Tracking Pathogen Transmission at the Human-Wildlife Interface: Banded Mongoose (*Mungos mungo*) and *Escherichia coli* as a Model System in Chobe, Botswana

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ABSTRACT

Anthropozoonotic diseases, defined as infectious diseases caused by pathogens transmitted from humans to wildlife, pose a significant health threat to wildlife populations. Many of these pathogens are also able to move from wildlife reservoirs to humans, termed zoonotic diseases, creating the possibility for bi-directional transmission between humans and wildlife. Recent studies show that a significant proportion of emerging infectious diseases in humans originate in wildlife reservoirs and that the frequency of emergence is increasing, yet the specific transmission pathways still remain speculative in most cases. Human fecal waste is persistent across human-modified landscapes and has been identified as a potential source of disease exposure for wildlife populations living near humans. As part of a long-term study of banded mongoose (Mungos mungo) that live in close association with humans and human fecal waste I used Escherichia coli and banded mongoose (Mungos mungo) for evaluating exchange of fecal waste-borne microorganisms at the human-wildlife interface. Antibiotic resistance was found in 57.5% ± 10.3% (n=87) of mongoose fecal samples and 37.2% ± 5.9% of isolates (n=253). Multidrug resistance was detected in 13.8% ± 4.2% of isolates (n=253). Mongoose and human fecal waste isolates consistently clustered together in phylogenetic analyses and statistical analysis of genetic variation showed no significant differences (p=0.18) between E. coli from human and mongoose populations. These results suggest that human fecal waste contamination is an important mechanism for the transmission of pathogens to both humans and animals, including the spread of antibiotic resistance in the environment, an emerging global health threat.
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LIST OF ABBREVIATIONS

Rep-PCR = repetitive polymerase chain reaction
MLST-PCR = multi-locus sequence type polymerase chain reaction
HIV = human immunodeficiency virus
TB = tuberculosis
AMOVA = analysis of molecular variance
$F_{ST}$ = fixation index
Chapter 1: Introduction and Literature Review

1. Emerging Infectious Disease

Throughout history, infectious diseases such as cholera and tuberculosis have played a major role in shaping human civilizations through endemic and epidemic routes of infection. Despite modern medical advancements, this is still much the case today. Emerging infectious diseases are an important threat to global health. A disease is considered ‘emerging’ if it is entirely new to the human population, if it has recently expanded significantly into broader geographic regions (dispersion), or if it was present in humans historically but is undergoing modern-day resurgence. A recent study on trends in infectious diseases suggest the frequency of emergence is increasing, posing significant threat to human, domestic animal and wildlife health globally. Annually, emerging infectious diseases are responsible for more than 25% of human deaths worldwide. The threat of infectious diseases is not limited to underdeveloped nations, but is seen increasingly in developed nations. This threat was apparent in the recent outbreaks of severe acute respiratory syndrome (SARS), avian influenza (H5N1), and swine influenza (H1N1), where human communities were threatened globally irrespective of gross domestic product (GDP) or national infrastructure. Increasingly important components of infectious disease emergence are multi-host pathogens that can be transmitted between animals and humans, termed zoonotic infectious disease. Of all emerging infectious diseases, 60% are zoonotic and 70% of those originate specifically from wildlife reservoirs, demonstrating the significance of wildlife in emerging threats to human health. Not only is disease transmission a threat to human health but diseases transmitted from humans to animals (termed anthropozoonotic diseases) are a threat to wildlife health. Sensitive species which are threatened or endangered can be further burdened by disease. For example, anthropozoonotic diseases are
gravely affecting primate conservation and are seen as an additional hurdle for wildlife conservation.

Key to this discussion is the development of an understanding of the process of pathogen spillover, which is defined as the transmission and successful host invasion of a pathogen from one host species to another host species.

Certainly, characteristics of human-wildlife-environmental interactions support pathogen invasion in the novel animal or human host. It is clear that human health influences animal and ecosystem health. In turn, these changes will influence human health and result in successful pathogen transmission and emergence of infectious disease. In such instances where two complex systems such as human and natural systems become interdependent, this is termed coupled dynamics. The actual identification of coupling points where these systems connect, allowing pathogens to move between human and animal hosts, is elusive and is often articulated in more theoretical terms than from actual empirical studies. So, while we know that certain pathogens are transmitted between wildlife and humans, the mechanisms and circumstances supporting transmission in these systems is yet unknown and remains speculative with few empirical studies addressing this important gap in our knowledge.

Understanding transmission coupling points at the human-wildlife interface is a primary challenge in the management of zoonotic and anthrozoonotic infectious disease. Taylor and Latham (2001) attempted to classify the types of pathogens and routes of transmission most likely to cause an outbreak of emerging infectious disease. Their findings support the idea that diseases with a zoonotic origin are twice as likely to be associated with emergence than non-zoonotic diseases. However, they also acknowledged that there is substantial bias in reporting, recognition, and research interest in the different taxa and geographical regions associated with
the available transmission data. Consequently, despite attempts to develop a predictive understanding of the transmission dynamics involved in zoonotic disease transmission, we still know little about the emergence process. What are the transmission pathways that link wildlife and human populations in emerging zoonotic disease? What conditions (host, pathogen, and environmental) influence these processes? There is an urgent need to identify the nature of these system-couplings in order to develop a mechanistic understanding of pathogen transmission at the human-wildlife interface. With human population growth exceeding 7 billion, overlap between humans, domestic animals and wildlife will only increase, and along with it, the potential for pathogen spillover and infectious disease emergence.

2. Drivers of Disease Emergence

Pathogen spillover is dependent upon dynamics of exposure, invasion and sustained transmission. Pathogen life-history characteristics, host population dynamics, transmission dynamics, and the environment all have important roles in this process. These host-pathogen dynamics are embraced in the concept of “microbial traffic”. This terminology attempts to encompass the factors underlying the appearance of EIDs, many of which are exacerbated by anthropogenic influences such as human landscape alteration. Human landscape alteration can have both immediate and long term effects on transmission through divergent influences that might impact genetic and biological factors, aspects of the physical environment, ecological dynamics, and other socioeconomic factors that can modify the characteristics of host(s)-pathogen interactions. Disruption of ecosystem balance by human modification of the environment therefore often precedes the emergence of disease. For example, the emergence of Nipah virus in Malaysia was associated with increased contact between wildlife, livestock,
and farmers due to intensifying agricultural practices. Deforestation provides another example of anthropogenic change, where remote regions of forest were made accessible, increasing interaction between humans and wildlife. Such access is thought to have contributed, in part, to the emergence of human immunodeficiency virus (HIV) and Ebola in human populations. Disease transmission can, in fact, be bidirectional as a pathogen might spillover from wildlife into susceptible human populations and then spillback from humans to susceptible wildlife species, cycling in and out of any number of species in the system. Human landscape alterations can therefore facilitate interaction between seemingly independent populations, creating a network of pathogen movement in the environment.

Human waste is one aspect of anthropogenic change that is persistent across all human-modified environments. There is widespread evidence of human waste-borne pathogen contamination of the environment which is especially frequent in developing nations with growing populations in which regulated waste management or sanitation practices are lacking. Fecal waste, or sewage, in the form of sludge or wastewater hosts an array of pathogenic bacteria, viruses, protozoa and helminthes which can lead to outbreaks of disease. Contamination of drinking water by sewage effluent is a recurring cause of human gastroenteritis, leading to morbidity and mortality worldwide. Disease transmission through environmental fecal contamination is not only a threat to human health, but also to wildlife health. Increasingly, human fecal waste is identified as a potential source of disease exposure for wildlife populations living near humans. Human fecal waste is therefore a key aspect of anthropogenic modification to the environment, frequently posing a health risk to humans, domestic animals and wildlife, as well as providing a potential pathway for waste-borne pathogen transmission at the human-wildlife interface.
3. *Escherichia coli as a Model Organism*

Once we accept that anthropogenic changes such as waste play a role in human and wildlife health, how can we identify specific pathways through which disease transmission occurs? In comparison to other ecological phenomena, the occurrence of disease spillover from an animal reservoir to a human host or vice versa is a relatively rare event, with the subsequent onset of sustained transmission being a subset of these rare events. Since transmission events are difficult to detect and surveillance and reporting systems are generally lacking, we are often not aware of an emerging infectious disease until there is an outbreak. Therefore, a model organism is useful for tracking the movement of microorganisms at the human-wildlife interface in order to identify transmission pathways and factors influencing invasion potential.

A useful model for assessing waste-borne pathogen transmission dynamics is the microorganism *Escherichia coli* because it is ubiquitous in the gastrointestinal tract among mammals and can be commensal or pathogenic (*Shigella spp.* strains for example), allowing simulation of both infectious and non-infectious disease transmission pathways among hosts and environments. The most common route of transmission of *E. coli* is the fecal-oral route. For this reason, *E. coli* is regarded as the global standard in biological indicators for water quality assessment. In addition, *E. coli* is extremely well studied with advanced detection and differentiation techniques available making it a versatile tool for analysis.

