ZOONOTIC DISEASES OF TEXAS SKUNKS (MAMMALIA: MEPHITIDAE) WITHIN A ONE HEALTH FRAMEWORK

A Dissertation

by

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Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

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May 2021

Major Subject: Wildlife and Fisheries Sciences

ABSTRACT

Zoonotic diseases of wildlife can have wide-ranging effects on public health, wildlife and conservation management, and ecosystem functions. Consequently, any investigation into the dynamics of these complex, adaptive systems necessitates a One Health approach. This thesis evaluates the disease ecology of Texas skunks (Mammalia: Mephitidae) within a One Health framework, the results of which can be practically applied to public health strategies and conservation planning for the promotion of both animal and human health. To this end, field surveillance of *Trypanosoma cruzi* (the etiological agent of Chagas disease) and the pathogenic agents of dermatophytosis were carried out in wild Texas skunks and assessed in relation to their impact on wildlife populations and human and domestic animal health. To ensure maximum extraction of DNA from the blood filter papers used in the *T. cruzi* surveillance of skunks, the extraction procedure for whole blood stored on Nobuto strips was optimized. Employing the resulting methodology, a PCR-based surveillance of *T. cruzi* in 235 wild skunks, representing four species, was conducted across 76 counties and ten ecoregions in Texas, USA, along with an evaluation of potential disease risk factors. An overall *T. cruzi* prevalence of 17.9% for all mephitid taxa was recovered, and the first cases of Chagas disease were reported in eastern spotted (*Spilogale putorius*), western spotted skunks (*S. gracilis*), and American hog-nosed skunks (*Conepatus leuconotus*). Although not statistically-significant, trends for juveniles to exhibit greater disease risk and for differential sex biases in *T. cruzi* prevalence between taxa were also recovered.

Concordantly, an evaluation of cutaneous infections was performed in a population of eastern spotted skunks, from which the zoonotic fungal pathogen *Microsporum canis* was cultured. These first records of *T. cruzi* and *M. canis* were then incorporated into a comprehensive review of infectious diseases of eastern spotted skunks. The implications of these pathogens for current and future population health of the species were addressed with reference to the influences of anthropogenic processes. This thesis contributes key data for informing population viability analyses and epidemiologic models in addition to providing a One Health evaluation of skunks that can serve as a baseline for future disease ecology studies.

ACKNOWLEDGEMENTS

I would like to thank my committee co-chairs, Dr. Lacher and Dr. Brightsmith, and my committee members, Dr. Kyle, Dr. Lopez, and Dr. Stronza, for their guidance, patience, and support throughout the course of this research. I would also like to thank Dr. Murray without whom I would have never been able to complete this endeavor. By welcoming me into her lab, Dr. Murray provided me with the best facilities and training opportunities that I could have ever hoped for, paired with an inspiring, encouraging, and supportive group of lab members and friends, to all of whom I am also greatly indebted. I especially want to thank Dr. Rodion Gorchakov and Rebecca B. Kairis for all the training, advice, laughs, and patience that they've shared with me over all these years and time zones.

Thanks also go to my friends and colleagues from the Applied Biodiversity Sciences program at Texas A&M University, especially Dr. Jason Martina who went above and beyond as a mentor, friend, and therapist to me through the ups and downs of my graduate program.

Finally, last but far from least, I have no words to express my gratitude to my husband and love of my life, Dr. Anton Wroblewski. Through all my graduate school struggles, he has been a constant supply of love, encouragement, chocolate, and caffeine. I will never be able to thank him enough for being so generous, patient, and supportive after everything he's endured along with me these last four years.

Specifically, regarding Chapter Two: I thank Melinda D. Luper, DVM, of Fur &

Feather Veterinary Hospital for the donation of canine WB used in our spiking experiment. I am also grateful to Dr. R.C. Dowler and the Angelo State Natural History Collections for access to archived skunk WB samples.

Specifically, regarding Chapter Three: I am extremely grateful to the following individuals and institutions for access to and donations of skunk WB samples: the Angelo State Natural History Collections; Angelo State University Skunk Day participants; Stephanie Brady (Wild West Wildlife Rehabilitation Center); Stephanie Brewer; Michelle Camara (Southern Wildlife Rehab, Inc.); Diane Cook; Dove Key Ranch Wildlife Rehabilitation, Inc.; Alexandra A. Gibson; Daniel L. Heare; Melinda D. Luper, DVM; Victoria Mann; J. Clint Perkins and all of the Texas Eastern Spotted Skunk research project members; and Anton F.-J. Wroblewski. Skunk silhouettes provided by Anton F.-J. Wroblewski.

Specifically, regarding Chapter Four: I thank W. Newman, F. Collins, and the Katy Prairie Conservancy for facilitating access to properties in order to conduct this field survey.

Specifically, regarding Chapter Five: Discussions with D.J. Brightsmith, T. Erickson, G.T. Kyle, T.E. Lacher, Jr., R.R. Lopez, M.D. Luper, M. Mora-Zacarias, K.O. Murray, A. Stronza, and A.F.J. Wroblewski improved the quality of this work.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a dissertation committee consisting of Chair Dr. Thomas E. Lacher, Jr. of the Department of Wildlife and Fisheries Sciences, Co-chair Dr. Donald J. Brightsmith of the Department of Veterinary Pathobiology, Dr. Gerard T. Kyle of the Department of Recreation, Parks, and Tourism Sciences, Dr. Roel R. Lopez of the Department of Wildlife and Fisheries Sciences, and Dr. Amanda Stronza of the Department of Recreation, Parks, and Tourism Sciences. Dr. Kristy O. Murray of the Department of Pediatrics-Tropical Medicine at Baylor College of Medicine and the Center for Human Immunobiology at Texas Children's Hospital also supervised the performance of this research.

The analyses described in Chapter 2 were conducted in part by Rebecca B. Kairis, Dr. Rodion Gorchakov (currently of the Health, Safety and Environment Department of King Abdullah University of Science and Technology of the Department), and Anna Wheless (currently of the Department of Biochemistry and Biophysics, University of North Carolina School of Medicine) within the laboratory of Dr. Kristy Murray at Baylor College of Medicine and Texas Children's Hospital. Melinda D. Luper, DVM, of Fur & Feather Veterinary Hospital donated the canine WB used in the spiking experiment. Dr. R.C. Dowler and the Angelo State Natural History Collections provided access to archived skunk WB samples.

The DNA extraction and PCR analyses described in Chapter 3 were conducted in part by Rebecca B. Kairis and Dr. Rodion Gorchakov (currently of the Health, Safety and Environment Department of King Abdullah University of Science and Technology) within the laboratory of Dr. Kristy Murray at Baylor College of Medicine and Texas Children's Hospital. The following individuals and institutions provided access to and donations of skunk WB samples for analysis: the Angelo State Natural History Collections; Angelo State University Skunk Day participants; Stephanie Brady (Wild West Wildlife Rehabilitation Center); Stephanie Martinez-Brewer; Michelle Camara (Southern Wildlife Rehab, Inc.); Diane Cook; Dove Key Ranch Wildlife Rehabilitation, Inc.; Alexandra A. Gibson; Daniel L. Heare; Melinda D. Luper, DVM; Victoria Mann; J. Clint Perkins and all of the Texas Eastern Spotted Skunk research project members; and Anton F.-J. Wroblewski.

In regards to Chapter 4, Alexandra A. Gibson (currently of the One Health Institute, School of Veterinary Medicine, University of California Davis), J. Clint Perkins (currently of the Department of Natural Resources Management, Texas Tech University), and Dr. R.C. Dowler of the Department of Biology, Angelo State University assisted with specimen collection. Dr. Melinda D. Luper of Fur & Feather Veterinary Hospital aided in the culturing and identification of samples.

All other work conducted for the dissertation was completed by the student independently.

Funding Sources

The work described in Chapter 2 and Chapter 3 was part of a project supported by NIAID award number RO3AI123650. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of NIAID. A CVM Merit Scholars Fellowship from the College of Veterinary Medicine & Biomedical Sciences, Texas A&M University funded two years of coursework.

NOMENCLATURE

ASK	Angelo State Natural History Collections
CDC	Centers for Disease Control and Prevention
CDV	Canine Distemper Virus
Ct	Cycle Threshold
DKRWR	Dove Key Ranch Wildlife Rehabilitation, Inc.
DNA	Deoxyribonucleic Acid
EID	Emerging Infectious Diseases
EPA	United States Environmental Protection Agency
ESS	Eastern Spotted Skunk
ESSCSG	Eastern Spotted Skunk Cooperative Study Group
IACUC	Institutional Animal Care and Use Committee
IAS	Invasive Alien Species
IUCN	International Union for Conservation Nature
KPC	Katy Prairie Conservancy
OMB	Office of Management and Budget
PCR	Polymerase Chain Reaction
PPE	Personal Protective Equipment
qPCR	Quantitative Polymerase Chain Reaction
RT-PCR	Real Time Polymerase Chain Reaction
SKAV	Skunk-Specific Amdoparvovirus

TDSHS	Texas Department of State Health Services
USDA	United States Department of Agriculture
USFWS	United States Fish and Wildlife Service
WB	Whole Blood
WHO	World Health Organization
WNV	West Nile Virus

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1. INTRODUCTION

An estimated one million plant and animal species are at high risk for extinction, the majority of them threatened directly by the consequences of human activity (Díaz et al. 2020). This escalating loss of biodiversity threatens not only the world's ecosystems, but also their provision of functions and services on which social-ecological systems (SES) rely (Ostrom 2009, Díaz et al. 2020). Burgeoning human population growth, habitat destruction and fragmentation, intensifying animal exploitation, human-induced climate change, and the rise of globalized travel and trade, all contribute directly or indirectly to biodiversity loss, ecosystem collapse, human-wildlife conflict, the proliferation of invasive alien species, and the rise of emerging and re-emerging zoonoses (Daszak et al. 2000, Díaz et al. 2020).

Infectious diseases contribute most markedly to the decline and extinction of wildlife species in cases where spillover or emerging novel pathogens affect naïve populations and/or endemic disease dynamics are altered by environmental or demographic shifts (Daszak et al. 2000). In order to assess the effects any pathogenic agent imposes on a population, subspecies, or species, an understanding of the abiotic and biotic drivers contributing to the pathogen's interactions with the taxon must be evaluated across geographic and temporal intervals. In particular, interrelationships between host behavior and demography, determinants of pathogen virulence, and disease transmission cycles regulate population and species level disease outcomes. The introduction of novel pathogens and epidemiological changes in pre-existing disease

dynamics of wildlife can be significantly influenced by anthropogenic processes, especially for populations overlapping the wildlife-domestic interface of habitats.

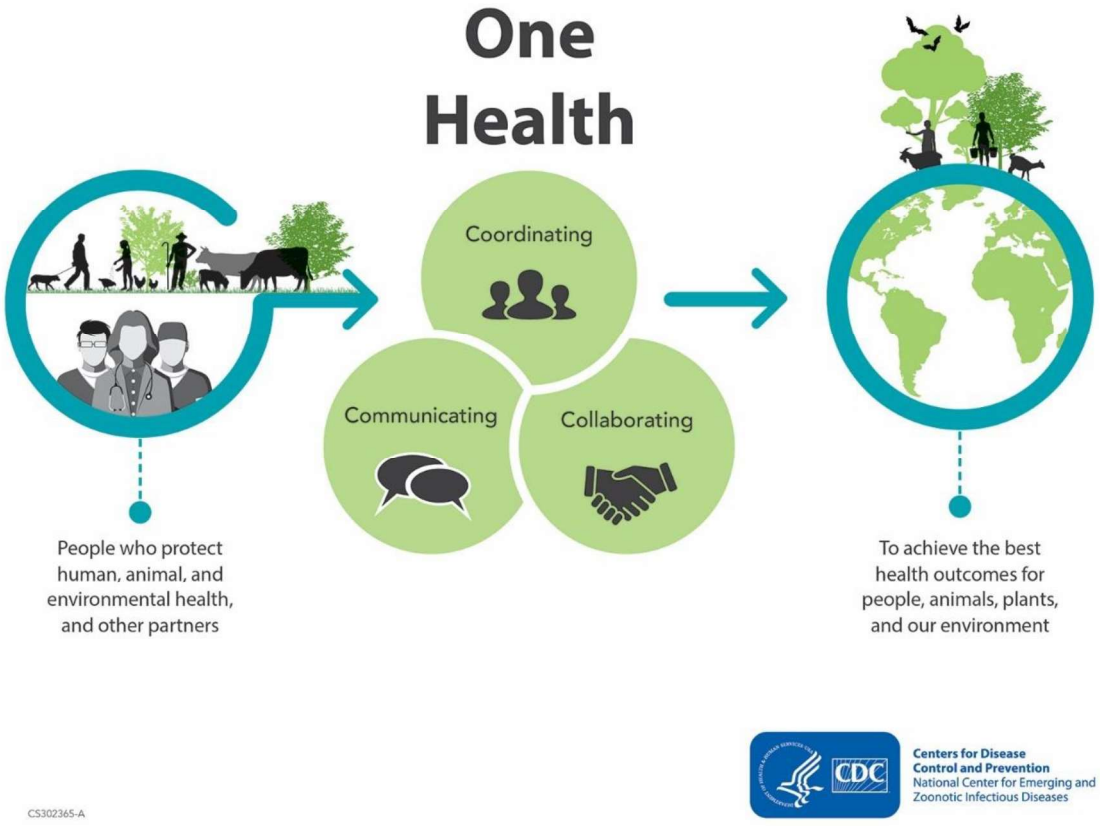
1.1. One Health and Zoonotic Diseases

Although the interconnection of human, animal, and environmental health has been recognized from the earliest historical times, the integrative framework of the One Health movement arose more recently from Schwabe's "one medicine" concept of the 20th century (Zinsstag et al. 2011, Rabinowitz 2018). One Health recognizes the inextricable links between the health of humans, wildlife, domestic animals, plants, and the ecosystems in which we all live (Zinsstag et al. 2011, Rabinowitz 2018). As such, the One Health approach is a collaborative, transdisciplinary approach that acts across geographic (i.e., local, regional, national, and global) and temporal (i.e., past, present, future) scales, bringing together a diversity of partners from a wide variety of fields (e.g., veterinarians, public health personnel, wildlife professionals, botanists, sociologists, economists, anthropologists, epidemiologists, ecologists, agriculturalists) to improve the quality of health for humans, animals, and their environments (Zinsstag et al. 2011, CDC 2018, Rabinowitz 2018; Figure 1.1).

The complex adaptive networks underlying the transmission cycles of zoonotic pathogens make these diseases especially disposed to investigation within One Health frameworks (Zinsstag et al. 2011, Rabinowitz 2018). Humans, wildlife, and synanthropic animals may operate within bridged domestic/peridomestic and sylvatic disease transmission cycles with varying consequences for veterinary and human medicine (Daszak et al. 2000, Northover et al. 2018, Silk et al. 2019). Consequently, it

is imperative to structure any exploration of zoonotic diseases within a One Health framework, incorporating environmental, domestic animal, wildlife, and public health (Daszak et al. 2000, Zinsstag et al. 2011, Rabinowitz 2018). Indeed, infectious disease management strategies with the greatest chance of real-world success are those employing One Health approaches (Cunningham et al. 2017, CDC 2018).

Figure 1.1. One Health framework as implemented in practice for improved health of humans, other animals, and the environment. Reprinted from Centers for Disease Control and Prevention (CDC 2018).



1.2. Skunks and Zoonotic Diseases

The North American members of the mammalian family Mephitidae (*Conepatus leuconotus*, *Mephitis mephitis*, *M. macroura*, *Spilogale angustifrons*, *S. gracilis*, *S.*

putorius, and *S. pygmaea*) are model taxa with which to explore the conservation implications of One Health-framed research on their ecological interrelationships with zoonotic pathogens (Figure 1.2). Owing to their omnivorous diet, den site habits, foraging activities, mating behaviors, and other pertinent habitat use and natural history traits, skunks are exposed to a wide variety of pathogenic agents (Medellín et al. 1998, Elbroch and Rinehart 2011, Ceballos 2014, ESSCSG 2019). Mephitids' potential exposure to and role within the transmission cycle of zoonotic diseases is exacerbated by anthropogenic processes, especially for populations that cross the wildlife-domestic interface such as those that exploit agricultural, peridomestic, and domestic settings (Medellín et al. 1998, Elbroch and Rinehart 2011, Ceballos 2014, ESSCSG 2019). Skunks may effectively dilute vector-borne zoonotic pathogens within a system as demonstrated by striped skunks (*M. mephitis*) and Lyme disease in New York (Levi et al. 2016). In addition, mephitid species hold promise for use as sentinels in surveillance strategies aimed at the early detection of epidemics or epizootics, on-going monitoring of infectious disease, and/or evaluations of the efficaciousness of various disease control measures (McCluskey 2003, Neo and Tan 2017). In practice, skunks are already effectively incorporated into rabies surveillance programs conducted by public health agencies across their North American range.

Figure 1.2. Skunk species (Mammalia: Mephitidae) endemic to Texas, U.S.A. (A) American hog-nosed skunk (Conepatus leuconotus); (B) American hog-nosed skunk, frontal view; (C) Striped skunk (Mephitis mephitis); (D) Western spotted skunk (Spilogale gracilis), photograph provided by National Park Service; (E) Eastern spotted skunk (S. putorius), (F) Hooded skunk (M. macroura), photograph provided by National Park Service.

The conservation medicine importance of zoonoses is particularly important for imperiled skunk species. The eastern spotted skunk (ESS) (*S. putorius*) and the pygmy spotted skunk (*S. pygmaea*) are both listed as vulnerable by the International Union for Conservation of Nature, while populations for American hog-nosed (*C. leuconotus*) and western spotted (*S. gracilis*) skunks are declining across their ranges (Cuarón et al. 2016, Gompper & Jachowski 2016, Helgen 2016, Helgen et al. 2016). Since these threatened taxa as well as the stable populations of more common skunks are all victims of HWC, the potential contribution of zoonotic diseases to the origination, maintenance, and/or

mitigation of HWC is of significant import for the strategic planning and implementation of mephitid conservation strategies (Cuarón et al. 2016, Gompper & Jachowski 2016, Helgen 2016, Helgen et al. 2016).

Minimizing the negative effects of infectious disease and HWC on skunks serves to protect ecosystem services that extend well beyond biodiversity preservation and zoonotic disease control. Mephitid taxa are critical components of the social-ecological systems in which they participate. The ability of some skunk populations to not only live but thrive in proximity to people and within highly fragmented habitats includes mephitids among the few wildlife taxa that can significantly boost biodiversity in urban and suburban areas and provide ecosystem services within heavily-compromised habitats. For instance, striped skunks are critical for the toxicant-free control of "nuisance" species, such as venomous snakes, rodents, and domestic and crop arthropod pests in human-habitated landscapes (Verts 1967). Skunks provide further economic and cultural benefits to people through their harvesting by fur trappers and their use in traditional medicines (Lantz 1917, Alonso-Castro, 2014).

1.3. Chagas Disease and Skunks

Chagas disease is a likely cause of morbidity and mortality in wild skunks across the United States and throughout Latin America. The parasitic protozoan *T. cruzi* is maintained in domestic, peridomestic, and sylvatic transmission cycles by a diversity of triatomine vectors and mammalian hosts (Bern et al. 2019). This neglected tropical parasite infects an estimated 6-7 million people across the Americas, making the zoonosis one of the most significant in terms of disease burden and public health

importance within the New World (WHO 2020). All species of North American skunk have ranges that overlap the geographic region in which Chagas disease has been recovered either from triatomine insects, domestic animals, wildlife reservoirs, or humans (Snowden and Kjos 2012, Bern et al. 2019). The ability of wild striped skunks, including those in Texas, and South American hog-nosed skunks (*Conepatus* spp.) to act as hosts for Chagas disease in both sylvatic and domestic transmission cycles has been well-established (McKeever et al. 1958a, Burkholder et al. 1980, Navin et al. 1985, Pietrokovsky et al. 1991, Wisnivesky-Colli et al. 1992, Ceballos et al. 2006, Charles et al. 2013, Roellig et al. 2013, Kramm et al. 2019).

T. cruzi infections commonly cause acute and chronic cardiac disorders in their mammalian hosts, including *M. mephitis* (Davis et al. 1980, Ryan et al. 1985, Barr et al. 1989, Barr 2009, Snowden and Kjos 2012, Bern 2015, Malik et al. 2015, Vitt et al. 2016). Chagas disease has also been linked to increased risk of mortality in domestic dogs and humans, who contract the disease from their infected mother via vertical transmission or lactation (Carlier and Torrico 2003, Snowden and Kjos 2012, Howard et al. 2014, Grinnage-Pulley et al. 2016). In mephitid taxa, decreased reproductive success due to either reduced fitness from cardiac disease and/or direct transmission of *T. cruzi* to offspring may result from Chagas disease. In following, the adverse effects of *T. cruzi* on wild skunk populations potentially threatens conservation objectives, public health initiatives, and the ecological, economic, and cultural benefits derived from these taxa. Consequently, research into the prevalence of active *T. cruzi* infections in skunks, characterizing factors contributing to disease risk (e.g., sex, species, age, location), and

assessing the degree to which mephitids serve as hosts, "dilutors," and sentinels for Chagas disease holds significance across multiple disciplines and for diverse sectors of society.

1.4. