmingham, Alabama. Information for these birds, including age, weight, diet, and housing, are presented in Table 1.

For the antimicrobial experiments, excrement samples were collected from the birds at two time points (Table 2). A total of 8 excrement samples were obtained across

all vultures (Table 2). Fresh droppings were collected with a sterile cotton swab (Cat.# 76407-738, VWR International) from the floor of each bird's enclosure. Each sample swab was placed into a separate pre-labeled biohazard collection bag (Cat.# 11215-684, VWR International) and transported on an ice pack in a Styrofoam cooler for downstream antimicrobial experiments.

For microbiome analysis, excrement samples were collected from each bird using the outlined procedure above across a 5-day period (Table 3). A total of 9 excrement samples were obtained across all vultures (Table 3). Each sample was stored immediately at -20°C in a freezer until transported on dry ice in a Styrofoam cooler for downstream microbiome analysis.

Antimicrobial Experimentation

Following collection and transport, excrement samples were processed within 8-10 hours of collection. Antimicrobial properties of black vultures' excrement were examined through a series of zone of inhibition tests using bacteria species (or Kirby-Bauer Test) and survival experiments using nematodes (*Caenorhabditis elegans*) (Martí et al., 2018; Park et al., 2017; Sutphin & Kaeberlein, 2009). The general setup for the zone of inhibition assay is shown in Figure 4. All experiments were carried out in a BSL-2 level biological safety cabinet (LabGard ES NU-540 Class II Type A2 Biosafety Cabinet) and personal protective equipment was worn including N95 masks, gloves, and lab coats.

Each vulture excrement sample was mixed into 10 mL of sterilized peptone water (peptone water was made using l00 mL deionized water, 1 g peptone, and 0.5 g sodium

chloride) (Cat.# 700011-270, VWR International) in a 50 mL Falcon tube. This excrement sample solution was divided into two separate 50 mL Falcon tubes for examining whole or filtered excrement downstream. Forty pre-sterilized 6 mm antibiotic assay disks (Cat.# 89007-048, VWR International) were added to 5 mL of whole or filtered excrement sample solution and soaked for 15 minutes (*M100 PERFORMANCE STANDARDS FOR ANTIMICROBIAL SUSCEPTIBILITY TESTING*, 2023). Next, the moist excrement-soaked disks were removed from the sample solution and placed in a sterile Petri dish lid and allowed to air dry for 30 minutes inside the biological safety cabinet. Three dried excrement disks were then placed onto each inoculated bacteria plate equal distance from the center of the plate for the zone of inhibition assay. To generate the filtered excrement sample solution, 5 mL of fresh vulture excrement sample solution was vacuum filtered through a 0.22 μm filter (150mL Filter Unit, Fisher Scientific, Cat.# FB 12566500).

Five bacterial species were selected to test in zone of inhibition experimentation (Table 4). Each bacterial species was grown in liquid cultures of nutrient broth (nutrient broth was made using 8 g of nutrient medium mixed with 1 L distilled water) (Cat.# 785361, Carolina Biological) shaking at 200 RPM at 37 °C overnight for 12 hours in a shaking biological incubator. 1.5 mL of each liquid bacterial culture was inoculated on a 100 mm nutrient agar plate, introduced to whole or filtered excrement sample disks, and incubated at 37 °C for 72 hours in a biological incubator. There was a total of 5 vulture excrement samples collected across 3 birds. Each excrement sample, whole or filtered, was tested against each bacterial species on 2 100 mm plates with 3 dried excrement

samples per plate. In total, this included 5 biological excrement samples and 6 technical replicates per biological excrement sample.

The following experimental modifications were carried out for the vulture excrement samples collected at the second time point (Table 2). For whole excrement sample solutions, all bacterial species were tested again as described above with the addition of 1 inoculated plate of *Bacillus coagulans* with a single soaked excrement disk per excrement sample per plate. Additionally, filtered excrement samples were only screened against *B. coagulans*.

Photos of each bacterial plate were taken using a Nikon camera (Cat.#NID56001855, B&H) and photo box (Cat.# FOSIAB1616, B&H). Identified zones of inhibition were quantified through measurements taken with digital calipers (Cat.# 36934-154, VWR International) and a ruler.

Nematode survival experiments were carried out using the common wildtype lab strain of *C. elegans*, N2, obtained from the Caenorhabditis Genetics Center (CGC). *Escherichia coli* OP50-1 was obtained from the CGC and grown in liquid cultures of LB broth. Nematode growth media (NGM) plates seeded with live OP50-1 were used to grow nematode populations and were made following standard protocols (Stiernagle, 2006). All nematodes were maintained at 20 °C in a refrigerated incubator.

Survival analyses were carried out with time synchronized L1 hermaphrodite animals or eggs. Populations were synchronized by picking young adult animals onto fresh plates for egg lay. For experiments in which L1 animals were exposed to vulture

excrement, NGM plates were spotted with 100 µL of whole or filtered excrement sample solution or peptone water. For experiments in which eggs were exposed to vulture excrement, NGM plates seeded with OP50-1 were spotted with four 30 µL spots of whole or filtered excrement sample solution or peptone water around the circular OP50-1 lawn in the center of the plate. All experiments were carried out at 20 °C in a refrigerated incubator. Animals were observed and scored every other day until day 5 for time synchronized L1 animals or day 4 for time synchronized eggs. Animals that died due to walling were censored. The results represent the survival rate from a minimum of four biological replicates with 20 animals per replicate. Photos of each experimental group were taken using a dissecting microscope and Moticam S3 microscope camera (Cat.# MoticamS3, Motic).

Microbiome Analysis

Excrement samples were collected following the protocol outlined above. Samples were collected from each bird across a 5-day period (day 1, day 3, and day 5). All samples were stored immediately at -20°C in a freezer and transported on dry ice in a Styrofoam cooler to the UAB Microbiome Core at the University of Alabama at Birmingham in Birmingham, Alabama.

Microbiome analysis of the black vulture excrement samples was carried out through 16S ribosomal RNA sequencing at the UAB Microbiome Institutional Research Core. Three excrement samples were taken from each vulture across a 5-day period (n = 9 samples). DNA was isolated from samples that was then carried through a standardized protocol for 16S rRNA sequencing on the Illumina MiSeq platform and bioinformatic data analysis as previously described (Kumar et al., 2014). Importantly, amplicon

sequence variants (ASVs) were utilized from this high-throughput analysis which allowed classification of groups of species based on DNA sequence variation.

Statistical Analysis

Statistical analyses were performed as indicated in the figure legends. A value of $P<0.05$ was considered statistically significant. Statistical analyses were performed using GraphPad Prism 9 (Version 9.4.0, GraphPad Software).

Figure and Thesis Creation

Figures were created using Adobe Photoshop 2023 (Version 25.5.0, Adobe) and Adobe Illustrator 2023 (Version 27.6.1, Adobe). Thesis document was composed using Microsoft Word (Version 16.74, Microsoft 365 Subscription) and compiled using Adobe Acrobat Pro (Version 2023.001.20177).