In some regions, *E. coli* has been shown to reproduce appreciably and persist for long periods of time (possibly up to a year) in the environment. Additionally, recent studies may upstage our understanding of *E. coli*’s life history as a mammalian gut-associated bacterium based on the discovery of environmentally adapted, non-host associated clades within *Escherichia*. Without whole-genome phylogenetic analysis, these strains are virtually
indistinguishable from the other host-associated strains because their genome contains the entirety of the *E. coli* genome as it is the root strain from which these evolved. For this reason, many scientists are actively searching for alternative, perhaps more sensitive fecal indicator organisms to include in analysis of fecal contamination\(^{35}\). One such alternative option is host-specific *Bacteriodes* which is an emerging champion of fecal indicator organisms because of a human-specific genetic marker in the 16S region which can definitively identify a human source of origin. While the outlook is promising for *Bacteriodes* as a host-specific tool for identifying the source of fecal contamination, there have been very few studies conducted on the survivability of *Bacteriodes* in environments outside of the host in relation to other fecal indicators and the pathogens for which they are attempting to model\(^{36}\). Additionally, host-specific 16S sequences have only been identified for a few species thus far and therefore *Bacteriodes* may not be applicable for determining fecal contamination from other animal sources\(^{36}\). Finally, one of the celebrated benefits of *Bacteriodes* as a fecal indicator is that culturing the organism is not required for analysis because detection is done genetically with PCR. The downfall of DNA-based PCR methods as opposed to culture is that it is difficult to differentiate viable and non-viable organisms. Therefore, *E. coli* still stands firmly as a useful fecal indicator bacterium and, for most studies, would not be easily replaced by an alternative organism.

Several recent studies have demonstrated the utility of *E. coli* as a model for human fecal waste contamination\(^{37-43}\) at the human-wildlife interface. While results of these studies suggest that transmission links between humans and wildlife exist, they do not promote or explore a mechanistic understanding of pathogen transmission pathways. In previous studies,\(^{37,39}\) antibiotic screening approaches were not incorporated into the study design and thus there is an
increased potential that perhaps multiple wildlife species other than humans were the source of microorganism transmission to the target (primates, for example) and the human host population under study, an important potentially confounding study element. Clearly, it is important to identify the mechanisms of microorganism exchange in order to inform and strengthen public and animal health strategies. Should *E. coli* strains be shared between mongoose and humans, the proposed banded mongoose – *E. coli* – human fecal waste model can be used to structure further hypothesis-driven research directed at identifying the influence of landscape, host- and pathogen-level influences on transmission and invasion dynamics at the human-wildlife interface, and the role of waste in this process. In this study, I evaluate the hypothesis that microorganisms are exchanged between humans and banded mongoose in Chobe, Botswana and assess the utility of this system for evaluating infectious disease transmission dynamics at the human-wildlife interface.

4. Study Site

Within the Chobe district of Botswana, we have a long-term ecological study of banded mongoose and the emergence of *Mycobacterium mungi*, a novel *M. tuberculosis* type pathogen. Within this study system major trucking routes connect people throughout Sub-Saharan Africa. The region is a hot spot of biodiversity, with a growing tourism industry attracted to the unique wildlife populations identified in the region. The study site includes the river-front area of the Chobe National Park, and is adjacent to Victoria Falls, Zimbabwe, each attracting millions of visitors each year. Surrounding the national park, there are dedicated forest reserves and small-scale agricultural operations. Collectively, the protected land, agriculture, tourism and high traffic make Chobe a multi land-use system comprising a nexus of human-wildlife and domestic...
animal interactions. In addition, human communities in this region currently are undergoing a major health crisis, with nearly 25% prevalence of HIV/AIDS in adults\textsuperscript{45} and a majority of the population contracting tuberculosis (TB) by adulthood\textsuperscript{46}. The overlap of human and wildlife populations in Chobe in conjunction with epidemic immunosuppressive disease presents a unique opportunity to study infectious disease transmission at the human-wildlife interface.

5. Study Subject

Banded mongoose (\textit{Mungos mungo}) are social, fossorial viverids that feed on invertebrates and small mammals and scavenge,\textsuperscript{47} particularly in human waste when the opportunity presents itself. In a long-term study from 2000-2010, Alexander and colleagues studied recurring outbreaks of a novel species within the \textit{Mycobacterium tuberculosis} (TB) complex, \textit{Mycobacterium mungi}\textsuperscript{44}. Banded mongoose den in French drains (septic tanks with a soak-away) at ecotourism facilities or homes and could be exposed to effluent flowing through these systems daily. Spatial overlap coupled with a preference for anthropogenic features puts banded mongoose in close proximity with the focal source of waste-borne pathogen transmission, which is a key factor in sentinel choice\textsuperscript{48} and establishes them as a unique sentinel of health and pathogen exposure at the human-wildlife interface.

6. Hypothesis

\textbf{H}_0: Banded mongoose (\textit{Mungos mungo}) utilizing human-modified environments in Chobe National Park and surrounding townships do not harbor strains of \textit{E. coli} that originate human fecal waste.
**H₀-₁:** *E. coli* isolates from mongoose living in human modified environments do not demonstrate resistance to antibiotics.

**H₀-₂:** *E. coli* isolates from banded mongoose are not genetically similar to those isolated from sources of human fecal waste in rep-PCR fingerprint analysis of short, repetitive, sequences in non-coding regions of genome.

**H₀-₃:** *E. coli* isolates from banded mongoose are not genetically similar those isolated from sources of human fecal waste in MLST-PCR sequence based analysis of seven, highly conserved housekeeping genes in coding regions of the genome.

**Hₐ:** Banded mongoose (*Mungos mungo*) utilizing human-modified environments in Chobe National Park and surrounding townships harbor strains of *E. coli* that originate from human fecal waste.

**Hₐ-₁:** *E. coli* isolates from mongoose living in human modified environments demonstrate resistance to antibiotics.

**Hₐ-₂:** *E. coli* isolates from banded mongoose are genetically similar to those isolated from sources of human fecal waste in rep-PCR fingerprint analysis in short, repetitive, sequences in non-coding regions of genome.

**Hₐ-₃:** *E. coli* isolates from banded mongoose are genetically similar those isolated from sources of human fecal waste in MLST-PCR sequence based analysis of seven, highly conserved housekeeping genes in coding regions of the genome.
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1. ABSTRACT

**Background:** A primary challenge to managing emerging infectious disease (EID) is identifying coupling points where pathogen transmission occurs at the human-wildlife-environmental interface. Anthropogenic changes function as drivers of EID and human waste is the most ubiquitous disturbance. While there is growing interest in understanding and managing the public health threat of EID emanating from multi-host pathogens, little is known regarding transmission pathways that link humans and animals. Are microorganisms being exchanged between humans and wildlife and what transmission pathways link these populations? **Methods:** I used *Escherichia coli*, a model organism, and banded mongoose (*Mungos mungo*) for evaluating exchange of waste-borne microorganisms at the human-wildlife interface. Fecal *E. coli* isolates were collected from five mongoose troops that live closely with humans as well as one troop with limited human contact. Samples of human fecal waste in the environment were collected from bush latrines and sewage. Only antibiotic-resistant mongoose isolates were further analyzed, as resistance has been demonstrated as a marker for isolates of human origin. Phylogenetic relationships were determined through rep-PCR fingerprinting and MLST-PCR sequence analysis. **Results:** Antibiotic resistance was found in 57.5% ± 10.3% of mongoose fecal samples (n=87) and 37.2% ± 5.9% of *E. coli* isolates (n=253) from study troops. Multidrug resistance detected among 13.8% ± 4.2% of isolates (n=253). Mongoose and human isolates consistently clustered together in repetitive polymerase chain reaction (rep-PCR) and multilocus sequence type polymerase chain reaction (MLST-PCR) phylogenetic analyses demonstrating that
transmission events are ongoing. Analysis of molecular variance (AMOVA) and fixation indexes ($F_{ST}$) values ($F_{ST}=0.0027$) showed no significant differences between *E. coli* from human and mongoose populations ($p=0.18$), therefore confirming that these populations are readily exchanging resistant strains of *E. coli* and that human fecal waste is an important link for microorganism transmission. **Conclusions:** Microorganism transmission does occur at the human-wildlife interface through human fecal waste, identifying an important mechanism for the transmission of pathogens of concern to both public and animal health. In this study, human fecal waste was also identified as an important mechanism for the spread of antibiotic resistance in the environment, an emerging global health threat.

