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Emerging Zoonotic Pathogens at the Human-Wildlife Interface in Protected Areas: Game in the Southeastern United States and Bushmeat in Northern Uganda

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ABSTRACT

The emergence of zoonotic pathogens through contact with animal reservoirs is a well-documented phenomenon and growing concern for public health. Particularly in light of the ongoing Ebola epidemic in the Democratic Republic of the Congo and the coronavirus pandemic, the need to understand mechanisms of contact and disease transmission at the human-wildlife interface and to understand which infectious agents may reside within wildlife reservoirs crucial. In this project, we investigated the potential introduction of zoonotic cestode *Echinococcus* canadensis to public lands in Tennessee subsequent to elk translocation effort and aimed to identify whether a transmission cycle was established in this area. We further aimed to elucidate drivers of zoonotic infections in the bushmeat trade in northern Uganda by assessing the phenomenon of 'species deception', evaluate social factors influencing participation in the bushmeat trade and risk for zoonosis exposure, and describe bacterial microbial diversity in market bushmeat in the area. We confirmed the presence of *E. canadensis,* with histological confirmation in 75% of elk included in our study and PCR confirmation in 50% of elk. Our findings in bushmeat in northern Uganda demonstrate nearly 30% mismatch between what bushmeat species are sold as in market and the true identity of these species based on PCR and Sanger sequencing. Surveys of hunters and cooks in communities adjacent to Murchison Falls National Park revealed that both hunters and cooks have the highest awareness of monkeypox and gastrointestinal illness as diseases that wildlife can carry. Self-reported injuries while cooking or butchering bushmeat were reported to be infrequent among both hunters and cooks. While cooks believed that hunters and dealers never described primate meat as another kind of animal, hunters reported usually doing this. Microbial diversity among wildlife samples was found to be high, regardless of tissue condition or wildlife species. Furthermore, 16s rRNA signatures of numerous Select Agent bacterial genera associated with significant human illness were detected in these samples. Microbial composition suggests that bushmeat microbiota is comprised of a combination of endogenous infections, environmental contamination, and spoilage associated bacteria. Regardless, the potential health consequences of unmitigated exposure to these microbes presents a clear risk to individual and global health. The findings of this project underscore the need for practical and culturally appropriate educational strategies to help hunters both in the United States and Uganda enact proper handling and butchering techniques to minimize contact with bodily tissues of wild animals.

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CHAPTER I

Introduction

Bushmeat and Zoonoses Risk in a Global Context

The term ‘bushmeat’ is a blanket term that refers to any non-domesticated animal species, or wildlife, including terrestrial mammals, reptiles, amphibians, and avian species (also commonly referred to as “game meat” or “wild-meat”). ‘Bushmeat harvest’ or ‘bushmeat hunting’ describes the intentional extraction of wildlife from its natural habitat, regardless of means or purpose. A key point of this is that these bushmeat species are frequently harvested at an unsustainable rate and often through illicit measures. Bushmeat is hunted for several reasons, including food and income, traditional and medicinal use, trophy hunting, and exotic pet trade, and plays an important role in many local economies, cultural identities, international trade, and in community nutrition [1-3]. Most commonly in the literature, and hereafter for the purposes of this dissertation, the term bushmeat will refer to the hunting of wildlife for consumption or sale at local markets to improve livelihood; however, increases in migration of rural populations into metropolitan areas has amplified commercial demand for bushmeat and given rise to transboundary movement of bushmeat [4, 5].

Bushmeat hunting is practiced worldwide, although the term is more frequently associated with the harvest of wildlife in tropical and subtropical ecosystems. A 2018 estimate of households dependent on bushmeat as a meat source surpasses 150 million households in developing countries [6]. Nielson et al. report that 39% of households in 24 countries across Asia, Africa, and Latin America reported engaging in bushmeat harvest in the past one year, with 89% of that harvest directly applied to dietary needs of the household [6, 7]. Globally, the bushmeat trade is a multibillion-dollar market, with trade values for the Republic of Côte d'Ivoire alone estimated at US $150 million in the year 2000 [1, 8]. In other, non-tropical regions, bushmeat is still hunted; however, in these areas hunting is not so often a necessity for financial or nutritional security, but largely recreational or for sport and is subject to stricter regulation.

Bushmeat in North America

In the United States and Canada, hunting of wildlife is largely a recreational activity in which wildlife is harvested for sport, trophy and meat [25]. In 2016, the U.S. Fish and Wildlife

Service reported 11.5 million individuals (>16 years of age) hunted over 184 million days with hunting expenditures estimated at US$26.2 billion [26]. Hunters targeting big game species including deer, wild turkey, elk, and bear totaled 9.2 million hunters and were the most populous group among US hunters [26]. Small game species hunters totaled 3.5 million hunters, targeting squirrel, rabbit/hare, quail, ptarmigan, and grouse/prairie chicken. Nearly 2.4 million hunters pursued migratory birds, including ducks, doves, and geese. Over 1.3 million hunters pursued "other animals", including groundhogs, feral pigs, raccoons, foxes, and coyotes [26]. Although exact harvest numbers are not reported for all states, Flahter et al. reported in general, harvest rates of big game species are increasing nationally for elk, wild turkey, deer and black bear, but decreasing for pronghorn [27]. No data on harvest rates for small game were readily available. First Nations people and Native Americans are currently able to hunt unrestricted on public lands and case-by-case on privately owned land under the threatened statute of food sovereignty [28, 29]; outside of this, hunting is a permitted sport and most species have bag limits per season determined by state. There are exceptions to bag limits in certain areas where species are overrun and year-round open seasons exist, as is the case on a state-by-state basis for feral hogs.

Bushmeat in sub-Saharan Africa

The harvest and consumption of bushmeat in sub-Saharan Africa has long been acknowledged as necessary for food security and nutrition, and income security, particularly in rural communities [9-12]. The magnitude of bushmeat harvest is difficult to quantify because studies are sporadic and not uniform in metrics. The magnitude of bushmeat harvest varies substantially among ecological habitats and assemblages, among socioeconomic and cultural gradients, in response to agricultural harvest, and among political boundaries. Commonly cited estimates for Nigeria and Cameroon [13], Ghana [14], Republic of Côte d'Ivoire [15], and the Congo Basin [8, 16] range from 12,000 tons to 4.9 million tons annually. Barnett et al. reported that in Tanzania, over 2000 tons of bushmeat valued at more than 50 million USD are confiscated by the government annually; nearly 60,000 tons of bushmeat are sold in market in the Central African Republic annually; and up to 365,000 tons are consumed annually in Mozambique [3, 5]. Estimates for annual bushmeat harvest are predicted to continue to rise in sub-Saharan Africa with increased demand and increased access into protected areas with the construction of roadways

despite attempts by many governments to limit and regulate hunting through restrictions, quotas and permitting systems [5, 17-19].

Data informing hunting methods, temporality, and prey preference are similarly erratic, incomplete, and vary widely among and within countries. Lindsey et al. 2007 report that snares, a non-specific hunting method, are mostly used, and that decreases in hunting are noted during agricultural seasons, presumably when the labor of hunters is required for farming [20]. Market differences also vary widely among countries. Although open markets with bushmeat carcasses on display to consumers are more common in forested areas in West and Central Africa, this is not the case in our study area, northern Uganda.

In Uganda, hunting, possession, and sale of bushmeat are illegal and are punishable by penalties from fines to imprisonment [21]. Bushmeat is still frequently hunted and sold in local markets; however, the transactions are necessarily more secretive and occur person-to-person. With the exception of baboons, vervet monkeys, and bush pigs on a land owners' property, wildlife is legally protected against hunting [22-24]; still, according to Nielson et al., 71% of Ugandan households have hunted wildlife at one point in the past one year [6]. Additionally, in the course of preliminary logistical work for this project, the concept of 'species deception' in the bushmeat market emerged, in which meat of one species is sold as the meat of another species. The motivation behind this misrepresentation may be related to market price, market demand, (or escape from the legal arm) or simply lack of knowledge of the true species which the seller possesses; regardless, this aspect of the bushmeat chain imposes an additional level of exposure, and potentially inadvertent exposure, and risk of zoonoses to consumers.

Conservation

One of the major concerns surrounding bushmeat trade is the impact that unregulated and unsustainable harvest has on the survivability of wildlife populations. The bushmeat trade is widely indicted as one of the major and most immediate threats to wildlife biodiversity. Megafauna are considered especially susceptible to overhunting due to low fecundity, slower movement, more obvious tracking, and greater payoff for effort [30]. Over-exploitation of wildlife is implicated in the endangerment and extirpation of numerous wildlife populations [30, 31]. This trend is notable in Ghana, where a 76% decrease in the biomass of 41 mammalian species was observed over a 30-year period resulting in the local extinction of up to 45% of these species, and

has been similarly demonstrated in both savanna and tropical forest ecosystems across central Africa [32, 33]. Several regional ecosystems that are similar in assemblage to Murchison Falls National Park, the study site for this dissertation, have experienced over-hunting that has locally decimated wildlife species, such as the local extinction of red hartebeest in the Serengeti ecosystem in Tanzania and severe endangerment of zebras, wildebeest, and rhinoceros in South Africa's Dwea and Cwebe reserves [34-37]. These examples are only a few of this widespread, but poorly documented occurrence, across sub-Saharan Africa. The impacts of decreased wildlife populations further pose a challenge to sustainable ecotourism, one of the most often proposed avenues for alleviating poverty in communities that border protected areas, as these operations rely on high wildlife density for tourist satisfaction [16]. Beyond impacts to the ecosystem health, the unsustainable hunting of wildlife will have severe negative consequences on the human populations that depend on bushmeat for protein and livelihood as threatened species are unable to recruit quickly enough to maintain a healthy population [38].

Zoonoses and Public Health

Beyond ecological impacts, the threat of emerging zoonotic diseases has been thrust into the public eye over the past few decades. More than 60% of emerging infectious diseases affecting human populations are zoonotic and over 71% of those zoonoses resulted from contact with wildlife [39]. Epidemic outbreaks of viral diseases like Ebola virus, Marburg virus, and henipaviruses have garnered international attention and illuminated the devastating medical and financial consequences of pathogen spillover [40, 41]. Reports of hunters contracting primate T-lymphotropic viruses in Cameroon underscore the dynamic nature of viral cross-species transmission events and make clear the need for surveillance and detection efforts [42]. Furthermore, endemic diseases such as anthrax, brucellosis, rabies, yellow fever, and enteric diarrheal illnesses pose a constant burden on at-risk populations [41, 43]. Infections contracted through ingestion can have high consequences, are largely underdiagnosed and undertreated, and are being demonstrated to be more prevalent than previously believed [44]. Katani et al. 2019 confirmed the presence of bacterial DNA signatures of *Brucella, Coxiella,* and *Bacillus* on bushmeat samples procured from the Serengeti [44].

The nature of bushmeat trade presents ample routes of opportunity for transmission of zoonotic pathogens. There are airborne and bloodborne hazards during the hunting process and

butchering of carcasses, as well as foodborne hazards present with improper food handling and poorly cooked meat. In many bushmeat markets, there is poor refrigeration capacity in the consumer chain, promoting proliferation of common foodborne diarrheal pathogens. Limited infrastructure for disease reporting and healthcare access remains common in the areas most dependent on bushmeat, further increasing risk.

For North America, the American Veterinary Medical Association has compiled a set of resources targeting hunters specifically to prevent specific zoonotic diseases associated with hunting. This disease list includes anaplasmosis, avian influenza, babesiosis, brucellosis, campylobacteriosis, chronic wasting disease (to be monitored for zoonotic potential), deer parapoxvirus, hydatid disease, ehrlichiosis, equine encephalitis virus, *E. coli*, hantavirus, leptospirosis, Lyme disease, rabies, plague, Q fever, *Baylisascaris procyonis* infection, Rocky Mountain Spotted Fever, salmonellosis, *Sarcoptes*, toxoplasmosis, trichinellosis, tuberculosis, tularemia, and West Nile Virus as potential exposures (https://www.avma.org/resources/public-health/disease-precautions-hunters). Through aggressive educational efforts that are tied to the permitting process, many of these diseases have decreased and are maintained at manageable incidences. Still, changes in spatial distribution or host range may occur and present opportunity for sporadic cases of these infections among hunters.

Research Significance

In this project, we examined the presence and risk of emergence of zoonotic diseases from hunted wildlife in both North America and East Africa. First, we aimed investigate the introduction of the zoonotic cestode, *Echinococcus granulosus,* into a region with no previous documentation of this disease in the United States. Second, we aimed to investigate factors that may contribute to exposure to zoonotic pathogens in bushmeat in northern Uganda, such as preference and knowledge in hunters and cooks, and factors in market such as 'species deception.' Lastly, we aimed to assess bacterial pathogen diversity in bushmeat species in market, with the goal to establish baseline data for this region, filling crucial gaps in the literature and laying groundwork for future investigations. .

For the North America data, results of this study will be used in public health and conservation initiatives to inform key high-risk groups of handling precautions and provide insight in further wildlife translocation efforts. Determination of baseline prevalence and ecology data for

this pathogen will begin to establish an understanding of *E. granulosus* and its changing distribution. This data is vital to informing wildlife management policy and public health efforts because of zoonotic potential of this pathogen. Similarly, for our East Africa data, we aim to clarify drivers of participation of local community members in the bushmeat trade as well as determine the degree of awareness of zoonotic disease risk associated with bushmeat handling. We also determined estimates of the rate of deception of bushmeat species at the point of sale. These data will serve as a resource to better understand bushmeat trade in our study area, which will hopefully inform development of local policies and interventions. Furthermore, insights gained from this data should be used to empower local community members, district leaders and public health stake holders to take action to increase safety measures to prevent zoonotic and foodborne infections in their own communities through increased food hygiene.

Hypotheses & Objectives

1. We hypothesize that *Echinococcus granulosus* has been introduced to east Tennessee through elk translocations in the early 2000's and that a sylvatic transmission cycle has been established in wildlife;
    a. Collect and evaluate tissue samples from elk and intestinal contents from coyotes for *Echinococcus granulosus* using histology, fecal examination, and PCR with Sanger sequencing
2. We hypothesize that a notable proportion of bushmeat samples collected in northern Uganda are being misrepresented in market by hunters and dealers unbeknownst to most consumers;
    a. Sample market bushmeat intended for human consumption to and perform PCR and Sanger sequencing identify the most common species hunted and sold at market in communities in northern Uganda,
    b. And perform PCR and Sanger sequencing on bushmeat tissue to compare reported species to identify rate of species deception in market.
3. We hypothesize that there are opportunities for improvement in hygiene and safety in the handling of bushmeat tissue from hunting to preparation within communities in northern Uganda and that preference as well as opportunity influence participation in the bushmeat trade;

a. Deploy questionnaires to self-identified hunters in communities in northern Uganda to assess disease knowledge and elucidate common hunting and dealing practices, including deception of bushmeat species.
b. Objective: Deploy questionnaires to female cooks in communities in northern Uganda to assess disease knowledge and factors influencing choice and risk in handling bushmeat.

4. And we hypothesize that there is considerable detectable bacterial microbial diversity in market-acquired bushmeat samples in northern Uganda.
    a. Apply next generation sequencing to evaluate microbial communities present in market bushmeat intended for human consumption and compare these communities across wildlife species and bushmeat tissue condition.

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CHAPTER II

Retrospective investigation of *Echinococcus canadensis* emergence in translocated elk (*Cervus canadensis*) in Tennessee and examination of definitive canid hosts

Disclosure

This chapter has been accepted for publication in *Parasites and Vectors*. This chapter appears within this text as published with minor modifications to formatting. Co-authors include Shelley J. Newman, Kathryn Purple, Brad Miller, Edward Ramsay, Robert Donnell and Richard W. Gerhold, all of whom provided substantial contributions to conceptualization, sample acquisition, analysis, and manuscript revision.

Abstract

Background: Few reports of *Echinococcus* spp. have been described in the Unites States; however, the geographical distribution of *Echinococcus* spp. in wild hosts is increasing consequent to human activities. In the early 2000's, 253 elk (*Cervus canadensis*) originating from Alberta, Canada were released into the Great Smoky Mountains National Park and North Cumberland Wildlife Management Area in an effort to re-establish their historical range.

Methods: We investigated the prevalence of *Echinococcus* spp. in re-established elk populations in the North Cumberland Wildlife Management Area and the Great Smoky Mountains National Park via a retrospective analysis of banked elk tissues and helminth examinations on intestinal contents from coyotes (*Canis latrans*) from the North Cumberland Wildlife Management Area.

Results: Four elk were PCR and sequence positive for *E. canadensis*. Each sequence had 98% or greater coverage and identity to multiple *E. canadensis* genotypes in Genbank. Adult *Echinococcus* spp. were not detected in any of the coyotes examined in this study.

Conclusions: Continued surveillance of this disease in susceptible species in these areas is warranted, and these data further underscore the risk of zoonotic pathogen introduction secondary to wildlife translocation.

Background

Echinococcus spp. are zoonotic cestode parasites responsible for cystic Echinococcosis (CE), one of the designated neglected tropical diseases by the World Health Organization [1]. The parasite cycles between intermediate ungulate hosts and canid definitive hosts as hydatid cysts in various organs and adult worms in the small intestines, respectively. Humans become incidentally infected with the parasite following ingestion of infective eggs shed in the feces of definitive canid hosts. The resulting pulmonary and hepatic cysts, termed hydatid cysts, are difficult to diagnose and treat in intermediate animal hosts and aberrant human hosts, cause substantial economic loss, and can be fatal as cysts compress host tissues or rupture within the host [2].

There are currently 10 recognized genotypes (G1-G10) which correspond to distinct species within the *Echinococcus granulosus* sensu lato (s.l.) complex. Each species differs in its host specificity, phenotypic and genetic characteristics, and pathogenicity patterns. The *E. granulosus* sensu stricto complex (G1-G3) includes the sheep strain, the Tasmanian sheep strain, and the buffalo strain, respectively and typically involves domestic livestock and domestic canines in its lifecycle. *E. equinus* (G4) is the horse stain and is specific to equids and *E. ortleppi* (G5) is the cattle strain, and typically cycles between cattle and dogs. *E. intermedius* (G6-G7), which are grouped with *E. canadensis* under some classification schemes, includes the camel and pig strains. *E. canadensis* (G8-G10) encompasses the American cervid strain and the Fennoscandian cervid strain, and cycles between cervids including moose, elk, and reindeer and canids. [4, 5, 6]. Members of *E. granulosus* sensu stricto are most frequently implicated as the causative agents of CE; however, *E. ortleppi* (G5), *E. intermedius* (G6-7), and *E. canadensis* (G8, G10) are also known contribute to the global burden of human disease [4,7,8].

In 2000, the Tennessee Wildlife Resources Agency (TWRA) implemented a re-establishment plan for elk (*Cervus canadensis*) into the Sundquist Wildlife Management and Royal Blue Wildlife Management Area (WMA) public lands in Campbell, Scott, Morgan, Claiborne, and Anderson Counties of Tennessee [9,10,11]. Royal Blue WMA has since been absorbed into the North Cumberland Wildlife Management Area (NCWMA). Additionally, in 2001, the National Parks Service reintroduced elk into the Cataloochee Valley area of the Great Smoky Mountains National Park (GSMNP). In both locations, elk had been extirpated since the mid-1800s [12]. From 2000 to 2008, a total of 201 elk were released into the NCWMA, and from 2001 to 2002, 52 elk were released into the GSMNP [9,10,13]. A 2016 TWRA survey documented 349 elk within

NCWMA, suggesting that the reintroduction was successful to date, and populations have remained steady in subsequent years [13]. In both locations, re-introduced elk were originally sourced from Elk Island National Park (EINP) in Alberta, Canada due to the park's history of testing animals for disease and having the Manitoban subspecies (*C. c. manitobensis*), which is considered the closest genetic stock to the extinct eastern elk (*C. c. canadensis*). A portion of the imported elk came from Land Between the Lakes (LBL) National Recreation Area, Kentucky; however, all LBL elk were originally sourced from EINP. Prior to translocation, elk were screened for major pathogens, including brucellosis, bovine tuberculosis, Johne's disease, anaplasmosis, vesicular stomatitis, bluetongue, epizootic hemorrhagic disease, infectious bovine rhinotracheitis/bovine viral diarrhea, and several strains of leptospirosis [10]. However, antemortem testing for *Echinococcus* was not available. *Echinococcus granulosus* s.l. is not currently considered endemic in GSMNP or NCWMA, but since the reintroduction of elk, the *E. granulosus* s.l. strain G10 (i.e. *E. canadensis*) has been presumptively diagnosed in one elk at necropsy. Moreover, an *E. granulosus* s.l. infection has been suspected in several other elk [14]. No previous reports of echinococcosis in wildlife in this region exist, although it is well documented in wildlife in Canada [15,16].

With the reintroduction of elk into the NCWMA and GSMNP ecosystems, a pathway for the maturation and spread of *Echinococcus* was newly recreated. It is an emerging concern that the transmission of *Echinococcus* from the translocated animals into wild or domestic canine populations and other sympatric cervids has occurred, thereby establishing a sustainable transmission cycle and reservoir for the disease. This creates a public health risk, as the GSMNP hosted 12.5 million recreational visitors in 2019 [17]. Similarly, NCWMA is a multi-purpose public land that hosts large numbers of visitors and issues 15 elk harvest permits annually [18]. Due to the high tourist load in these recreational areas and the presence of wild canids (coyotes, foxes) and free-roaming domestic dogs, both of which can serve as definitive hosts, there is increased opportunity for wildlife and domestic animal contact, as well as zoonotic transmission [19].

This study describes *E. granulosus* s.l. lesions and molecular characterization from necropsied elk from NCWMA and GSMNP and investigates parasite transmission in the NCWMA by examining coyote intestinal samples for eggs or protoscoleces. The establishment of a baseline prevalence and ecology data of this pathogen will help fill a critical void in the current awareness

of the parasite. Due to the zoonotic potential of this pathogen, this information is vital to informing wildlife management policy, clinical medical and veterinary medical practice, and public health efforts [20].

Methods

A retrospective search of the University of Tennessee College of Veterinary Medicine (Knoxville, Tennessee) pathology archive spanning 17 years (2000-2017) was conducted to find all necropsy cases of suspected *E. granulosus* s.l. in elk. Archived histology slides of all selected cases were reviewed by a board-certified pathologist (S.J. Newman) to confirm the presence of *E. granulosus* s.l. organisms or characteristic hydatid cysts and brood capsules within archived tissue.

Tissue samples were cut from paraffin blocks from all identified cases with lesions consistent with *E. granulosus* s.l. for DNA extraction and subsequent PCR testing to confirm presence of *E. granulosus* s.l. An additional histology slide was cut after the 10 µm tissue PCR slices and then stained to determine if organisms had been uncovered at the depth of the corresponding PCR sample. Separate microtome blades were used for each block, and microtomes were cleaned thoroughly with DNA AWAY (Fisher Scientific) between blocks. Extraction of DNA was performed using QIAGEN DNeasy Blood & Tissue® extraction kit, according to manufacturer instructions. PCR was completed using COX-1 primers targeting the parasite mitochondrial cytochrome c oxidase subunit 1 gene with sequences as follows: COI-F: 5′-TTTTTTGGGCATCCTGAGGTTTAT-3′ and COI-R: 5'TAAAGAAAGAACATAA TGAAAATG-3' [21]. Cycling conditions for PCR were performed in an automatic thermocycler under the following conditions: after an initial denaturation for 1 minute at 95°C there were 40 cycles consisting of 1 minute at 95°C, 1 minute at 50°C, and 1 minute at 72°C, with a final extension step for 10 minutes at 72°C. Both DNA extraction and PCR negative controls were used in PCR reactions to detect contamination. The PCR products were examined using gel electrophoresis in 1.5% agarose gel. Bidirectional sequencing of amplicons was performed at the University of Tennessee sequencing facility (Knoxville, TN). The obtained sequences were compared in GenBank using Basic Local Alignment Search Tool (BLAST). One sample was obtained from the pluck of a freshly killed elk on the same day it was admitted to the University of Tennessee necropsy service (SP 17-465; Fig 1A). For this specimen, hydatid cysts were observed grossly in the elk lung. Tissue from the cyst wall and fluid from within the cyst were

sampled with a sterile scalpel and syringe, respectively, and used for the PCR reaction as described above. In addition, the fluid from the cyst was examined by light microscopy for characteristic findings of *Echinococcus* spp. protoscoleces (Fig 1B).

Coyote carcasses from within NCWMA were provided by TWRA for examination. Restricted necropsies limited to the gastrointestinal tract were performed. Fecal samples were collected directly from the large intestine of the animals. Fecal flotations using Sheather's sugar solution with a water step were performed on ~1 gram of feces to identify any helminth eggs and coccidian-type oocysts. The gastrointestinal tract from the pylorus of the stomach to the cecum was removed and sieved using Grainger mesh sieves down to the 400 μm mesh. Sieved intestinal contents were preserved in 70% ethanol and examined under a dissecting scope to morphologically identify helminths. Any Taeniidae eggs or protoscoleces were subject to PCR using COX-1 gene for molecular identification [21,22].

Results

Of 103 elk necropsy records examined, 14 (13.6%) reports matched selected search criteria based on gross examination. Of these, seven of the 14 cases (50%) that were examined by the pathologist showed histologic findings consistent with or suggestive of *Echinococcus* infection (Fig. 2). The other 7 cases were excluded from further study based on a lack of histologic evidence of *Echinococcus* infection. Of the seven archived necropsy cases, only four cases demonstrated identifiable brood capsules or protoscoleces. All seven cases showed evidence of non-specific cyst wall present within lung tissue. Cause of death was not attributed to *Echinococcus* infection in any of the seven cases.

Three of the seven (42.9%) paraffin-embedded tissue sections were PCR positive using the COX-1 gene target (Table 1). The single sample obtained from elk SP 17-465 at necropsy was PCR positive. Of these four PCR positive samples, three had histologic evidence of *E. granulosus* s.l. parasites. Two of the four archived cases with histologic evidence of infection were PCR negative. Sequence analysis of the four consensus sequences via NCBI Genbank disclosed at least 98% coverage and 98% identity to multiple *E. canadensis* genotypes. Nucleotide sequences were submitted to NCBI Genbank for each of our four samples. Accession numbers and BLAST result metadata are described in Table 1. Phylogenetic alignment of the COX-1 region resulted in a 324-bp alignment with 305 bp being invariant, resulting in a 94.1% conserved identity among the 4

samples. Elk NE 03-2586 and elk SP 17-465 were the most closely related with a p-distance of 0.0062, while elk 04-420 and elk and 05-331 had the furthest relationship with a p-distance of 0.059. Three of the four samples (SP 17-465, NE 04-420, NE 03-2586) clustered with *E. canadensis* G10 isolates on construction of a phylogenetic tree using the neighbor-joining method. Weak neighbor-joining bootstrap values (47%) support this conservation. Elk NE 05-331 grouped with *E. canadensis* G8 isolates, supported by a strong neighbor-joining bootstrap value of 100% [23,24]. Phylogenetic relationships among the four *Echinococcus* samples can be seen in Figure 3.