III. RESULTS

Wild Vulture Sample Collection

Sample collection of wild samples was unsuccessful. Bait was observed for 4 days at the first location (North 33.8330513, West -85.7672834) (Figure 3C). The bait was initially placed under power lines (Figure 3A). Due to lack of vulture activity, power lines, and high foot traffic of hikers on the Chief Ladiga Trail the tarp was moved further into the field to the second location (North 33.8329101, West -85.7697980) (Figure 3D). The second location had little to no foot traffic and no powerlines to interfere with baiting. The second location was observed for 3 days, but no vultures approached the tarp or bait during daylight observations.

Captive Vulture Sample Collection

Captive vulture sample collection was successful. We collected a total of 8 excrement samples from 3 captive black vultures at the Birmingham Zoo at 2 collection time points for the downstream antimicrobial study (Table 2). We collected a total of 9 excrement samples from the same 3 captive vultures across a five-day period for the downstream microbiome analysis (Table 3).

Antimicrobial Experimentation

The antimicrobial study yielded positive results for detected zones of growth inhibition for 2 out of the 5 bacterial species when introduced to the black vulture excrement. For the excrement samples collected during the first captive collection, it was observed that the whole, unfiltered sample solution-soaked disks yielded zones of growth inhibition for *Bacillus coagulans* (Table 5; Figure 5). The presence of zones and measurements for *B. coagulans* was significantly different compared to the lack of zones

with 4 other bacterial species (Figure 7A). For the excrement samples collected during the second captive collection, it was observed that the whole, unfiltered sample solution-soaked disks yielded zones of growth inhibition for *Staphlococcus aureus* (Table 6; Figure 6). The presence of zones and measurements for *S. aureus* was significantly different compared to the lack of zones with 4 other bacterial species (Figure 7B).

The antimicrobial study yielded negative results for survival analysis of N2 *C. elegans* when introduced to the black vulture excrement. For L1 animals exposed at day 1, there was no significant difference for survival of animals across the experimental groups, including peptone water (control), whole excrement sample solution, and filtered excrement sample solution (Figure 8A). For eggs exposed at day 0, there was no significant difference for survival of animals across the experimental groups (Figure 8B).

Microbiome Analysis

A total of 31 bacterial species were identified across 9 classes within the collected black vulture excrements. Clostridia is the most prominent bacterial class identified within the excrement samples making up 85.64% of the total reads (Figure 9). The next most abundant bacterial class is Fusobacteria with 7.13% of the total reads (Figure 9). The bacterial profile identified within and across excrement samples taken from three birds across a 5-day period was conserved (Figure 10). Excrement samples from vulture 3 appeared to have a distinct bacterial distribution compared to other two birds with higher percentage of identified reads for Fusobacteria.

IV. CONCLUSIONS

In the attempts to collect excrement samples from wild black vultures, there are numerous variables that need to be considered that could have resulted in unsuccessful observations, baiting, and sample collection. The black vultures might have been deterred from the area due to human activity and sensing our observational presence. Initially, the deer rib cages were placed on the tarp under powerlines near the end of the field close to the Chief Ladiga Trail with a lot of human foot and bicycle traffic. The tarp and bait were then moved to a more remote location in the field, however there was still no activity. Black vultures have a heightened sense of smell, and it is possible that they could smell our presence on the field and were deterred (Santos et al., 2023). Turkey vultures have an even more sensitive sense of smell than black vultures, and black vultures often rely on the presence of turkey vultures by tracking the turkey vultures with their keen eyesight in order to find their food source (Grigg et al., 2017). If the turkey vultures smelled our presence this could have disturbed the whole cycle of black vultures finding carrion. Additionally, the deer meat did not produce much of a smell for the turkey vultures or black vultures to pick up on. During the observations, vehicles were parked down the road and binoculars were used to observe the tarp. However, the black vultures may have seen the vehicles and been deterred from approaching the tarp. Additionally, the reflection of the sunlight off the plastic tarp could have discouraged birds from lighting on or around the tarp and exploring the bait. There is little mention of using plastic tarps for sample collection from birds in the wild in the literature, which could be due to unique challenges that this approach entails. Additionally using fresh, rotting bait might attract more scavengers. The bait we obtained was stored in a chest freezer and thawed

upon its use. Lastly, during sample collection, the area was under a wind advisory with high winds throughout the collection period. Black vultures are described as stable in flight, but sometimes wobbling slightly in strong winds (*Hawkwatch International - Black Vulture*, n.d.). This could have potentially prevented the black vultures from flying and discovering the bait.

Black vulture excrement does appear to have the ability to prevent some microbes from growing. The antimicrobial studies revealed positive and negative results screening against 5 bacterial species and 1 nematode species. The whole excrement sample solution led to zones of growth inhibition for *B. coagulans* and *S. aureus*. Notably, both bacterial species are Gram-positive bacteria. There were no detected zones of inhibition for any Gram-negative bacteria species exposed to the excrement-soaked disks. These findings concur with a previous study's findings where they specifically isolated lactic acid bacteria (LAB) from the excrement of Griffon vultures and tested those against various bacteria species (Arbulu et al., 2016). They found that the isolated LAB from the excrement inhibited the growth of some bacterial species, specifically the Gram-positive ones and not the Gram-negative ones. The vulture excrement may be inhibiting the growth of the Gram-positive bacteria species due to the structural difference between the two that ultimately results in their Gram stain difference. Gram-positive bacteria have a thicker peptidoglycan layer and no outer lipid membrane while Gram-negative bacteria have a thinner peptidoglycan layer surrounded by an outer membrane containing lipopolysaccharide (Silhavy et al., 2010). This difference could be allowing sensitivity of the Gram-positive bacteria to the vulture excrement. With regards to the antimicrobial effects of the black vulture excrement on nematodes, the whole excrement sample

solution did not negatively impact *C. elegans* survival, as they thrived and appeared to be consuming live bacteria within the excrement.

Although *B. coagulans* and *S. aureus* both showed zones of inhibition with the whole excrement samples, there was difference across the two captive sample collections (Table 2). For the first collection we observed that *B. coagulans* had positive zones of inhibition around the excrement disk while the *S. aureus* had no zones of inhibition. From the second collection approximately 1 month later, *B. coagulans* did not present zones of inhibition with exposure to excrement disks, while *S. aureus* did have positive zones of inhibition. This could be the result of the natural changes that occur within an organism's gut microbiome across time (Jones et al., 2021). Excrement samples were collected across a 5-day period for the microbiome analysis for this reason to get a thorough representation of the black vulture microbiome.

There was a difference observed between the whole and filtered experimental groups across the zone of inhibition tests. In both the *B. coagulans* and *S. aureus*, the whole excrement sample solution produced zones of inhibition where the filtered excrement sample solution did not result in zones. This suggests that the microbes within the excrement are resulting in the observed antimicrobial properties as opposed to it solely being metabolites produced by these microbes. One possible explanation is the microbial interaction and communication between bacterial cells, known as quorum sensing, stimulates the production of metabolites that lead to the zones of inhibition (Mukherjee & Bassler, 2019). Future studies of this complex microbial communication and quorum sensing could lead to the discovery of new antibiotics (McCormack, 2006).