2. INTRODUCTION

Pathogen transmission between humans and animals threatens human health and wildlife conservation. Zoonotic diseases, or those infectious agents that can be transmitted from animals to humans, are responsible for more than 60% of emerging infectious diseases threatening human health worldwide and of these, 70% originate from wildlife reservoirs $^9$. The threat of zoonotic disease is not limited to developing nations but is seen increasingly in developed nations $^5$ as evidenced by recent outbreaks of pathogens such as swine influenza (H1N1) in the United States. Annually, emerging infectious diseases are responsible for more than 25% of human deaths worldwide $^6,49$. Diseases transmitted from humans to wildlife, termed anthropozoonotic diseases, can cause morbidity and mortality in wildlife populations which is especially challenging to conservation efforts for sensitive species $^{11}$. For example, a recent study identified human metapneumovirus (HMPV) associated with ecotourism activities as the pathogen responsible for killing several protected mountain gorillas (*Gorilla beringei beringei*) in Rwanda $^{50}$. Other
studies similarly demonstrated anthropozoonotic pathogen transmission as a significant health threat to non-human primates \(^{51,52}\). Although non-human primate conservation has been the focus of most of the research conducted on this topic thus far, the health threat of anthropozoonotic disease is not limited to primates. Anthropozoonotic diseases can infect a variety of host species causing morbidity and mortality \(^{53-55}\) meaning human disease transmission is a serious concern for conservation.

While it is evident from the examples above that pathogen transmission is occurring between humans and wildlife, the actual mechanisms of transmission are elusive and few studies have targeted the transmission pathways that link these populations. What is the connection between these populations that allow pathogen transmission to occur? What factors (host, pathogen, environmental) influence these processes? It is important to note that many infectious diseases (for example tuberculosis, salmonellosis, and brucellosis) have the ability to be zoonotic and anthropozoonotic potentially moving bi-directionally between human and wildlife hosts. An enhanced understanding of human-wildlife disease transmission dynamics could therefore have significant influence on human and wildlife health management globally. To achieve this, a model system is needed to identify mechanisms of disease transmission and further identify factors influencing host-pathogen interactions at the human-wildlife interface.

This study was conducted within the Chobe district of Botswana along the river-front area of the Chobe National Park and its associated townships of Kasane and Kazungula. Chobe is considered a biodiversity hotspot and boasts a growing tourism industry surrounding the Chobe National Park. Protected land and commercial development coupled with small-scale agriculture make Chobe a multi land-use system characterized by human, wildlife, and domestic animal interaction. In this region, we have a long-term ecological study of banded mongoose (Mungos
mungo) and the emergence of Mycobacterium mungi, a novel M. tuberculosis pathogen. The local human population in Chobe is also suffering from a high prevalence of HIV/AIDS and tuberculosis. The interface of human and wildlife interaction in Chobe in conjunction with epidemic immunosuppressive disease presents a unique opportunity to study microorganism exchange between humans and wildlife.

Human modification of the environment, or anthropogenic change, often precedes the emergence of disease by creating novel aspects of direct and indirect contact between humans and wildlife that can serve as pathways for pathogen transmission. Human fecal waste is one consequence of the human presence that is persistent across varying human-modified landscapes. There is widespread evidence of human fecal waste-borne pathogen contamination of the environment and this is especially true in developing nations with growing populations in which regulated waste management or sanitation practices are lacking. Fecal waste, or sewage, in the form of sludge or wastewater hosts an array of bacteria, viruses, protozoa and helminthes which can lead to outbreaks of disease. Therefore, human fecal contamination of the environment is one link between humans and wildlife that presents a possible pathway for disease transmission.

Banded mongoose are social, fossorial viverids that feed on invertebrates and small mammals and therefore often dig in soft, moist soils or fecal material looking for prey. Banded mongoose in Chobe have a high tolerance for human presence. The artificially watered lawns of tourist lodges and private homes in Chobe present an attractive habitat for foraging. Banded mongoose have been shown to scavenge particularly in from human waste (kitchen scraps and sewage) when the opportunity presents itself. In addition, they often choose to den in French drains (septic tanks with a soak-away) in ecotourism facilities and homes or other anthropogenic
structures as opposed to natural habitats where they can be exposed to human fecal waste. Spatial overlap coupled with a preference for anthropogenic features puts banded mongoose in close proximity with the focal source of fecal waste-borne pathogen transmission. This establishes banded mongoose as a unique wildlife sentinel for the potential health risks associated with exposure to human fecal microorganisms.

*Escherichia coli* is a useful model organism for assessing fecal waste-borne transmission dynamics for this study because it is essentially ubiquitous among the gastrointestinal tracts of mammals and is regarded as the global standard for indicating fecal contamination of water. Additionally, recent studies have demonstrated the utility of *E. coli* as a model for microorganism exchange at the human-wildlife interface\(^{37-43,57}\). *E. coli* can be pathogenic in humans and wildlife (*Shigella* spp. strains for example) allowing simulation of bacterial infectious disease transmission. *E. coli* is relatively simple to isolate non-invasively from feces and an extensive breadth of literature exists on detection and differentiation techniques making *E. coli* a versatile tool for analysis.

Previous research on microorganism exchange at the human-wildlife interface suggests that transmission pathways linking humans and wildlife exist because these populations share genetically similar strains of *E. coli* but these studies did not empirically investigate pathways of transmission. However, it is imperative that we begin to understand how pathogens move between human and wildlife hosts if we are to appropriately manage human and animal health threats from multi-host pathogens. The goal of this study was to determine whether the banded mongoose – *E. coli* – human fecal waste model successfully illustrates a potential pathway for microorganism exchange between humans and wildlife. The proposed model could then be used
to structure further research directed at identifying landscape-, host-, and pathogen-level influences on transmission dynamics at the human-wildlife interface.

3. METHODS

3.1 Fecal and Environmental Sample Collection

Five troops of banded mongoose (CCL, CGL, CSL, KUB, SEF; Figure 1) living in close association with humans at tourist lodges within and around Chobe National Park in the townships of Kasane and Kazungula were sampled. These troops are part of a long-term study with documented, routine utilization of anthropogenic structures associated with human fecal waste at tourist lodges and in town. I also sampled one troop (HP) not associated with a tourist lodge residing in a natural area of the Chobe National Park with limited human contact to examine the potential impacts of tourism and microorganism exchange. Feces from banded mongoose were passively collected during their morning latrine behavior (n=6 troops). Most banded mongoose defecate each morning upon leaving their den thus it is possible to collect fecal samples from individuals in each troop without replication.
Repeat fecal sampling events from morning latrines were conducted from study troops in an attempt to increase sampling coverage of individuals over time. To ensure that orally consumed material was not repeatedly sampled (i.e. to achieve independent sampling events), sampling intervals were determined by fecal clearance time. Fecal clearance time, defined here as the time it takes to completely pass material through the gut from consumption to excretion, was assessed using a distinctive fecal dye and captive mongoose held at the CARACAL Biodiversity Centre in Kasane, Botswana (IACUC: 07-146-FIW and 10-154-FIW). We used blue food coloring in powder form containing FD&C Blue No.1 dye (Linnea’s, Inc., Kent, Ohio) as a visual fecal marker. It is easy to detect and safe for consumption. 4.5g of powdered dye was added to 200g of canned dog food, homogenized and fed to four captive mongoose (one female, three males). Feces were collected and inspected every two hours thereafter. The initial detection
of blue dye occurred within two hours of feeding, maximized at 24hrs, and was no longer visible after 80hrs. The minimum period between repeat samplings was thus set to 8 days to address possible variation in gut transit time related to behavioral or diet differences in wild and captive mongoose.

Human feces were collected from bush latrines (i.e., defecation directly into the environment) found in the home range of study mongoose. Samples were also collected from sewage treatment facilities for the area, as well as sewage sludge/wastewater leakage within mongoose home ranges. Samples from natural soils not known to be contaminated with waste also were collected.

All feces were collected in sterile tubes shortly after defecation to prevent UV damage or loss of moisture (with the exception of human feces collected opportunistically) and frozen at -20°C within 8hrs of collection until processing, to reduce alteration of the microbial community diversity. In order to avoid environmental contamination, care was taken to sample from the center of the feces which did not come in contact with the soil. For mongoose feces, soil contamination was not a concern because they consume soil while foraging and therefore it is expected that their feces would contain soil. Additionally, by nature of their diet, mongoose feces are often filled with exoskeletons, sand, and vegetative material making the specimen very lightweight and delicate. Therefore it was not possible to sample from the center as either this provided inadequate sample volume or destroyed the structure of the sample. In the case of liquid samples such as wastewater, sterile gauze was swirled in the source to catch particulates.
3.2 Bacterial Isolation

Samples were homogenized and a 1g portion diluted in bacteriological-grade Peptone (Sigma-Aldrich, St. Louis, MO USA)(1:10 dilution, concentration 1mg/mL), spread on MacConkey Agar (Becton Dickinson Company, Franklin Lakes, NJ, USA) plates, and incubated for 24hrs at 37ºC. Up to six putative *E. coli* colonies were picked from mongoose fecal plates, while up to fifteen were chosen from environmental plates because, by definition, sewage waste represents an array of individual sources and therefore underrepresentation is more of a concern than overrepresentation. Given the limited opportunities to collect human fecal samples from bush latrines and their possible degradation by exposure to the elements, up to fifteen colonies were picked from these plates. All colonies were grown in tubes containing 10mL tryptic soy broth (Becton Dickinson Company, Franklin Lakes, NJ, USA) and aliquoted into 1.5mL tubes for further DNA extraction and antibiotic susceptibility testing. Samples were stored at room temperature for up to six months prior to antibiotic susceptibility testing; however, any samples stored over 12 weeks were pelleted and fresh media provided.