Eleven adult coyotes were necropsied and examined. Adult *E. granulosus* s.l. parasites were not detected on gross inspection of intestinal content in any of the coyotes included in this study on complete helminth examination. No Taeniidae-like eggs were identified on fecal floatation from any coyotes included in this study. Sediment of fecal floatation material that was recovered and then centrifuged in water was also PCR-negative for *E. granulosus* s.l. DNA.

Discussion

The findings in this study demonstrate a public health concern for potential zoonotic transmission of *Echinococcus granulosus* s.l. (i.e. *E. canadensis*) for the areas in and surrounding GSMNP and NCWMA. Introduction of this parasite into a region with no previous documentation of a sylvatic transmission cycle and no public education or prevention strategies creates abundant opportunity for wildlife, domestic animals, and humans to become exposed with little to no recognition of the risks. Furthermore, private agricultural land abuts much of the park, allowing for contact with domestic canids and livestock and the humans that frequent these areas. Concern should be high for the overlap of sylvatic and domestic transmission cycles, as alternative viable intermediate and definitive hosts exist in proximity to reintroduction areas. *Echinococcus granulosus* s.l. has been previously documented in the southeastern United States in hogs, cattle, and domesticated dogs, although there have previously been no sylvatic cycles documented in the region [6,25,26].

The genetic distance between samples in this study suggest that there is some heterogeneity among sequences in Tennessee. For at least three of these samples (SP 17-465, NE 04-420, NE 03-2586) the differences are minor with no genotype differences, which suggests they may be similar or the same strain of *E. canadensis* G10. Elk NE 05-331 exhibited greater genetic distance

from other samples and its phylogeny suggested closer relation to *E. canadensis* G8 strains. This may suggest multiple introduction events or introduction of distinct strains of *Echinococcus* in individuals from different geographic sourcing. Further research in translocated elk is warranted to investigate these differences among Tennessee isolates to clarify which strains have been introduced and to establish their origin. Continued surveillance of viable canid hosts for *Echinococcus* may provide insight into which strains are present. Although COX-1 is a well-established target for looking at interspecies variation, future studies may benefit from multi-locus or whole genome analysis to provide better resolution of *Echinococcus* isolates.

Four of the samples were PCR negative for *Echinococcus* /cestode DNA despite two of these samples having characteristic histologic evidence of *Echinococcus* infection. There are several possible explanations for these negative PCR results in the samples with demonstrable protoscoleces and brood capsules, including possible cross-linked DNA secondary to prolonged formalin fixation, which has been previously shown to inhibit DNA amplification [27,28]. It is also possible that the cestodes were too mineralized and degraded within the cysts to allow DNA extraction, particularly if there was a protracted latency between the death of the animals and the submission to necropsy. Alternatively, samples taken from the archived paraffin blocks did not capture sections of cyst or parasite DNA.

No canids included in this investigation were positive for Taeniidae eggs or protoscoleces on intestinal or fecal examination or PCR from intestinal content for *Echinococcus* spp. Positive canids would support the hypothesis of sustained *Echinococcus* transmission in the reintroduction areas in addition to being present in elk imported from Canada. Coyotes were opportunistically sampled by TWRA from areas adjacent to and within the elks' range. All coyotes necropsied were either killed on private property or found dead. Our sample size for surveillance of definitive canid hosts was small and only included coyotes. Future surveillance should include other canids active in both areas, including red foxes (*Vulpes vulpes*), gray foxes (*Urocyon cinereoargenteus*) and potentially domesticated dogs. There are no thriving populations of red wolves (*Canis rufus*) in GSMNP, following a failed reintroduction program [29]. Although the canid sample size was small in this study, if the negative fecal results are truly representative of the canid population, the lack of a large canid predator in GSMNP may be protective against the establishment of an efficient transmission cycle. However, further intensive canid helminth research in the areas is needed to determine if this association is accurate. An active sampling strategy and recruitment of multiple

stakeholders (e.g. landowners, resource agencies, wildlife biologists, etc.) to provide specimens may prove useful in the future to more concretely rule out the establishment of an ongoing transmission cycle. In future studies, PCR on fecal homogenate, even in the absence of taeniid eggs on floatation, may be considered as an adjunct diagnostic tool [30].

Three of the four *Echinococcus* positive elk (NE 03-2586, NE 04-420, NE 05-331) were confirmed to have been part of the stock imported to the region by ear tag number. We suspect that one of the *Echinococcus* positive elk (SP 17-465) was the offspring of one of the originally translocated elk, but we were unable to definitively confirm this. This individual was potentially born in Tennessee, as the last elk was imported to the region in 2008. This suspicion warrants further examination of various intermediate and definitive hosts for this parasite in the region. If this elk were to be a confirmed offspring, this would provide compelling evidence for the establishment of a sylvatic transmission cycle in an area with no previous documentation of the disease, even in the absence of *Echinococcus* positive canid definitive hosts in this study, as this parasite is not vertically transmitted.

Conclusions

Wildlife translocations have remained a popular and often successful conservation tool to re-establish or augment declining or extirpated populations; however, relatively little emphasis has been placed on disease risk until recently. This neglect is in spite of many documented cases of introduction of novel diseases secondary to translocation efforts, such as with parvoviral enteritis in raccoons (*Procyon lotor*) in West Virginia, rabies from translocated raccoons to local skunks (*Mephitis mephitis*) in West Virginia, brucellosis and tuberculosis in translocated plains bison (*Bison bison*) in Montana, and *Echinococcus multilocularis* in European beavers (*Castor fiber*) in the United Kingdom [29-35]. Furthermore, translocation of animals inherently includes numerous stressors, including transport, handling, capture, confinement, diagnostic screening, and release into unfamiliar environments; it is well documented that increases in these stressors are associated with diminished immune function [36]. Potential alterations in immune function during the translocation process may increase the opportunity for infectious diseases to establish in the hosts and allow the introduction of novel pathogens into immunologically naïve populations with potentially serious consequences to the native wildlife, domestic animals and humans. The findings of this study underscore the need for thoughtful, evidence-based best practices in

weighing the benefits of reintroduction efforts against the risk of novel pathogen introduction, and a robust process to identify and appropriately mitigate potential disease risks in the translocation of wildlife species.

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Appendix II

Tables and Figures for Chapter II

Table 2-1. Summary of histological presence of protoscolex or brood capsule in lung tissue or liver tissue and PCR results of elk specimens with *Echinococcus* lesions from Tennessee 2002-2017. Assigned GenBank accession numbers for submitted sequences are provided. Closest match queried from GenBank and metadata for the respective sequences are provided.

Specimen ID	Accession Year	Histologic Evidence	PCR	GenBank Accession	First BLAST Result	Host Species	Reference
NE 02-3628	2002	-	-	---	---	---	---
NE 03 2586	2003	+	+	MN833319	*Echinococcus canadensis* mitochondrion G10 (AB777927.1)	*Alces alces*	Konyaev et al. 2013 [39]
NE 04-420	2004	-	+	MN833320	*Echinococcus canadensis* mitochondrion G10 (MG597240.1)	*Bos grunniens*	Wu et al. 2018 [40]
NE 04-800	2004	+	-	---	---	---	---
NE 05-331	2005	+	+	MN833321	*Echinococcus canadensis* mitochondrion G8 (MG574827.1)	*Canis latrans*	Schurer et al. 2018 [41]
NE 07-1	2007	+	-	---	---	---	---
NE 08-46	2008	-	-	---	---	---	---
SP 17-465	2017	+	+	MN833322	*Echinococcus canadensis* mitochondrion G10 (MG597240.1)	*Bos grunniens*	Wu et al. 2018 [40]

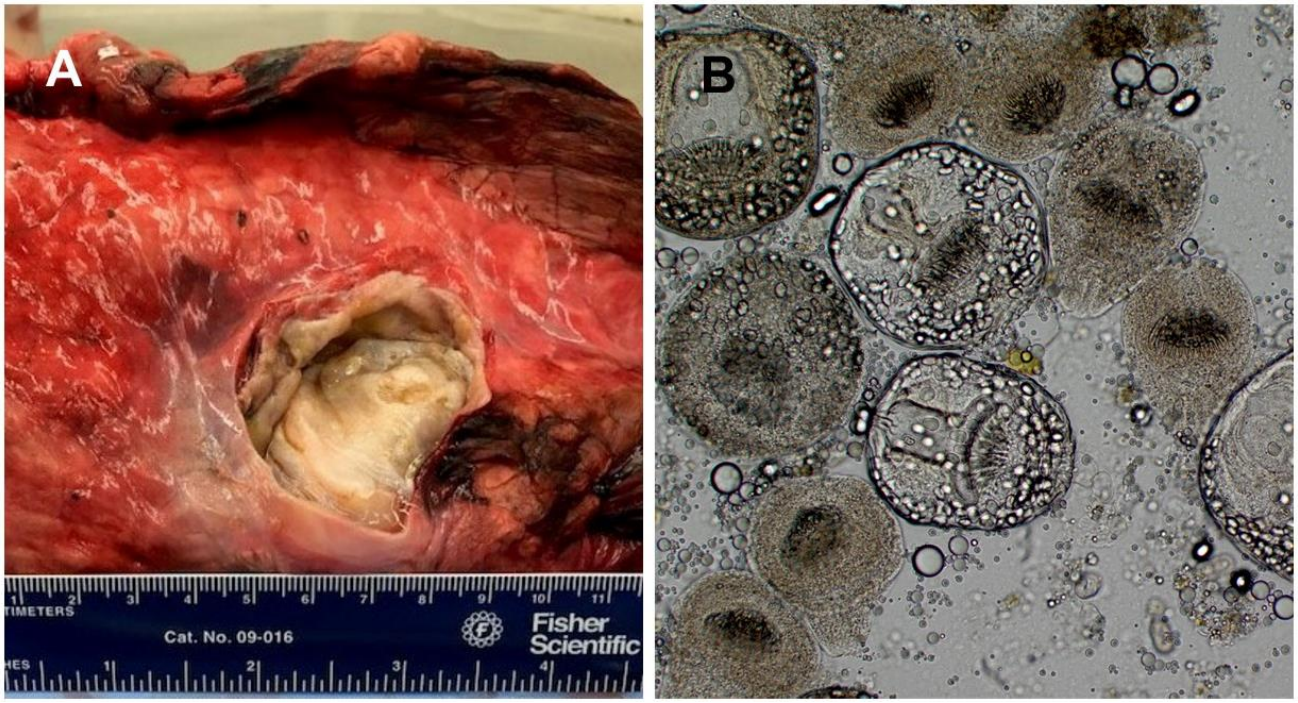

Figure 2-1. A) Photograph of a hydatid cyst within the lung tissue of elk SP 17-465 at gross necropsy at the University of Tennessee, 2017. Ruler with inches and centimeters for scale included in photograph. B) Microscopic image of invaginated protoscoleces isolated from within aspirate taken from a hydatid cyst of elk SP 17-465 at gross necropsy. Image provided by Heidi Wyrosdick.

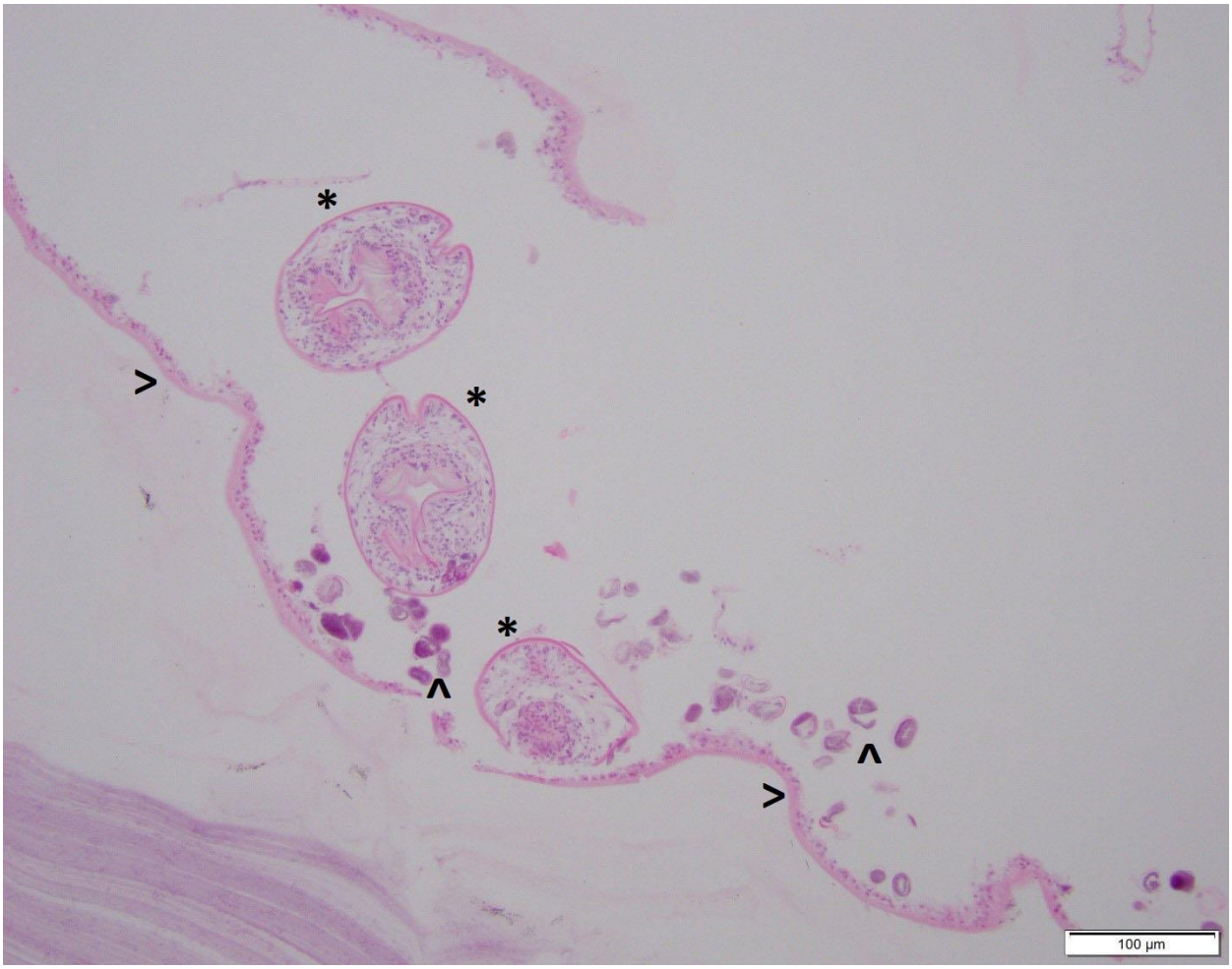

Figure 2-2. Histologic section of a hydatid cyst from elk 07-1. The brood capsule (>) containing three characteristic protoscoleces (*) and mineralized concretions [calcareous corpuscles] (^) can be seen.

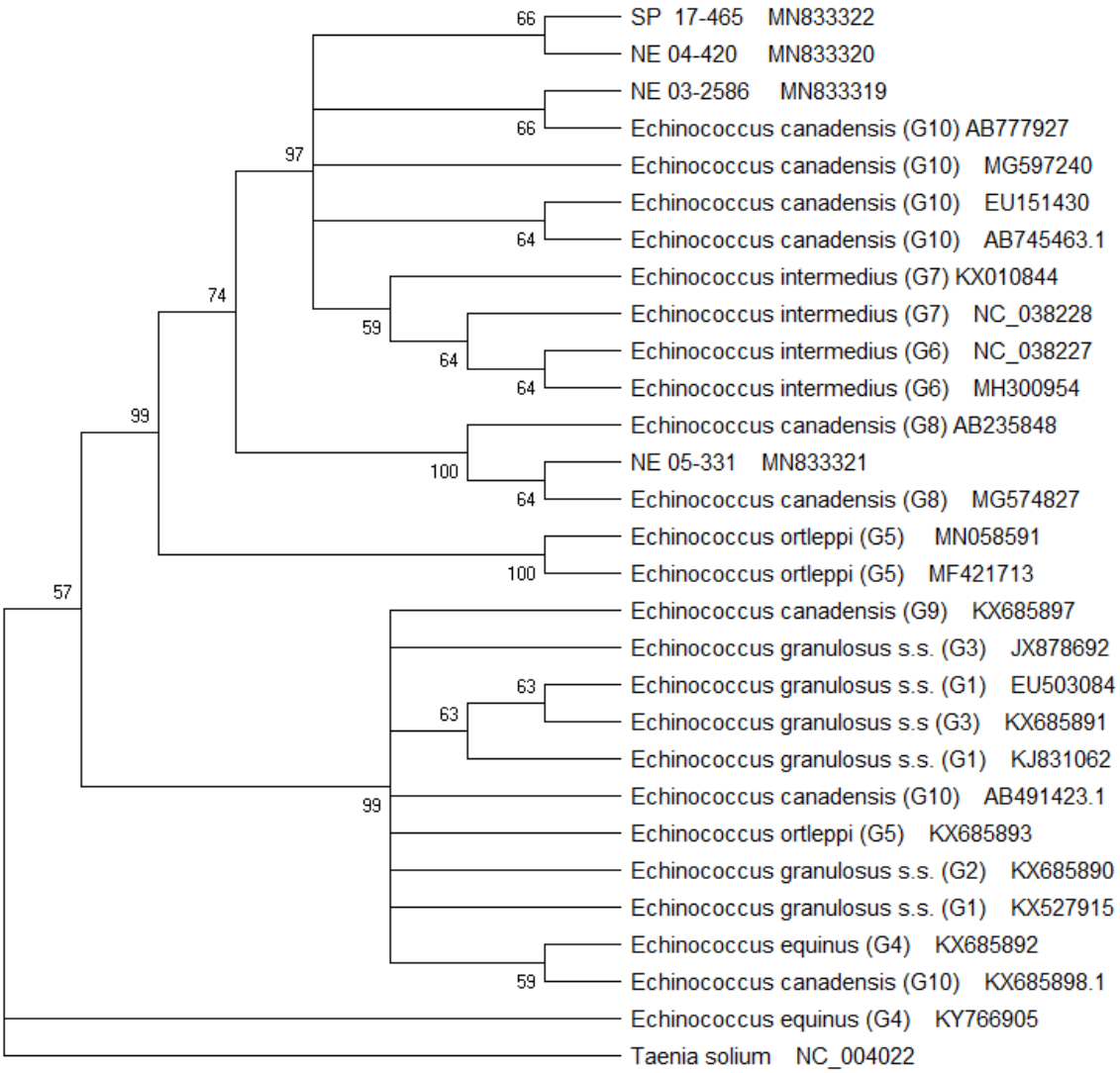

Figure 2-3. Evolutionary relationship of four *Echinococcus canadensis* isolates from elk (NE 04-420, NE 05-331, NE 03-2586, and SP 17-465) based on COX-1 sequences. Evolutionary history was inferred by the Neighbor-joining method using the program MEGA. Percentage of replicate trees in which associated taxa cluster together >50% of times in the bootstrap test displayed at nodes (1000 replications). *Taenia solium* serves as the outgroup.

CHAPTER III

Market Deception: Molecular Identification of Bushmeat Species in Northern Uganda

Disclosure

This chapter is under review for publication in *Zoonoses and Public Health*. This chapter appears within this text as submitted with minor modifications to formatting.

Abstract

Spillover of zoonotic diseases from wildlife to humans is believed to occur most often from contact with 'high risk' wildlife, such as primates, rodents, and bats in regions where bushmeat is commonly hunted, such as Asia and sub-Saharan Africa. In Northern Uganda, consumption of bats and primates is not widely culturally accepted. However, preliminary reports from hunters indicate that baboons are often hunted and sold as culturally desirable species by dealers and hunters. This deception in the market subverts the ability of community members to make informed choices about the risks involved in consuming bushmeat. We collected 229 bushmeat samples from 23 communities adjacent to Murchison Falls National Park. Reported species was recorded at point of sale for each sample. PCR targeting mammalian *cyt b* and 12s rRNA genes and sequencing were performed to identify samples to the lowest taxonomic unit using NCBI BLAST. Overall, 27.9% (61/219) of samples had disparate results between species reported and BLAST analysis. Thirty-four species were identified, with the most frequent wildlife being waterbuck (31.5%), warthog (13.7%), black rat (5.9%). These data indicate a public health risk for bushmeat consumers in Northern Uganda as they cannot assess species-related risk when purchasing bushmeat, thereby increasing potential exposure to zoonotic pathogens. This data also provides insight into regional hunter prey preference and market preference of local community members.

Background

Bushmeat harvest and consumption is a well-described practice in sub-Saharan Africa and has long been acknowledged as important in food security and nutrition, income security, and crop protection, particularly in rural communities (Fa et al. 2003; Starkey 2004; Davies et al. 2007). However, even within the framework of economic provision, the issue of bushmeat harvest presents two major concerns: the public health risk to communities through contact with zoonotic pathogens and the controversy surrounding the conservation of protected species. Since the 1970's, over 60% of emerging infectious diseases affecting human populations have been zoonotic in nature, with 71.8% of those zoonotic events resulting from contact with wildlife species (Jones et al. 2008). Within the last several decades, Uganda has been home to numerous zoonotic disease events resulting from contact with wildlife species, including anthrax, Ebola virus, Marburg disease virus, rabies virus, yellow fever, and HTLV/STLV-1 (Adjemian et al. 2011; Nabukenya et al. 2014; Kurpiers et al. 2016). Certain wildlife species have been identified as having higher inherent risk of zoonotic disease emergence, particularly bats, non-human primates (NHPs), ungulates, and rodents (Cleaveland et al. 2007).

Quantification of bushmeat harvest has been described for some sub-Saharan African countries, particularly those in West and Central Africa. Estimates for Nigeria and Cameroon (Fa et al. 2006), Ghana (Ntiamoa-Baidu 1998), Cote d'Ivoire (Caspary 1999), and the Congo Basin (Wilkie and Carpenter 1999; Fa et al. 2002) range from 12,000 tons to 4.9 million tons annually; however, few reports are available for Uganda (Olupot et al. 2009). Murchison Falls National Park in northern Uganda is the oldest and largest protected area in Uganda and is recognized for its biodiversity. Wildlife species within the park are highly susceptible to hunting since many of the park's borders are directly adjacent to local communities, increasing potential for human conflict with wildlife, as well as increasing opportunity and incentive to hunt.

In Uganda, all hunting of wildlife species is illegal except for vervet monkeys (Chlorocebus pygerythrus), olive baboons (*Papio anubis*), and bushpigs (*Potamochoerus larvatus*) (Kato and Okumu 2008; Travers et al. 2017). Hunting of these species is permitted without penalty when they are found to depredate crops on farmers' property (Lamprey 2002). Despite the legal restrictions on hunting, bushmeat harvesting is a common and an accepted practice, with meat being used for both food and as an additional source of income. During preliminary communications, hunters claimed to conduct 'species deception' at market, where species that

were culturally unacceptable to consume (like NHPs) were opportunistically hunted and disguised as culturally desirable/acceptable species, such as antelopes, warthogs, and bushrats (Willcox, Personal Communications). Due to the clandestine nature of bushmeat hunting in Uganda, there are not open markets where carcasses are displayed for purchase, but rather person-to-person transactions take place.

We hypothesized that species deception exists and occurs most frequently when baboons and vervet monkeys are legally hunted and disguised as other species. This study aimed to describe the most frequently hunted species and quantified the rates of species deception in markets to identify potential opportunities for transmission of food borne zoonoses.

Methods

Study Area

Samples were collected from 23 villages within the Nwoya district in northern Uganda (Figure 1). The Nwoya district is composed of 4 sub-counties, Purongo, Anaka, Alero, and Koch Goma, and it forms the northern border of the Murchison Falls Conservation Area (MFCA). The MFCA is Uganda's largest continuous protected area, consisting of the 3,893 km^2 Murchison Falls National Park (MNFP) to the north, the 748 km^2 Bugungu Wildlife Reserve (BWR) to the southwest, and the 720 km^2 Karuma Falls Wildlife Reserve (KFWR) to the southeast. Villages where bushmeat samples were collected are shown in Figure 1.

Sampling

Initial contact with hunters and dealers in the communities were made through Ugandan community liaisons and research associates. Bushmeat samples were purchased from hunters, dealers, and women within study communities from July to August 2016 and from June to July 2017 for the price of 10,000 Ugandan shillings (equivalent of approximately $3 USD) per sample. Species reported, condition of meat (fresh, smoked, hard-smoked), and village where purchased were recorded for each sample. Tissue was considered fresh when harvested from bushmeat and no treatment of meat was applied other than storage. Tissue was considered smoked if the meat was harvested and noted to be smoked but was soft and the internal portion was differently textured and colored. Tissue was considered hard smoked if the meat was smoked, hard to the touch, and homogenous in texture and color. Once collected, an interior section of each bushmeat tissue was

excised using a sterile scalpel blade. Samples 91 through 226 were placed immediately into RNA*later*™ Stabilization Solution (Thermo Fisher Scientific) in sterile Eppendorf conical tubes to preserve the genomic DNA and RNA due to additional funding that allowed for viral sequencing. Samples were transported to storage facilities in Gulu, Uganda and placed in a freezer (-18 °C) until transported to Makerere University, Kampala for long-term storage at -80 °C.

Molecular Techniques

DNA extraction was performed on all samples using the DNeasy® Blood & Tissue Extraction Kit (QIAGEN) according to manufacturer's instructions. The success of DNA extraction was confirmed by gel electrophoresis on 2% agarose stained with ethidium bromide. A polymerase chain reaction was performed on extracted DNA using two universal mammalian primers and cycling conditions summarized in Table 1. MTCB-F/MTCB-R universal mammalian primers targeting the mitochondrial cytochrome *b* gene were used first (Naidu et al. 2012). If this procedure was unable to provide clean sequences, L1085/H1259 universal vertebrate primers targeting the 12s rRNA gene were used (Kitano et al. 2007) instead. Gel electrophoresis was performed on all PCR products on a 2% agarose gel stained with ethidium bromide. PCR products were purified using QIAquick® PCR Purification Kit (QIAGEN) according to manufacturer's instructions. Purified PCR products were sent to Macrogen, Inc. for Sanger dideoxy chain termination sequencing. The forward and reverse chromatogram strands were aligned in Sequencher 5.46 software (GeneCodes Corporation) and the overhanging strands trimmed to create a consensus nucleotide sequence. Resultant consensus sequences were queried against the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) to identify mammalian species to the lowest possible taxonomic unit and the highest percentage identity.

Analysis

BLAST results were compared to species reported by bushmeat providers at point of sale to calculate the crude rate of mismatch within our samples. Deception was coded as 0 (no mismatch) if the molecular results matched to species level or if reported species and molecular result were within the same clade. For example, if "kob" was reported but the molecular result was waterbuck, both are antelope species and no deception was recorded. Species were coded as 1

(mismatch) if reported species results did not match to species level and were not within the same clade.

Comparison of proportions of deception among bushmeat source groups was performed in SPSS® using the Bonferroni method. These results were confirmed with a two-sided test of proportions (prtest function) using STATA®. Logistic regression was performed with Deception as the binary outcome variable and sample source, village, and molecularly identified species as predictor variables using IBM SPSS version 25.

Results

Sample Collection

Bushmeat samples (n = 229) were collected from 22 communities. Eighty-nine samples were collected in 2016 and 140 in 2017. Samples were obtained from villages within Anaka, Koch Goma, and Purongo sub-counties. One hundred twenty-seven (58%) samples were provided by hunters compared to dealers (n = 37; 16.9%) or cooks (n = 55; 25.1%). These data are shown in Table 2. Data on species reported by sample source are shown in Table 3. Thirty-eight different species were reported by bushmeat providers, with two samples reported as "unknown bushmeat species." Kob was the most frequently reported species (n = 63; 28.8%). Only seven samples were reported as vermin species, including baboon (n = 5), bushpig (n = 1), and vervet monkey (n = 1). The condition of bushmeat samples ranged from fresh to hard-smoked, with 112 (48.9%) fresh, 104 (45.4%) smoked, and 13 (5.7%) hard-smoked.

Molecular Results

Ten samples were omitted from the final analysis due to degraded tissue from excessive meat smoking, resulting in 219 viable samples. Consensus sequences ranged from 85-918 bp in length, and the results are summarized in Table 4. Thirty-four different species were identified using NCBI BLAST. One sample could only be identified to genus level. Identity of samples to first BLAST result ranged from 90% to 100%. The most frequently identified species by molecular methods was waterbuck (*Kobus ellipsiprymnus*), with 69 samples (31.5%). In total, 108 (49.3%) samples were antelope species. Only 3 samples were found to be one of the three legal species to hunt: 2 olive baboons and one bushpig. Twenty-three (10.5%) of the samples were found to be domestic species (cow, goat, and sheep).

Statistical Analysis Results

The overall rate of species deception/misrepresentation among samples was 27.9%, with 61/219 samples not matching what was reported based on sequencing. Samples acquired from hunters had the highest rate of deception among the three sources of bushmeat with 36.2% being misreported. Women and dealers did not significantly differ from each other in proportions of deception, but hunters differed significantly from both women and dealers in proportions of misrepresented samples ($p = 0.002$) (Table 2.) No predictor variables were found to be significant in the logistic regression model.

Discussion

Incorrect identification of bushmeat species intended for human consumption presents a potential public health issue because it subverts the ability of bushmeat consumers to know what they are handling and consuming. For example, most bushmeat consumers living in our study area should have little contact with primates or bats, as it is culturally unacceptable to eat these animals. However, when deception occurs, these animals may infiltrate the food supply chain. Additionally, accurate knowledge of the species purchased may lead to differences in the precautions used to prepare different meats, and, therefore, could potentially lead to increased exposure to zoonotic pathogens.

Certain species are considered to be at an inherently higher risk for cross-species transmission of zoonotic pathogens, including bats, rodents, ungulates, and non-human primates (Cleaveland et al. 2007). Unpublished research (Dell et al.) indicates that community members in Nwoya district are aware that certain species carry zoonotic pathogens and present greater risk of zoonotic disease than others; therefore, the phenomenon of species deception at market may hinder the effectiveness of targeted educational efforts of safe handling and cooking of wild meats if consumers are misled about the species they are handling. Hunting, butchering, cleaning, and cooking of meat places handlers in direct contact with tissue and fluids from wildlife where they may be exposed to zoonotic organisms. In 2017, the government of Uganda collaborated with the Global Health Security Agenda to identify seven priority zoonotic diseases: anthrax, influenza viruses, brucellosis, viral hemorrhagic fevers, plague, and rabies; each of these can be transmitted through contact with wildlife hosts (Sekamatte et al. 2018).

Over a quarter of bushmeat samples included in this study were being sold as a species that was not the true harvested species. There are several potential explanations for this trend. One explanation is that hunters and dealers may not know or remember which species was harvested at the point of sale. Increased efforts by the Uganda Wildlife Authority (UWA) to patrol for and prevent hunting activity has forced the harvest and sale of bushmeat to become increasingly clandestine (Lamprey 2002). Anecdotal evidence collected from hunters in the field suggests that some of the misrepresentation observed in this study may not be intentional deception to consumers, but rather the result of efforts to hide hunting activity while in the field. Several hunters reported that when wildlife is successfully captured, the carcasses are quickly butchered in the field in such a way that the bones may be discarded and left behind (Dell et al, unpublished). This practice is performed so that hunters are less likely to be incriminated if caught and questioned by UWA officers.

An alternative explanation for this rate of species misrepresentation is the intentional disguise of meat to match market demand and increase profit. Although guns were a prominent tool used in hunting during a report in 1984 (Oneka 1990), the domestic conflict and insurgency in Northern Uganda from the mid-1990s to 2000s fortified the ban on civilian owned fire-arms, forcing a greater dependence of hunters on non-specific hunting methods, like snares or pitfall traps. These hunting methods likely result in the capture of non-target bushmeat species for which there is poor market demand. This would in turn increase the motivation to misrepresent the species of bushmeat. There has been previous documentation of bushmeat hunters and dealers misrepresenting the type of meat to increase profit at sale (Adeyoju et al. 2010).

Our finding that bushmeat hunters have a lower proportion of correct sample identity than cooks and dealers (who had statistically similar proportions) are contrary to the findings in Bityani et al. 2012 in bushmeat from the Serengeti, which reported that samples collected from hunters had the greatest identification accuracy. This may be due to the differences in butchering practices between sites, the variation in law enforcement, and the perceived severity of consequences if caught. For example, in Tanzania, a game cropping strategy was introduced to the Serengeti that provided legal bushmeat to villages bordering the park, attempting to decrease illegal hunting activity and to allow for increased transparency in the bushmeat market (Rentsch and Damon 2013).

In addition to public health and emerging zoonoses concerns, conservation concerns surrounding the practice of unregulated bushmeat harvest include the decline or extirpation of wildlife species, which has been documented in several countries (Fa et al. 2015; Lindsey et al. 2015; Rogan et al. 2015). In northern Uganda, the illegality of firearms has also led to increased use of opportunistic harvest practices and non-specific capture methods. While this may decrease the frequency of hunting large-bodied wildlife, which are most vulnerable and often present in the fewest numbers, and documented to be preferred as prey by hunters, it presents difficulty in predicting which species may be most at risk from bushmeat-related activities (Bodmer 1995). Although bushmeat harvest may be locally sustainable in some areas, extra-local demand for bushmeat and unregulated harvest increase pressures on the wildlife populations in protected areas (Bitanyi et al. 2012). The over-exploitation of species geographically confined to protected areas not only threaten the survival of the species, but may also increase the density of infectious diseases in wildlife populations, including endemic zoonotic diseases, facilitating their emergence in human populations who come in contact with these wildlife populations (Smith et al. 2015).

Our findings are consistent with previous reports of the most commonly poached species within MFCA (Oneka 1990; Olupot et al. 2009). All but one of the species identified in this study are currently listed with the International Union for Conservation of Nature as "Not Threatened" (NT) or "Least Concern" (LC). Only one species (hippopotamus) is currently listed as vulnerable, and no species are listed as endangered or critically endangered. Molecular identification of animal tissue confiscated from apprehended poachers may serve as a useful tool to identify which species are most commonly hunted and which need the greatest investment in conservation.

There are limitations to the results found in this study. Two hundred and twenty-nine samples were obtained in the field, but ten of these samples were unable to yield readable DNA sequences. Each of these 10 samples were either "smoked" or "hard smoked" and likely had DNA of compromised and degraded quality. Additionally, the collection of bushmeat samples was not performed year-round. There may be differences among the most commonly hunted species based on seasonality. Due to restricting the sampling periods to late summer for both years, any potential differences were not identified in this study.

Four samples indicated blue wildebeest (C*onnochates taurinus*) as the first sequence match through BLAST; this species does not have a geographic range in Uganda. Identity of these matches ranged from 92% to 100%. All samples whose first BLAST result was wildebeest were

analyzed using the L1085/H1259 primer set. This primer set uses a shorter target sequence than the MTCB primers, which yielded higher success during PCR with samples that were more heavily smoked. However, the shorter target sequence may result in a less specific BLAST result and capture of closely related species. In each of the 4 cases of wildebeest BLAST result, hartebeest (*Alcelaphus* spp.) was a match result with a lower identity and cover. It is likely that these samples were hartebeest, which have a natural range in Uganda, and these four samples were not excluded from analysis.

Molecular analysis showed that 25 of our bushmeat samples were actually from domesticated animals commonly found on subsistence farms in the area. It is likely that locals provided samples of already-butchered domestic meat to community liaisons after learning through word of mouth that researchers were offering compensation for bushmeat samples. Although it is possible these samples were sold deceptively to obtain the compensation offered, we cannot exclude the possibility that the domesticated species found in this study were also being sold to community members as bushmeat. Bushmeat has been documented to be more expensive than domestic meats in market, a finding that was confirmed to be true in our study area as well (Dell et al. in preparation; Moore 2001; Loibooki et al. 2002; Rentsch and Damon 2013).

Conclusions

The findings in this paper underscore the potential risks for unknown exposure to potential zoonotic pathogens. Not only do our findings confirm the widespread bushmeat trade within sampled communities, but they also demonstrate the grossly under recognized issue of market deception to consumers of hunted wildlife. The findings in this paper may establish the need for further surveillance of bushmeat trade in areas with similar regulations and social norms. Targeted educational programs focused on safe handling and food safety practices with wild animal tissues may be indicated to reduce exposure to infected tissue and to increase the appropriate precautions taken during food handling and preparation.

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Appendix III

Tables and Figures for Chapter III

Table 3-1. Primers, PCR cycling conditions, and literature sources used for DNA extraction and PCR of bushmeat samples collected from Nwoya district, Uganda, 2016-2017.

Primer	PCR product size (bp)	Primer Sequence 5' to 3'	DNA Target	Cycling Conditions	Reference
MTCB-F	~1420	CCHCCATAAATAGGNGAAGG	cyt *b*	95°C/45 sec, 55°C/60 sec, 72°C/2 min, 35 cycles	Naidu et al. 2012
MTCB-R	~1420	WAGAAYTTCAGCTTTGG	cyt *b*	95°C/45 sec, 55°C/60 sec, 72°C/2 min, 35 cycles	Naidu et al. 2012
L1085	215	CCCAAACTGGGATTAGATACCC	12S rRNA	94°C/30 sec, 55°C/30 sec, 72°C/30 sec, 35 cycles	Kitano et al. 2007
H1259	215	GTTTGCTGAAGATGGCGGTA	12S rRNA	94°C/30 sec, 55°C/30 sec, 72°C/30 sec, 35 cycles	Kitano et al. 2007

Table 3-2**.** Sample source and accuracy of species identification given by providers of bushmeat samples obtained from Nwoya district north of Murchison Falls National Park, Uganda, 2016-2017. Subscripts denote proportions of accurately identified samples by source that do not differ significantly from each other at a 0.05 significance level using the Bonferroni method. P = 0.002.

		Number and percentage of samples provided	Number and percentage of correctly identified samples using molecular typing
Source	Hunter	127 (58%)	81 (63.8%) $_{a}$
	Women	55 (25.1%)	45 (81.8%) $_{b}$
	Dealer	37 (16.9%)	33 (89.2%) $_{b}$
Total		219 (100%)	159 (72.6%)

Table 3-3. Bushmeat species reported by hunters at time of sampling, including frequency (n), percentage (%), and accuracy of reporting of identified species among bushmeat samples obtained from Nwoya district, Uganda, 2016-2017.

Species Reported	Number (n) and Percentage (%) of Reported Species	Number (n) and Percentage (%) of Reported Species Identified Correctly by Molecular Testing
Kob	63 (28.8%)	60 (95.2%)
Warthog	32 (14.6%)	22 (68.8%)
Waterbuck	22 (10%)	17 (77.3%)
Bush rat	14 (6.4%)	10 (71.4%)
Dik dik	10 (4.6%)	7 (70.0%)
Buffalo	7 (3.2%)	2 (28.6%)
Impala	7 (3.2%)	7 (100%)
Antelope	6 (2.7%)	5 (83.3%)
Squirrel	6 (2.7%)	5 (83.3%)
Hippopotamus	6 (2.7%)	6 (100%)
Baboon	5 (2.3%)	2 (40.0%)
Bushbuck	5 (2.3%)	3 (60.0%)
Bat	4 (1.8%)	3 (75.0%)
Oribi	3 (1.4%)	0 (0%)
Wild rabbit	3 (1.4%)	1 (33.3%)
Monkey	2 (0.9%)	1 (50%)
Rat	2 (0.9%)	2 (100%)
Unknown	2 (0.9%)	0 (0%)
Cane rat	2 (0.9%)	2 (100%)
Aardvark	1 (0.5%)	1 (100%)
Acholi rat	1 (0.5%)	0 (0%)
Black & white colobus monkey	1 (0.5%)	0 (0%)
Black & white okello	1 (0.5%)	0 (0%)
Bushpig	1 (0.5%)	0 (0%)
Civet	1 (0.5%)	0 (0%)
Crested porcupine	1 (0.5%)	0 (0%)
Greater pangolin	1 (0.5%)	0 (0%)
Hartebeest	1 (0.5%)	0 (0%)
Ober rat	1 (0.5%)	1 (100%)
Patas monkey	1 (0.5%)	0 (0%)
Porcupine	1 (0.5%)	1 (100%)
Rhinoceros	1 (0.5%)	0 (0%)
Rodent	1 (0.5%)	0 (0%)
Serval	1 (0.5%)	0 (0%)
Spotted hyena	1 (0.5%)	0 (0%)

Table 3-3 Continued.

Species Reported	Number (n) and Percentage (%) of Reported Species	Number (n) and Percentage (%) of Reported Species Identified Correctly by Molecular Testing
Striped hyena	1 (0.5%)	0 (0%)
Vervet monkey	1 (0.5%)	1 (100%)
Total	219 (100.0%)	

Table 3-4. Number and percentage of total bushmeat samples that were molecularly identified to correct species compared to bushmeat species reported by hunter obtained from Nwoya district, Uganda, 2016-2017.

Scientific Name	Common Name	Number (n) and Percentage (%) of Total Bushmeat Samples Molecularly Identified to Correct Species	Number (n) and Percentage (%) of Identified Samples Correctly Reported
Kobus ellipsiprymnus	Waterbuck	69 (31.5%)	61 (88.4%)
Phacochoerus africanus	Common warthog	30 (13.7%)	24 (80.0%)
Capra hircus	Domestic goat	14 (6.4%)	0 (0%)
Rattus rattus	Black rat	13 (5.9%)	8 (61.5%)
Kobus leche	Lechwe	11 (5.0%)	11 (100.0%)
Kobus kob	Kob	9 (4.1%)	9 (100.0%)
Hippopotamus amphibius	Hippopotamus	8 (3.7%)	6 (75.0%)
Bos taurus	Domestic cow	7 (3.2%)	0 (0%)
Cricetomys gambianus	Gambian pouched rat	5 (2.3%)	5 (100.0%)
Xerus erythropus	Striped ground squirrel	5 (2.3%)	3 (60.0%)
Connochaetes taurinus	Blue wildebeest	4 (1.8%)	2 (50.0%)
Lepus microtis	African savanna hare	4 (1.8%)	1 (25.0%)
Ourebia ourebi	Oribi	4 (1.8%)	4 (100.0%)
Pelea capreolus	Grey rhebok	4 (1.8%)	4 (100%)
Chlorocebus tantalus	Tantalus monkey	3 (1.4%)	2 (66.7%)
Sylvicapra grimmia	Common duiker	3 (1.4%)	2 (66.7%)
Syncerus caffer	African buffalo	3 (1.4%)	2 (66.7%)
Arvichernanthis niloticus	African grass rat	2 (0.9%)	2 (100.0%)
Epomophorus minor	Minor epauletted fruit bat	2 (0.9%)	1 (50.0%)
Ovis aries	Domestic sheep	2 (0.9%)	0 (0%)
Papio anubis	Olive baboon	2 (0.9%)	2 (100.0%)
Tatera guinea	Guinea gerbil	2 (0.9%)	1 (50.0%)
Alcelaphus buselaphus	Hartebeest	1 (0.5%)	1 (100.0%)
Cephalophus silvicultor	Yellow-backed duiker	1 (0.5%)	1 (100.0%)
Chaerephon pumilus	Little free-tailed bat	1 (0.5%)	1 (1000%)
Epomophorus gambianus	Gambian epauletted fruit bat	1 (0.5%)	0 (0%)
Felis sylvestris	Wildcat	1 (0.5%)	0 (0%)
Hystrix cristata	Crested porcupine	1 (0.5%)	1 (100.0%)
Madoqua kirkii	Kirk's dik dik	1 (0.5%)	1 (100.0%)
Mastomys spp.	Multimammate mouse	1 (0.5%)	1 (100.0%)
Megaderma lyra	Greater false vampire bat	1 (0.5%)	1 (100.0%)
Orycteropus afer	Aardvark	1 (0.5%)	1 (100.0%)

Table 3-4 Continued.

Scientific Name	Common Name	Number (n) and Percentage (%) of Total Bushmeat Samples Molecularly Identified to Correct Species	Number (n) and Percentage (%) of Identified Samples Correctly Reported
Redunca arundinium	Southern reedbuck	1 (0.5%)	1 (100.0%)
Sus scrofa	Bushpig	1 (0.5%)	0 (0%)
Tatera leucogaster	Bushveld gerbil	1 (0.5%)	0 (0%)
Total		219 (100%)	158

Supplementary Table 3-5. NCBI BLAST first results, including cover, identity, and accession of bushmeat samples collected from Nwoya district, 2016 and 2017.

Sample Number	Query Length	Query Cover	Identity	Accession Number	First Match	Scientific Name
1	529	99%	98%	AJ314548.1	Phacochoerus africanus mitochondrial cyt-B gene for cytochrome b, isolate Pafr3	*Phacochoerus africanus*
2	472	100%	100%	AJ314548.1	Phacochoerus africanus mitochondrial cyt-B gene for cytochrome b, isolate Pafr3	*Phacochoerus africanus*
3	851	100%	98%	JN632593.1	Alcelaphus buselaphus isolate CYTO mitochondrion, complete genome	*Alcelaphus buselaphus*
4	438	100%	100%	AJ314548.1	Phacochoerus africanus mitochondrial cyt-B gene for cytochrome b, isolate Pafr3	*Phacochoerus africanus*
5	155	100%	97%	KU682700.1	Chlorocebus tantalus isolate C10 mitochondrion, complete genome	*Chlorocebus tantalus*
6	570	100%	96%	AF052939.1	Kobus kob cytochrome b (cytb) gene, mitochondrial gene encoding mitochondrial protein, complete cds	*Kobus kob*
7	557	100%	100%	AJ314548.1	Phacochoerus africanus mitochondrial cyt-B gene for cytochrome b, isolate Pafr3	*Phacochoerus africanus*
8	142	98%	99%	KJ192730.1	Xerus erythropus isolate XeryT891 12S ribosomal RNA gene, partial sequence; mitochondrial	*Xerus erythropus*
9	148	100%	98%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
10	156	100%	97%	JX446401.1	Kobus leche mitochondrion, complete genome	*Kobus leche*
11	142	100%	99%	KU682700.1	Chlorocebus tantalus isolate C10 mitochondrion, complete genome	*Chlorocebus tantalus*
12	427	100%	98%	JF728771.1	Kobus ellipsiprymnus isolate TS011 cytochrome b (cytb) gene, complete cds; mitochondrial	*Kobus ellipsiprymnus*
13	143	100%	98%	JN632684.1	Pelea capreolus isolate South mitochondrion, complete genome	*Pelea capreolus*
14	172	98%	98%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
15	788	100%	97%	AF052939.1	Kobus kob cytochrome b (cytb) gene, mitochondrial gene encoding mitochondrial protein, complete cds	*Kobus kob*
16	140	100%	99%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
17	139	100%	98%	JN632651.2	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
18	141	95%	98%	JN632651.3	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
19	138	98%	100%	JN632701.1	Sylvicapra grimmia isolate SUN mitochondrion, complete genome	*Sylvicapra grimmia*
20	757	99%	97%	AF052939.1	Kobus kob cytochrome b (cytb) gene, mitochondrial gene encoding mitochondrial protein, complete cds	*Kobus kob*

Supplementary Table 3-5 Continued.

Sample Number	Query Length	Query Cover	Identity	Accession Number	First Match	Scientific Name
21	136	100%	99%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
22	137	100%	100%	DQ409327.1	Phacochoerus africanus mitochondrion, complete genome	*Phacochoerus africanus*
23	918	100%	99%	DQ409327.1	Phacochoerus africanus mitochondrion, complete genome	*Phacochoerus africanus*
24	823	100%	97%	AF052939.1	Kobus kob cytochrome b (cytb) gene, mitochondrial gene encoding mitochondrial protein, complete cds	*Kobus kob*
25	674	100%	96%	AF052939.1	Kobus kob cytochrome b (cytb) gene, mitochondrial gene encoding mitochondrial protein, complete cds	*Kobus kob*
26	137	100%	100%	AP003425.1	Hippopotamus amphibius mitochondrial DNA, complete genome	*Hippopotamus amphibius*
27	730	99%	97%	AP003425.1	Hippopotamus amphibius mitochondrial DNA, complete genome	*Hippopotamus amphibius*
28	834	100%	99%	JN632701.1	Sylvicapra grimmia isolate SUN mitochondrion, complete genome	*Sylvicapra grimmia*
29	139	98%	99%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
30	142	100%	100%	KP681245.1	Sus scrofa breed wild boar mitochondrion, complete genome	*Sus scrofa*
31	590	99%	96%	JN632701.1	Sylvicapra grimmia isolate SUN mitochondrion, complete genome	*Sylvicapra grimmia*
32	135	100%	99%	AP003425.1	Hippopotamus amphibius mitochondrial DNA, complete genome	*Hippopotamus amphibius*
33	859	99%	97%	AF052939.1	Kobus kob cytochrome b (cytb) gene, mitochondrial gene encoding mitochondrial protein, complete cds	*Kobus kob*
34	697	100%	99%	JQ235527.1	Syncerus caffer isolate 9083 mitochondrion, complete genome	*Syncerus caffer*
36	734	99%	99%	JQ235527.1	Syncerus caffer isolate 9083 mitochondrion, complete genome	*Syncerus caffer*
38	143	100%	100%	DQ409327.1	Phacochoerus africanus mitochondrion, complete genome	*Phacochoerus africanus*
39	868	100%	100%	AJ314548.1	Phacochoerus africanus mitochondrial cyt-B gene for cytochrome b, isolate Pafr3	*Phacochoerus africanus*
40	784	100%	97%	AF052939.1	Kobus kob cytochrome b (cytb) gene, mitochondrial gene encoding mitochondrial protein, complete cds	*Kobus kob*
41	159	100%	100%	DQ409327.1	Phacochoerus africanus mitochondrion, complete genome	*Phacochoerus africanus*
42	140	100%	100%	DQ409327.1	Phacochoerus africanus mitochondrion, complete genome	*Phacochoerus africanus*

Supplementary Table 3-5 Continued.

Sample Number	Query Length	Query Cover	Identity	Accession Number	First Match	Scientific Name
43	911	100%	99%	JF728771.1	Kobus ellipsiprymnus isolate TS011 cytochrome b (cytb) gene, complete cds; mitochondrial	*Kobus ellipsiprymnus*
44	807	100%	100%	AP003425.1	Hippopotamus amphibius mitochondrial DNA, complete genome	*Hippopotamus amphibius*
45	871	100%	99%	AP003425.1	Hippopotamus amphibius mitochondrial DNA, complete genome	*Hippopotamus amphibius*
46	381	100%	92%	JF728771.1	Kobus ellipsiprymnus isolate TS011 cytochrome b (cytb) gene, complete cds; mitochondrial	*Kobus ellipsiprymnus*
47	145	100%	99%	JQ235547.1	Syncerus caffer isolate 655 mitochondrion, complete genome	*Syncerus caffer*
48	853	99%	99%	AJ314548.1	Phacochoerus africanus mitochondrial cyt-B gene for cytochrome b, isolate Pafr3	*Phacochoerus africanus*
49	860	100%	99%	JF728771.1	Kobus ellipsiprymnus isolate TS011 cytochrome b (cytb) gene, complete cds; mitochondrial	*Kobus ellipsiprymnus*
50	138	100%	100%	KU682699.1	Chlorocebus tantalus isolate C9 mitochondrion, complete genome	*Chlorocebus tantalus*
52	626	100%	99%	AP003425.1	Hippopotamus amphibius mitochondrial DNA, complete genome	*Hippopotamus amphibius*
53	827	95%	93%	JN675528.1	Rattus rattus isolate 21RrI_15 cytochrome b (cytb) gene, partial cds; mitochondrial	*Rattus rattus*
55	353	99%	95%	KT221828.1	Rattus rattus isolate Rr17 cytochrome b (cytb) gene, partial cds; mitochondrial	*Rattus rattus*
56	148	100%	95%	KJ192730.1	Xerus erythropus isolate XeryT891 12S ribosomal RNA gene, partial sequence; mitochondrial	*Xerus erythropus*
58	138	100%	100%	MF004246.1	Ovis aries isolate KarM breed Karadi mitochondrion, complete genome	*Ovis aries*
59	145	100%	99%	AJ851241.1	Tatera leucogaster mitochondrial 12S rRNA gene	*Tatera leucogaster*
61	685	100%	98%	KP229147.1	Ovis aries isolate QL27 cytochrome b (CytB) gene, complete cds; mitochondrial	*Ovis aries*
64	164	98%	99%	MF663794.1	Bos taurus voucher CDM20170726 mitochondrion, complete genome	*Bos taurus*
65	440	90%	98%	JQ410201.1	Cricetomys gambianus isolate OGBCRIC1 cytochrome b gene, partial cds; mitochondrial	*Cricetomys gambianus*
66	830	100%	99%	KY366506.1	Capra hircus cretica isolate 96Chc cytochrome b gene, partial cds; mitochondrial	*Capra hircus*

Supplementary Table 3-5 Continued.

Sample Number	Query Length	Query Cover	Identity	Accession Number	First Match	Scientific Name
67	178	97%	98%	AJ430551.1	Tatera guinea mitochondrial 12S rRNA gene	*Tatera guinea*
69	142	100%	100%	MF573068.1	Capra hircus mitochondrion, complete genome	*Capra hircus*
70	140	99%	100%	MF573068.2	Capra hircus mitochondrion, complete genome	*Capra hircus*
71	121	100%	100%	AJ430551.1	Tatera guinea mitochondrial 12S rRNA gene	*Tatera guinea*
72	140	100%	99%	KJ192475.1	Cricetomys gambianus isolate CspT1320 12S ribosomal RNA gene, partial sequence; mitochondrial	*Cricetomys gambianus*
73	176	98%	99%	MF573068.1	Capra hircus mitochondrion, complete genome	*Capra hircus*
74	761	100%	100%	KR059156.1	Capra hircus isolate 11_Ch44 haplogroup A2a mitochondrion, complete genome	*Capra hircus*
75	140	100%	98%	KJ192730.1	Xerus erythropus isolate XeryT891 12S ribosomal RNA gene, partial sequence; mitochondrial	*Xerus erythropus*
76	142	100%	98%	KJ192475.1	Cricetomys gambianus isolate CspT1320 12S ribosomal RNA gene, partial sequence; mitochondrial	*Cricetomys gambianus*
77	136	100%	98%	KJ192475.1	Cricetomys gambianus isolate CspT1320 12S ribosomal RNA gene, partial sequence; mitochondrial	*Cricetomys gambianus*
78	746	100%	100%	KY366506.1	Capra hircus cretica isolate 96Chc cytochrome b gene, partial cds; mitochondrial	*Capra hircus*
79	742	100%	95%	KF282339.1	Rattus rattus haplotype 47 cytochrome b gene, partial cds; tRNA-Thr and tRNA-Pro genes, complete sequence; and D-loop, partial sequence; mitochondrial	*Rattus rattus*
80	133	100%	99%	KX381445.1	Rattus rattus isolate M1358 12S ribosomal RNA gene, partial sequence; mitochondrial	*Rattus rattus*
81	142	100%	100%	MF573068.1	Capra hircus mitochondrion, complete genome	*Capra hircus*
82	140	100%	100%	MF573068.2	Capra hircus mitochondrion, complete genome	*Capra hircus*
83	621	99%	99%	KF282339.1	Rattus rattus haplotype 47 cytochrome b gene, partial cds; tRNA-Thr and tRNA-Pro genes, complete sequence; and D-loop, partial sequence; mitochondrial	*Rattus rattus*
84	664	100%	100%	KR059156.1	Capra hircus isolate 11_Ch44 haplogroup A2a mitochondrion, complete genome	*Capra hircus*

Supplementary Table 3-5 Continued.

Sample Number	Query Length	Query Cover	Identity	Accession Number	First Match	Scientific Name
85	573	100%	99%	KR059156.1	Capra hircus isolate 11_Ch44 haplogroup A2a mitochondrion, complete genome	*Capra hircus*
86	130	100%	100%	KX381445.1	Rattus rattus isolate M1358 12S ribosomal RNA gene, partial sequence; mitochondrial	*Rattus rattus*
87	677	100%	100%	KR059156.1	Capra hircus isolate 11_Ch44 haplogroup A2a mitochondrion, complete genome	*Capra hircus*
88	667	100%	99%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
89	164	100%	100%	MF573068.1	Capra hircus mitochondrion, complete genome	*Capra hircus*
90	151	94%	90%	KT963027.1	Epomophorus gambianus mitochondrion, complete genome	*Epomophorus gambianus*
91	138	97%	99%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
92	144	100%	99%	DQ409327.1	Phacochoerus africanus mitochondrion, complete genome	*Phacochoerus africanus*
93	135	100%	95%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
94	137	100%	92%	Y08810.1	H.amphibius mitochondrial 12S rRNA gene	*Hippopotamus amphibius*
95	143	100%	99%	JN632628.1	Connochaetes taurinus isolate SUN70 mitochondrion, complete genome	*Connochaetes taurinus*
96	131	100%	100%	JN632628.1	Connochaetes taurinus isolate SUN70 mitochondrion, complete genome	*Connochaetes taurinus*
97	138	98%	99%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
98	168	98%	91%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
99	139	100%	99%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
100	144	100%	98%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
101	154	100%	97%	AP003425.1	Hippopotamus amphibius mitochondrial DNA, complete genome	*Hippopotamus amphibius*
102	144	100%	99%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
103	152	100%	100%	AY495454.1	Chaerephon pumila 12S ribosomal RNA, tRNA-Val, and 16S ribosomal RNA genes, complete sequence; mitochondrial	*Chaerephon pumilus*
104	172	100%	100%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
105	150	100%	100%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
106	143	100%	99%	DQ409327.1	Phacochoerus africanus mitochondrion, complete genome	*Phacochoerus africanus*

Supplementary Table 3-5 Continued.

Sample Number	Query Length	Query Cover	Identity	Accession Number	First Match	Scientific Name
107	147	98%	99%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
108	147	100%	100%	DQ409327.1	Phacochoerus africanus mitochondrion, complete genome	*Phacochoerus africanus*
109	141	100%	98%	KJ192475.1	Cricetomys gambianus isolate CspT1320 12S ribosomal RNA gene, partial sequence; mitochondrial	*Cricetomys gambianus*
111	183	97%	99%	AF141282.2	Mastomys huberti 12S ribosomal RNA gene, partial sequence; mitochondrial gene for mitochondrial product	*Mastomys spp.*
112	149	100%	99%	DQ409327.1	Phacochoerus africanus mitochondrion, complete genome	*Phacochoerus africanus*
113	140	96%	100%	DQ409327.1	Phacochoerus africanus mitochondrion, complete genome	*Phacochoerus africanus*
114	148	100%	100%	KT875880.1	Epomophorus minor isolate Epomino51 12S ribosomal RNA gene, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA gene, partial sequence; mitochondrial	*Epomophorus minor*
115	141	100%	100%	KX381445.1	Rattus rattus isolate M1358 12S ribosomal RNA gene, partial sequence; mitochondrial	*Rattus rattus*
116	144	99%	97%	JX446401.1	Kobus leche mitochondrion, complete genome	*Kobus leche*
117	142	100%	100%	KX381445.1	Rattus rattus isolate M1358 12S ribosomal RNA gene, partial sequence; mitochondrial	*Rattus rattus*
118	161	96%	95%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
119	137	99%	97%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
120	145	100%	98%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
121	139	100%	96%	JX446401.1	Kobus leche mitochondrion, complete genome	*Kobus leche*
122	147	99%	98%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
123	175	96%	96%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
124	143	100%	99%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
125	144	94%	100%	U87000.1	Kobus kob 12S ribosomal RNA gene, mitochondrial gene for mitochondrial RNA, partial sequence	*Kobus kob*
126	166	96%	92%	JN632628.1	Connochaetes taurinus isolate SUN70 mitochondrion, complete genome	*Connochaetes taurinus*
127	134	100%	100%	DQ409327.1	Phacochoerus africanus mitochondrion, complete genome	*Phacochoerus africanus*

Supplementary Table 3-5 Continued.

Sample Number	Query Length	Query Cover	Identity	Accession Number	First Match	Scientific Name
128	144	99%	99%	DQ409327.1	Phacochoerus africanus mitochondrion, complete genome	*Phacochoerus africanus*
129	173	100%	97%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
130	141	95%	98%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
131	140	100%	98%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
132	128	100%	97%	JX446401.1	Kobus leche mitochondrion, complete genome	*Kobus ellipsiprymnus*
133	141	93%	97%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
134	129	100%	98%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
135	168	95%	93%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
136	147	100%	100%	DQ409327.1	Phacochoerus africanus mitochondrion, complete genome	*Phacochoerus africanus*
137	144	100%	98%	JN632684.1	Pelea capreolus isolate South mitochondrion, complete genome	*Pelea capreolus*
138	148	100%	98%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
139	175	99%	95%	JN632651.2	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
140	174	100%	98%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Epomophorus minor*
141	192	65%	96%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
142	143	100%	97%	JX446401.1	Kobus leche mitochondrion, complete genome	*Kobus leche*
143	178	96%	98%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
144	172	98%	94%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
145	178	97%	97%	JN632651.2	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
146	127	98%	92%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
147	147	99%	99%	KJ192730.1	Xerus erythropus isolate XeryT891 12S ribosomal RNA gene, partial sequence; mitochondrial	*Xerus erythropus*
148	136	100%	98%	JX446401.1	Kobus leche mitochondrion, complete genome	*Kobus leche*
149	135	100%	100%	MF663794.1	Bos taurus voucher CDM20170726 mitochondrion, complete genome	*Bos taurus*

Supplementary Table 3-5 Continued.

Sample Number	Query Length	Query Cover	Identity	Accession Number	First Match	Scientific Name
150	140	100%	91%	AF069538.1	Megaderma lyra 12S ribosomal RNA gene, complete sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA gene, complete sequence; mitochondrial genes for mitochondrial RNAs	*Megaderma lyra*
151	144	100%	99%	MF663794.1	Bos taurus voucher CDM20170726 mitochondrion, complete genome	*Bos taurus*
152	146	100%	100%	DQ409327.1	Phacochoerus africanus mitochondrion, complete genome	*Phacochoerus africanus*
153	138	100%	98%	JN632684.1	Pelea capreolus isolate South mitochondrion, complete genome	*Pelea capreolus*
154	141	100%	98%	JN632684.2	Pelea capreolus isolate South mitochondrion, complete genome	*Pelea capreolus*
155	165	99%	96%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
156	135	99%	98%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
157	137	100%	100%	JN632651.2	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
158	169	100%	97%	KJ192608.1	Orycteropus afer isolate OafeT1350 12S ribosomal RNA gene, partial sequence; mitochondrial	*Orycteropus afer*
159	175	99%	99%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
160	123	100%	100%	DQ409327.1	Phacochoerus africanus mitochondrion, complete genome	*Phacochoerus africanus*
161	142	100%	100%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
162	140	100%	100%	DQ409327.1	Phacochoerus africanus mitochondrion, complete genome	*Phacochoerus africanus*
163	132	99%	98%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
164	168	99%	96%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
165	149	100%	95%	KX002032.1	Felis silvestris 12S ribosomal RNA gene, partial sequence; mitochondrial	*Felis sylvestris*
166	127	100%	100%	MF573068.1	Capra hircus mitochondrion, complete genome	*Capra hircus*
167	143	100%	99%	DQ409327.1	Phacochoerus africanus mitochondrion, complete genome	*Phacochoerus africanus*
168	135	97%	96%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
169	138	98%	99%	JN632651.2	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
170	127	98%	98%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
171	166	96%	96%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*

Supplementary Table 3-5 Continued.

Sample Number	Query Length	Query Cover	Identity	Accession Number	First Match	Scientific Name
172	179	95%	97%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
173	134	100%	100%	DQ409327.1	Phacochoerus africanus mitochondrion, complete genome	*Phacochoerus africanus*
174	109	100%	100%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
175	162	99%	94%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
176	137	99%	99%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
177	145	100%	98%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
178	175	97%	97%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
179	386	45%	99%	DQ409327.1	Phacochoerus africanus mitochondrion, complete genome	*Phacochoerus africanus*
180	93	98%	98%	JX446401.1	Kobus leche mitochondrion, complete genome	*Kobus leche*
181	146	100%	100%	DQ409327.1	Phacochoerus africanus mitochondrion, complete genome	*Phacochoerus africanus*
182	177	96%	96%	AY093659.1	Hystrix cristata 12S ribosomal RNA gene, partial sequence; mitochondrial gene for mitochondrial product	*Hystrix cristata*
183	124	100%	100%	DQ409327.1	Phacochoerus africanus mitochondrion, complete genome	*Phacochoerus africanus*
184	149	100%	97%	JX446401.1	Kobus leche mitochondrion, complete genome	*Kobus leche*
185	141	100%	100%	DQ409327.1	Phacochoerus africanus mitochondrion, complete genome	*Phacochoerus africanus*
186	145	100%	97%	JX446401.1	Kobus leche mitochondrion, complete genome	*Kobus leche*
187	139	99%	100%	DQ409327.1	Phacochoerus africanus mitochondrion, complete genome	*Phacochoerus africanus*
188	137	100%	98%	JN632680.1	Ourebia ourebi isolate South mitochondrion, complete genome	*Ourebia ourebi*
189	147	100%	99%	JN632680.2	Ourebia ourebi isolate South mitochondrion, complete genome	*Ourebia ourebi*
190	143	100%	98%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
191	139	100%	98%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
192	139	100%	97%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
193	175	98%	96%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
194	142	99%	99%	JN632680.1	Ourebia ourebi isolate South mitochondrion, complete genome	*Ourebia ourebi*

Supplementary Table 3-5 Continued.

Sample Number	Query Length	Query Cover	Identity	Accession Number	First Match	Scientific Name
195	134	95%	98%	JN632654.1	Madoqua kirkii isolate SUN mitochondrion, complete genome	*Madoqua kirkii*
196	144	100%	99%	KJ192554.1	Lepus microtis isolate LvicT1295 12S ribosomal RNA gene, partial sequence; mitochondrial	*Lepus microtis*
197	139	100%	99%	KJ192554.1	Lepus microtis isolate LvicT1295 12S ribosomal RNA gene, partial sequence; mitochondrial	*Lepus microtis*
198	146	99%	96%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
199	128	100%	97%	JN632680.1	Ourebia ourebi isolate South mitochondrion, complete genome	*Ourebia ourebi*
200	152	100%	100%	DQ409327.1	Phacochoerus africanus mitochondrion, complete genome	*Phacochoerus africanus*
201	146	100%	99%	KJ192554.1	Lepus microtis isolate LvicT1295 12S ribosomal RNA gene, partial sequence; mitochondrial	*Lepus microtis*
202	153	100%	99%	KJ192554.1	Lepus microtis isolate LvicT1295 12S ribosomal RNA gene, partial sequence; mitochondrial	*Lepus microtis*
203	165	95%	97%	AF141259.2	Arvicanthis niloticus 12S ribosomal RNA gene, partial sequence; mitochondrial gene for mitochondrial product	*Arvivanthis niloticus*
204	173	98%	96%	AF141259.2	Arvicanthis niloticus 12S ribosomal RNA gene, partial sequence; mitochondrial gene for mitochondrial product	*Arvivanthis niloticus*
205	178	97%	99%	MF573068.1	Capra hircus mitochondrion, complete genome	*Capra hircus*
206	182	96%	99%	EU273707.1	Rattus rattus isolate RNZRrTit01 mitochondrion, complete genome	*Rattus rattus*
208	180	100%	96%	EU273707.1	Rattus rattus isolate RNZRrTit01 mitochondrion, complete genome	*Rattus rattus*
209	180	97%	99%	EU273707.1	Rattus rattus isolate RNZRrTit01 mitochondrion, complete genome	*Rattus rattus*
210	141	98%	99%	KJ192730.1	Xerus erythropus isolate XeryT891 12S ribosomal RNA gene, partial sequence; mitochondrial	*Xerus erythropus*
211	180	99%	97%	EU273707.1	Rattus rattus isolate RNZRrTit01 mitochondrion, complete genome	*Rattus rattus*
212	146	100%	98%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
213	148	100%	98%	KX381445.1	Rattus rattus isolate M1358 12S ribosomal RNA gene, partial sequence; mitochondrial	*Rattus rattus*
214	139	98%	97%	JX446401.1	Kobus leche mitochondrion, complete genome	*Kobus leche*

Supplementary Table 3-5 Continued.

Sample Number	Query Length	Query Cover	Identity	Accession Number	First Match	Scientific Name
215	136	99%	99%	U87000.1	Kobus kob 12S ribosomal RNA gene, mitochondrial gene for mitochondrial RNA, partial sequence	*Kobus kob*
216	162	100%	92%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
217	138	100%	96%	JX446401.1	Kobus leche mitochondrion, complete genome	*Kobus leche*
218	143	100%	98%	JN632694.1	Redunca arundinum isolate MBP12 mitochondrion, complete genome	*Redunca arundinium*
219	149	100%	99%	JX946196.2	Papio anubis isolate east mitochondrion, complete genome	*Papio anubis*
220	143	92%	96%	AF154262.1	Cephalophus silvicultor 12S ribosomal RNA gene, partial sequence; mitochondrial gene for mitochondrial product	*Cephalophus silvicultor*
221	135	95%	95%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
222	173	100%	96%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
223	149	100%	95%	JN632628.1	Connochaetes taurinus isolate SUN70 mitochondrion, complete genome	*Connochaetes taurinus*
224	104	100%	97%	JX946196.2	Papio anubis isolate east mitochondrion, complete genome	*Papio anubis*
225	132	94%	94%	JX446401.1	Kobus leche mitochondrion, complete genome	*Kobus leche*
226	172	98%	96%	JN632651.1	Kobus ellipsiprymnus isolate Niger mitochondrion, complete genome	*Kobus ellipsiprymnus*
227	180	98%	99%	MF663794.1	Bos taurus voucher CDM20170726 mitochondrion, complete genome	*Bos taurus*
228	173	98%	100%	MF663794.1	Bos taurus voucher CDM20170726 mitochondrion, complete genome	*Bos taurus*
229	174	99%	98%	MF663794.1	Bos taurus voucher CDM20170726 mitochondrion, complete genome	*Bos taurus*
230	181	97%	96%	MF663794.1	Bos taurus voucher CDM20170726 mitochondrion, complete genome	*Bos taurus*

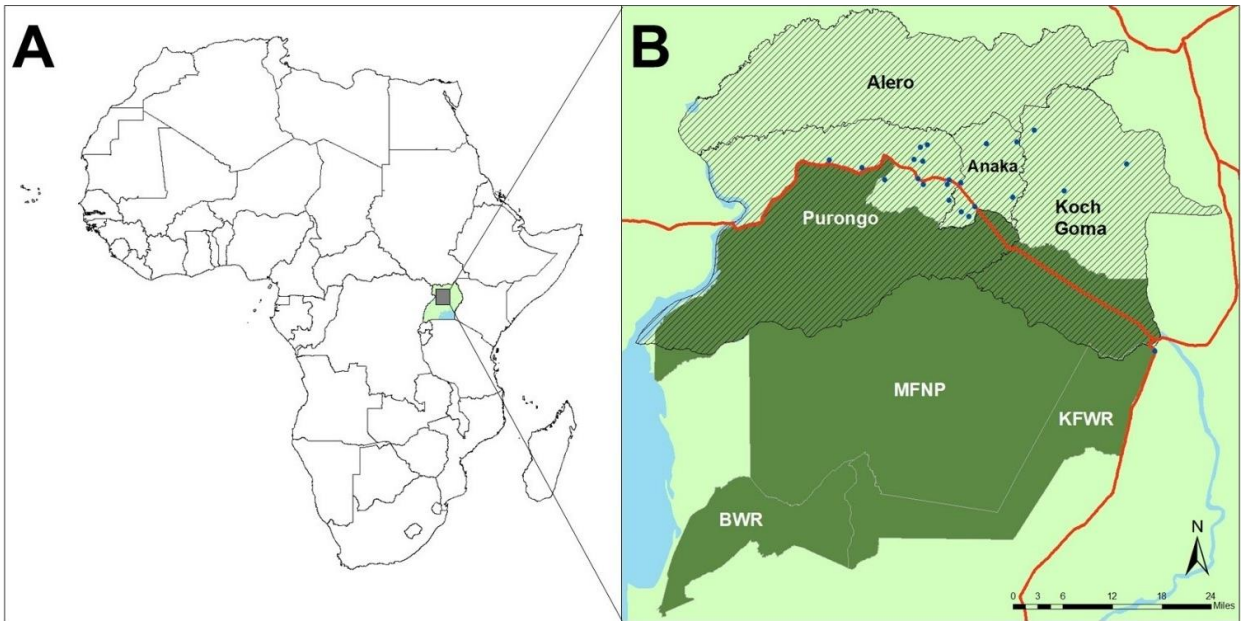

Figure 1. (A) Map of Africa showing the location of Uganda and Murchison Falls Conservation Area (MFCA), and (B) Nwoya District and its sub-counties (black hatched area) and the MFCA protected area (dark green area) Murchison Falls National Park (MFNP), Bugungu Wildlife Reserve (BWR) and Karuma Falls Wildlife Reserve (KFWR) with the major highways (red line) and sub-counties. Blue dots represent villages where samples were collected.

CHAPTER IV

Attitudes, practices and awareness of zoonoses in community members involved in the bushmeat trade near Murchison Falls National Park, northern Uganda

Disclosure

This chapter is under review for publication in *PLOS One*. This chapter appears within this text as submitted with minor modifications to formatting.

Abstract

The harvest and consumption of bushmeat is a widespread practice in the tropics and sub-tropics. Often in these communities, there is a dependence on bushmeat for both food security and basic income needs. Despite the importance of bushmeat for many households worldwide, the practice raises concern for transmission of zoonotic pathogens through hunting, food preparation, and consumption. In Uganda, harvest of wildlife is illegal, but bushmeat hunting, especially in communities bordering protected areas, is commonplace. We interviewed 292 women who cook for their households and 180 self-identified hunters from 21 villages bordering Murchison Falls National Park in northern Uganda to gain insights into bushmeat preferences, opportunity for zoonotic pathogen transmission through injury or consumption, and awareness of common wildlife-associated zoonoses. We found that both hunters and cooks considered primates to be the most likely wildlife species to carry diseases humans can catch. Among common zoonotic pathogens, the greatest proportions of cooks and hunters believed that gastrointestinal pathogens, followed by monkeypox, can be transmitted by wildlife. Neither cooks nor hunters report frequent injury during cooking, butchering, or hunting, and few report taking precautions while handling bushmeat. Three of the five most preferred meat choices reported by cooks were domestic meats, while four of five for hunters were wildlife species. The majority of cooks believe that hunters and dealers never to rarely disguise primate meat as another kind of meat in market, while the majority of hunters report that they usually disguise primate meat as another kind of meat. These data play a crucial role in our understanding of potential for exposure to and infection with zoonotic pathogens in the bushmeat trade. Expanding our knowledge of awareness, perceptions and risks enables us to identify opportunities to mitigate infections and injury risk and promote safe handling practices.

Background

The hunting and consumption of bushmeat is a widespread practice in tropical and subtropical ecosystems, often to provide food security and supplement basic income for participating households. Estimates for households dependent on bushmeat as a meat source surpass 150 million in the Global South (Nielsen, Meilby et al. 2018). In recent studies, 39% of surveyed households in 24 countries reported hunting bushmeat and 89% of that harvest was directly applied to dietary needs (Nielsen, Pouliot et al. 2017, Nielsen, Meilby et al. 2018). Additionally, bushmeat hunting tends to be most prevalent in areas with greater biodiversity indices, which frequently align with regions experiencing higher poverty and food insecurity (Adams, Aveling et al. 2004, Fisher and Christopher 2007, Cawthorn and Hoffman 2015). In Uganda alone, over 71% of households reported having participated at some point in bushmeat harvest and/or consumption (Nielsen, Pouliot et al. 2017). The widespread dependence of populations on bushmeat for nutritional and financial security raises concern for the sustainability of hunting practices for wildlife populations where bushmeat harvest is prevalent and for the risk of exposure of hunters and consumers to emerging, reemerging, and endemic zoonotic diseases during hunting, preparation, and consumption (Brashares, Arcese et al. 2004, Wolfe, Daszak et al. 2005, Kurpiers, Schulte-Herbrüggen et al. 2016).

Human contact with wildlife is a major pathway for emerging and endemic infectious diseases, with 62% of all newly emerging infectious diseases being zoonotic and over 70% of those zoonoses implicating wildlife reservoirs (Jones, Patel et al. 2008). The bushmeat trade presents numerous routes of opportunity for transmission of zoonotic pathogens, including airborne and blood-borne during hunting and the butchering of carcasses, as well as foodborne risks associated with preparation and consumption. Consumption-related risks are especially relevant in areas where there is suboptimal storage of meat in the consumer chain, allowing proliferation of bacterial pathogens (Paulsen, Nagy et al. 2008, Bachand, Ravel et al. 2012, Kuukyi, Amfo-Otu et al. 2014). Moreover, information about the effects of hunting and associated diseases remain limited largely due to poor healthcare access and reporting in many regions where bushmeat hunting and consumption is common. Recent epidemics have instilled zoonotic diseases into the global consciousness following large-scale and highly publicized outbreaks such as the 2015 and ongoing Ebola virus epidemics and the recent COVID-19 pandemic; each of these infectious agents originated from contact with wildlife species (Pigott, Golding et al. 2014, Saéz, Weiss et al. 2015,

Ahmad, Khan et al. 2020, Kannan, Ali et al. 2020, Rothan and Byrareddy 2020). Less highly publicized, but arguably more pervasive in many local communities is the presence of endemic zoonotic bacterial pathogens in hunted wildlife such as *Shigella, Campylobacter, Listeria, Pseudomonas, Staphylococcus, Salmonella*, *Shigella, E. coli,* and *Brucella* among others (Kayode and Kolawole 2008, Alexander, Blackburn et al. 2012, Bachand, Ravel et al. 2012, Kagambèga, Lienemann et al. 2013, Awaiwanont, Pongsopawijit et al. 2014, Chaber and Cunningham 2016, Kurpiers, Schulte-Herbrüggen et al. 2016). Diarrheal and other foodborne illnesses are still a significant cause of mortality, disability, and economic loss in many countries (Käferstein, Motarjemi et al. 1997, Donovan, Bailey et al. 2003, von Witzke, Kirschke et al. 2005).

An additional concern is that pathogens from hunted wildlife may also be brought into contact with domestic animal species. African swine fever, avian influenza, rabies, anthrax, tuberculosis, brucellosis, and Rift Valley Fever are among some of the most well-studied diseases that can be transmitted from wildlife to livestock with contact. These infections result in poor animal health outcomes, resulting in negative impacts to farmer livelihoods, and may continue to circulate between livestock and wildlife through these animals' contact networks (Craft 2015, Wiethoelter, Beltrán-Alcrudo et al. 2015, Kukielka, Jori et al. 2016). Many of these multi-host animal pathogens may also spillover from livestock to cause sporadic cases or outbreaks of disease in humans (Alexander, Blackburn et al. 2012, Kanouté, Gragnon et al. 2017, Muturi, Gachohi et al. 2018, Mwakapeje, Høgset et al. 2018). Risk for human cases of these diseases may increase substantially in subsistence farm settings, where extensive contact with domestic animals and handling of animal products occurs daily.

Despite increasing interest in wildlife-acquired zoonoses, much of the information we have on the prevalence and practice of bushmeat in communities comes from geographically-limited surveys of hunters and small-scale studies reporting market observations, which give limited insight to the bushmeat markets in other communities, even within the same region or country (Taylor, Scharlemann et al. 2015). Bushmeat serves as a vital resource in many rural lower-income regions of sub-Saharan Africa, but more research on the prevalence and drivers of the bushmeat trade has been conducted in West Africa and Central Africa than in East Africa. Estimates attribute nearly 90% of consumed animal protein in West and Central Africa to bushmeat, with daily wild meat consumption ranging from 0.008kg/day in Libreville, Gabon to up to 0.22kg/day in Campo, Cameroon (Ntiamoa-Baidu 1997, Pearce 2005, Nasi, Brown et al. 2008). The widespread

dependence of households on bushmeat is generally accepted as fact but only sporadically documented, with data particularly lacking in East Africa. Because the cultural, legal, and sociopolitical differences among communities engaged in bushmeat trade are distinct, there are gaps in our understanding of what drives the bushmeat trade. This limitation reduces our ability to understand how to effectively mitigate the associated risks of bushmeat hunting and consumption.