With the evidence of antimicrobial properties from whole excrement samples, the microbes from the excrement can be isolated and tested against various pathogenic bacteria, such as the ESKAPE pathogens (Rice, 2008). This allows for the potential discovery of new antibiotic and antimicrobial agents. Additionally, if the microbes that produce these agents are found to be pathogenic to humans, then it still allows a better understanding of the larger biological world, how all organisms interact, and could pose further questions into how these black vultures can survive with major human pathogens in their guts.

From this project, it was observed that the black vulture excrement did not alter *C. elegans* development and early survival. They appeared to be consuming live bacteria within the excrement. From previous studies it has been shown that altering the bacterial diets of *C. elegans* alters lifespan and healthspan (Stuhr & Curran, 2020). Since the vulture excrement contains a wide array of bacterial microbes, nematodes that consume it as food would likely have altered lifespan and/or healthspan as well. The current nematode experiments were only carried out to examine the impact of the excrement on *C. elegans* development and early survival. Further experiments could be carried out to examine the impact of this altered microbial diet on nematode lifespan and healthspan in the laboratory.

Analysis of 16S rRNA sequences is a common method for the taxonomic identification of bacterial strains in a microbiome study (Bukin et al., 2019). An amplicon sequence variant (ASV) was used to analyze the black vulture excrement samples in this study. This method was used over operating taxonomic units (OTUs) as ASVs provide a

higher distinction between species (Callahan et al., 2017). ASVs resolve sequencing differences with a single nucleotide change, allowing for the classification of groups of species based on their DNA sequences (García-López et al., 2021). Similar to the findings by Roggenbuck (2014), the microbial flora within the black vulture's excrement was highly conserved within and across individuals with only 31 microbial species across 9 classes (Figure 10). Bacterial classes of Clostridia and Fusobacteria found within the black vulture's excrement is highly pathogenic to most other vertebrates, but these vultures may have adapted to house them in their gut system to help them digest rancid material such as carcasses (Callaway, 2014).

The microbiome observed for vulture 3 varied more from vultures 1 and 2. This could be due to the difference in housing across these organisms. Vultures 1 and 2 were housed in private, individual ambassador animal enclosures consisting of a concrete floor, chain-link fence with some sun coverings, and tree perches. Vulture 3 was housed in a more public location within the zoo's petting area within a barn. Although this animal was in an enclosure not to be petted, its housing had more exposure with other animals housed in the barn area including sheep, goats, and donkeys and the many visitors coming through the area. This difference in housing conditions could explain the difference in microbe abundance across the birds' microbiomes.

The ability of black vulture excrement to inhibit Gram-positive bacteria growth does suggest that the vultures might be gaining an antimicrobial benefit from their excrement by defecating on their legs. Additional bacterial species could be tested to examine the scope of this inhibition of growth.

For further investigation of the antimicrobial properties of vulture excrement, a larger variety of New World vulture species and their excrement need to be examined to see if this is solely the property of black vulture excrement or if it is consistent between all New World vulture species. It would also be interesting to compare the microbial flora of wild versus captive black vultures to examine the potential impact of diet and habitat, as we studied captive birds at a zoo for this project. In a previous study, it was observed that the hindgut microbial flora between wild and zoo vultures was highly conserved (Roggenbuck et al., 2014). Additionally, it would be interesting to test if storks, *Ciconiidae*, have anti-microbial properties in their excrement as they were previously believed to be in the same family as New World vultures and they also excrete on their legs for urohidrosis (Cabello-Vergel et al., 2021). Understanding how these birds protect themselves from harmful pathogens is essential to continue to learn about these animals and keep their populations safe as they continue to help keep our environments scavenged and clean.

REFERENCES

Arbulu, S., Jiménez, J. J., Gútiez, L., Campanero, C., del Campo, R., Cintas, L. M., Herranz, C., & Hernández, P. E. (2016). Evaluation of bacteriocinogenic activity, safety traits and biotechnological potential of fecal lactic acid bacteria (LAB), isolated from Griffon Vultures (Gyps fulvus subsp. Fulvus). *BMC Microbiology*, *16*(1), 228. https://doi.org/10.1186/s12866-016-0840-2

Bird Life International. (2016). *Coragyps atratus: The IUCN Red List of Threatened Species 2016: e.T22697624A93624950* [dataset]. https://doi.org/10.2305/IUCN.UK.2016-3.RLTS.T22697624A93624950.en

Bird Life International. (2018). *Cathartes aura: The IUCN Red List of Threatened Species 2018: e.T22697627A131941613* [dataset]. https://doi.org/10.2305/IUCN.UK.2018-2.RLTS.T22697627A131941613.en

Buechley, E. R., & Şekercioğlu, Ç. H. (2016a). The avian scavenger crisis: Looming extinctions, trophic cascades, and loss of critical ecosystem functions. *Biological Conservation*, *198*, 220–228. https://doi.org/10.1016/j.biocon.2016.04.001

Buechley, E. R., & Şekercioğlu, Ç. H. (2016b). Vultures. *Current Biology*, *26*(13), R560–R561. https://doi.org/10.1016/j.cub.2016.01.052

Bukin, Yu. S., Galachyants, Yu. P., Morozov, I. V., Bukin, S. V., Zakharenko, A. S., & Zemskaya, T. I. (2019). The effect of 16S rRNA region choice on bacterial community metabarcoding results. *Scientific Data*, *6*(1), 190007. https://doi.org/10.1038/sdata.2019.7

Cabello-Vergel, J., Soriano-Redondo, A., Villegas, A., Masero, J. A., Guzmán, J. M. S., & Gutiérrez, J. S. (2021). Urohidrosis as an overlooked cooling mechanism in long-legged birds. *Scientific Reports*, *11*(1), 20018. https://doi.org/10.1038/s41598-021-99296-8

Callahan, B. J., McMurdie, P. J., & Holmes, S. P. (2017). Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *The ISME Journal*, *11*(12), 2639–2643. https://doi.org/10.1038/ismej.2017.119

Callaway, E. (2014). Microbes help vultures eat rotting meat. *Nature*, nature.2014.16345. https://doi.org/10.1038/nature.2014.16345

Carlson, E. (2021). *Cathartidae (New World vultures and condors)*. Animal Diversity Web. https://animaldiversity.org/accounts/Cathartidae/

Energy Conserving and Heat Dissipating Mechanisms of the Turkey Vulture. (1970). *The Auk*, *87*(1), 111–124. https://doi.org/10.2307/4083662

García-López, R., Cornejo-Granados, F., Lopez-Zavala, A. A., Cota-Huízar, A., Sotelo-Mundo, R. R., Gómez-Gil, B., & Ochoa-Leyva, A. (2021). OTUs and ASVs Produce Comparable Taxonomic and Diversity from Shrimp Microbiota 16S Profiles Using Tailored Abundance Filters. *Genes*, *12*(4), 564. https://doi.org/10.3390/genes12040564

Graves, G. R. (2019). Urohidrosis and tarsal color in Cathartes vultures (Aves: Cathartidae). *Proceedings of the Biological Society of Washington*, *132*(1), 56. https://doi.org/10.2988/19-00002