3.3 Antibiotic Susceptibility Testing

DNA was extracted as follows: approximately 1.6mL of live culture broth was placed in a 5mL tube followed by the addition of 1mL of 50% liquid dish detergent. Tubes were vigorously shaken and then incubated at 65-70ºC for 15 minutes. DNA was precipitated using 1.6mL of 100% ethanol and residue was eliminated with a wash step before re-suspension in 200uL of 1xTE buffer (TEKnova, Hollister, CA, USA). Positive identification of *E. coli* was determined using genera-specific primers for the gene malB using PCR as previously described. The efficacy of these primers for correctly identifying *E. coli* from feces in this study was
confirmed by sequencing the 16S rDNA amplicons of samples that were presumptively *E. coli* based on *malB* PCR. Sanger sequencing was completed by the Virginia Bioinformatics Institute Core Laboratory Facility. The resulting sequences were processed and the function BLAST n used to search the results against the NCBI database. Only isolates positively identified as *E. coli* were further analyzed.

Isolates collected in this study were tested for susceptibility to 10 antibiotics (number in parenthesis refers to the concentration in μg): ampicillin (AM10), chloramphenicol (C30), ciprofloxacin (CIP5), doxycycline (D30), gentamycin (GM10), neomycin (N30), streptomycin (S10), tetracycline (TE-30), sulfamethoxazole-trimethaprim (SXT), and ceftiofur (XNL) (BBL, Becton Dickinson Company, Franklin Lakes, NJ, USA). Antimicrobials chosen for this study were based on those selected from previous studies conducted in Africa39,42,61. Antibiotic susceptibility was measured using a field-modified Kirby-Bauer disc diffusion method with breakpoints indicated by the Clinical Laboratory Standards Institute (CLSI) 62. Because these experiments were performed in a field laboratory with limited equipment, it was not possible to adjust the cultures to McFarland turbidity standards. However, quality results were ensured in several ways. First, *E. coli* ATCC 25922 culture, a quality assurance indicator strain recommended by CLSI, was treated identically to sample isolates and results remained consistently within required limits for each test batch. Second, any isolates demonstrating intermediate resistance were accepted as susceptible for conservative reporting. Third, since stage of growth can affect resistance, all cultures tested were greater than 24hrs of age (which corresponds to stationary phase) in tryptic soy broth at the time of testing. Using this method, the absorbance values were equivalent between samples because the organism measured (*E. coli* confirmed by *malB* PCR as discussed above) was consistent among samples and the growth
stage was consistent; therefore, the size of the cells on which absorbance was determined was consistent. These modifications made it possible to apply antibiotic susceptibility testing to isolates in a field setting. In conjunction with samples analyzed in this study, human antibiotic resistance data for the region were extracted from the local Kasane Primary Hospital (no human ID, data blinded) from routine testing of clinically isolated pathogens on pre-established antibiotic panels that were used to determine susceptibility of human bacterial microorganisms (2007-2011).

3.4 Genetic Profiling

Antibiotic resistance was used as a filter prior to further analyses, and therefore only mongoose isolates resistant to at least one antibiotic were used for phylogenetic analysis. Because antibiotic resistance is indicative of human origin, using only resistant isolates reduced the possibility that other, possibly multiple wildlife species (not humans) were the source of microorganism transmission to the target animal and human populations being evaluated, avoiding an important potential confounding element. Genetic relationships were determined by repetitive polymerase chain reaction (rep-PCR) and multi-locus sequence type polymerase chain reaction (MLST-PCR). Rep-PCR has been shown to be an advantageous method for fingerprinting microbes such as *E. coli* in this context, and the evolutionary trees generated have been shown to reliably represent true phylogenetic distances between strains. Fingerprint profiles were generated using densitometric (curve-based) genotype determination as opposed to band-based methods with the FPQuest ver 4.5 program (BioRad, Hercules, CA, USA). The resulting phylogenetic trees were built by cluster analysis, Pearson’s correlation, neighbor-joining algorithm, and optimization parameters set from previously published guidelines.
tree was rooted with the Rep-PCR fingerprint of the *E. coli* K-12 strain. Analysis of molecular variance (AMOVA) was performed on the resulting genotypes converted to binary output (“band-match” procedure in FPQuest) to determine the genetic diversity among and within populations of *E. coli* from mongoose and sources of human fecal waste.

Rep-PCR analysis was optimized from previously published methods and performed on both mongoose isolates that demonstrated antibiotic resistance and all sources of human waste. In summary, reactions were performed as 25μL volumes by adding 2μL DNA template (of standardized concentration 100-300ng/μL) to 2.5U Phire *Taq* (Finnzymes, Vantaa, Finland) and 1X of 5X Phire *Taq* Buffer (containing 1.5mM MgCl2, 500μM of dNTP mix), 1μM of BOX AIR primer (5’-CTACGGCAAGGCGACGCTGACG-3’) and an additional 1.5mM of MgCl2. Cycling was performed using a BioRad MyCycler (Bio-Rad, Hercules, CA, USA) at 98ºC for 3 min; followed by 35 cycles of 98ºC for 1 min, 64ºC for 8 min, and 71ºC for 1 min. Final extension was 15 min at 71ºC, followed by holding at 4ºC. Samples were electrophoresed in an Owl D3-14 wide gel box (ThermoScientific, Waltham, MA, USA) at 45V for 10hrs at 4-8ºC in a 2% gel using 1X TAE buffer. After every tenth sample and at either end of the gel, 10μL of molecular weight marker (100bp and 1kb, New England Biolabs, Ipswitch, MA, USA) was loaded. Gels were stained for 60min using 400mL 1x TAE containing 30μL of 1% EtBr (Fisher Scientific, Waltham, MA, USA), then destained for 40min and photographed immediately using a Gel Doc XR system (Bio-Rad, Hercules, CA, USA).

To confirm the genetic relationships generated from fingerprint analysis, a subset of isolates representing each clade were amplified and sequenced for MLST-PCR to construct a tree based on composite genotypes at different loci in a single isolate ensuring that conclusions on strain origin and population differentiation were robust. The seven housekeeping loci (*aspC*, ...
*clpX, fadD, icdA, lysP, mdh, uidA* shown in Table 1 were chosen for analysis based on previous publications that demonstrated that these polymorphic informative sites can reliably discern strains of *E. coli* \(^{67}\). These genes, while highly conserved, are still variable enough to discern phylogenetic relationships \(^{67}\). The resulting phylogenetic tree was built using the Tamura-Nei distance model, neighbor-joining algorithm, and 70% support threshold. The tree was rooted with the composite sequence of the same seven loci from *E. coli* K-12 available from the EcMLST database \(^{67}\).

<table>
<thead>
<tr>
<th>Locus</th>
<th>Product</th>
<th>Size (bp)</th>
<th>PCR Primers</th>
<th>Amplicon (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>aspC</td>
<td>aspartate aminotransferase</td>
<td>1191</td>
<td>5' - GTTTCTGTCGGATGAAACGTC - 3'&lt;br&gt;5' - AAACCTGGTAAGCGAGATC - 3'</td>
<td>594</td>
</tr>
<tr>
<td>clpX</td>
<td>ATP-dependent Clp protease</td>
<td>1275</td>
<td>5' - CTTCCGAGTCGGTGATACAA - 3'&lt;br&gt;5' - GACAACCGGAGAGGACACAA - 3'</td>
<td>672</td>
</tr>
<tr>
<td>fadD</td>
<td>cyl-CoA synthetase</td>
<td>1686</td>
<td>5' - GCTGCCGCTGTATGCTTACACATTT - 3'&lt;br&gt;5' - GCAGGAAATTTCTTTTCATAT - 3'</td>
<td>580</td>
</tr>
<tr>
<td>icdA</td>
<td>isocitrate dehydrogenase</td>
<td>1251</td>
<td>5' - CTGCGAAGCACTGAGAATC - 3'&lt;br&gt;5' - ACGTGGGTGGCTTCAAACA - 3'</td>
<td>669</td>
</tr>
<tr>
<td>lysP</td>
<td>lysine-specific permease</td>
<td>1470</td>
<td>5' - CTTACGCGGTAATTAAAGG - 3'&lt;br&gt;5' - GGTCTCCTGGAAGAGAAG - 3'</td>
<td>628</td>
</tr>
<tr>
<td>Mdh</td>
<td>malate dehydrogenase</td>
<td>939</td>
<td>5' - GTCGATCTGAGCCATATCCCTAC - 3'&lt;br&gt;5' - TACTGACCGTGCCTCCTACCA - 3'</td>
<td>650</td>
</tr>
<tr>
<td>uidA</td>
<td>beta-D-glucuronidase</td>
<td>1812</td>
<td>5' - CATTACGCGCAAGTAGTGCTTCAAT - 3'&lt;br&gt;5' - CCATGCAGCGTTATCGAATCTCT - 3'</td>
<td>658</td>
</tr>
</tbody>
</table>