In Uganda, hunting of all wildlife species by citizens is illegal and a punishable offence under the Uganda Wildlife Act of 2000 (Uganda 2000). There is exception to this if a vermin species depredates crops on private land, in which case the animal can be disposed under the permission and supervision of the Uganda Wildlife Authority (UWA) (Naughton-Treves 1999, Saj, Sicotte et al. 2001, Schroth, Fonseca et al. 2004, Tweheyo, Hill et al. 2005, Olupot, McNeilage et al. 2009, Hill, Webber et al. 2017). There are currently three recognized vermin species: bushpigs (*Potamochoerus larvatus*), vervet monkeys (*Chlorocebus pygerythrus*), and olive baboons (*Papio anubis*) (Uganda 2000). Despite legal restrictions on hunting wildlife, bushmeat harvest is widespread and culturally accepted (Moreto and Lemieux 2015, Pomeroy, Tushabe et al. 2017). The illegal nature of the practice has resulted in a covert market with person-to-person exchanges rather than open markets supporting consumer choice. Furthermore, in initial communications with Ugandan collaborators on this project, the concept of "species deception" in market emerged, in which bushmeat is sold to consumers by either hunters or dealers as a different species than the true species. Dell et al. (in review) demonstrated nearly 30% of bushmeat sold in these same communities are misrepresented as another species of bushmeat. This practice adds an additional degree of risk to the bushmeat chain, as certain species of wildlife, such as primates, bats, and rodents, are more often implicated as reservoirs for zoonotic diseases of consequence than species like warthog or antelope, which are more culturally desirable to consume and lower risk animals for zoonotic spillover events (Han, Kramer et al. 2016, Olival, Hosseini et al. 2017).

In this paper, we present bushmeat hunting and handling survey data collected from hunters and cooks in 21 communities adjacent to protected areas in northern Uganda. Cooks and hunters were chosen as they represent the population subsets in greatest contact with bushmeat and most in control of implementing practices that might minimize exposure to zoonotic pathogens. Our research objectives for this study were to elucidate drivers of participation in the bushmeat trade by hunters and cooks, gain insight into hunting practices in our study area, and to establish an understanding of the level of local knowledge of zoonotic disease risk from participation in these

activities. These data serve as an important resource to begin to understand this ubiquitous, but clandestine, practice and to inform policy and community engagement to prevent both emerging and endemic zoonotic illnesses in these communities. Furthermore, insights gained from these data should be used to empower local community members, district leaders and public health stakeholders to increase safety measures that prevent and reduce the incidence of zoonotic infections resulting from contact with bushmeat.

Methods

Study area

The Murchison Falls Conservation Area (MFCA) is Uganda's largest and oldest continuous protected area and its most visited national park, comprised of the 3,893 km² Murchison Falls National Park (MNFP) to the north, the 748 km² Bugungu Wildlife Reserve to the southwest, and the 720 km² Karuma Falls Wildlife Reserve to the southeast. The park was initially founded in 1926 as a game reserve to preserve the savannah, forests, and Murchison Falls, a major tourist attraction for its high flow rate and beauty, and then gazetted as a national park in 1952 following the National Parks Act (Authority 2020). The existing protected area sits at the northern terminus of the Albertine Rift and is notable for its high biodiversity of both mammalian and avian species (Plumptre, Davenport et al. 2007). The MFCA is managed and operated by the Uganda Wildlife Authority and is used primarily for conservation and ecotourism. MFNP is the second most visited national park in the country with 75,360 visitors (30.7% of all national park visits) reported by the Ministry of Tourism, Wildlife, and Antiquities in 2016. Of these visitors, 29,868 are non-residents and foreigners. Estimated revenue from entrance to all Ugandan protected areas and related recreational activities for UWA is UGX 92,628,231,456 (Antiquities 2018). Revenue sharing at 20% of tourism to MFNP resulted in disbursement of UGX 8,421,310,000 (USD 2,285,945.79) to the surrounding communities for livelihood projects "geared towards management of human wildlife conflicts, livelihood improvement, and common good in the frontline parishes" from 2012-2018 and UGX 10,290,101,500 (USD 2,793,225.07) total since 2005 (Antiquities 2018). Projects funded by revenue sharing in bordering MFNP have included classroom block construction and school staff accommodation, health unit construction, sanitation projects, and livestock-based income-generating activities (such as goat, poultry, and rabbit rearing and bee-keeping) (Manyindo and Makumbi 2005).

Human population density in the areas surrounding MFNP has increased from an estimated 18 individuals/km² in 1959 to 111 individuals/km² reported on the 2014 census (Hartter, Dowhaniuk et al. 2016). Our study was conducted in villages in Nwoya district in northern Uganda. Nwoya district is composed of 4 sub-counties, Purongo, Anaka, Alero, and Koch Goma, and forms the northernmost border of Murchison Falls Conservation Area (MFCA) The population of Nwoya district in the 2014 census was 133,506, with a projected population in 2019 of 214,200 (Statistics 2016). Nwoya district reports a population density of 23 individuals/km² and an average household size of 5 individuals (Statistics 2016). A map of the study area can be seen in Figure 4-1.

Survey design

Our survey was constructed in cooperation with our partners at Makerere University and our governmental partner, the private secretary in charge of veterinary affairs in the State House of Uganda. The survey instrument was designed to gain insight to the attitudes, practices, perceived risk, and preferences surrounding the bushmeat trade in the greater MFNP region so that appropriate educational and disease prevention measures could be implemented with increased efficacy. The survey contained questions about meat preference, perceived risk of injury and disease during activities involving bushmeat, knowledge of zoonotic diseases, availability of species in market, and demographic information. Questions were presented in a variety of formats, including multiple choice, ordered response, free response, and battery-type statements with Likert-type response choices.

We constructed the survey in English and translated it into Acholi. The Acholi survey was then back-translated to ensure clarity and understanding of survey items. We pilot tested the hunter survey instrument using cognitive interviews with three Acholi-speaking hunters and two Ugandan veterinary professionals (Dillman, Smyth et al. 2014). We pilot tested the female cook survey instrument using a group cognitive interview of seven female Acholi-speaking community members and separate cognitive interviews with three Ugandan academic colleagues to ensure questions were appropriate and easily understood. If a question contained language that was not easily understood or conveyed a meaning that was not intended, the question was rewritten and rechecked with pilot group members before being deployed in the field. All survey materials and research procedures were approved by the University of Tennessee's Office of Research and Engagement's Institutional Review Board (protocol number UTK IRB-16-03109-XM & UTK IRB

16-3158-XM) and the Uganda National Council for Science and Technology (research registration number HS 3013). Site-specific permissions were secured through oral consent by local leaders. Local field staff obtained oral informed consent for voluntary individual participation. The iSurvey iPad application (Harvestyourdata 2016 & 2017) was used to administer the questionnaire in the field and store response data locally on the tablets and then uploaded to the program's data cloud each evening.

On-site interviews

Hunter interviews were conducted over a two-week period in July 2016 in 10 villages in Nwoya district with individuals who self-identified as having hunted wildlife in MFNP. We selected villages based on their proximity and accessibility to MFNP and expected participation in the bushmeat trade as identified by our local collaborators. Initial hunter respondents in each village were identified by our community liaisons. The liaisons for this research period were two men who were local community members with a demonstrated history of involvement in scientific research with collaborators at Makerere University, fluency in Acholi, and knowledge and familiarity with local hunters and bushmeat markets. We obtained subsequent interviews through word-of-mouth among hunters and through a snowball sampling technique in which initial respondents recruited other hunters (Sadler, Lee et al. 2010). This method was utilized since illegal hunting is a sensitive topic with potential to carry penalties to those involved if participants were implicated. Moreover, this method is used routinely in studies focused on populations that may be difficult to identify (Bernard and Bernard 2013). Respondents were assured anonymity and all respondents participated voluntarily and were not incentivized to participate in this study with gifts or monetary payment.

Interviews with female cooks were conducted over a 3-week period in July 2017 in 21 villages and communities in Nwoya district. The same 10 villages as in 2016, as well as additional sub-communities of the original villages in which women worked, were sampled. We attempted to interview every woman involved in household food preparation in each village included in the study area. One to four days before interviewing in a village, our community liaison traveled to that village to describe our study to women living in the community and arrange a time at which interested cooks could gather for interviews. Interviews were conducted one-on-one in Acholi, except in instances when participants were uncomfortable responding to questionnaires alone. In

these cases, groups of two to three women would be asked questionnaire items in proximity and each individual participant's response would be recorded separately. In this case, printed paper questionnaires were used to record responses from each respondent and later entered into iSurvey by researchers. All paper survey results were entered manually the same day interviews were conducted and checked for data entry errors. As with hunter surveys, all participants participated voluntarily and were not incentivized to participate in the study with gifts or monetary payment.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics 25. We used descriptive statistics to summarize survey data. Comparisons of proportions between hunters and cooks were assessed with z-tests (Bonferroni correction). Statistical significance was concluded at $P \leq 0.05$ for all tests. Constructs of hunters' and cooks' perceived risk of zoonotic diseases through contact with bushmeat were assessed using principal components factor analysis with a Varimax rotation (Hartel, Carlton et al. 2015). Factors were extracted based on Eigenvalues greater than 1 and confirmed via Monte-Carlo parallel analysis (Kaiser 1991, Hayton, Allen et al. 2004, Watkins 2006, Matsunaga 2010). Threshold for retention of variables in final analysis was 0.5. Variables below this were removed and the factor analysis was re-run. Cronbach's α was used to assess the final extracted factor reliability (Santos 1999).

Results

Descriptive statistics and socio-demographics

Demographic information for cooks and hunters is summarized in Table 4-1. We interviewed 180 self-identified hunters in 10 communities adjacent to MFNP. Hunters were generally younger adults ($\bar{x}$ ± SD; 33 years±10.9), ranging from 18 years to 74 years old. The majority of hunters reported having lived in the community since birth (n=110; 60.8%). Most hunters reported primary school as their highest level of education (n=137; 76.1%), and most were married (n=158; 87.8%). An overwhelming majority of hunters reported their primacy occupation as farmer (n=167; 92.8%), while only three respondents (1.7%) identified their primary occupation as hunter.

We interviewed 292 women who cook for their households from 21 communities. The mean age of cooks was 37 (±14.2) years, ranging from 18 years to 81 years old. Unlike hunters,

most cooks did not live in the community since birth, with only 22 (7.5%) of respondents being born in the respective study villages. Cooks' mean length of time spent living in the community was 13 (±14.1) years, ranging from one year to 70 years. The majority of cooks reported primary school as their highest level of education (n=175; 59.9%), and most were married (n=193; 66.1%). The most common primary occupation among cooks was farmer (n=222; 76.0%); however, more than one primary occupation was reported by twenty-six respondents (8.9%).

Hunting techniques and practices

Hunters indicated that the African buffalo (*Syncerus caffer caffer*) is the most dangerous wild animal to hunt (44.2%, n=80) and the most dangerous to trap (48.6%, n=88). Hunters used spears to hunt more than once per week (1.40 ±1.06), and dogs (2.0±1.5), wire snares (3.1±2.7), and sticks/clubs (2.4±1.9) less frequently, where 1=nearly every day, 2= at least 3 times per week, 3=once a week, 4=several times per month, 5=several times per year, and 6=never. When asked about the safety of hunting techniques, hunters perceived bow hunting as the most dangerous hunting technique (3.4±1.0), followed by trapping (2.6±1.0), spear hunting (2.5±0.9), and hunting with dogs (2.5±0.8), where 1= very safe, 2=safe, 3=neither safe nor dangerous, 4= dangerous and 5=very dangerous. Hunters reported being wounded most frequently during butchering (3.1±1.1), followed by trapping (2.2±1.1), spear hunting (1.9±1.0), then hunting with dogs (1.8±1.1), where1= never, 2= rarely, 3=sometimes, 4=frequently, 5= every time. Fifty-eight percent (n=105) of hunters reported having harvested, hunted, or trapped baboons or monkeys (69.1%, n=125) and bats (63.5%, n=115). Only 5% (n=9) of hunters reported taking any kind of safety precaution when hunting, trapping, or handling bushmeat. The most frequently reported precaution taken was to "leave bones in bush" (n=4). One respondent described wearing plastic bags on his hands as gloves.

Food preparation practices

A greater proportion of cooks reported taking precautions when preparing domestic meats (n=79; 27.1%) compared to when preparing bushmeat (n=68; 23.3%). Most cooks reported sometimes being wounded while preparing or cooking meat (n=163; 55.8%), then rarely (n=67; 22.6%), never (n=45; 15.4%), frequently (n=16; 5.5%), and usually (n=1; 0.3%). The mean number of adults cooked for on a daily basis was 3.6 (SD ±2.2), ranging from one to 16 adults per

single respondent; mean number of children cooked for on a daily basis was 4.9 (SD ±3.3), ranging from one to 40 children per single respondent.

Meat preference and market value

Meat preference data are displayed in Figure 4-3. Overall, hunters preferred the taste of bushmeat over domestic meats. However, on an animal-by-animal basis, hunters reported that the most delicious animal was domestic chicken (n=31, 17.2%), followed by antelope and warthog (each n=28, 15.6%), hippopotamus (n=22, 12.2%), and goat and edible bush rat (each n=21, 11.7%). Antelope was the most frequently reported most delicious wild meat when only wild meat options were listed (n=49, 27.2%,). Most (n=95, 52.8%) hunters preferred to eat meat from either wildlife or domestic species overall compared to either fish (n=27, 15.0%) or beans/vegetables (n=58, 32.2%).

Generally, cooks preferred the taste of domestic meats to bushmeat. Chicken (n=116; 38.5%) was ranked the most delicious meat, followed by goat (n=89; 29.6 %), beef (n=53; 17.6%), warthog (n=9; 3.3%), and pork (n=9; 3.3%). Cooks also selected domestic meat choices as the most nutritious, indicating chicken (n=146; 48.5%), goat (n=77; 25.6%), and beef (n=33; 11%) as the most nutritious meats. Cooks identified bushmeat (4.05±0.9) as being more expensive in market than domestic meat choices (3.0±1.006), where 1= very cheap, 2= cheap, 3=neither cheap nor expensive, 4 expensive, and 5=very expensive. The majority of cooks reported that they "never" knowingly consumed baboons (n=270; 90%), monkey species (n=271; 90%), chimpanzees (n=279; 92.7%), or bats (n=279; 92.7%).

Disease knowledge/food safety

When queried about knowledge of major diseases being carried and spread to humans by wildlife, hunter responses were varied. Stomachache and other diarrheal illnesses were most acknowledged for their zoonotic potential at 74.6% (n=135) followed by 62.2% (n=112) for monkeypox. Marburg virus (35.9%; n=65) and brucellosis (40.3%; n=73) had the least zoonotic potential awareness. Cook responses to this question were similar to hunters', with the most awareness for stomachache and diarrheal illness (69.5%; n=203) and monkeypox (67.1%; n=196) and the least for Marburg virus (26.4%; n=77). Cook and hunter response proportions differed significantly from each other for Marburg virus, monkeypox, brucellosis, and scabies, but not for

Ebola virus or stomachache and diarrheal illness. These data are summarized in Figure 4-2. Furthermore, hunters indicated that they believed wildlife were most likely to carry diseases livestock could catch (3.6±1.2), followed by people (3.5±1.2), and least likely to carry disease that hunting dogs could catch (3.4±1.3), where 1=very unlikely, 2= unlikely, 3=neither unlikely nor likely, 4= likely, and 5=very likely.

Cooks considered domestic meat consumption (cow, pig, chicken, goat) overall safer (3.1±0.8) than bushmeat species (2.6±0.1), where 1=very dangerous, 2= dangerous, 3=neither safe nor dangerous, 4= safe, 5= very safe. Baboons (3.5±0.9), chimpanzees (3.5±0.9), goat (3.4±0.9), monkeys (3.4±0.9), pigs (3.3±0.9), and bats (3.3±0.9) were perceived by cooks to be the most likely to make a person sick when consumed, where 1=very unlikely, 2=unlikely, 3=neither unlikely nor likely, 4= likely, and 5= very likely. Cooks identified edible bush rats as the least likely meat to make people sick when consumed (2.3±1.0), followed by beans and vegetables (2.3±1.0 and chicken (2.5±1.0). The perceived likelihoods that wildlife carried diseases that hunting dogs (3.5±0.9) or domestic livestock (3.5±0.9) could catch were comparable. Cutting and butchering meat during food preparation and active hunting were considered to carry the greatest risk of disease from wildlife (3.4±0.9) and (3.3±0.9) respectively, compared to trapping methods (3.1±1,0). Cooking was perceived to carry notably less risk of disease than these activities (2.5±1.0). All above questions were scaled 1=very unlikely, 2=likely, 3=neither unlikely nor likely, 4=likely, 5=very likely.

Species deception in market

Species deception data for hunter and cooks are summarized in Figure 4-4. A notable majority of hunters (n=156; 86.2%) report that they “usually” disguise primate meat as some other kind of meat in market. Furthermore, 95% (n=172) of hunters report that dealers “usually” disguise primate meat as some other kind of meat in market. Cooks responded most frequently that they believed bushmeat hunters disguised primate (baboon, monkey, chimpanzee) as some other kind of meat to sell to never occur (n=151; 50.2%), with virtually no cooks (n=2; 0.7%) believing that it usually occurs. When asked how often market sellers or dealers disguise primate meat as some other kind of meat to sell, the majority of cooks again reported that this never happened (n=255; 84.7%) and only one cook reported that they believed it usually occurs (n=1; 0.3%); moreover, most cooks believe that baboons (n=24l; 79.7%), monkeys (n=250; 83.1%), chimpanzees (n=264;

97.7%), and bats (n=278: 92.4%) are "never" available in market to purchase. Independent t-tests confirm a significant difference in mean responses between cooks and hunters for both questions about hunters ($t_{437.8}$ = -35.3, $p < 0.001$) and dealers ($t_{392.0}$ = -63.3) disguising primate meat as another kind.

Perceived disease risk from bushmeat taxa

Principal components factor analysis results for the question "how likely it is that each wildlife species carry disease that humans can catch?" are summarized in Table 4-2. Both cooks' and hunters' responses grouped into 3 variables for these. Each animal was rated on a scale of 1-5 according to 1= very unlikely, 2=unlikely, 3=neither unlikely nor likely, 4= likely, and 5= very likely; a lower number represents a perception of lower risk of contracting a zoonoses from that species/group. For cooks, primates (monkeys, baboons, chimpanzees) grouped together with the highest means (group $\bar{x}$ = 3.7), all domesticated animals eaten for meat grouped with the next highest means (group $\bar{x}$ =3.4), and non-bat, non-primate wildlife grouped together for the lowest means (group $\bar{x}$ =3.1). Bats did not fit into any of the factor reduction groupings for cooks ($\bar{x}$ = 3.4). For hunters, primates and bats grouped together (group $\bar{x}$ = 3.80), non-bat, non-primate wildlife species grouped together ($\bar{x}$ =2.1), all domesticated animals (group $\bar{x}$ = 2.4), and edible bush rat did not group into any other factor ($\bar{x}$ =1.6). Based on our threshold value of 0.5, porcupine was removed from the variable list for both hunters and cooks in the final analysis. Edible bush rats also fell below our threshold value for hunters and was removed from the final analysis.

Discussion

The findings of this study emphasize important areas of concern for public health and conservation measures from the bushmeat trade in northern Uganda. Most of our respondents in both hunter and cook surveys reported their primary occupation as farming, which is consistent with other studies in sub-Saharan Africa where hunting is seen as supplemental to agricultural activities rather than a primary occupation (Wilkie, Curran et al. 1998, Marfo, Anchirinah et al. 2002, Odonkor, Gbogbo et al. 2007, Subramanian 2012, Alexander, McNamara et al. 2015). Bushmeat hunting is thought to be primarily done as a source of supplemental income or to ensure household food security. Interviews of UWA law enforcement officers in Queen Elizabeth National Park corroborate the need for bushmeat for both personal consumption and generation of

basic income, citing poverty and lack of economic opportunity as the main reasons for poaching (Moreto and Lemieux 2015). Still, our findings indicate that preference for wild animal meat may play a role in bushmeat utilization, as four of the five top preferred meats by hunters were wild animals rather than domestic choices (Wilkie, Starkey et al. 2005, Schenck, Effa et al. 2006, Mwakatobe, Røskaft et al. 2012). This finding is not mirrored by the reported preferences of cooks, who generally preferred domestic meat options and believed domestic meat choices to be more nutritious than bushmeat, which may indicate that male household members may have more influence over household food choices.

Based on responses to our questions about diseases that wildlife carry, almost all respondents were aware that there is a real and present risk of disease spillover from wildlife to people. Epidemics in recent years may contribute to this knowledge, but for hunters this awareness does not appear to influence or motivate any precautionary behaviors during the harvest of wildlife as virtually no respondents reported taking precautions. Rather, the precautions that were reported were related to the potential for legal or financial repercussions if caught by authorities for poaching. The most reported precaution was "butchering in the field" and "leaving the bones behind" to minimize evidence of poaching. Similar to studies in Central Africa, these responses suggest that risk of illness or injury from bushmeat hunting does not outweigh the incentive of financial profit from the sale or use value of the harvested bushmeat (Monroe and Willcox 2006).

Previous research has shown that there is nearly a 30% discrepancy between what species bushmeat is being sold as by hunters and dealers and what species are actually being sold in Uganda (Dell, in review). The data in this paper substantiate that this deception may be intentional by hunters in many cases. Most hunters interviewed reported that they usually disguised primate meat as another species and that they knew dealers of bushmeat would often do the same; however, cooks' responses to the same question indicate they do not believe that this deception occurs. Although only disguising primates was asked about in our surveys, data form Dell et al. reveal that this intentional deception is not restricted to species that are taboo to consume and includes the disguise of species that were most preferred in this study as other kinds of bushmeat. This incongruity is potentially harmful because it subverts the ability of bushmeat consumers to make informed choices about their diets. Moreover, the way that cooks responded to the question about diseases humans can catch from wildlife indicates that there is awareness that certain species carry more inherent risk for zoonoses transmission than others. If we assume that this translates to

differences in precautionary practices in food preparation and handling, then consumers may be inadvertently exposing themselves and others consuming the meals to zoonotic pathogens due to this misrepresentation. Most cooks we interviewed noted that they did not eat bats and primates; this should thereby confer a degree of 'cultural immunity'. The phenomenon of market deception and hunters admitting to eating bats and primates in the bush may challenge the degree that preference and choice protect community members from exposure to zoonotic pathogens carried by species with a high risk of spillover.

Hunters have arguably the greatest amount of contact with animal tissue through the process of hunting itself. Even with snares and traps, the risk of injury during these events is high, particularly if the animals are not found dead when the traps are checked, and the wounded animal must be killed at close range. Inhalation of aerosolized particles on fur or urine of wildlife, inadvertent fecal-oral transmission when handling the carcass, bloodborne transmission during the killing and butchering process, as well as the potential for transmission through saliva via a bite during the kill all pose serious threats to the health of hunters (LeBreton, Prosser et al. 2006). Although the majority of hunters did not report frequently being injured during hunting, trapping, and butchering, multiple hunters did admit to butchering wildlife carcasses hastily in the field to leave behind the bones which may reasonably lead to increased incidence of injury. Injury remains a common experience as part of bushmeat harvest, with incidence of injury to bushmeat hunters in a community in western Uganda at over 13% and nearly 60% of those injured seeking medical care for their injuries (Paige, Frost et al. 2014). Hunting using firearms may reduce contact with live animals if hunters are accurate shots, however, civilian-owned firearms in Uganda are strictly regulated through fire-arm certificates and stringently enforced. We did not ask about hunting with firearms on the advice of our colleagues in Uganda. The sensitive nature of this subject led us to believe that self-reporting of use would be inaccurate or discourage study participation. Although it is not reported in our study, hunting with firearms is common in other areas of sub-Saharan Africa (Batumike, Imani et al. , Holmern, Mkama et al. 2006, Alexander, McNamara et al. 2015, Ávila, Tagg et al. 2019).

Hunters most reported trapping using wire neck- or leg-hold snares. This and the other non-selective hunting measures most frequently reported in our study are consistent with commonly used methods across the tropics and subtropics for their relative ease of use, but pose a particular threat to wildlife (Noss 1998, Noss 1998). Non-selective hunting methods result in substantial

bycatch of non-target species which leads to decomposition or scavenging, may disproportionately impact threatened species, and may result in intentional wasting if traps are inconveniently located to hunters or if less profitable species are snared (Noss 1998, Newing 2001, Ripple, Abernethy et al. 2016). This practice poses a threat to the sustainability of wildlife populations, particularly wildlife populations in border zones of these protected areas where human populations are dense and access to protected areas is convenient (ref). In our study, wasting due to capture of non-target species may be less an of issue since hunters reported bringing back meat that was already butchered in the field and is presumably more likely to be passed off as more in-demand meats or meats that will fetch a higher market price (Dell, in review).

In both hunter and cook groups, primates were considered to present a higher risk of zoonotic disease transmission than other species. For hunters, bats grouped with primates as the highest-risk species. Cooks responses grouped primates together as the highest-risk species, but bats did not group with them and had a lower mean response. This difference may be explained by the fact that many women married into the community and may have come from nearby mountainous regions where bats are more often consumed and are not considered a high-risk animal for disease spillover (Dell and Willcox Personal Communications). During interviews, both cooks and hunters indicated that in the more mountainous regions nearby, larger bat species are commonly consumed, whereas in Nwoya district, most did not report that they considered bats edible or a preferred species (Dell and Willcox Personal Communications). Cooks considered domesticated animals, rather than wildlife, to have the next greatest zoonotic risk, where hunters considered what broadly grouped as other wildlife to have the next greatest zoonotic risk. Veterinary outreach efforts to promote vaccination and domestic animal health in Nwoya district historically tended to target the women in the household, as livestock rearing and farming is typically their responsibility (Dell Personal Communications). This increased awareness of domestic animal health and disease may contribute to cooks' responses, indicating that educational campaigns may be an effective strategy for mitigating food-related infections.

Few cooks reported taking special precautions when preparing either bushmeat or domestic meat. Moreover, a greater proportion of cooks reported taking precautions when handling domestic meat than bushmeat. This is consistent with the belief that domestic species are more likely to cause disease in people than most wildlife. Cooks responses indicated that although most of them have a level of concern about diseases from bushmeat at the time of purchase, that concern

decreases during cooking/preparation, and decreases even further at the time of consumption. This finding either speaks to confidence in appropriate food safety technique or is an example of awareness of an abstract issue, like emerging zoonotic diseases, that has little relevance to them on a practical and day to day level.