Grigg, N. P., Krilow, J. M., Gutierrez-Ibanez, C., Wylie, D. R., Graves, G. R., & Iwaniuk, A. N. (2017). Anatomical evidence for scent guided foraging in the

turkey vulture. *Scientific Reports*, *7*(1), 17408. https://doi.org/10.1038/s41598-017-17794-0

Hawkwatch International—Black Vulture. (n.d.). Retrieved June 28, 2023, from https://hawkwatch.org/learn/factsheets/item/808-black-vulture

Johnson, C. K., Kelly, T. R., & Rideout, B. A. (2013). Lead in Ammunition: A Persistent Threat to Health and Conservation. *EcoHealth*, *10*(4), 455–464. https://doi.org/10.1007/s10393-013-0896-5

Jones, J., Reinke, S. N., Ali, A., Palmer, D. J., & Christophersen, C. T. (2021). Fecal sample collection methods and time of day impact microbiome composition and short chain fatty acid concentrations. *Scientific Reports*, *11*(1), 13964. https://doi.org/10.1038/s41598-021-93031-z

Kiff, L., Peakall, D., & Wilbur, S. (1983). *Eggshell thickness and DDE residue levels in vulture eggs* (pp. 440–458).

Kumar, R., Eipers, P., Little, R. B., Crowley, M., Crossman, D. K., Lefkowitz, E. J., & Morrow, C. D. (2014). Getting Started with Microbiome Analysis: Sample Acquisition to Bioinformatics. *Current Protocols in Human Genetics*, *82*(1). https://doi.org/10.1002/0471142905.hg1808s82

M100 PERFORMANCE STANDARDS FOR ANTIMICROBIAL SUSCEPTIBILITY TESTING (33rd ed.). (2023). Clinical and Laboratory Standards Institute.

Martí, M., Frígols, B., & Serrano-Aroca, A. (2018). Antimicrobial Characterization of Advanced Materials for Bioengineering Applications. *Journal of Visualized Experiments*, *138*, 57710. https://doi.org/10.3791/57710

McCormack, J. (2006). Quorum sensing, bacterial communication and new antibiotics. *Internal Medicine Journal*, *36*(12), 757–758. https://doi.org/10.1111/j.1445-5994.2006.01232.x

Migratory Bird Treaty Act, Pub. L. No. 16 U.S.C. 703-712 (1918). https://www.govinfo.gov/content/pkg/USCODE-2020-title16/pdf/USCODE-2020-title16-chap7-subchapII-sec703.pdf

Moore, C. (n.d.). *Black Vulture*. Outdoor Alabama. Retrieved May 17, 2023, from https://www.outdooralabama.com/herons/black-vulture

Mukherjee, S., & Bassler, B. L. (2019). Bacterial quorum sensing in complex and dynamically changing environments. *Nature Reviews Microbiology*, *17*(6), 371–382. https://doi.org/10.1038/s41579-019-0186-5

Park, H.-E. H., Jung, Y., & Lee, S.-J. V. (2017). Survival assays using Caenorhabditis elegans. *Molecules and Cells*, *40*(2), 90–99. https://doi.org/10.14348/molcells.2017.0017

Pugh, K. (n.d.). *Turkey Vulture*. Outdoor Alabama. Retrieved May 31, 2023, from https://www.outdooralabama.com/herons/turkey-vulture

Rice, L. B. (2008). Federal Funding for the Study of Antimicrobial Resistance in Nosocomial Pathogens: No ESKAPE. *The Journal of Infectious Diseases*, *197*(8), 1079–1081. https://doi.org/10.1086/533452

Rideout, B. A., Stalis, I., Papendick, R., Pessier, A., Puschner, B., Finkelstein, M. E., Smith, D. R., Johnson, M., Mace, M., Stroud, R., Brandt, J., Burnett, J., Parish, C., Petterson, J., Witte, C., Stringfield, C., Orr, K., Zuba, J., Wallace, M., & Grantham, J. (2012). PATTERNS OF MORTALITY IN FREE-RANGING

CALIFORNIA CONDORS (GYMNOGYPS CALIFORNIANUS). *Journal of Wildlife Diseases*, *48*(1), 95–112. https://doi.org/10.7589/0090-3558-48.1.95

Roggenbuck, M., Bærholm Schnell, I., Blom, N., Bælum, J., Bertelsen, M. F., Sicheritz-Pontén, T., Sørensen, S. J., Gilbert, M. T. P., Graves, G. R., & Hansen, L. H. (2014). The microbiome of New World vultures. *Nature Communications*, *5*(1), 5498. https://doi.org/10.1038/ncomms6498

Santos, L. H., Santos, C. D., & Da Silva, M. L. (2023). The limits of olfactory perception in black vultures: A field experiment. *Ethology Ecology & Evolution*, *35*(3), 340–347. https://doi.org/10.1080/03949370.2022.2062617

Sauer, J. R., Niven, D. K., Hines, J. E., Ziolkowski, Jr., D. J., Pardieck, K. L., Fallon, J. E., & Link, W. A. (2014). *North American Breeding Bird Survey Results and Analysis 1966-2013 | U.S. Geological Survey*. https://www.usgs.gov/tools/north-american-breeding-bird-survey-results-and-analysis

Silhavy, T. J., Kahne, D., & Walker, S. (2010). The Bacterial Cell Envelope. *Cold Spring Harbor Perspectives in Biology*, *2*(5), a000414–a000414. https://doi.org/10.1101/cshperspect.a000414

State of the Birds 2019. (2019). State of the Birds 2019. https://www.stateofthebirds.org/2019

Stiernagle, T. (2006). Maintenance of C. elegans. *WormBook*. https://doi.org/10.1895/wormbook.1.101.1

Stuhr, N. L., & Curran, S. P. (2020). Bacterial diets differentially alter lifespan and healthspan trajectories in C. elegans. *Communications Biology*, *3*(1), 653. https://doi.org/10.1038/s42003-020-01379-1

Sutphin, G. L., & Kaeberlein, M. (2009). Measuring Caenorhabditis elegans Life Span on Solid Media. *Journal of Visualized Experiments*, *27*, 1152. https://doi.org/10.3791/1152

The Black Vulture (Coragyps atratus) | MSEM in the Field. (2017, May 28). https://sites.stedwards.edu/mseminthefield/2017/05/28/black-vulture-coragyps-atratus/

USDA APHIS | Species. (2020, June 2). https://www.aphis.usda.gov/aphis/ourfocus/wildlifedamage/operational-activities/sa-damage-management/ct-species-by-name?sp=Vultures

Vulture | San Diego Zoo Animals & Plants. (n.d.). Retrieved June 27, 2023, from https://animals.sandiegozoo.org/animals/vulture

Wink, M. (1995). Phylogeny of Old and New World Vultures (Aves: Accipitridae and Cathartidae) Inferred from Nucleotide Sequences of the Mitochondrial Cytochrome b Gene. *Zeitschrift Für Naturforschung C*, *50*(11–12), 868–882. https://doi.org/10.1515/znc-1995-11-1220

Wünschmann, A., Armién, A. G., Höfle, U., Kinne, J., Lowenstine, L. J., & Shivaprasad, H. L. (2018). Birds of Prey. In *Pathology of Wildlife and Zoo Animals* (pp. 723–745). Elsevier. https://doi.org/10.1016/B978-0-12-805306-5.00030-4

APPENDIX A: TABLES

Table 1

Demographics and information for black vultures at the Birmingham Zoo

	Age (years)*	Weight (kg)	Diet	Habitat
Vulture 1	2	2	Frozen/thawed prey items (mice, rats, and rabbit) and horse meat	Covered area with rotating perches, travels through zoo for programming
Vulture 2	28	2.3	Frozen/thawed prey items (mice and rats) and horse meat	Covered area with rotating perches, travels through zoo for programming
Vulture 3	28	2.1	Frozen/thawed prey items (mice and rats) and horse meat	Indoor mew and large outdoor netted enclosure

*Ages are estimates

Table 2

Captive black vulture excrement sample collection for antimicrobial experiments

	Collection Time Points	
	First – 28/03/2023	*Second – 25/04/2023*
Vulture 1	1 sample	1 sample
Vulture 2	2 samples	1 sample
Vulture 3	2 samples	1 sample
Subtotal	5 samples	3 samples
Total	**8 samples**	

Note: Collection time points are displayed as day/month/year

Table 3

Captive black vulture excrement sample collection for microbiome analysis

	Collection Time Points				
	29/03/2023	*30/03/2023*	*31/03/2023*	*01/04/2023*	*02/04/2023*
Vulture 1	1 sample	--	1 sample	--	1 sample
Vulture 2	1 sample	--	1 sample	--	1 sample
Vulture 3	--	2 samples	--	1 sample	--
Subtotal	2 samples	2 samples	2 samples	1 sample	2 samples
Total	**9 samples**				

Note: Collection time points are displayed as day/month/year

Table 4

Bacterial species selected and tested against vulture excrement

Bacteria Species	**Gram-status**	**ATCC Catalog Strain (Kwik-Stik)**
Bacillus coagulans	Positive	7050
Enterobacter cloacae	Negative	BAA-2341
Escherichia coli	Negative	33876
Pseudomonas aeruginosa	Negative	27853
Staphylococcus aureus	Positive	BAA-1026

Table 5

Zone of inhibition experimental results of black vulture excrement samples from first captive collection

Sample	***B. coagulans***								***E. cloacae***			
	Whole						*Filtered*		*Whole*		*Filtered*	
	3 disks			*3 disks*			*3 disks*	*3 disks*	*3 disks*	*3 disks*	*3 disks*	*3 disks*
Vulture 1-1	Zones of inhibition present						No zones	No zones	No zones		No zones	
	21.1 mm	10.8 mm	19.4 mm	12.7 mm	12.3 mm	13 mm						
Vulture 2-1	No zones			Zones of inhibition present			No zones	No zones	No zones		No zones	
				**	16.3 mm	19.9 mm						
Vulture 2-2	Zones of inhibition present						No zones	No zones	No zones		No zones	
	6.6 mm	5.6 mm	11.3 mm	3.8 mm	12 mm	9.4 mm						
Vulture 3-1	No zones; lawn with fungal growth			No zones; lawn with fungal growth			No zones; fungal growth	No zones; fungal growth	No zones; fungal growth		No zones	
Vulture 3-2	Zones of inhibition present						No zones	No zones	No zones		No zones	
	9.4 mm	10.9 mm	11.1 mm	13.8 mm	17.8 mm	16.4 mm						

Note: Table includes qualitative and quantitative data for detected zones of inhibition, no zones of inhibition, and/or presence of fungal growth across experimental and control groups for vulture samples and bacterial species.

Table 5 CONTINUED

Zone of inhibition experimental results of black vulture excrement samples from first captive collection

Sample	*E. coli*				*P. aeruginosa*				*S. aureus*			
	Whole		*Filtered*		*Whole*		*Filtered*		*Whole*		*Filtered*	
	3 disks	*3 disks*	*3 disks*	*3 disks*	*3 disks*	*3 disks*	*3 disks*	*3 disks*	*3 disks*	*3 disks*	*3 disks*	*3 disks*
Vulture 1-1	No zones		No zones		No zones		No zones		No zones		No zones	
Vulture 2-1	No zones		No zones		No zones		No zones		No zones		No zones	
Vulture 2-2	No zones		No zones		No zones		No zones		No zones		No zones	
Vulture 3-1	No zones; fungal growth		No zones; fungal growth		No zones	No zones; fungal growth	No zones		No zones; fungal growth		No zones; fungal growth	
Vulture 3-2	No zones; fungal growth		No zones		No zones; fungal growth		No zones		No zones		No zones	

Note: Table includes qualitative and quantitative data for detected zones of inhibition, no zones of inhibition, and/or presence of fungal growth across experimental and control groups for vulture samples and bacterial species.

Table 6

Zone of inhibition experimental results of black vulture excrement samples from second captive collection

Sample	***B. coagulans***				***E. cloacae***		***E. coli***	
	Whole		*Filtered*		*Whole*		*Whole*	
	1 disk	*3 disks*	*1 disk*	*3 disks*	*3 disks*		*3 disks*	
Vulture 1-1	No zone; lawn	No zones; lawn with 3 large colonies	No zone; lawn with 1 large colony	No zones; lawn with 2 large colonies	No zones; lawn		No zones; lawn with 2 large colonies	
Vulture 2-1	No zone; lawn	No zones; lawn with 6 large colonies	No zone; lawn with 3 large colonies	No zones; lawn with 3 large colonies	No zones; lawn with pinpoint colonies		No zones; lawn with fungal growth	
Vulture 3-1	No zone; lawn with 3 large colonies	No zones; lawn with 10 large colonies	No zone; lawn with 2 large colonies	No zones; lawn with 2 large colonies	No zones; lawn with pinpoint colonies		No zones; lawn with fungal growth	
	Seeded plate		*3 disks*		*Seeded plate*	*3 disks*	*Seeded plate*	*3 disks*
Control Bacteria Plate	Lawn with 4 large colonies		No zones; lawn with 2 large colonies		Lawn	No zones; lawn	Lawn	No zones; lawn

Note: Table includes qualitative and quantitative data for detected zones of inhibition, no zones of inhibition, and/or presence of fungal growth across experimental and control groups for vulture samples and bacterial species.

Table 6 CONTINUED

Zone of inhibition experimental results of black vulture excrement samples from second captive collection

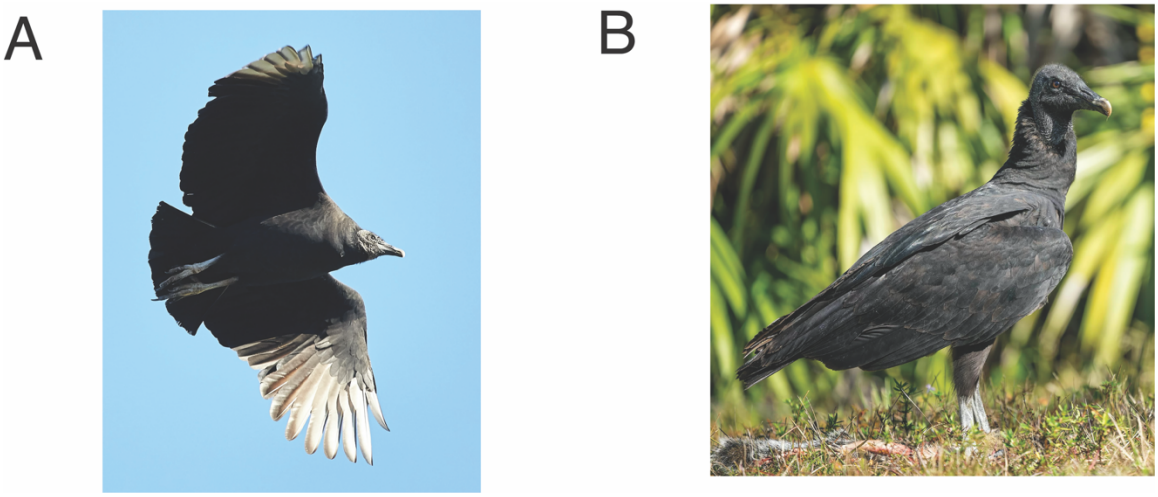
Sample	***P. aeruginosa***		***S. aureus***		
	Whole		*Whole*		
	3 disks		*3 disks*		
Vulture 1-1	No zones; lawn		Zones of inhibition present		
			11 mm	12 mm	9 mm
Vulture 2-1	No zones; lawn with fungal growth		Zones of inhibition present		
			11 mm	11 mm	Fungal
Vulture 3-1	No zones; lawn		Zones of inhibition present		
			9 mm	6 mm	9 mm
	Seeded plate	*3 disks*	*Seeded plate*	*3 disks*	
Control Bacteria Plate	Lawn	No zones; lawn	Lawn	No zones; lawn	

Note: Table includes qualitative and quantitative data for detected zones of inhibition, no zones of inhibition, and/or presence of fungal growth across experimental and control groups for vulture samples and bacterial species.

APPENDIX B: FIGURES

Figure 1

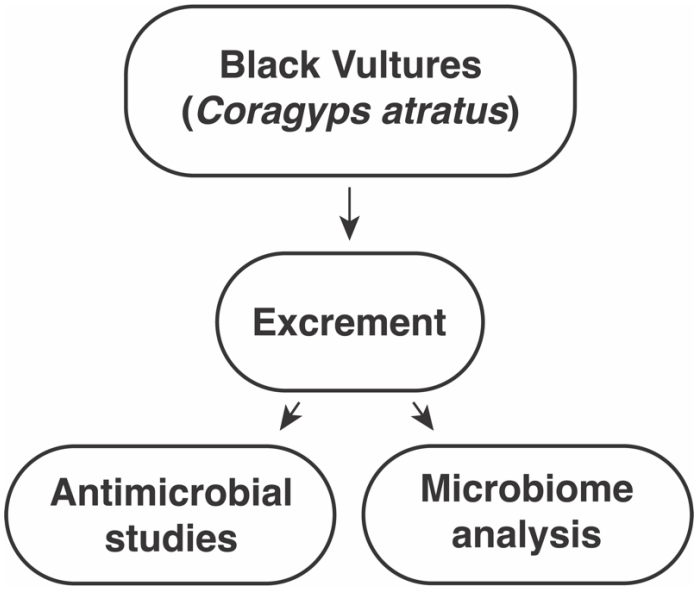
Images of black vultures (Coragyps atratus).



Representative images of a black vulture flying (A) and on the ground (B). Panel A is a photo by Matthew Schwartz on Unsplash. Panel B is a photo by Philip Brown on Unsplash. Images published on Unsplash are made to be used freely (https://unsplash.com/license).

Figure 2

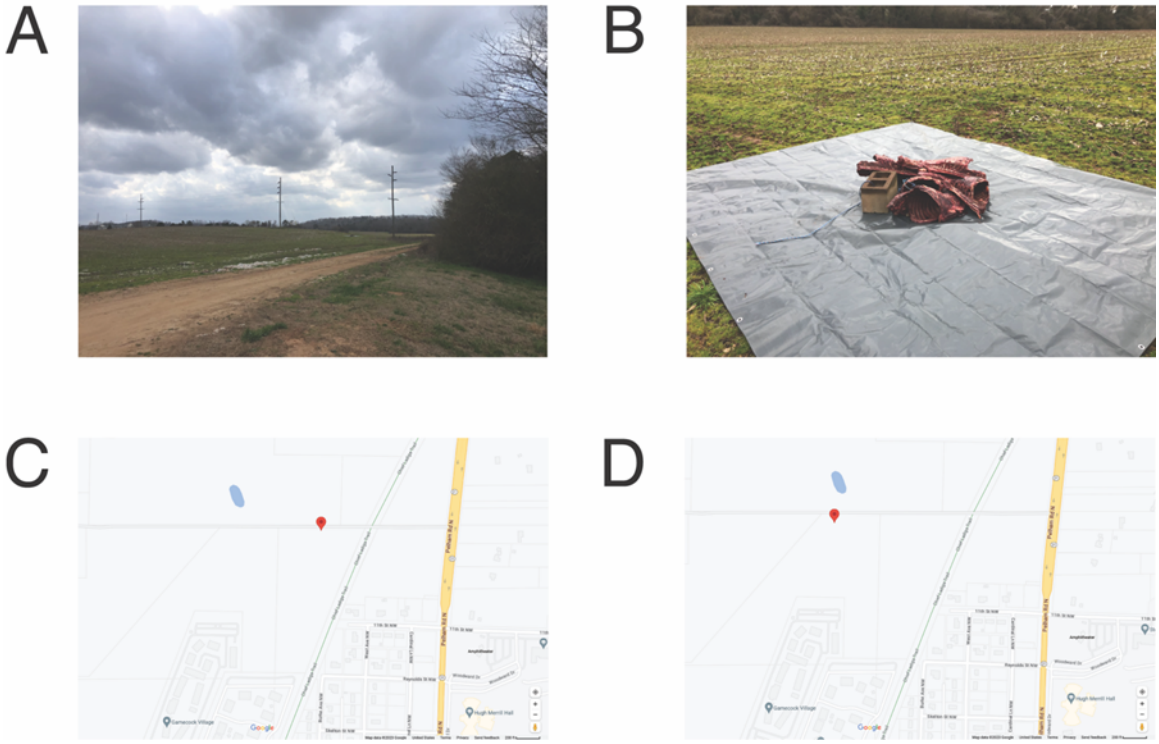
Experimental design.



Flow chart of experimental design showing the two arms of the project.

Figure 3

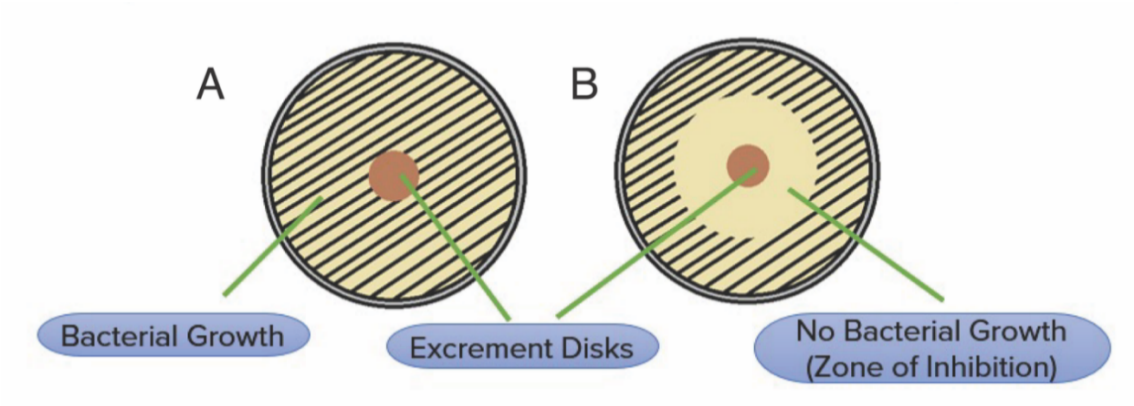
Wild black vulture excrement sample collection.



Excrement sample collection from wild black vultures was attempted in a cotton field near Jacksonville State University campus in Jacksonville, AL (A). A plastic tarp and bait were placed the field to attract vultures (B). The deer rib cages were weighed down with a concrete block on the tarp. Baiting took place in two locations within the field (C) first location and (D) second location. The red dot pinpoints the location.

Figure 4

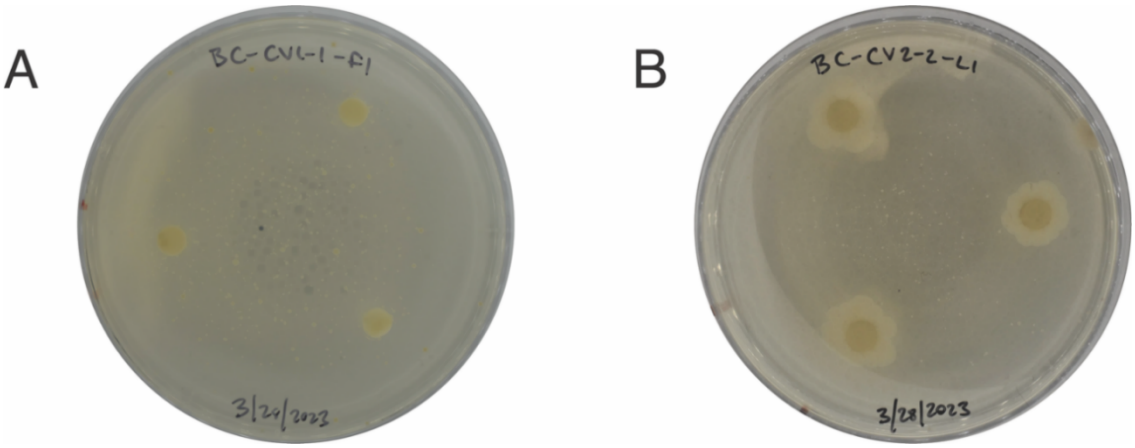
Antimicrobial assay setup with vulture excrement disks.



This diagram depicts the setup using vulture excrement disks in the zone of inhibition assay, a petri dish demonstrating no zone of growth inhibition of plated bacteria (A) and a petri dish demonstrating a zone of growth inhibition of plated bacteria (B). Both have a filter paper disk soaked in vulture excrement.

Figure 5

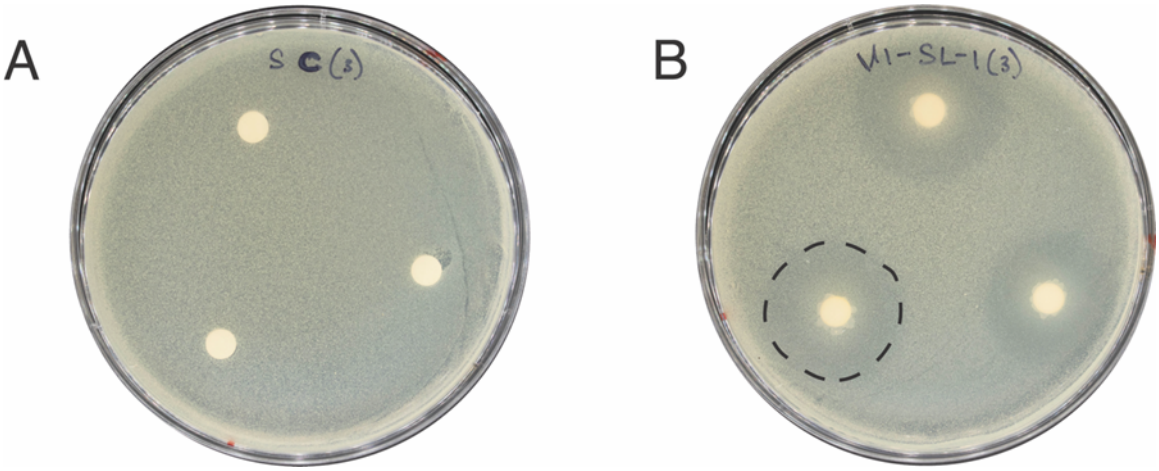
Images of Bacillus coagulans whole and filtered plates from the zone of inhibition experiment using excrement samples from first captive collection.



A) Experimental plate with filtered excrement-soaked disks on a plate inoculated with *Bacillus coagulans* with no zones of inhibition. B) Plate inoculated with *Bacillus coagulans* and whole, unfiltered excrement-soaked disks demonstrating zones of inhibition. Zones were detected and measured during the experiment; however, zones were not visible and clear in plate images due to camera settings.

Figure 6

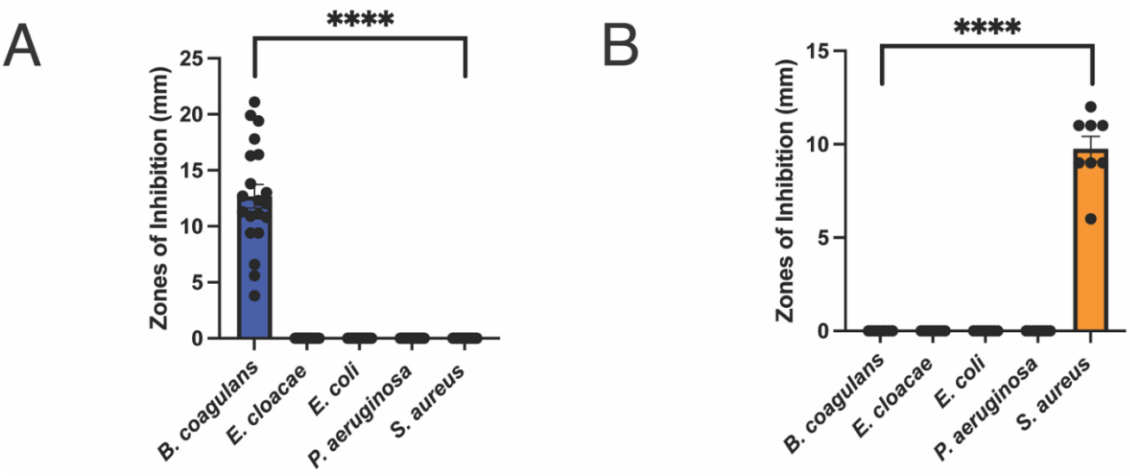
Images of Staphylococcus aureus control and experimental plates from zone of inhibition experiment using excrement samples from second captive collection.



A) Control plate with peptone water-soaked disks on a plate inoculated with *Staphylococcus aureus* with no zones of inhibition. B) Plate inoculated with *Staphylococcus aureus* and whole, unfiltered excrement-soaked disks demonstrating zones of inhibition. Zones were detected and measured during the experiment; importantly, zones were visible and clear in plate images due to optimized camera settings. The black dashed circle indicates the zone of inhibition present for *Staphylococcus aureus* for one of the three whole, unfiltered excrement-soaked disks. All three whole, unfiltered excrement-soaked disks had zones present and visible.

Figure 7

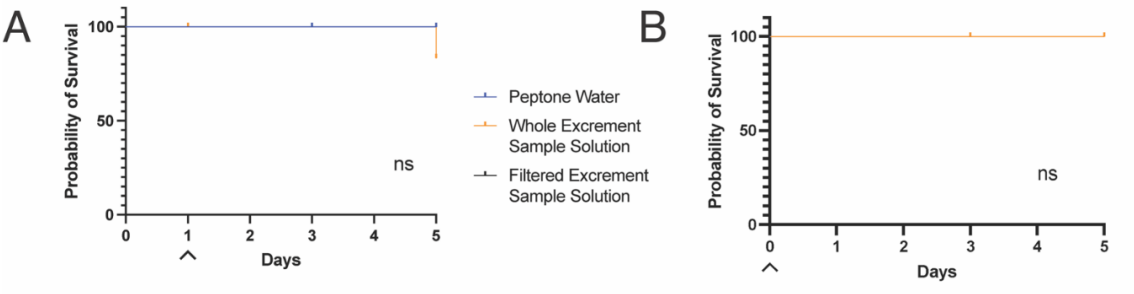
Comparison of antimicrobial experiments for zones of inhibition from black vulture excrement exposure across bacterial species.



There is a significant difference in zones of inhibition present across the 5 bacterial species tested against the whole black vulture excrement sample solution from the first captive sample collection (A) and the second captive sample collection (B). ****P<0.0001 versus experimental groups by a Kruskal-Wallis test. Data are presented as mean±SEM.

Figure 8

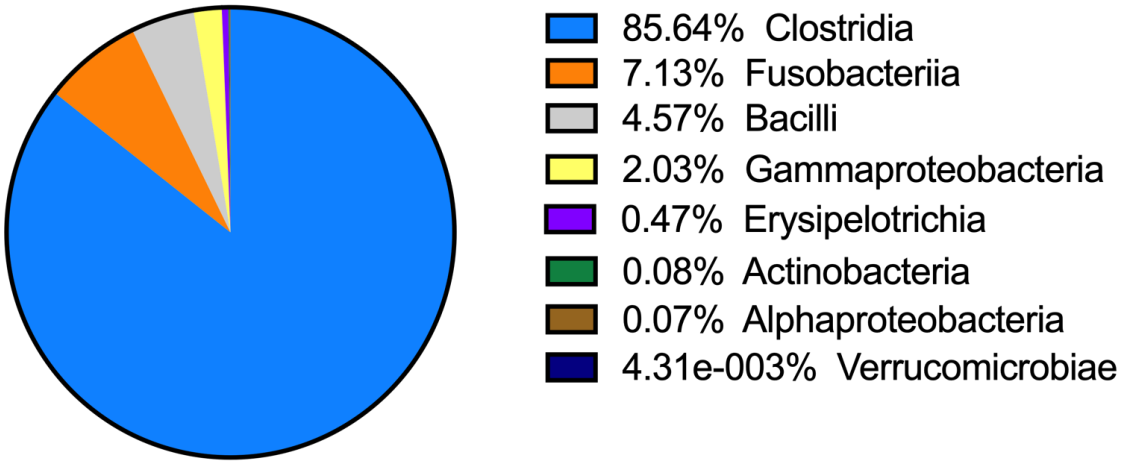
Kaplan-Meier survival curves of N2 C. elegans exposed to black vulture excrement.



Exposure to whole excrement sample solution or filtered excrement sample solution did not impact animal survival compared to peptone water, from the L1 stage (A, ns=not significant, P=0.9394) or the egg stage (B, ns=not significant, P>0.9999). The log-rank Mantel-Cox test was used to assess differences in overall lifespans. The results represent the survival of 80 animals per experimental group. The caret indicates start of exposure. Tick marks indicate times of censored observations.

Figure 9

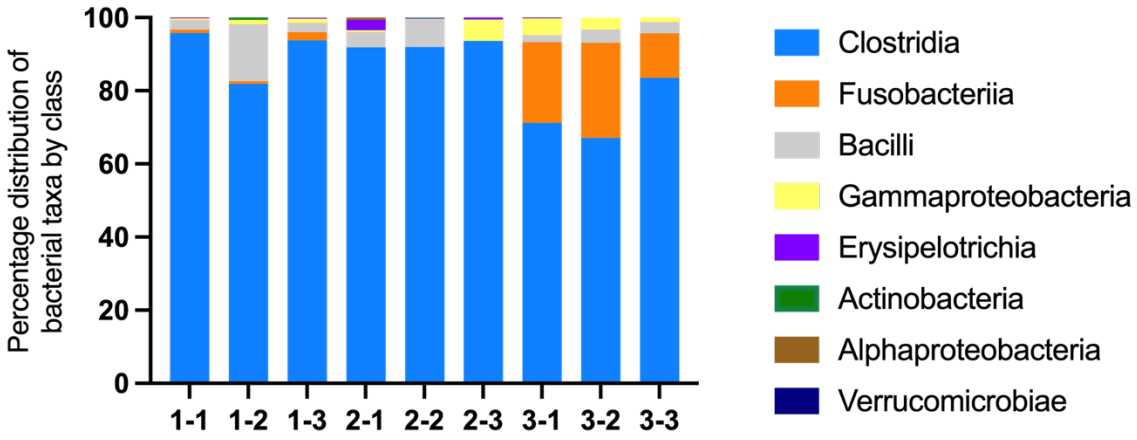
Excrement microbial flora of captive black vulture is unique.



Mean bacterial class distribution found in the excrement of black vultures (3 individuals, n = 9 excrement samples, 3 samples/individual across a 5-day period). All ASVs having similar taxonomic composition are grouped together for all samples and the results are presented at the taxonomic level of class. All 9 classes classified are displayed, with data ranging from 0.00431 to 85.64% of total reads.

Figure 10

Taxonomic bacterial profile at class level within each excrement sample from black vultures.



There was a total of 9 excrement samples from 3 individuals (3 samples/individual across a 5-day period). All ASVs having similar taxonomic composition are grouped together for each sample and the results are presented at the taxonomic level of class. Vultures (1-3) along with the 3 samples from each individual are displayed on the x-axis, such that vulture 1 excrement samples are 1-1, 1-2, and 1-3. Data is displayed as percentage of total reads within each individual's sample.