MLST-PCR reactions were performed in 25\(\mu\)L volumes by adding 1\(\mu\)L DNA template (of standardized concentration 100-300ng/\(\mu\)L) to 2.5U Phire *Taq* (Finnzymes, Vantaa, Finland) and 1X of 5X Phire *Taq* Buffer containing 1.5mM MgCl\(_2\), 500\(\mu\)M of dNTP mix, 0.5\(\mu\)M of forward and reverse primers for seven MLST loci as previously described \(^{67}\) and an additional 1.5mM of MgCl\(_2\). Cycling was performed using a BioRad MyCycler (BioRad, Hercules, CA, USA) at 98°C for 3min; followed by 35 cycles of 98°C for 1 min, 56°C for 1 min, and 72°C for 30sec. Final extension was 5min at 72°C, followed by holding at 4°C. PCR products were
cleaned using ExoSAP-IT (USB Corp, Cleveland, OH, USA) and sequenced using the same forward primers as in PCR amplification. Sequencing services were provided by the Virginia Bioinformatics Institute at Virginia Tech (Blacksburg, VA, USA) and BioBasic, Inc. (Markham, Ontario, Canada).

3.5 Data Analysis

Statistical analysis in prevalence of antibiotic resistance were determined by Fisher’s Exact test and Chi square test using the statistical software JMP, version 8.0 (SAS Institute, Cary, NC USA). Fingerprint gel profiles were analyzed and stored in the FPQuest program ver 4.5 (BioRad, Hercules, CA USA) based on densitometric (curve-based) analysis and bacterial genotypes were converted to binary variables using the “bandmatch” method in FPQuest to perform Analysis of Molecular Variance (AMOVA) and calculate fixation indexes, or FST, with the program Arlequin ver 3.1 64. Amplicons of each loci for MLST were sequenced by the Virginia Bioinformatics Institute (Blacksburg, VA, USA) and BioBasic, Inc. (Markham, Ontario, Canada). Resulting DNA sequences were aligned and analyzed using the program Geneious 5.4.2 68 (Biomatters, Ltd, Auckland, New Zealand).

4. RESULTS

4.1 Isolates of Escherichia coli

A total of 230 samples were collected from mongoose, sources of human fecal waste, and the natural environment. Of the samples collected, 34.8% failed to successfully culture any bacterial growth. Samples from natural environments not known to be contaminated with fecal
waste did not yield any successful culture growth for analysis. In mongoose and human fecal waste samples that did exhibit cultural growth, *E. coli* was positively identified in 48.8% of the colonies isolated yielding a total of 367 isolates of *E. coli* for analysis in this study (Table 2). A majority of samples obtained from each mongoose troop were collected during one sampling event, followed by only minimal additional samples collected on repeat attempts (Table 3).

Table 2: Description of *E. coli* isolates collected from banded mongoose and sources of human fecal waste. a) Total numbers of samples and isolates collected by source over multiple sampling events, b) Total numbers of successfully cultured samples and *E. coli* positive isolates by source over multiple sampling events

<table>
<thead>
<tr>
<th>Source</th>
<th>Samples N</th>
<th>Isolates N</th>
<th>E. coli N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mongoose1</td>
<td>174</td>
<td>520</td>
<td>274</td>
</tr>
<tr>
<td>Human2</td>
<td>6</td>
<td>53</td>
<td>48</td>
</tr>
<tr>
<td>Evaporation Ponds3</td>
<td>6</td>
<td>64</td>
<td>31</td>
</tr>
<tr>
<td>Waste4</td>
<td>31</td>
<td>115</td>
<td>14</td>
</tr>
<tr>
<td>Natural Environment5</td>
<td>13</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Totals</td>
<td>230</td>
<td>752</td>
<td>367</td>
</tr>
</tbody>
</table>

1 Fecal samples collected non-invasively, 2 Human fecal samples collected from bush latrine sites, 3 Samples collected from evaporation ponds at the local sewage treatment facility, 4 Sewage waste found within mongoose home range, 5 Soil and water samples collected from areas not known to be contaminated with sewage, * culture failure rate of 34.8%, ^* E. coli success rate

Table 3: Number of mongoose fecal samples collected from each troop by sampling event. For each sampling event, the N of feces collected is listed followed by the N of isolates collected (N/N).

<table>
<thead>
<tr>
<th>Troop</th>
<th>Est. Size ± Sampling 1 Sampling 2 Sampling 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N) (N/N) (N/N) (N/N)</td>
</tr>
<tr>
<td>CCL</td>
<td>42 25 / 103 8 / 25 -</td>
</tr>
<tr>
<td>CGL</td>
<td>53 24 / 70 6 / 36 6 / 6</td>
</tr>
<tr>
<td>CSL</td>
<td>42 49 / 79 9 / 7 -</td>
</tr>
<tr>
<td>KUB</td>
<td>19 26 / 72 -</td>
</tr>
<tr>
<td>SEF</td>
<td>6 4 / 7 4 / 0 -</td>
</tr>
<tr>
<td>HP</td>
<td>21 11 / 45 -</td>
</tr>
</tbody>
</table>

*Troop size estimates are based on visual counts during observations in the months when sampling occurred. These counts are loose approximations and are expected to vary frequently.
4.2 Antibiotic Susceptibility

4.2.1 Banded Mongoose Troops

Overall 40-55.6% of mongoose tested harbored resistant *E. coli* (Figure 2). Of samples from troop CCL screened for antibiotic susceptibility, 20.3-22.2% of *E. coli* isolates were resistant to at least one antibiotic. There was no significant difference in prevalence between sampling events (p=0.896 and p=0.538, respectively).

**Figure 2**: Troop CCL’s prevalence of antibiotic resistance (to at least one antibiotic) by sampling event, depicted in terms of individual mongoose fecal samples and *E. coli* isolates. Error bars are calculated based on 95% confidence. There was no significant difference between the two sampling dates (p=0.538 mongoose and p=0.896 for isolates) determined by Fisher’s Exact test. Data labels inside bars correspond to the number of either mongoose fecal samples or *E. coli* isolates screened.

Of samples from troop CGL screened for antibiotic susceptibility, 47.4-62.5% of *E. coli* isolates were resistant to at least one antibiotic meaning that 33.3-80% of mongoose tested harbored resistant *E. coli* (Figure 3). There was no significant difference in prevalence between sampling events (p=0.580 and p=0.187 respectively).
Figure 3: Troop CGL’s prevalence of antibiotic resistance (to at least one antibiotic) by sampling event, depicted in terms of individual mongoose fecal samples and *E. coli* isolates. Error bars are calculated based on 95% confidence. There was no significant difference between sampling dates (*p*=0.187 mongoose and *p*=0.580 for isolates) determined by Fisher’s Exact test. Data labels inside bars correspond to the number of either mongoose fecal samples or *E. coli* isolates screened.

Of samples from troop CSL screened for antibiotic susceptibility, 50-60% of *E. coli* isolates were resistant to at least one antibiotic, meaning that 50-68.8% of mongoose tested harbored resistant *E. coli* (Figure 4). There was no significant difference in prevalence between sampling events (*p*=0.677 and *p*=0.596, respectively).
Figure 4: Troop CSL’s prevalence of antibiotic resistance (to at least one antibiotic) by sampling event, depicted in terms of individual mongoose fecal samples and *E. coli* isolates. Error bars are calculated based on 95% confidence. There was no significant difference between sampling dates (p=0.596 mongoose and p=0.677 for isolates) determined by Fisher’s Exact test. Data labels inside bars correspond to the number of either mongoose fecal samples or *E. coli* isolates screened.

Of samples from troop SEF screened for antibiotic susceptibility, 0-100% of *E. coli* isolates were resistant to at least one antibiotic, meaning that 0-100% of mongoose tested at any given time harbored resistant *E. coli* (Figure 5). There was no significant difference in prevalence between these sampling events, however (p=0.083 and p=0.157, respectively), due to an extremely high error rate because of low sample sizes.
Figure 5: Troop SEF’s prevalence of antibiotic resistance (to at least one antibiotic) by sampling event, depicted in terms of individual mongoose fecal samples and *E. coli* isolates. Error bars are calculated based on 95% confidence. There was no significant difference between sampling dates (p=0.157 mongoose and p=0.083 for isolates) determined by Fisher’s Exact test. Data labels inside bars correspond to the number of either mongoose fecal samples or *E. coli* isolates screened.

Of samples from troop HP screened for antibiotic susceptibility, 77.8% of *E. coli* isolates were resistant to at least one antibiotic, meaning that 56.4% of mongoose tested harbored resistant *E. coli* (Figure 6). There was only one sampling event for this troop.
Figure 6: Troop HP’s prevalence of antibiotic resistance (to at least one antibiotic) depicted in terms of individual mongoose fecal samples and *E. coli* isolates. Error bars are calculated based on 95% confidence. Data labels inside bars correspond to the number of either mongoose fecal samples or *E. coli* isolates screened.

Of samples from troop KUB screened for antibiotic susceptibility, 30.8% of *E. coli* isolates were resistant to at least one antibiotic, meaning that 17% of mongoose tested harbored resistant *E. coli* (Figure 7). There was only one sampling event for this troop.
Figure 7: Troop KUB’s prevalence of antibiotic resistance (to at least one antibiotic) depicted in terms of individual mongoose fecal samples and *E. coli* isolates. Error bars are calculated based on 95% confidence. Data labels inside bars correspond to the number of either mongoose fecal samples or *E. coli* isolates screened.

There are no significant differences in prevalence across sampling events for any troop therefore, these data from events can be pooled (Figure 8). Also, for each troop, pooling of sampling events resulted in more conservative antibiotic resistance reporting. Antibiotic susceptibility testing was performed on a total of 253 of the 274 *E. coli* isolates collected from mongoose (n=87 fecal samples). Of these 253 mongoose isolates, 94 demonstrated resistance to at least one antibiotic (on average 37.2%, 95% CI ± 5.9%). This translates into 50 of the 87 individual fecal samples collected harboring resistant *E. coli* (on average 57.5%, 95% CI ± 10.3%). Across troops there was no significant difference in the prevalence of antibiotic resistance among individuals (p=0.253, Chi square test); however, prevalence of resistance among isolates was significantly different between troops (p=0.000, Chi square test).
Figure 8: Prevalence of antibiotic resistance (to at least one antibiotic) for each troop based on pooled sampling events. Prevalence is depicted in terms of individual mongoose fecal samples and *E. coli* isolates. Total N=253 isolates of *E. coli* and N= 87 mongoose. Data labels inside bars correspond to the number of either mongoose fecal samples or *E. coli* isolates screened from that troop. Error bars are calculated based on 95% confidence. Average resistance was 37.2% ± 5.9% of *E. coli* isolates and 57.5% ±10.3% of mongoose fecal samples. No significant difference in prevalence of resistance among mongoose between troops (p=0.253), however there were significant differences in prevalence of isolate resistance between troops (p=0.00) determined by Chi Square test.

Mongoose isolates were most commonly resistant to ampicillin (AM10), followed by doxycycline (D30), tetracycline (Te30), streptomycin (S10), sulfamethoxazole-trimethaprim (SXT), and chloramphenicol (C30) (Figure 9). A low number of isolates (n=5) demonstrated resistance to ceftiofur (XNL30), which is not used in human treatment. Troops exhibited differences in diversity of drug resistance. While troop CGL did not have the highest prevalence of overall resistance (Figure 8), it was the only troop to demonstrate resistance to all of the antibiotics screened (Figure 9). Troop HP had the highest prevalence of antibiotic resistance (Figure 8), but was predominantly resistant only to ampicillin (Figure 9).
Figure 9: The number of *E. coli* isolates in each troop of banded mongoose from pooled sampling events demonstrating resistance to a particular antibiotic. Antibiotics are listed as abbreviations followed by dosage in numbers. AM10 = ampicillin, Te30 = tetracycline, D30=doxycycline, SXT = sulfamethoxazole-trimethaprim, S10 = streptomycin, C30 = chloramphenicol, XNL30 = ceftiofur, GM10 = gentamycin, N30 = neomycin, CIP5 = ciprofloxacin.

4.2.2 Human Fecal Waste

Of the total 93 *E. coli* isolates collected from sources of human fecal waste to represent the human population in this study (Table 2), 75 were screened for antibiotic susceptibility and 82.6% (95% CI ± 8.5%) were resistant to at least one antibiotic. Isolates from human fecal waste collected in this study exhibited frequencies of drug resistance similar to data extracted from Kasane Primary Hospital laboratory records of antibiotic sensitivity testing for cultured microorganisms from the local human population (Figure 10). Similarly, banded mongoose were predominantly resistant to the same drugs as the human population, albeit at lower incidence (Figure 10).
4.2.3 Multidrug Resistance

Multidrug resistance, or resistance to more than one antibiotic, occurred in 13.8% (n=253, 95% CI ± 4.2%) of mongoose isolates screened among all troops and 64.9% (n=77, 95% CI ± 10.6%) of isolates from human fecal waste. Prevalence of multidrug resistance was significantly different between troops both in terms of mongoose fecal samples harboring multidrug resistant *E. coli* (p=0.032, Chi square test) and prevalence in isolates themselves (p=0.000, Chi square test) (Figure 11). Troop HP, from a natural environment not associated with tourist lodges, demonstrated the lowest levels of multidrug resistance. Troops SEF and CGL had the highest levels. Extreme drug resistance, defined here as resistance to five or more
antibiotics, was observed in 28.6% (95% CI ± 14.9%) of those mongoose isolates demonstrating multidrug resistance (n=35).

![Prevalence of Multidrug Resistance in Banded Mongoose](image)

**Figure 11**: Prevalence of multidrug resistance (to two or more antibiotics) for each troop based on pooled sampling events. Prevalence is depicted in terms of individual mongoose fecal samples and *E. coli* isolates. Total N=253 isolates of *E. coli* and N= 87 mongoose. Data labels next to bars correspond to the number of either mongoose fecal samples or *E. coli* isolates screened from that troop. Error bars are calculated based on 95% confidence. Average resistance was 13.8% ± 4.2% of *E.coli* isolates and 25.2% ± 9.1% of mongoose fecal samples. Prevalence of resistance between mongoose troops was significantly different (p=0.000 and p=0.032 respectively) determined by Chi square test.

4.3 Repetitive BOX-PCR

Analysis of molecular variance (AMOVA) revealed that genetic diversity was greater within these populations of *E. coli*, i.e., within humans or within mongoose, respectively (99.75% of the observed variation) than between populations (0.25% of the observed variation) therefore, no significant difference was found between *E. coli* isolated from human and mongoose populations (p=0.18). No significant differentiation in *E. coli* was found between
mongoose troops in natural areas (HP) when compared with those living in association with tourist lodges (CSL, CGL, SEF, KUB, CCL) (p=0.66).

The fixation index, or $F_{ST}$, calculated between *E. coli* isolated from banded mongoose and sources of human fecal waste was small ($F_{ST} = 0.0027$) indicating there is very little variation in genotype frequencies among these populations. The fixation index ($F_{ST} = 0$) calculated between *E. coli* isolated from troops living in natural, unmodified habitats (HP) and those living in association with tourist lodges (CSL, CGL, SEF, KUB, CCL) demonstrated that there is no difference, in terms of genotype frequencies, between these populations.

Antibiotic resistant isolates from all troops of mongoose, including HP from an unmodified, natural environment consistently clustered with those of humans and human fecal waste in rep-PCR analysis (Figure 12). Often multiple unique isolates from mongoose and human individuals clustered tightly as did multiple isolates from the local sewage system. However, clades containing exclusively mongoose or human isolates were not observed. Mongoose isolates did not cluster significantly by troop but instead were highly intermixed with isolates from human sources. Only one unique mongoose isolate branched by itself closest to the reference strain (*E. coli* K-12) and did not cluster with human fecal waste.
Figure 12: Phylogenetic tree based on rep-PCR fingerprint of isolates from human sources and antibiotic resistant mongoose (n=74 mongoose, n=87 human). Isolates not visibly differentiable were removed from this figure (n=133 unique fingerprints shown). Phylogenetic relationships built by cluster analysis, Pearson’s correlation, neighbor-joining algorithm, and optimization parameters set from previously published guidelines. Cophenetic correlation is a parameter expressing the consistency of a cluster. This method calculates the correlation between the dendrogram-derived similarities and the matrix similarities. The value is calculated for the whole tree to have an estimation of the faithfulness of a cluster analysis (and of each subcluster). Cophenetic correlations demonstrated 70% confidence in this cluster analysis. Tree is rooted with the fingerprint profile of E. coli K12.
4.4 Multi-locus Sequence Type - PCR

MLST-PCR performed on a subset of *E. coli* isolates (n=23) representing each of the clades observed in rep-PCR analysis revealed very slight genetic divergence (maximum <0.10 base substitutions per site) between isolates from banded mongoose and human fecal waste (Figure 13). *E. coli* isolated from banded mongoose and sources of human fecal waste consistently clustered together, forming one main, intermixed clade. Only one unique mongoose isolate branched by itself closest to the reference strain (*E. coli* K-12).

![Phylogenetic tree](image)

**Figure 13**: Phylogenetic tree based on n=23 *E. coli* composite sequences from seven housekeeping genes (*uidA*, *mdh*, *lysP*, *icdA*, *clpX*, *aspC*, *fadD*). Values alongside branches correspond to % confidence generated from 1000 bootstraps. Phylogenetic relationships built from seven consensus trees using Tamura-Nei distance model, neighbor-joining algorithm, and 70% support threshold. The tree is rooted with composite sequence of *E. coli* K12 from the EcMLST database. Scale is based on the number of base substitutions per site.
5. DISCUSSION

5.1 E. coli Isolate Collection

Yield of E. coli isolates from mongoose samples was low and highly variable. This was not unexpected, however, because of the nature of the mongoose diet; their feces are very dry with a great deal of vegetative matter and insect exoskeletons. Human feces yielded a high incidence of success for E. coli isolation. Sources of sewage leakage in the environment, such as sludge and wastewater, yielded 14 isolates. While these samples were predominantly of human origin as they are part of the sewage system, I cannot exclusively rule out contribution from other sources. For example, during wastewater treatment, sewage is stored in open-air ponds which could be contaminated with non-human fecal microorganisms from animals such as birds, amphibians or small mammals. In addition, once sewage has spilled into the environment, it is difficult to conclusively determine whether isolates from soils have become mixed with the sewage. However, natural soil samples from areas not known to be contaminated with sewage failed to culture any E. coli isolates. It is likely that if contamination from additional sources was occurring, these isolates existed in low numbers compared to those contributed by the human population.

5.2 Antibiotic Resistance

Levels of antibiotic resistance observed in banded mongoose in Chobe are appreciably high. A concern when pooling sampling events within troops across sampling periods is that replication and over-reporting the number of individuals harboring antibiotic resistant strains in each troop cannot be excluded. However, new individuals might well be sampled between times since initial sampling was not comprehensive (Table 3). Prevalence of resistance was reduced
when sampling events were pooled in comparison to prevalence of the single largest sampling event. Therefore, in this study, reporting pooled data was a more conservative measure and thus utilized. Another concern is that since isolates were not identified as genetically distinct prior to screening for antibiotic resistance, it is possible that identical isolates were counted multiple times there by exaggerating the prevalence of resistant isolates estimated in this study. Looking only at those unique isolates from the rep-PCR fingerprint analysis (which are all resistant), however, the prevalence of resistant isolates in banded mongoose still exceeds those reported in similar studies in primates in Uganda37.

While troop HP is from a natural area inside Chobe National Park, not associated with lodges, these mongoose demonstrated the highest overall prevalence of resistant individuals but predominantly only to ampicillin. Only one isolate from the troop was resistant to additional antibiotics. Consequently, this troop exhibited the lowest levels of multidrug resistance which is most often associated with transmission from a human source. Resistance only to ampicillin may be biologically significant in that it is possible there is some natural, environmental resistance to ampicillin. However, the local human population is also frequently resistant to ampicillin and these ampicillin resistant isolates from HP clustered with isolates from humans and human fecal waste in genetic fingerprinting and sequence analysis. This result may indicate that nearby troops are freely exchanging bacteria or that they are all coming in contact with the same human fecal waste contaminant. Therefore, even in supposedly natural environments, microorganism spread from human fecal waste is occurring, potentially through direct means such as human defecation in these areas, indirectly through dispersal of mongoose across the landscape, or contamination of river water used by mongoose.
Troops CGL and SEF demonstrated the highest levels of multidrug resistance; however, SEF only has six individuals in the group and with extremely low sample sizes (n=3 and n=2 respectively) it is therefore expected that prevalence would be inflated. Alternatively, troop CGL had robust sample sizes and yet still demonstrated high prevalence of resistance. In some troops, multidrug resistant isolates were aggregated to a few individual mongoose fecal samples (KUB, for example), but in CGL multidrug resistance was widespread across individuals and isolates. Troop CGL is also located within Chobe National Park; however, unlike HP it is associated with a tourist lodge and dens in the employee camp where there is a higher degree of cohabitation with humans than perhaps at other lodges.

The drug ceftiofur (XNL30) is not used in human treatment, only in veterinary medicine, and was included in this study for comparison. A small number of mongoose isolates (n=5) from different mongoose in different troops demonstrated resistance to ceftiofur. Although some farmers do engage in agriculture in Chobe, these are small-scale subsistence operations and therefore do not use antibiotics as growth promoters, so it is unlikely that agricultural practices are contributing to antibiotic resistance to ceftiofur. Four of these isolates demonstrated resistance to other drugs as well, two of which exhibited extreme drug resistance. It is possible, that these isolates carry a gene that resists ceftiofur’s mechanism of action for inhibiting growth even though they have never been exposed to the drug.

High incidence of antibiotic resistance in banded mongoose may be due to the lack of regulation of antibiotics in Chobe, where they can be freely accessed by the local community with little or no regulation regarding inappropriate use of antibiotics and the further development of resistance. Human fecal waste provides an important mechanism for the spread of antibiotic resistance to the environment, an emerging global health threat. In this study, antibiotic
resistance was identified in banded mongoose living in close association with humans at tourist lodges as well as natural areas with little human contact. This observation suggests that impacts of fecal waste, such as antibiotic resistance, may also extend beyond direct contact with human waste; perhaps providing an example of host-mediated environmental amplification.

Transmission of resistant isolates to environmental reservoirs is a growing concern for human and ecosystem health. Multidrug resistant pathogens, termed “superbugs,” cause high rates of mortality. Additionally, from an economic standpoint, the arms race with antibiotic resistance is costing society more than $35 billion a year. These concerns are especially poignant in a region like Chobe where rates of HIV/TB co-infection are some of the highest in the world creating a greater possibility that harboring resistant strains of *E. coli* and other micro-organisms could lead to resistance in other host-pathogens systems through horizontal gene transfer. This work identifies the need to include wildlife in assessments of antibiotic resistance in ecological systems. Most regulatory assessments are limited to domestic animals and humans, both groups potentially having direct antibiotic treatment as a matter of course. Inclusion of wildlife hosts in the surveillance approach will enhance the identification of environmental accumulation and contamination of antibiotic resistance.

5.3 Phylogenetic Analysis

Neither mongoose nor human isolates formed exclusive clades in phylogenetic analysis based on either fingerprint or sequence data. Genetic relationships between isolates interpreted through branching patterns were not identical between fingerprint- and DNA sequence-based analyses in this study, even when using optimization parameters published by previous studies. It is not unexpected that results from these two genetic analyses may yield slightly different
phylogenetic relationships, as they are examining two very different sections of the *E. coli* genome. Rep-PCR amplifies non-coding regions which are free from evolutionary pressures to remain conserved between strains and therefore, vary widely from isolate to isolate; hence, the utility of this analysis in generating unique fingerprints for each isolate. Consequently, due to the extreme variability of these non-coding regions, rep-PCR is not as sensitive a tool for evolutionary relationships. MLST-PCR, however, amplifies critical loci in coding regions which tend to be highly conserved across strains. This analysis tool does not have the same ability to identify individual strains, but is extremely useful in determining evolutionary relationships. Logically then, when drawing conclusions on phylogenetic relationships from two such disparate assays, it is entirely reasonable that there would be minor differences in branching patterns. Regardless, the ultimate conclusions concerning the genetic similarities of *E. coli* isolated from banded mongoose and human fecal waste in this study are the same for both analyses: banded mongoose in Chobe National Park and surrounding townships harbor strains of *E. coli* that are genetically very similar to strains isolated from human fecal waste.

Interestingly, the single outlying samples clustering closest to the reference sequence, *E. coli* K-12, on both rep-PCR and MLST-PCR phylogenetic analysis were isolated from the same mongoose fecal sample. During collection, the fecal specimen was noted as loose and containing a large proportion of green mucous suggesting the individual was sick. No clinical information is available on the individual mongoose that provided the specimen. No additional analysis was performed on the sample.
5.4 Banded mongoose – E. coli – Human fecal waste Model

In this study, the banded mongoose – E. coli – human fecal waste model was found to be useful for detecting the exchange of fecal-orally transmitted microorganisms between humans and wildlife through fecal contamination of the environment. One, perhaps confounding or compounding factor that needs consideration in future use of this model is a single, unifying contaminant source between humans and wildlife such as water. Fecal contamination of the Chobe River could mean the river acts as a vehicle for microorganism transmission, whereby all animals utilizing this water source are exposed to human fecal waste-borne microorganisms. Conversely, contamination of a unifying water source also creates the potential for bidirectional transmission, perhaps even in a cyclical fashion, between humans and wildlife as humans also heavily utilize this waterway.

Developing models like the banded mongoose – E. coli – human fecal waste model allow us to move from theoretical concepts to an empirical framework for exploring pathogen dynamics at the human-wildlife interface. This model can be utilized for future research regarding the impacts of fecal-orally transmitted microbial traffic on human and wildlife population health; i.e., to determine how land-use, host or pathogen characteristics can contribute to pathogen exposure in human-modified environments and whether there are far-reaching effects of anthropozoonotic diseases in national parks or other protected areas. The importance of the latter process is becoming increasingly evident with recent cases of mortality in endangered species due to human pathogen transmission, as in gorillas in Uganda and Rwanda\(^{50,52,78}\).

Increased micro-organismal traffic through fecal waste identifies a venue for other, perhaps pathogenic, fecal-orally transmitted microorganisms to be exchanged across a wide array of susceptible wildlife, animal and human hosts. The potential for sewage waste to act as a
repository for microorganism transmission was illustrated in a recent metagenomics study in which over 234 known plant, insect and algal viruses (including 17 that infect humans) as well as bacteriophages, were found in sewage samples, identifying this material as the most diverse viral metagenome of environmental origin. How much undetected fecal waste-borne microbial traffic of pathogens is occurring at this interface? Even if *E. coli* is unique in its ability to be sustained and transmitted environmentally among a variety of hosts, it is unlikely that it is the only microorganism able to follow such a transmission pathway. Even so, *E. coli* itself can be pathogenic in humans and is one of the leading causes of diarrheal-related deaths worldwide.

Despite the importance of emerging infectious disease as a primary threat to human and animal health, our understanding of the mechanisms of pathogen transmission between humans and wildlife is limited. This knowledge gap presents a key barrier in developing a more predicative capacity in the management and control of emerging infectious disease. This study demonstrates that there is ongoing transmission of *E. coli*, a model microorganism at the human-wildlife interface, even in protected areas within the Chobe National Park.

Banded mongoose are able to successfully occupy a gradient of environments from protected wildlife areas to human settlements connecting host communities across land uses. Banded mongoose are not the only such species, however; warthogs (*Phacoecerus aethiopicus*), baboons (*Papio ursinus*), and vervets (*Cercopithecus aethiops*) have the potential to behave this way as well. Species with similar adaptability exist in North America, for example raccoon (*Procyon lotor*), skunk (*Mephitis mephitis*), Virginia opossum (*Didelphis virginiana*), and coyote (*Canis latrans*); in England, fox (*Vulpes v. crucigera*) and badger (*Meles meles*), and in Australia, the brushtail possum (*Trichosurus vulpecula*). These species can potentially transfer fecal micro-organisms across landscapes, allowing the potential for disease transmission from
human waste to extend beyond areas of human environmental contamination. I term this process of spreading environmentally transmitted microorganisms as host-mediated, environmental amplification. Species that can contribute to environmental amplification represent important sentinel species for surveillance and detection programs for pathogens of public and animal health significance.

In wildlife health, detection and surveillance for infectious disease is more difficult than surveillance conducted on humans and domestic animals. Often we are not aware of an outbreak of infectious disease in wildlife unless numerous animals are found dead. Even then, carcasses are rapidly consumed, and it is often difficult to obtain samples for diagnostic purposes. Pathogen impacts on a host may be more subtle, influencing host demography as through pathogen-induced decreased fitness and increased predation or decreased reproduction and survivorship. Even though human surveillance systems are relatively superior to animal systems, detection of emerging disease in humans can be hampered by a variety of factors including infrastructural support, health-care seeking behavior and the presence of dominating outbreaks such as malaria or HIV/AIDS. Then, to what degree is microbial traffic influencing human and wildlife health in the region? Do certain life-history strategies of animals (herbivore, predator) or trophic levels in wildlife communities increase the likelihood of exposure to microorganisms of human origin? What are the factors that influence host-pathogen-environmental interactions which allow pathogen spillover at the human-wildlife interface? These are critical questions on the way to a better understanding of the risks to human and wildlife health imposed by microorganism exchange at the human-wildlife interface.
6. REFERENCES


49 57 rwego ib, gillespie tr, isabirye-basuta g et al. 2008. High rates of escherichia coli transmission between livestock and humans in rural uganda. journal of clinical microbiology 46: 3187-3191.
61 rolland rm, hausfater g, marshall b et al. 1985. antibiotic-resistant bacteria in wild primates - increased prevalence in baboons feeding on human refuse. applied and environmental microbiology 49: 791-794.
63 rademaker j, louws f and de bruijn f. 2004. characterization of the diversity of ecologically important microbes by rep-pcr genomic fingerprinting. in: (ed akkermans adl vej, de bruijn fj, eds. molecular microbial ecology manual.3.4.3. kluwer academic publishers.netherlands.
64 goldberg tl, gillespie tr and singer rs. 2006. optimization of analytical parameters for inferring relationships among escherichia coli isolates from repetitive-element pcr by maximizing correspondence with multilocus sequence typing data. applied and environmental microbiology 72: 6049-6052.
68 drummond aj ab, buxton s, cheung m, cooper a, duran c, field m, heled j, kearse m, markowitz s, moir r, stones-havas s, sturrock s, thierer t, wilson a. geneious v. 5.3. 2010.
69 aminov ri. 2010. a brief history of the antibiotic era: lessons learned and challenges for the future. frontiers in microbiology 1: 134.
70 allen hk, donato j, wang hh et al. 2010. call of the wild: antibiotic resistance genes in natural environments. nature reviews microbiology 8: 251-259.


CONCLUSIONS

The banded mongoose – *E. coli* – human fecal waste model was able to successfully demonstrate microorganism exchange is occurring between humans and banded mongoose in Chobe and that fecal contamination of the environment is a potential pathway for disease transmission at the human-wildlife interface. This model has the potential for use in future studies to determine how land use or host-pathogen interaction can influence the risk of exposure of wildlife to human fecal waste-borne pathogens. Results of this study have significant implications for the management of ecosystem health in Chobe and with further study, this model could be used as a sentinel system for detecting fecal pathogens posing health risk to humans and wildlife.

Human fecal waste contamination as a possible mechanism of disease transmission could be explored at other human-wildlife interfaces to improve conservation efforts. For instance, this could be extremely valuable to managing anthroponotic disease transmission to endangered primate populations in Uganda and Rwanda where similar studies have demonstrated bacterial exchange is occurring between humans and wildlife. Steps could be taken to limit fecal contamination and consequently reduce the likelihood of transmission of anthroponotic pathogens to wildlife.
SUMMARY

Goals for this study were successfully achieved by demonstrating the utility of the proposed model in microorganism exchange at the human-wildlife interface and by addressing the hypothesis outlined in the introduction. Recommendations for future use of this model include:

- Primers designed to positively identify *E. coli* could be optimized to target clades of *E. coli* specifically associated with the human gut to reduce the chance of detecting environmentally adapted strains.

- Multiple sampling events as part of an attempt to gain greater coverage of mongoose troops proved unfruitful since a majority of samples came from a single collection event for each troop. Multiple sampling events also meant dealing with the possibility of increased error due to repeat sampling of individuals and isolates. It would be more beneficial to process samples from a single collection date which produced substantial sample numbers.

- The increase in sampling intensity of colonies grown from human feces (up to 15 isolates instead of 6) to accurately represent as many unique *E. coli* isolates from the human gut as possible proved redundant. A majority of the isolates were identical in fingerprint analysis. A maximum of six putative *E. coli* isolates would have been sufficient for all sample types collected in this study.

- Since a unifying resource such as the Chobe River could be a confounding “third party” factor in the similarities found between *E. coli* isolates from humans and mongoose, it would be advantageous to include analysis of water isolates in future experiments.
• While alternative fecal indicators are not suitable replacements for *E. coli* in this model at this stage, the model could be enhanced by the inclusion of an additional bacterium such as *Bacteriodes* which can be more definitively linked to its originating host. This allows the model to capitalize on the benefits of both fecal indicators.

• The model could be enhanced by including other species with various life history strategies to better judge the risk of fecal waste-borne pathogen exposure to different categories of wildlife (herbivores vs carnivores, water-associated vs inland, diurnal vs nocturnal, social vs solitary, etc). No doubt that since banded mongoose are very social animals and choose to den in septic systems they may over-represent the risk of exposure for other wildlife, but that does not mean there is no risk. Understanding how these life history characteristics influence exposure is important for managing the risk to wildlife health.

• Since antibiotic susceptibility data from the Kasane Primary Hospital was not available during the design stage of this study, the inclusion of more locally available, more human-specific antibiotics in future experiments could prove even more informative than the panel used here. For example, widespread resistance to Ampicillin as a naturally derived antibiotic means that resistance to this drug in our study is not particularly informative. Inclusion of antibiotics such as Clindamycin and Vancomycin would mean greater overlap with the panel used by the hospital and perhaps generate tighter correlations in resistance signature between humans and wildlife.
Sampling mongoose in geographic regions further from human fecal waste and comparing the genetic similarities between *E. coli* in those individuals versus the *E. coli* from humans and wildlife in this study could demonstrate whether or not a gradient effect occurs in relation to human proximity.
REFERENCES


STEC Center: A Reference Center to Facilitate the Study of Shiga Toxin-Producing *Escherichia coli*. EcMLST System. <http://shigatox.net/stec/cgi-bin/index>.


APPENDIX

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