The complexity of the issue of bushmeat presents challenges to efforts to adapt data about the practice into useful and practical intervention strategies. Engaging our target population involves communicating that the risk of zoonotic disease spillover and threats to conservation are both relevant and of consequence to them specifically. Even if this is achieved, evidence to support awareness and concern as adequate motivation to elicit behavioral changes, especially when these changes are impractical or costly, is not well supported (McCaffrey 2004, Monroe and Willcox 2006). Further data suggest that intervention strategies that depend on informal societal mores and local-level institutions may have greater buy-in than governmental level regulations (Ostrom, Burger et al. 1999, Colding and Folke 2001).

It is important to consider that hunting in our study area remains an illicit activity and the threat of discovery or implication of participation in poaching may have deterred participation of both hunters and cooks. The illegality of hunting may have also biased responses of those who participated in the study, leading to underestimations of participation. Additionally, questions about disguising meat as another kind may have bias in responses, as cooks acknowledging that this occurs directly implicates members or their communities in deceptive behavior. Similarly, responses by cooks about preferred meat choices may underrepresent a preference for bushmeat, due to issues surrounding its legality.

Conclusions

We have provided important insights into awareness of zoonoses and occupational injury for community members involved in the bushmeat commodity chain, as well as patterns of meat preference and market availability of bushmeat in villages bordering MFCA. These data clarify points in the bushmeat commodity chain, namely butchering, trapping, and contact with incorrectly specified bushmeat tissue, where cooks and hunters are most susceptible to injury and exposure to infectious agents. More detailed evaluations of subjective cultural characteristics of this community, such as beliefs, attitudes, and social norms of the community as a whole rather than hunters and cooks alone, will help in understanding determinants, practices, and preferences in the

bushmeat trade. This will ultimately lead to the development of more successful and appropriate conservation tactics for wildlife species in MFNP. Furthermore, increasing community engagement and advancing community understanding of the interplay between wildlife species and their own health may inform approaches by public health entities that ultimately increase the communities perceived control of mitigating their own disease risk.

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Appendix IV

Tables and Figures for Chapter IV

Table 4-1. Demographic information of interviewed cooks and hunters from communities in Nwoya District, Uganda 2016-2017.

Hunters (n=180)		Cooks (n=292)	
Age ($\bar{x}$±SD)	33.0 ± 11.0	**Age ($\bar{x}$±SD)**	37.3 ± 14.4
Marital Status		**Marital Status**	
Married	158 (87.3%)	Married	199 (66.1%)
Divorced	7 (3.9%)	Divorced	23 (7.6%)
Widowed	2 (1.1%)	Widowed	58 (19.3%)
Never married	14 (7.7%)	Never married	21 (7%)
Education Level		**Education Level**	
Technical/trade school	1 (0.6%)	Technical/trade school	4 (1.3%)
Secondary school	38 (21.0%)	Secondary school	36 (12.0%)
Primary school	138 (76.2%)	Primary school	183 (60.8%)
College or university	4 (2.2%)	Informal/no schooling	78 (25.9%)
Years Lived in Community		**Years Lived Community**	
1-5 years	37 (20.6%)	1-5 years	103 (35.3%)
6-10 years	20 (11.1%)	6-10 years	85 (29.1%)
11-20 years	13 (7.2%)	11-20 years	42 (14.4%)
21+ years	109 (60.6%)	21+ years	62 (21.2%)
Primary Occupation		**Primary Occupation**	
Farmer	167 (92.8%)	Farmer	220 (75.3%)
Businessman	3 (1.7%)	Vendor	28 (9.6%)
Hunter	3 (1.7%)	Businesswoman	14 (4.8%)
Motorcycle taxi	3 (1.7%)	Food service worker	8 (2.7%)
Quarry worker	1 (0.6%)	No occupation	7 (2.4%)
Mechanic	1 (0.6%)	Tailor	5 (1.7%)
Teacher	1 (0.6%)	Hairdresser	5 (1.7%)
Surveyor	1 (0.6%)	Hotel owner	2 (0.7%)
		Childcare giver	1 (0.3%)
		Teacher	1 (0.3%)
		Savings group chair	1 (0.3%)

Table 4-2. Principal components analysis with Varimax rotation of cook and hunter perceptions of zoonotic disease risk from various wildlife species, Uganda 2016-2017. Bolded figures represent the highest factor loadings and the meaningful groups created from these loadings.

Hunters (n=180)						Cooks (n=292)					
Animal type	$\bar{x}$	SE	Primates & Bats	Other wildlife	Domestic animals	Animal type	$\bar{x}$	SE	Primates	Other wildlife	Domestic animals
Baboon or monkey	3.99	0.092	**0.819**	0.139	0.063	Monkey	3.59	0.050	**0.778**	0.167	0.099
Bat	3.61	0.094	**0.732**	0.056	0.003	Baboon	3.75	0.042	**0.876**	0.100	0.156
Antelopes	1.68	0.081	-0.095	**0.732**	0.237	Chimpanzee	3.79	0.040	**0.909**	0.106	0.093
Buffaloes	2.33	0.105	0.076	**0.781**	0.076	Antelope	2.86	0.054	-0.010	**0.613**	0.295
Warthog or bushpig	2.54	0.105	0.291	**0.683**	0.068	Buffalo	3.17	0.053	0.162	**0.712**	0.160
Hippo	1.80	0.088	0.095	**0.769**	0.101	Bushpig	3.27	0.054	0.152	**0.865**	0.107
Cow	2.87	0.102	0.142	0.136	**0.773**	Warthog	3.17	0.055	0.178	**0.845**	0.0646
Chicken	2.12	0.102	0.003	-0.006	**0.716**	Edible bushrat	2.82	0.055	0.045	**0.586**	0.317
Goat	2.31	0.103	-0.056	0.288	**0.689**	Cow	3.62	0.050	0.328	0.129	**0.598**
						Chicken	3.32	0.059	-0.030	0.142	**0.734**
						Pig	3.63	0.045	0.119	0.184	**0.760**
						Goat	3.16	0.056	0.133	0.257	**0.751**
Eigenvalues			1.136	2.896	1.338	Eigenvalues			1.823	4.414	1.369
Variance explained (%)			12.62	32.17	14.87	Variance explained (%)			15.19	36.78	11.41
Cronbach's α			0.444	0.749	0.583	Cronbach's α			0.842	0.816	0.739

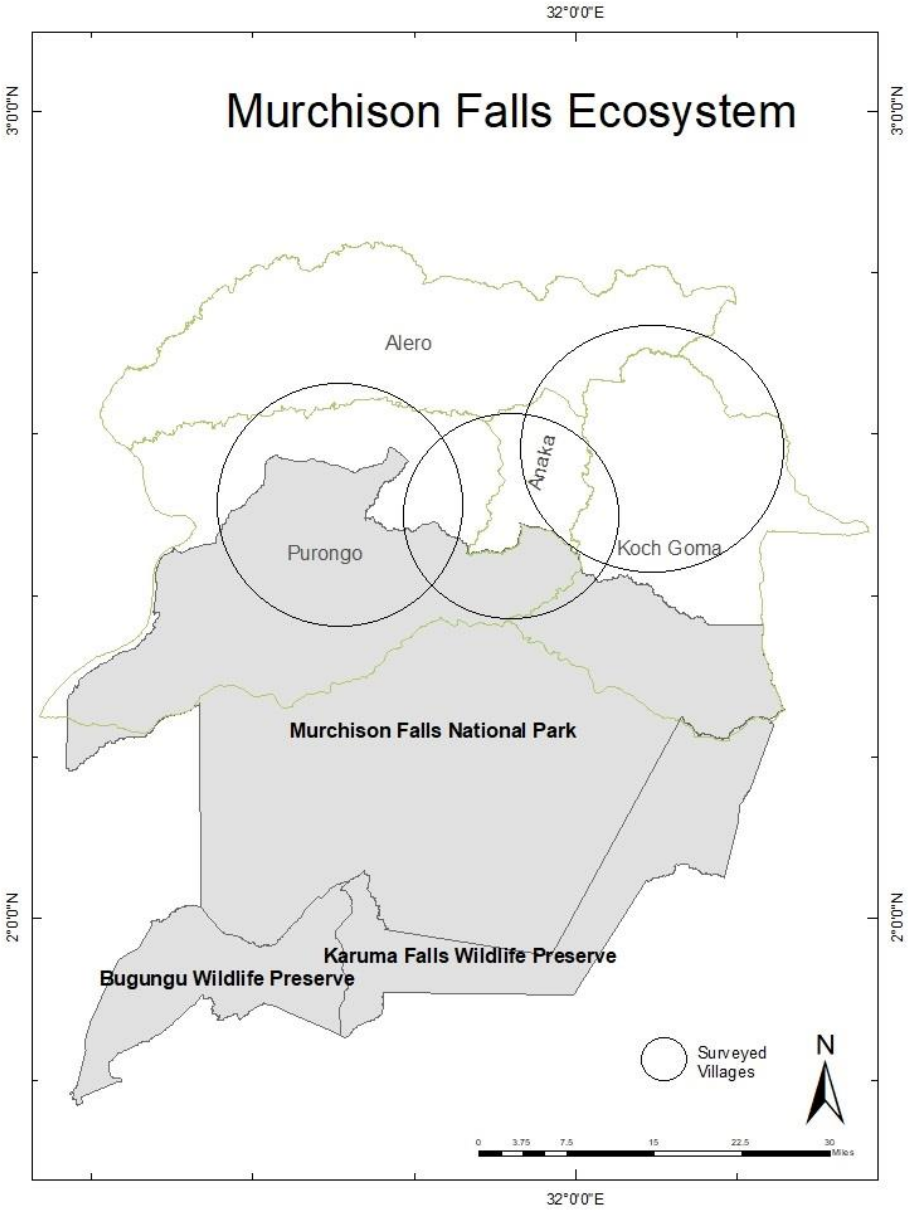
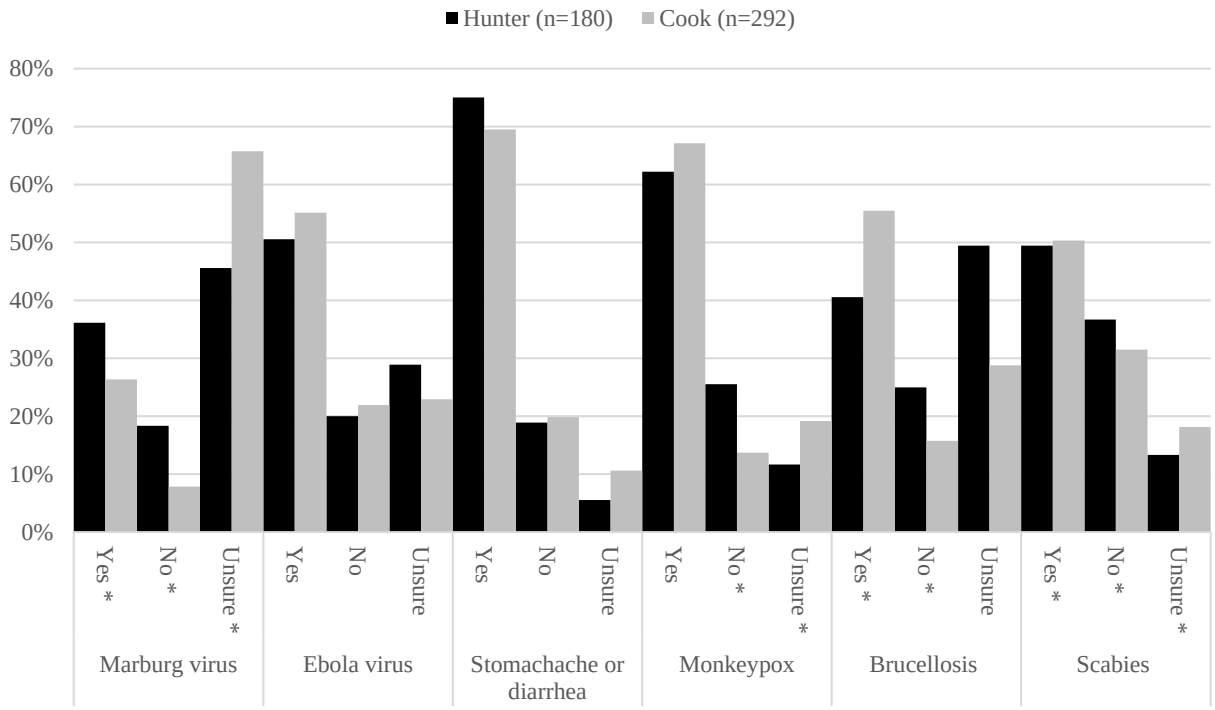


Figure 4-1. Map of the Murchison Falls Conservation Area (Bugungu Wildlife Preserve, Karuma Falls Wildlife Preserve, and Murchison Falls National Park) and the northern adjacent district, Nwoya. Nwoya district boundaries are delineated by the green borders and divided into its four subdistricts (Koch Goma, Anaka, Alero, and Purongo). Black circles indicate general undisclosed locations where interviews were conducted with hunters and cooks, 2016-2017.



a. Proportions of cooks and hunter participants sharing for response categories denoted by * differ significantly from each other at $P \leq 0.05$. Likelihood ratio

Figure 4-2. Cook and hunter responses to whether they believe wildlife species can carry select zoonotic diseases, Nwoya district, Uganda, 2016-2017.

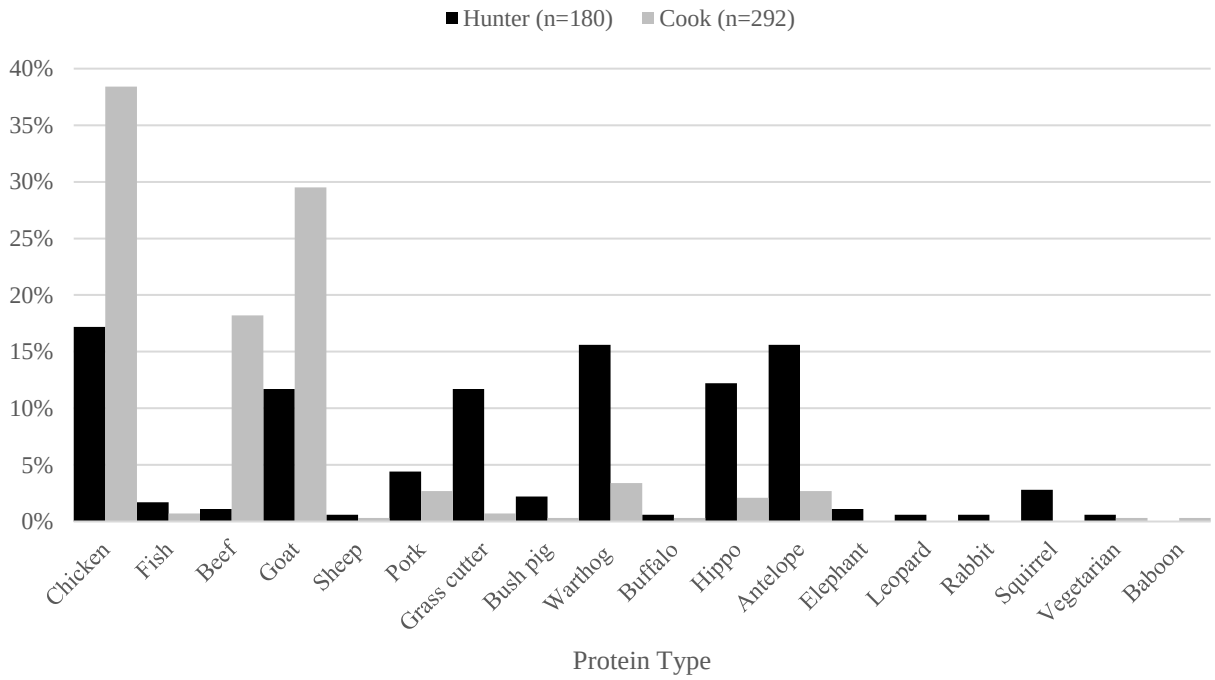


Figure 4-3. Cook and hunter responses to which type of meat they most prefer to eat from among wild and domestic choices in Nwoya district, Uganda, 2016-2017.

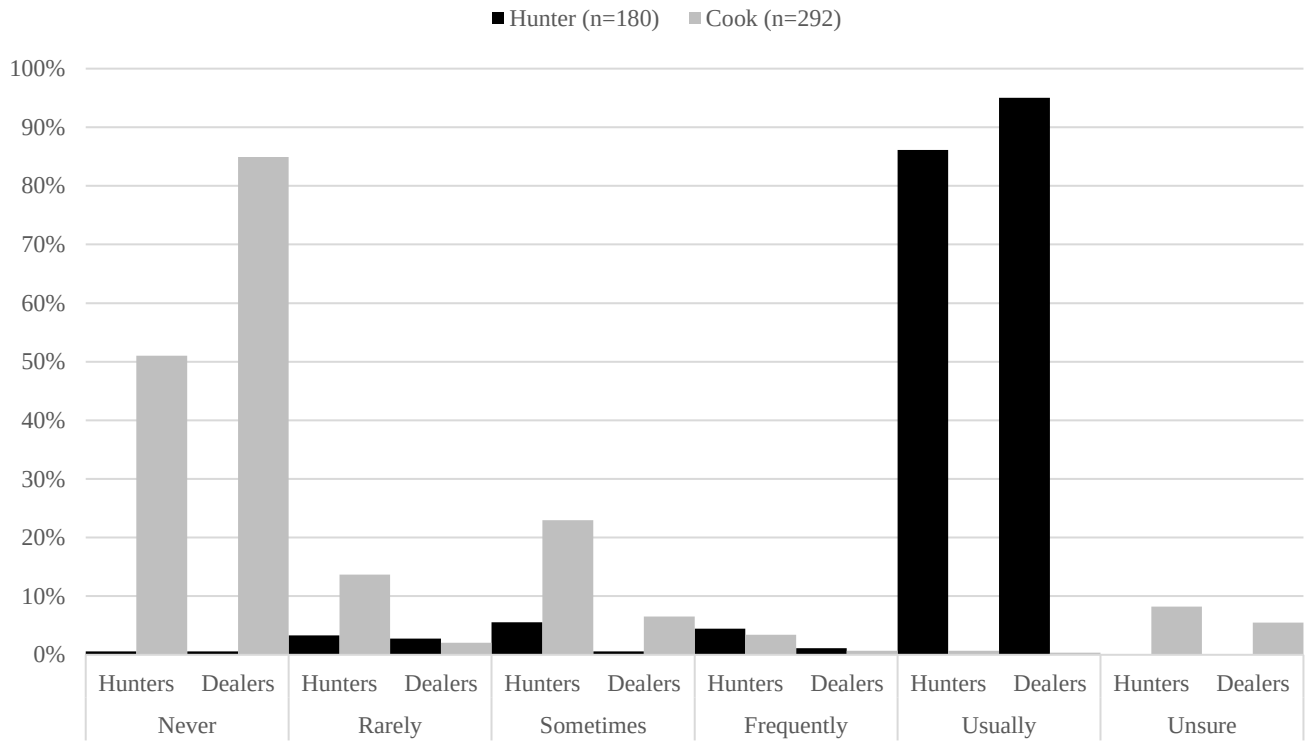


Figure 4-4. Cook and hunter responses to how often hunters and dealers disguise primate meat as another kind of meat to sell in Nwoya district, Uganda, 2016- 2017. Independent t-tests show a significant difference in mean responses between cooks and hunters for both questions about how frequently hunters disguise primate meat ($t_{437.8} = -35.3$, $p < 0.001$) and how frequently dealers disguise primate meat ($t_{392.0} = -63.3$).

CHAPTER V

Bacterial Microbial Diversity in Bushmeat from Murchison Falls Conservation Area

Abstract

The reliance of many rural communities bordering protected areas on bushmeat for nutrition and income is widespread, but bushmeat hunting, handling, and consumption carries high risk for zoonotic pathogen exposures. Emerging infectious disease epidemics resulting from contact with wildlife are increasing in frequency and pose a notable public health threat to individuals and the greater global population. In this study, we examined the microbiological composition of 137 bushmeat samples obtained from communities adjacent to Murchison Falls Conservation Area in Uganda. These samples represented 25 mammalian species in variable tissue conditions. Seventy-nine samples were analyzed using Sanger dideoxy chain termination sequencing targeting the conserved 16s rRNA gene. Fifty-eight samples were analyzed by 16s rRNA amplicon sequencing to evaluate the bushmeat microbiome composition. Sanger sequencing identified 22 genera representing 5 phyla and 14 families. *Proteus*, *Clostridium*, and *Macrococcus* were most frequently identified. The 16s rRNA amplicon sequencing identified over 35,000 unique operational taxonomic units (OTUs) within our samples, with dominant phyla including Firmicutes, Proteobacteria, and Bacteroidetes. No significant differences in alpha or beta diversity were noted for tissue condition or wildlife species group and both alpha and beta diversity were high among groups. Bacterial signatures of multiple USA Select Agents and human pathogens of consequence were detected within the samples. Our findings suggest that a combination of environmental contamination, endogenous infection, and meat spoilage contribute to bacterial microbiome composition of bushmeat and underscore the need to better understand factors influencing both bacterial composition of bushmeat and opportunities for exposure to these microbes. These findings provide useful data to inform food safety and injury prevention tactics needed to reduce bushmeat-associated disease emergence, both on the local and global scale.

Introduction

The term bushmeat refers to the tissues, typically muscle meat and organs, harvested from wildlife and represents a substantial source of protein in many countries. In Uganda, hunting and harvest of wildlife species is illegal except in select cases of species designated vermin species and carried out under the supervision of the Uganda Wildlife Authority [1]. Designated vermin species include bush pigs (*Potamochoerus larvatus*), vervet monkeys (*Chlorocebus pygerythrus),* and baboons (*Papio anubis*). Despite legal restrictions on hunting, it is a commonplace activity in many communities and especially in communities bordering protected areas where access to wildlife is readily accessible [2]. For many areas, including communities around Murchison Falls Conservation Area (MFCA) in northern Uganda, bushmeat represents a significant source of nutrition and household income [3]. Estimates for bushmeat utilization in Uganda are sparse compared to estimates from nearby regions, but over 71% of surveyed households reported consuming bushmeat at some point in time [4-7]. Quantification of bushmeat harvest for consumption is upwards of 2,200,000,000 total kg/yr, and 64.3kg/yr per person in the Congo Basin; in the Serengeti ecosystem of Tanzania, consumption of 2-5 bushmeat meals per household each week is estimated [8].

Due to the illicit nature of bushmeat harvest, bushmeat around MFCA is primarily obtained and sold on a person-to-person basis, either through middleman dealers to consumers or directly from hunter to consumer, rather than in open markets that are more common in western and central Africa. Bushmeat is either sold as fresh tissue or has been processed by smoking to preserve the meat. Although many bushmeat transactions occur locally and consumers should be familiar with the nature of bushmeat products, discrepancies exist between what bushmeat is being sold as to consumers and what the actual species of meat being is sold (Chapter III). Moreover, many self-identified hunters in the region report intentionally disguising less desirable species (like primates or bats) as other species during these transactions (Chapter IV).

Over 60% of newly emerging infectious diseases are zoonotic, and of those nearly 75% originated from human contact with wildlife [9]. Concern for zoonotic spillover events for novel and documented human pathogens should be high, particularly in the wake of the Ebola epidemics of the past decade and the 2019-2020 coronavirus pandemic, both of which emerged contact with wildlife [10-14]. Research suggests that most of the bushmeat harvested in the northern Uganda region remains locally consumed and for immediate dietary needs [15]; however, with increasing

population shifts to cities, extra-local demand for bushmeat has risen in urban centers [16]. Lack of precautions taken during the hunting, butchering, and food preparation put those involved in the bushmeat commodity chain at great risk of exposure to bacterial and viral pathogens through multiple exposure routes [17]. Furthermore, as the bushmeat commodity chain expands its geographic reach, concerns for transboundary spread of zoonotic pathogens raises concern for local and global public health risk. The social, economic, and public health impacts of unmitigated spread of zoonotic pathogens have been made evident with the increasing frequency of both bacterial and viral contemporary zoonotic epidemics [18-21].

The handling and consumption of bushmeat poses both individual and global health risks. In this study, we analyzed the bacterial microbial diversity of bushmeat samples acquired from markets in communities bordering the Murchison Falls Conservation Area, Uganda. These data serve to better understand the distribution of bacterial communities in bushmeat and gain insight into what factors contribute to the presence of high consequence bacterial exposure. These findings will help to predict patterns by which pathogens may infect people and under which conditions they are likely to emerge, as well as inform effective and practical preventive health measures.

Methods

Bushmeat Tissue Acquisition

Bushmeat tissue samples were obtained from 23 villages and trading centers within the Nwoya district in northern Uganda (Fig. 5-1). The Nwoya district is composed of 4 sub-counties, Purongo, Anaka, Alero, and Koch Goma, and forms the northern border of the Murchison Falls Conservation Area (MFCA). The MFCA is Uganda's largest continuous protected area, comprised of the 3,893 km^2 Murchison Falls National Park (MNFP) in the north, the 720 km^2 Karuma Falls Wildlife Reserve (KFWR) in the southeast, and the 748 km^2 Bugungu Wildlife Reserve (BWR) in the southwest.

Initial contact with hunters and dealers at sampling sites was facilitated through Ugandan community liaisons and research associates. Bushmeat samples were purchased from hunters, dealers, and women who cook within study sites from July to August 2016 and from June to July 2017 for the price of UGX 10,000 per sample. Species reported and condition of meat (fresh or smoked) were recorded at time of acquisition. Tissue was considered fresh when still raw, uncured, and no treatment was applied other than storage. Tissue was considered smoked if the meat was dried and processed by smoking. No other methods of preservation were observed. Once collected,

approximately 25-50 grams of the sample were placed in sterile Eppendorf conical tubes and placed on ice packs. Samples were transported to a temporary storage freezer (-18˚C) for the duration of fieldwork then transported to Makerere University for long-term storage in a -80˚C freezer. A subset of samples (n = 136) were immediately submerged into RNA*later*™ Stabilization Solution (Thermo Fisher Scientific) in sterile Eppendorf conical tubes at the time of acquisition to preserve the genomic DNA and RNA due to additional funding that allowed for 16s amplicon sequencing.

This study and all methods were approved by the University of Tennessee's Office of Research and Engagement's Institutional Review Board (protocol number UTK IRB-16-03109-XM & UTK IRB 16-3158-XM) and the Uganda National Council for Science and Technology (research registration number HS 3013). Oral consent and approval was obtained from all local leaders and decision-makers in communities included in this study.

Nucleic Acid Extraction

Two small internal sections of tissue weighing approximately 100 mg each were removed with a sterile disposable scalpel blade for each bushmeat sample. Nucleic acid extraction for bacterial sequencing was performed on all samples using the DNeasy® Blood & Tissue Extraction Kit (QIAGEN) according to manufacturer's instructions with minor modifications. For 136 samples obtained in 2017, homogenization was performed with the Omni International Bead Ruptor 12 bead mill homogenizer for 45 seconds at 6m/s in 2ml Hard Tissue Homogenizing Mix Nuclease & Microbial DNA Free pre-filled bead tubes. Adequacy of extracted DNA was confirmed with the Thermofisher Qubit 3 bioanalyzer.

Sanger Sequencing

PCR was performed on extracted DNA using the universal 16s rRNA primers Bact 16SF (5' CTACGGGGGGCAGCAG) and Bact 16SR (3' GGACTACCGGGGTATTT). PCR cycling conditions consisted of a single cycle of 95˚C for 30s, followed by 25 cycles of 95˚C for 30s, 55˚C for 30s, and 72˚C for 15s, followed by a final extension step at 72˚C for 7 min [22]. PCR was confirmed by gel electrophoresis of all PCR products on a 2% agarose gel stained with ethidium bromide. PCR products were purified using QIAquick® PCR Purification Kit (QIAGEN) according to manufacturer's instructions. Purified PCR products were sent to Macrogen, Inc.

(Seoul, South Korea) for Sanger sequencing. Raw sequencing data were returned in .fasta format. The forward and reverse strands were aligned using Sequencher 5.46 software (GeneCodes Corporation) to create a consensus nucleotide sequence. Overhanging ends of the forward and reverse strands were trimmed from the consensus sequence. Resultant consensus nucleotide sequences were queried against the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) to identify bacterial species to the lowest possible taxonomic unit (genus). Proportions of taxa present were evaluated by sample condition and wildlife species group by z-tests with Bonferroni correction. Statistical significance was assumed at $p<0.05$.

Microbiome 16S rRNA Amplicon Sequencing

For samples for which an alignment was not possible or that, once aligned, chromatograms did not contain clear nucleotide peaks, purified gDNA was sent to Macrogen, Inc. (Seoul, South Korea) for 16s V3-V4 hypervariable region amplicon library construction. Samples were shipped on dry ice. The V3-V4 hypervariable region of the 16s rRNA gene was amplified using the universal 16s rRNA paired primers 341F-V3 (5'CCTACGGGNGGCWGCAG) and 805R-V4 (3'GACTACHVGGGTATCTAATCC). Sequencing was run on the Illumina MiSeq platform with the Herculase II Fusion DNA Plymerase Nextera XT Index Kit v2 according to the Macrogen MiSeq protocol, with no modifications to the protocol noted. Adapter sequences were removed using programs Scythe (v0.994) and sequence reads shorter in length than 36bp were filtered to produce clean data output [23]. The accuracy of each nucleotide was reported by Phred Quality Score in final report. Resulting 16s microbiome libraries were delivered in .fastq format.

16s Microbiota Composition Bioinformatics and Statistical Analysis

Resultant bacterial microbiome libraries were processed using the open-source platform mothur following the MiSeq protocol and queried against a curated subset of the SILVA V138 database [24]. An oligo file was created to locate our specific primer set within the V3-V4 region and to trim the SILVA database to our region [25, 26]. Contigs were formed for all sequences and resultant sequences were screened to remove chimeras, excessively long homopolymers, and fragments overhanging the alignment. Operational taxonomic units (OTUs) were assigned to consensus sequences for each sample based on the trimmed SILVA reference database. OTUS

comprising less than 0.0001% relative abundance among samples were removed from subsequent analysis.

Alpha diversity was compared among samples using R packages “vegan” and “phyloseq” in R Studio [27-29]. Shannon, Simpson and Inverse Simpson diversity indices were calculated based on an average bootstrap at 1000 iterations. An average bootstrap of at 1000 iterations was also used to calculate beta-diversity matrices of Bray-Curtis dissimilarity.

Statistical analyses were performed in R Studio using the package vegan [28]. Kruskal-Wallis rank sums were performed based on Shapiro-Wilks tests of normality to test differences in Shannon diversity indices and Simpson diversity indices for the following comparisons: bushmeat condition (fresh or smoked) and bushmeat sample species (primate, rodent, antelope, warthog, or other wildlife). Adonis tests with 999 permutations were performed to test for effects of the same groups on beta diversity based on Bray-Curtis dissimilarity values. Beta dispersion was quantified by calculating multidimensional areas of minimum convex polygons fit to clusters of sample condition and wildlife species group using the ‘betadisper’ function in vegan. These areas were based on distances created from NMDS of previously calculated Bray-Curtis dissimilarity values. PERMANOVAs were used to test statistical differences in beta dispersion between sample condition and among wildlife species groups.

Percent OTU relative abundance by phylum was calculated and graphed between sample condition and among wildlife species group using “phyloseq” [27]. Significant indicator taxa, taxa representative of other taxa and environmental conditions of the microbial community for samples or groups, were identified for sample condition and wildlife species group using the “indicspecies” package in R [30]. All tests performed in this study for statistical analysis were performed with statistical significance at $p<0.05$.

Community visualization

Differences in alpha diversity of microbiomes were visualized through violin plots of the Shannon diversity index by sample condition and wildlife species group and through violin plots with inset boxplots using package “ggplot2” [31]. Beta diversity was visualized using NMDS plots based on Bray-Curtis distance matrices using packages “ggplot2” and “vegan”. Beta dispersion calculations were represented as boxplots with the packages “vegan” and “ggplot2”. Stacked bar charts used to display relative phyla abundance were calculated and created in package “phlyoseq”.

Results

Bushmeat samples included in analysis

226 bushmeat samples were obtained during June to July of 2016 (n=90 samples) and July of 2017 (n=136 samples). 209 samples yielded quality DNA for sequencing. Of these 209, bacterial DNA was successfully extracted from 161 samples and sent for Sanger sequencing. Twenty samples (12.4%) were excluded from further analysis due to being from non-target species (domestic farm animals). An additional 4 samples (2.5%) were excluded on the basis of poor DNA quality (< 50% quality score for at least one of the strands.) Fifty-eight of the remaining 137 samples contained high quality data with multiple chromatograms peaks at multiple positions or had more than 25% base pair ambiguities at alignment or would not align. These 58 samples were sent for 16s rRNA amplicon sequencing.

Twenty-five mammalian species were represented in the bushmeat samples, confirmed via PCR and Sanger sequencing (Chapter III). The most abundant species were waterbuck (*Kobus ellipsiprymnus*), warthog (*Phacocoerus africanus*), and lechwe (*Kobus leche*), although other wildlife species such as hippopotamus (*Hippopotamus amphibius*), black rat (*Rattus rattus*), Uganda kob (*Kobus kob*) and others were present in lower proportions. For downstream 16s rRNA amplicon analysis, wildlife species were condensed into 5 broad groups: antelope (which includes all antelope species), warthog, rodent (all rodents), primate (olive baboon), and other wildlife (including hippopotami, hares, buffalo and others). 25.5% of samples were collected in 2016 and 74.5% in 2017. Most (58.4%) samples included in this study were smoked and 41.6% were obtained fresh (not processed other than butchering and/or storage). Characteristics of all bushmeat samples included in this study are summarized in Table 1.

Sanger sequencing results

Seventy-nine Sanger sequencing results were queried against GenBank. Twenty-two bacterial genera representing 5 phyla and 14 families were detected among our samples. *Proteus* (22.8%), *Clostridium* (11.4%), *Macrococcus* (10.1%), and *Enterobacter* (7.6%) were the most frequently identified genera. Most bacterial species belonged to the Morganelleaceae (26.5%) and Clostridiaceae (17.7%) families. Over 85% of bacterial OTUs belonged to the phyla Proteobacteria (50.6%) or Firmicutes (36.7%). Bacterial taxa frequencies are shown in Fig. 5-2. No genera

included in the Select Agents list were detected by Sanger sequencing in our samples. Select Agents are agents determined by the Centers for Disease Control and Prevention and the United States Department of Agriculture to hold the potential to pose a "severe threat to both human and animal health, to plant health, or to animal and plant products." No statistically significant differences in proportions of genera, family, or phyla were detected by sample condition or by wildlife species group.

16s rRNA amplicon sequencing results

Fifty-eight samples were sent for 16s rRNA amplicon sequencing. Raw sequencing data recovered 13,305,715 sequences among the 58 samples. Following processing in mothur, 3,435,592 unique sequences were identified which were assigned to 34,566 OTUs. Sequence counts per individual sample ranged from 1,937 to 160,624 sequences. 613 OTUs remained when OTUs comprising less than 0.0001 relative abundance mean among samples were removed from analysis. Fifty-eight samples containing 613 OTUs were included in downstream visualization and analysis, containing 18 bacterial phyla and 171 genera. The most abundant phyla included Firmicutes (38.2%), Proteobacteria (30.0%), and Bacteroidetes (16.5%) (Fig. 5-3, Table 5-2).

The rodent group ($\bar{x}$ ±SD; 2.28±1.42) had greatest evenness and lowest diversity based on Shannon diversity index overall among wildlife species groups while the "other wildlife group" (1.62 ± 0.84) had the lowest evenness and greatest diversity among species; however, there were no statistically significant differences in alpha diversity among wildlife species groups (p=0.193) (Table 5-3). Fresh samples (2.38 ± 0.74) had greater average evenness and greater diversity overall than smoked samples (1.89 ± 0.76). This difference in alpha diversity was statistically significant (p=0.022) (Fig 5-4, 5-5, Table 5-4.)

Beta diversity was statistically different between bushmeat sample condition based on adonis analysis of Bray-Curtis dissimilarity values (p= 0.001) but was not statistically different among wildlife species groups with a p-value (p= 0.07.) Beta dispersion was significantly different among wildlife species groups (p=0.001); however, the group that demonstrated a difference was the primate group (Fig. 5-6). When the primate group was removed and the beta dispersion test was re-run, there was no statistical difference among the remaining groups of antelope, rodent, warthog, and other wildlife (p=0.379). Smoked samples had a greater beta dispersion than did fresh samples, but this difference was not statistically significant with a p value (p= 0.068). NMDS

ordination plots of Bray-Curtis dissimilarity values did not reveal any discernible patterns of clustering between fresh and smoked samples. Likewise, no distinct patterns were observed among wildlife species groups (Fig. 5-7).

Indicator species analysis produced 90 OTUs from nine phyla significantly distinguishing microbial communities of fresh bushmeat samples and eight OTUs from three phyla for smoked bushmeat samples (Table 5-5). Among wildlife species groups, indicator species were identified for rodents and primates, but none were statistically significant for warthogs, antelope, or other wildlife. Twelve OTUs representing four phyla were indicative of the primate group and thirteen OTUs representing three phyla were indicative of rodents (Table 5-6).

Of the bacterial Select Agents, five genera included in the list were identified. 248 OTUs characterized by *Clostridium*, 570 OTUs characterized by *Staphylococcus*, and 193 OTUs characterized by *Bacillus* were identified. *Mycoplasma* and *Burkholderia* represented 5 OTUs and 1 OTU each, respectively.

Discussion

Despite restrictions on hunting and removal of wildlife species in Uganda, bushmeat hunting is a common practice in and around protected areas, including Murchison Falls Conservation Area. Contact with wildlife has been associated with increased opportunity for zoonotic pathogen transmission and spillover events. Participation in the bushmeat trade presents multiple exposure routes to bloodborne, respiratory, and foodborne pathogens that have important health and economic impacts. Furthermore, hunting, trapping and butchering of wildlife carcasses carry notable inherent risk for injury to hunters and butchers and close proximity to pelts, blood, and salivary secretions, and thus increased opportunity for direct contact with these pathogens. This study aimed to fill a pressing knowledge gap and assess the microbiome of bushmeat samples from a variety of species and stages of processing in order to better understand risk of exposure to bacterial zoonoses during hunting, butchering, and consuming bushmeat for those living around MFCA.

Our Sanger sequencing data revealed the dominant presence of several genera of concern in our bushmeat samples, including *Clostridium, Escherichia,* and *Staphylococcus*. Several members of the genus *Clostridium* are human pathogens responsible for severe disease syndromes through oral exposure, including *C. botulinum*, *C. difficile,* and *C. perfringens*, and *C. tetani* though contact or wounding in addition to other *Clostridium* species emerging as pathogens. The

presence of this genus is particularly worrisome due to fact that butchering and consumption of bushmeat creates direct exposures for consumers and hunters; additionally, the increasing antimicrobial resistance of *Clostridium* species and sparser medical facilities in rural regions constitute significant barriers to treatment of clostridial infections [32]. *Staphylococcus*, although a ubiquitous genus and common commensal bacterium on human skin, is responsible for life-threatening and debilitating infections that are exacerbated by increasing antimicrobial resistance within this bacterial group [33, 34]. No significant associations between genera and condition or species group were found. Although this may be related to a relatively small sample size and uneven wildlife species groups, this finding is consistent with our 16s rRNA data, which also revealed limited statistical associations between taxa and these factors.

Results of 16s rRNA analysis indicated that Firmicutes, Proteobacteria, Bacteroidetes were the most abundant phyla, consistent across all samples included in this study. These phyla contain many common commensal and pathogenic bacteria and are often major contributors to microbiomes in many biotic systems. Findings in this study are consistent with other microbiome studies of bushmeat, demonstrating similar relative abundances of phyla [35]. Similar abundances were observed among 56 of 58 of our samples, even between fresh and smoked tissues with two samples demonstrating a lower total abundance of sequencing reads. Lower overall bacterial abundances were expected for smoked samples as this is used as a method of preserving bushmeat, but this was not observed in this study. It is possible that endogenous bacteria were inactivated or reduced by the smoking process, but that subsequent handling and transportation of bushmeat reintroduced environmental or commensal human bacteria. Additionally, it is important to consider that the presence of bacterial genetic material does not necessarily confirm infective capability and no culturing was performed as a part of this study. We did not inquire about approximate latency from hunting and harvest to time of collection for our samples. Neither did we gather information about how bushmeat was transported or by how many persons it was handled, so we were unable to control for these variables in this analysis. Assessing such variables may provide insight into factors influencing bacterial taxa abundance and microbial diversity.

All samples demonstrated high diversity regardless of species or sample condition. Samples were obtained from hunters, dealers, and consumers of bushmeat at different points in the commodity chain. This introduces some uncertainty about whether the bacterial microbiome composition seen in these samples represent microbiota endogenous to the wildlife hosts or

represent environmental contamination or food spoilage. We attempted to mitigate the effects of environmental contamination by sampling internal tissue from the samples; however, for those samples that had been removed from the host for a substantial amount of time, bacterial taxa associated with environmental contamination may still be present. Many activities in the bushmeat chain are carried out under non-hygienic conditions and with suboptimal to no cold-chain storage. Common meat spoilage bacteria include *Lactobacillus curvatus*, *Lactobacillus sake*, *Pseudomonas fiuorescens*, *Serratia liquefaciens*, *Brochothrix thermosphacta*, and *Carnobacterium piscicola*, while bacterial genera found in raw meat under variable refrigeration temperatures include countless bacteria, from *Escherichia* to *Klebsiella* to *Wiesella* [36, 37]. Nearly 40 of our OTUs were characterized by *Serratia* with fewer OTUs characterized by *Pseudomonas* and *Lactobacillus* identified. Long transit under variable conditions, both temporally and spatially, may increase the likelihood that environmental- and spoilage-associated bacterial taxa will be present on bushmeat. Environmental contamination encompasses a broad range of potential sources of contamination and bacterial taxa; however, the most abundant soil microbiome taxa include Acidobacteria, Verrucomicrobia, and Bacteroides [38]. Although each of these phyla were present within our samples, they were low in abundance relative to other phyla, such as Proteobacteria and Firmicutes. Only few hunters that we surveyed from the same area our bushmeat samples were collected reported using gloves when hunting or butchering (Chapter IV). It is possible that the microbiota of our bushmeat samples may be affected by the predominant commensal and pathogenic human skin bacteria. Cyanobacteria, Firmicutes, Bacteroidetes, and Proteobacteria are major phyla contributors to the human skin microbiome composition and are likewise dominant in our bushmeat samples [39-41].

Both the Shannon Diversity Index and Simpson Diversity Index were used to compare alpha diversity between sample conditions and among wildlife species groups. This analysis was performed at the phylum level and no statistically significant differences in alpha were detected at this taxon level for either alpha diversity metric. Nearly all wildlife species included in this study were herbivores that feed on similar plant sources and inhabit the same ecosystem, which is predominantly savannah in MFCA near our study sites. It is reasonable to suppose that animals had similar environmental exposures and may support similar microbial communities in their coats, skin, and gut. Several wildlife species in this study (baboons, hippopotamus, bats) have notably different feeding patterns and range in differing habitats than the grazing savanna-based

species that comprise most of our samples, but this did not appear to significantly influence alpha diversity. Beta diversity was similarly high overall among all samples. Ordination of sample groups by condition and wildlife species did not indicate any clustering patterns for either variable and was confirmed to have no statistical differences. Beta dispersion was found to be greater for the primate group; however, this should be interpreted cautiously as the primate group only contained one sample. It is interesting that there was no significant difference between fresh and smoked samples, and this may indicate that spoilage and environmental contamination of samples may play a larger role in bacterial exposure and food safety than endogenous bacterial infections. Smoking was used as a method of tissue preservation and perceived by many cooks to be a processing step that improves the safety of bushmeat by eliminating or decreasing the presence of harmful pathogens (Chapter IV).

Two of the top five indicator species for fresh samples were enteric bacteria, while several indicator species for smoked samples were *Corynebacterium*, the genera of the causative agent of diptheria. Although the primate group returned indicator taxa, this should be cautiously interpreted due to low sample size of this group. Indicator species are more conventionally used for traditional microbial community assessment (ie. skin or gut microbiomes) to assess a living, changing microbiome. Still, consideration of indicator species may prove useful in elucidating bacterial taxa associated with particular wildlife species, which may translate to practical recommendations such as identifying which wildlife should be avoided due to greater risk or increasing precautions taken when handling certain species.

Due to concern for zoonotic transmission during handling and consumption of bushmeat, samples were examined at the genus level for the presence of pathogens of particular interest to human health. Bacterial genera included in the Select Agents List that were present in our samples included *Clostridium*, *Staphylococcus, Mycoplasma, Burkholderia, Brucella,* and *Bacillus* (https://www.selectagents.gov/SelectAgentsandToxinsList.html). Additional bacterial pathogens of human consequence present in our samples included *Legionella, Escherichia, Streptococcus, Klebsiella, Vibrio* and *Bartonella*. Although we were able to confirm the presence of these genera, sequencing the 16s rRNA gene does not provide adequate resolution to identify bacteria to the species level. Many of the above genera are diverse and include species which are pathogenic to humans as well as species not known to cause human disease. For example, we confirmed the presence of *Bacillus*, but we cannot confirm whether this is *Bacillus anthracis*, a select agent, or

Bacillus cereus, a pathogenic foodborne agent, or one of the many benign species found ubiquitously in soil [42, 43]. Despite the lack of resolution, the presence of signatures for these genera of interest are sufficient to raise concerns regarding the safety of bushmeat and warrant more targeted sequencing efforts to identify pathogenic species.

Bushmeat hunting and consumption is a socially ingrained and often essential practice to many communities, but the risk of exposure and infection by zoonotic bacterial pathogens is clear. These data highlight the staggering quantity and diversity of bacteria present in bushmeat tissue intended for consumption. Our findings are suggestive of the presence of numerous known pathogens of human consequence and validate the need for further study of the diverse and elusive factors that shape bushmeat microbiomes. Findings in this study suggest that a combination of environmental contaminants, spoilage, and endogenous bacteria may contribute to microbial profiles of bushmeat and suggest that distinct bacterial taxa and bacterial loads are present at different stages in the bushmeat commodity chain. This has important implications for adopting food preparation safety strategies compared to handling and butchering safety recommendations to prevent infections. Improved understanding of these microbiomes is essential to providing effectual and accurate tactics to reduce zoonotic infections associated with the bushmeat trade and mitigating opportunities for epidemic events.

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Appendix V

Tables and Figures for Chapter V

Table 5-1. Summary of characteristics of bushmeat samples obtained from communities outside of Murchison Falls Conservation Area, Uganda, 2016-2017. Characteristics groupings include year collected, wildlife species, and sample condition (fresh or smoked).

Variable		Sanger	16s Amplicon
Wildlife Species	Waterbuck[a]	27	20
	Warthog[b]	12	10
	Hippopotamus[e]	2	5
	Lechwe[a]	7	4
	Grey rhebok[a]	0	3
	African buffalo[e]	0	2
	African grass rat[d]	0	2
	Common duiker[a]	1	2
	Kob[a]	4	2
	Striped ground squirrel[d]	1	2
	African savanna hare[e]	3	1
	Black rat[d]	5	1
	Wildebeest[a]	3	1
	Gambian pouched rat[d]	0	1
	Guinea gerbil[d]	0	1
	Olive baboon[c]	1	1
	Aardvark[e]	1	0
	Kirk's dik dik[a]	1	0
	Little free-tailed bat[f]	1	0
	Minor epauletted fruit bat[f]	1	0
	Multimammate mouse[d]	1	0
	Oribi[a]	4	0
	Tantalus monkey[c]	2	0
	Wild cat[e]	1	0
	Yellow-backed duiker[a]	1	0
Condition	Fresh	35	22
	Smoked	44	36
Year	2016	11	24
	2017	68	34

[a]Antelope group
[b]Warthog group
[c]Primate group
[d]Rodent group
[e]Other Widllife group
[f]Bat group

Table 5-2. Average % phylum abundance listed by wildlife species group (antelope, warthog, rodent, primate, other wildlife) and by bushmeat sample condition (fresh, smoked) in bushmeat samples obtained from northern Uganda, 2016-2017.

Phylum	**All**	**Fresh**	**Smoked**	**Antelope**	**Warthog**	**Primate**	**Rodent**	**Other**
Acidobacteria	0.02	0.06	0.0	0.0	0.0	0.0	0.27	0.0
Actinobacteria	0.59	1.00	0.33	0.34	0.45	4.0E-03	2.97	0.37
Bacteria_unclassified	0.07	0.10	0.06	0.04	0.01	0.56	0.30	0.09
Bacteroidetes	9.37	7.03	10.79	9.46	18.56	3.20	3.10	1.78
Candidate_division_ BRC1	0.02	0.04	0.0	0.0	0.0	0.0	0.18	0.0
Candidate_division_ OP10	4.0E-3	0.01	0.0	0.0	0.0	0.0	0.05	0.0
Candidate_division_ SR1	0.03	1.0E-3	0.05	0.06	0.0	0.0	0.0	0.0
Candidate_division_ TM7	0.02	0.04	1.0E-3	2.0E-03	2.0E-03	0.0	0.15	3.0E-04
Chlamydiae	1.0E-3	3.0E-3	0.0	0.0	0.0	0.0	0.01	0.0
Chloroflexi	0.01	0.02	0.0	2.26E-05	0.0	0.0	0.09	0.0
Deinococcus-Thermus	0.01	0.03	4.0E-03	0.0	0.0	0.0	0.14	0.02
Firmicutes	54.59	47.18	59.12	55.65	40.53	43.04	47.68	72.90
Fusobacteria	0.03	0.04	0.02	0.05	0.0	2.0E-03	0.0	0.0
Gemmatimonadetes	0.01	0.03	2.80E-03	3.37E-04	0.0	0.0	0.14	0.0
Lentisphaerae	0.01	7.4e-05	0.01	0.01	0.0	0.0	0.0	0.0
Planctomycetes	0.04	0.09	2.0e-03	0.0	0.0	0.0	0.41	0.01
Proteobacteria	35.18	44.30	29.61	34.39	40.45	53.19	44.41	24.84
Verrucomicrobia	0.01	0.03	5.1e-5	8.9e-05	0.0	0.0	0.12	0.0
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Table 5-3. Alpha diversity results based on Shannon Diversity index and Simpson/Inverse Simpson diversity index for bushmeat samples by wildlife species group. P-values were calculated using Kruskal-Wallis based on Shapiro-Wilk test of normality.

Alpha Diversity Measure	**Statistical Test**	**p-value**	**Antelope ($\bar{x}$ ± SD)**	**Warthog ($\bar{x}$ ± SD)**	**Rodent ($\bar{x}$ ± SD)**	**Primate ($\bar{x}$ ± SD)**	**Other Wildlife ($\bar{x}$ ± SD)**
Shannon Diversity Index	Kruskal-Wallis	0.193	2.24 ± 0.70	1.94 ± 0.53	2.28 ± 1.42	2.16	1.62 ± 0.84
Simpson/Inverse Simpson Diversity Index	Kruskal-Wallis	0.332	0.78 ± 0.18	0.76 ± 0.10	0.74 ± 0.16	0.84	0.63 ± 0.27

Table 5-4. Alpha diversity results based on Shannon Diversity index and Simpson diversity index for bushmeat samples by bushmeat sample condition. P-values were calculated using Kruskal-Wallis based on Shapiro-Wilk test of normality.

Alpha Diversity Measure	Statistical Test	p-value	Fresh ($\bar{x}$ ± SD)	Smoked ($\bar{x}$ ± SD)
Shannon Diversity Index	Kruskal-Wallis	0.022	2.38 ± 0.74	1.89 ± 0.76
Simpson/Inverse Simpson Diversity Index	Kruskal-Wallis	0.017	0.82 ± 0.10	0.70 ± 0.21

Table 5-5. List of top five indictor taxa significantly associated with each sample condition at time of acquisition in bushmeat samples collected in northern Uganda, 2016-2017 based on indicator species analysis. Ninety OTUs were significantly associated with fresh samples and eight with smoked.

Condition	**Statistic**	**p-value**	**Phylum**	**Genus**
Fresh	0.500	0.000	Proteobacteria	*Escherichia*
Fresh	0.417	0.000	Proteobacteria	*Enteric_bacteria_cluster*
Fresh	0.361	0.003	Firmicutes	*Vagococcus*
Fresh	0.351	0.001	Proteobacteria	*Stenotrophomonas*
Fresh	0.335	0.001	Bacteroidetes	*Empedobacter*
Smoked	0.332	0.006	Bacteroidetes	*Myroides*
Smoked	0.278	0.039	Actinobacteria	*Corynebacterium*
Smoked	0.268	0.030	Bacteroidetes	*Myroides*
Smoked	0.260	0.036	Proteobacteria	*Ignatzschineria*
Smoked	0.240	0.035	Actinobacteria	*Corynebacterium*

Table 5-6. List of top five indictor taxa significantly associated with each wildlife species group in bushmeat samples collected in northern Uganda, 2016-2017 based on indicator species analysis. Twelve OTUs were significantly associated with the primate group, thirteen with the rodent group. The antelope, warthog, and other wildlife groups were not found to have any statistically significant associated OTUs.

Species Group	**Statistic**	**p-value**	**Phylum**	**Genus**
Primate	0.999	0.012	Bacteria_unclassified	*Bacteria unclassified*
Primate	0.993	0.005	Proteobacteria	*Enterobacteriaceae_unclassified*
Primate	0.990	0.018	Firmicutes	*Clostridium*
Primate	0.985	0.001	Proteobacteria	*Plesiomonas*
Primate	0.993	0.005	Proteobacteria	*Enterobacteriaceae_unclassified*
Rodent	0.413	0.301	Actinobacteria	*Ornithinimicrobium*
Rodent	0.412	0.024	Proteobacteria	*Pantoea*
Rodent	0.412	0.035	Actinobacteria	*Proprionibacterium*
Rodent	0.411	0.042	Actinobacteria	*Corynebacterium*
Rodent	0.409	0.047	Proteobacteria	*Acinetobacter*

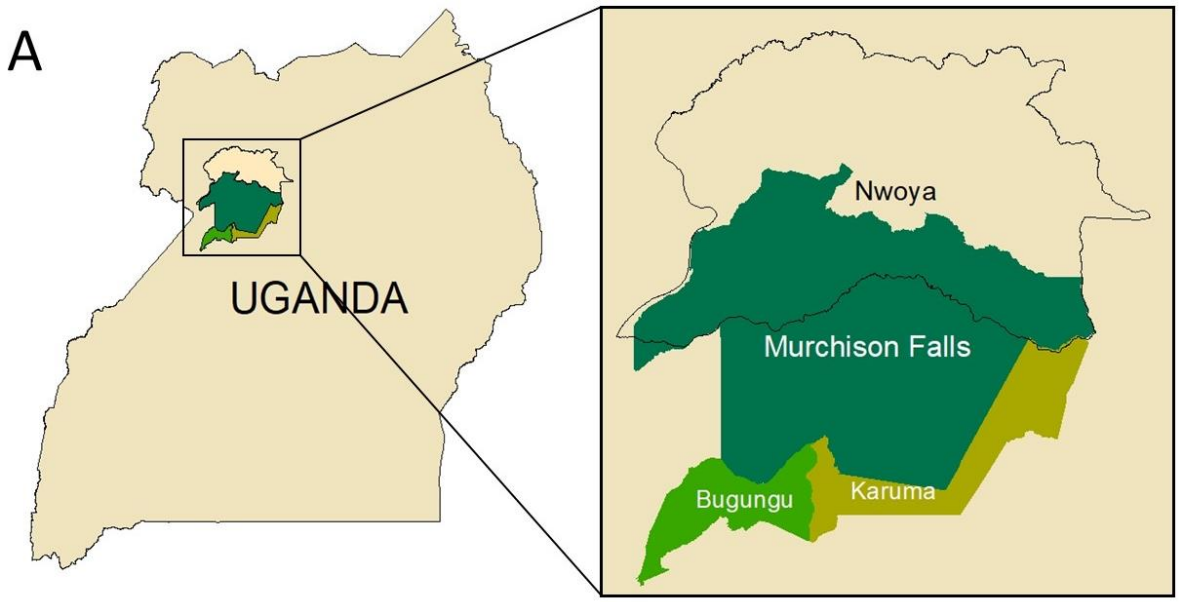

Figure 5-1. Map of Murchison Falls Conservation Area

Map of Murchison Falls Conservation Area in Uganda and Nwoya district at the northern border where samples in this study were collected between 2016-2017.

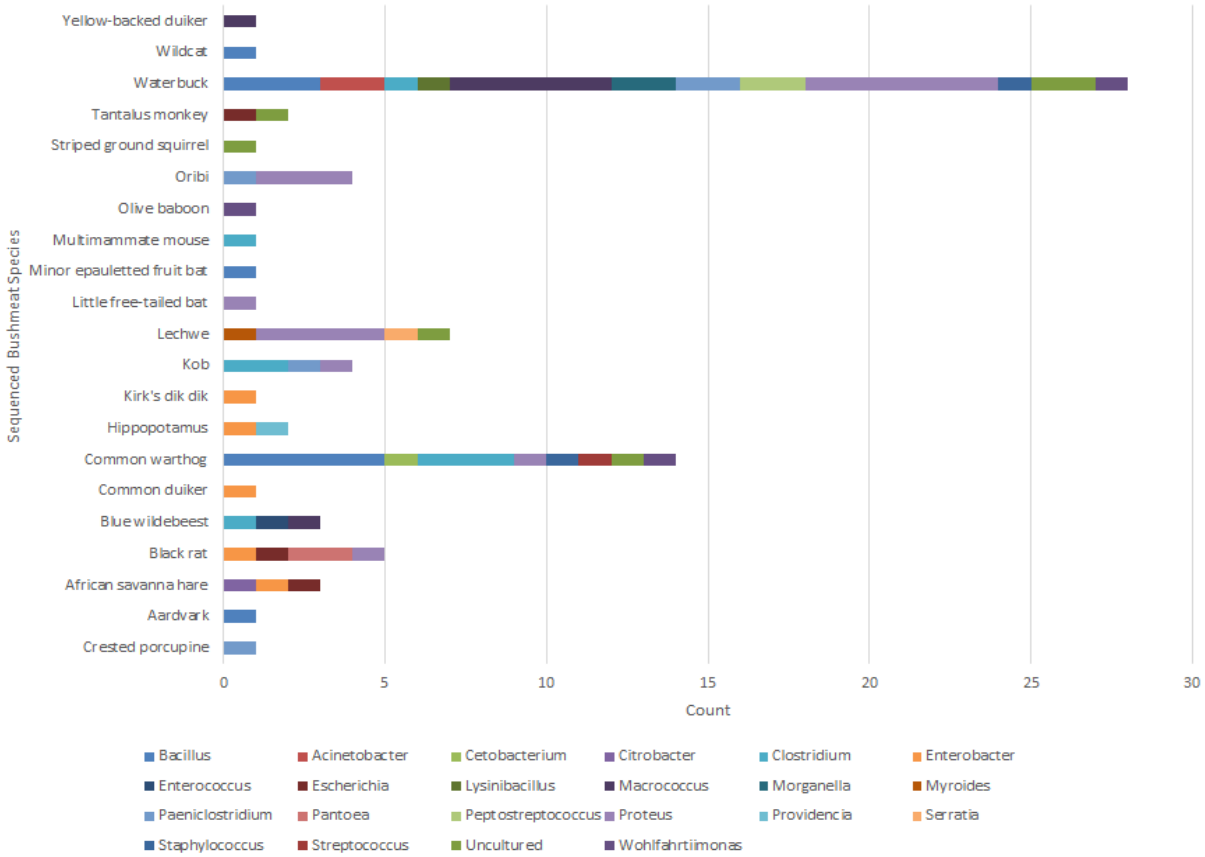

Fig 5-2. Sanger Sequencing Results by Genera

Bacterial genera identified by Sanger sequencing of the 16s rRNA gene and queried against NCBI Genbank in bushmeat samples from Murchison Falls Conservation Area, Uganda, 2016-2017.

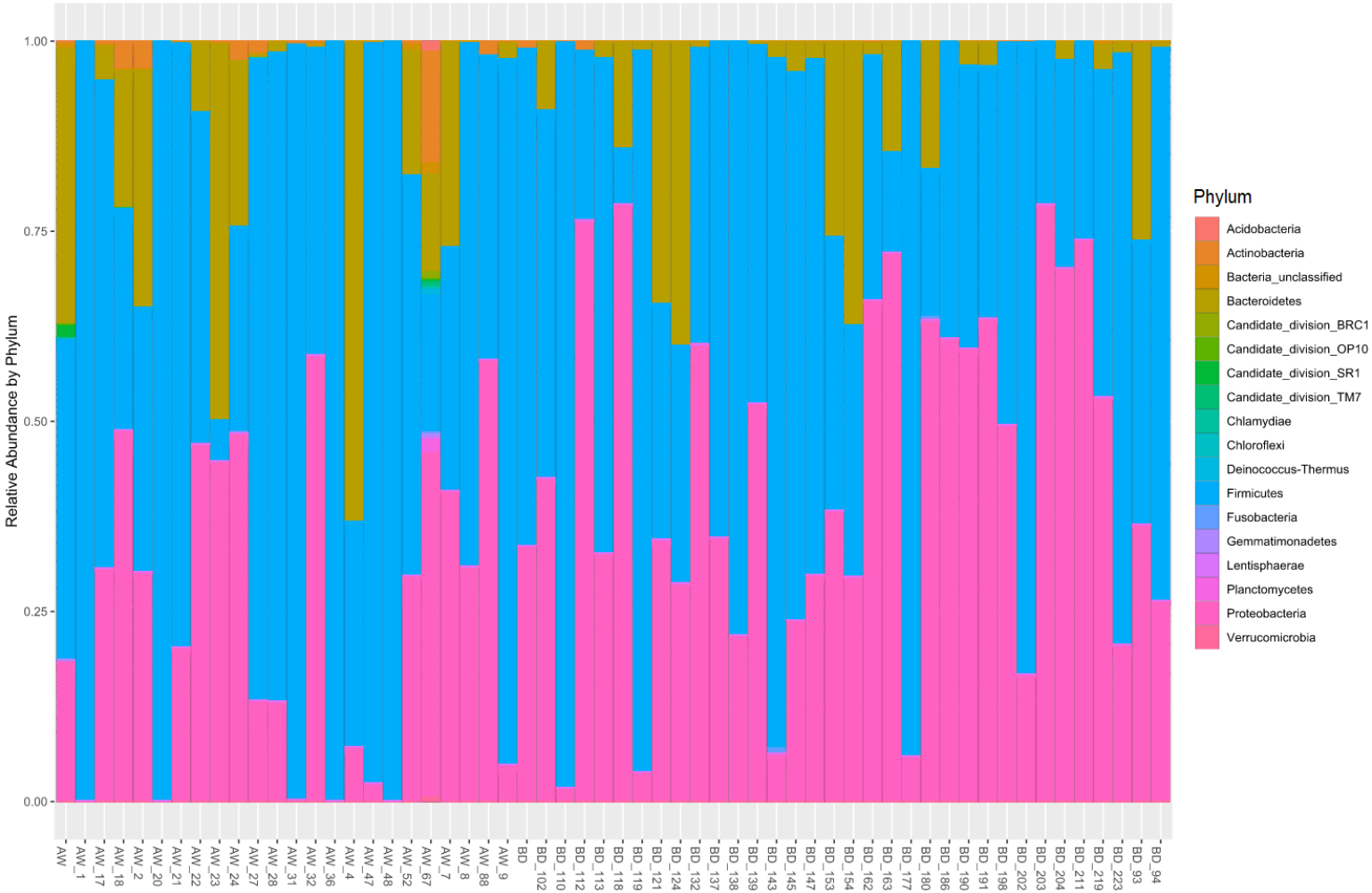

Fig 5-3. Relativized Phylum Abundance by Sample the reader cannot know what animal species BD_116 is. Or?

Relativized phylum abundance by individual bushmeat sample collected from northern Uganda, 2016-2017.

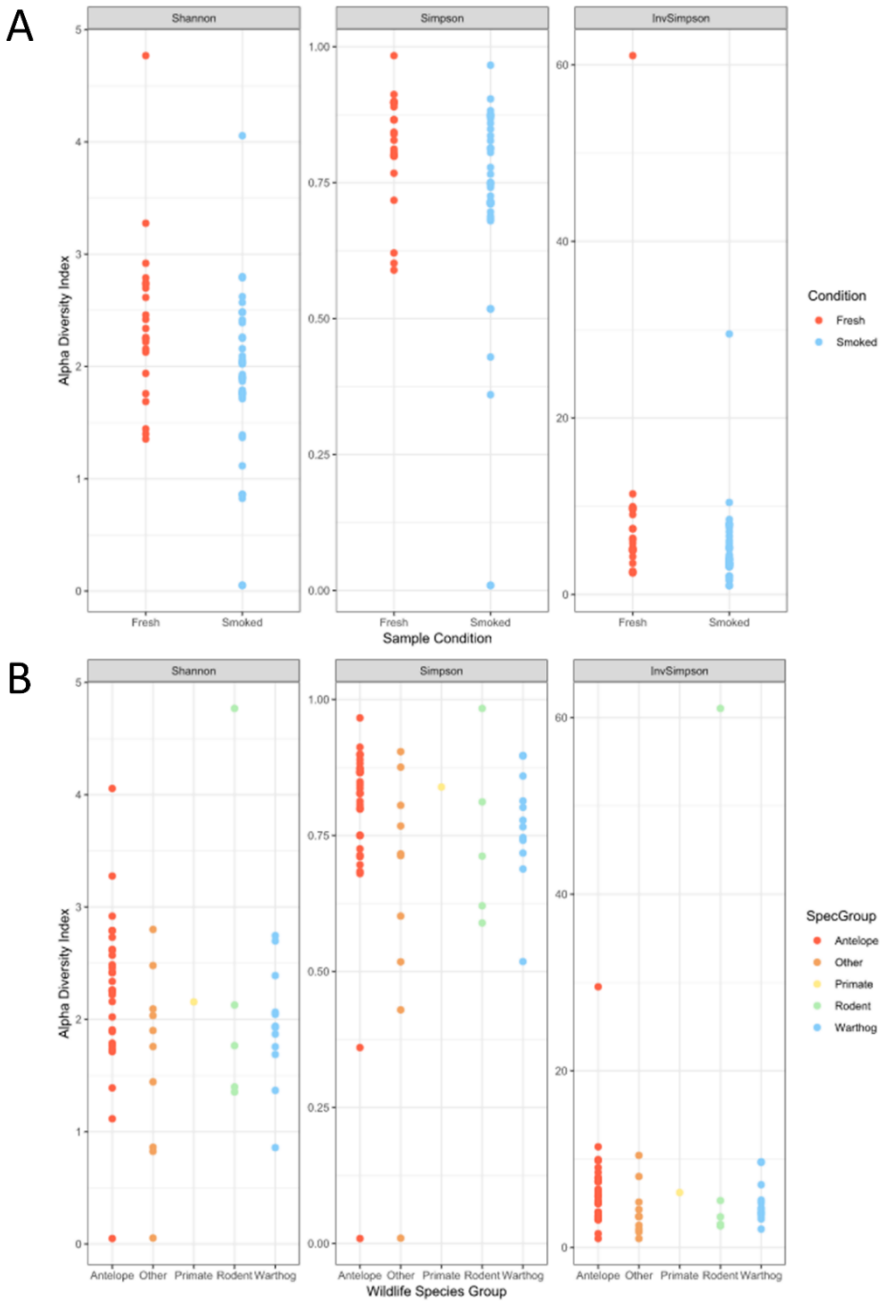

Figure 5-4. Alpha Diversity by Major Diversity Indices

Alpha diversity plots for Shannon Diversity index, Simpson index, and inverse Simpson index. A) illustrates differences between smoked and fresh samples, and B) illustrates differences among wildlife species groups.

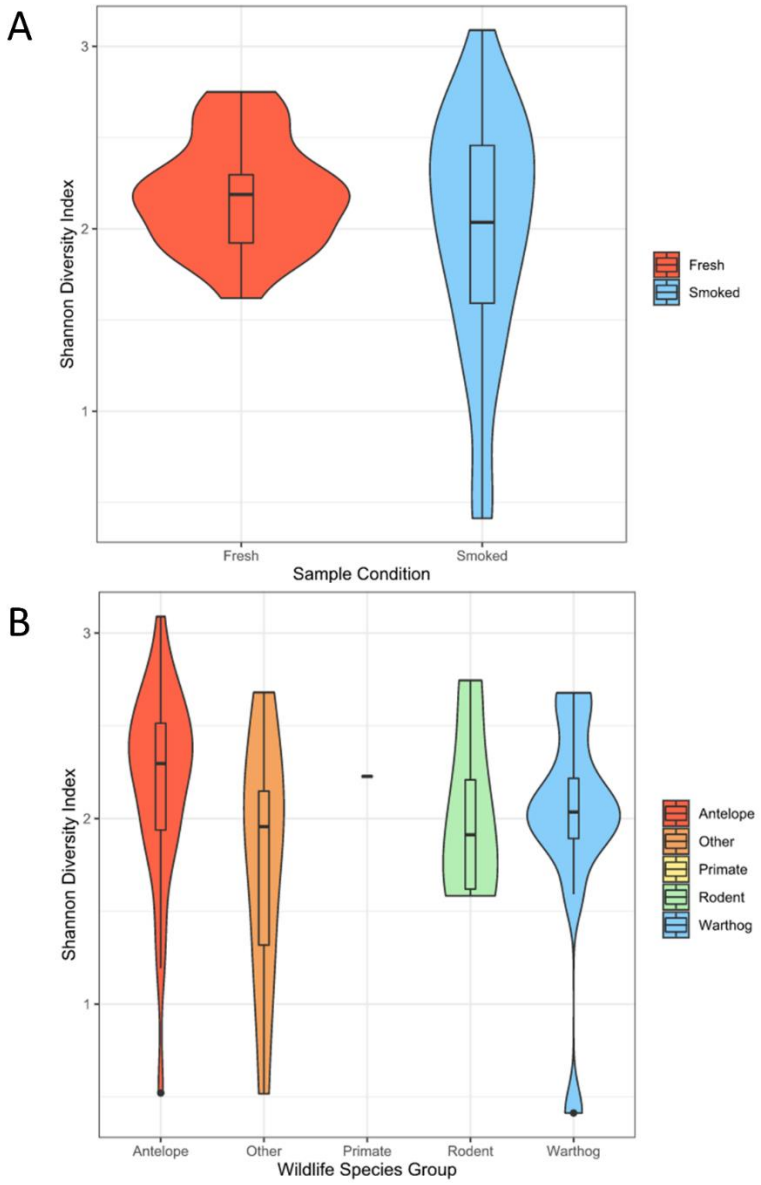

Figure 5-5. Alpha Diversity based on Shannon Index

Violin plots with inset box-and-whisker plots comparing the Shannon diversity index of bushmeat microbial diversity A) between sample condition of fresh (red) or smoked (blue) and B) among wildlife species groups of antelope (red), other wildlife (orange), primate (yellow), rodent (green), and warthog (blue) from bushmeat samples collected in northern Uganda, 2016-2017.

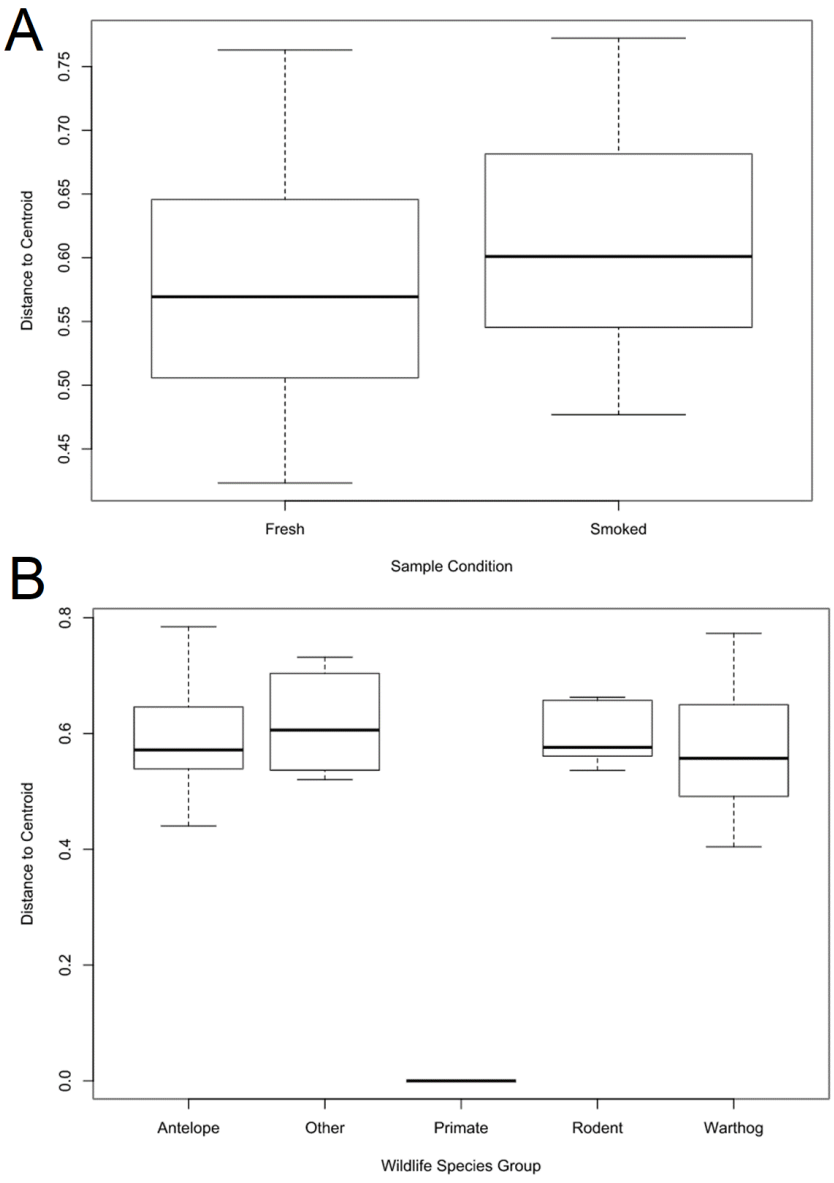

Figure 5-6. Beta Dispersion

Box plot illustrating beta dispersion of bushmeat biodiversity between A) sample condition (fresh or smoked) and B) among wildlife species groups from bushmeat samples collected in northern Uganda, 2016-2017.

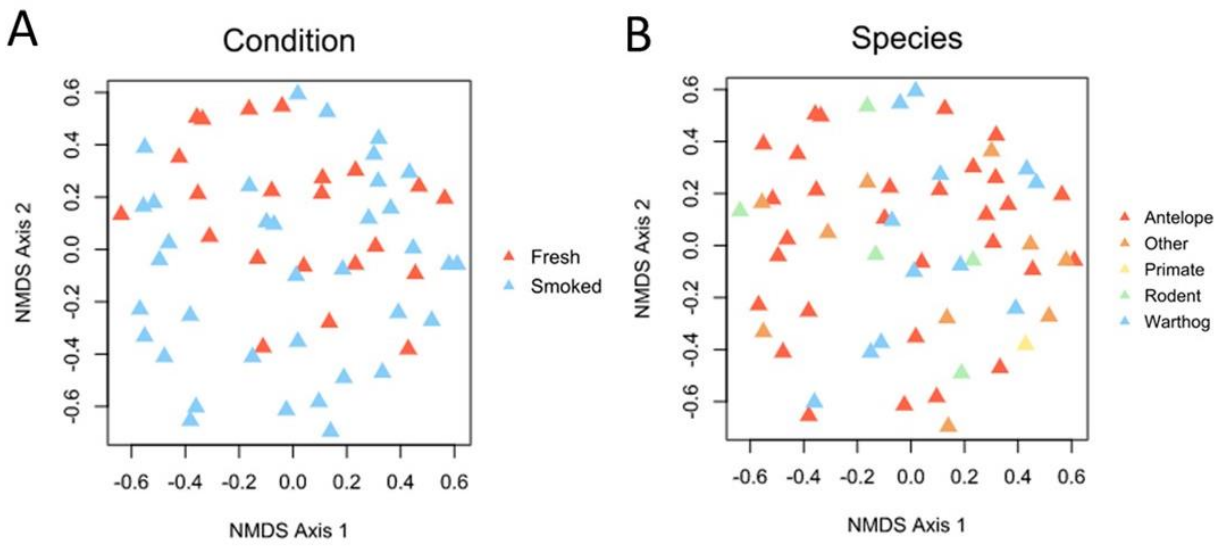

Figure 5-7. NMDS Ordination Plots Using Bray-Curtis Dissimilarity

NMDS ordination plots of bushmeat microbial diversity based on Bray-Curtis dissimilarity. Each point represents a single bushmeat sample. Ordination points in panel A are grouped by bushmeat sample condition of fresh (red) or smoked (blue). Ordination points in panel B are grouped by wildlife species group of antelope (red), other wildlife (orange), primate (yellow), rodent (green) or warthog (blue).

CHAPTER VI

Conclusion

The epidemiology of zoonotic infectious diseases is a topic of increasing importance as globalization and increased contact with infectious disease reservoirs allow for epidemic spread of emerging infectious diseases. As the factors that facilitate disease emergence become increasingly complex, non-traditional partnerships and approaches to describing and predicting these emerging disease events are necessary. In this dissertation, we have utilized multi-modal traditional and advanced diagnostic modalities to describe emerging infectious diseases from wildlife reservoirs in the United States on public lands and in protected areas in northern Uganda. We utilized social science and molecular diagnostics to create a more complete picture of factors contributing to disease emergence and individual risk of exposure so that effective, appropriate, and practical strategies can be implemented to reduce disease burden in people in close contact with wildlife and their tissues.

We documented the first molecular confirmation of zoonotic cestode parasite *Echinococcus canadensis* in translocated elk in the southeastern United States and lay the groundwork for future prevalence studies and continued surveillance of this pathogen within elk populations. This project also addresses the potential for establishment of a sylvatic transmission cycle, which has notable implications for recreationalists, both hunters and otherwise, who utilize these public lands. Although we could not definitively confirm infection of definitive canid hosts within the context of this study, we have emphasized the importance of an active surveillance strategy in viable definitive hosts. The establishment *E. canadensis* in this area is of notable public health consideration as a neglected tropical disease with chronic, potentially fatal health consequences in infected humans. Data from this study may serve to support the development of educational strategies for recreationalists to these areas to prevent fecal-oral infection with infective eggs.

This project also documents the phenomenon of species deception in market in northern Uganda, describe demographic and social drivers of bushmeat utilization and zoonoses awareness in communities in northern Uganda, and analyze the microbial diversity of bushmeat samples outside of Murchison Falls Conservation Area. Our findings demonstrate that within the bushmeat commodity chain there is deception to consumers about which species they handle and consume

and we confirm that at least some of this deception is intentional. The findings from this chapter present alarming findings, as this deception subverts the ability of consumers of bushmeat to make informed choices about their exposures. These data furthermore provide useful insight into the which wildlife species are most often harvested and provide valuable baseline data with implications for conservation and management strategies of wildlife in Murchison Falls National Park. Our social science findings demonstrated a degree of awareness of some common zoonotic pathogens in the area, although women tended to have higher awareness than hunters. Hunters are more involved in handling of more types wildlife tissues and involved in greater risk activities for injury than cooks, which presents an important opportunity for food handling and safety educational efforts. The microbial diversity findings reiterate the importance of good food handling practice, as they suggest that post-mortem contamination of bushmeat tissue, in addition to endogenous infections, play a significant role in the bacterial community ecology of bushmeat, and therefore present a much broader range of microbes to which hunters and consumers are exposed.

The findings of this project underscore the need for integrative and multidisciplinary approaches addressing the public heath priority of preventing and mitigating the potentially devastating effects of emerging zoonoses. Our findings further emphasize the knowledge gaps present among the diverse geographies and cultures in which hunting of wildlife is widespread and highlight the need for continued surveillance of pathogens in wildlife reservoirs. Appropriately adapted and practical efforts to improve food safety, reduce injury risk during these activities, and increase awareness of environmental contamination with zoonotic pathogens is warranted to continue to mitigate the emergence of viral, bacterial, and parasitic zoonotic infections.

VITA

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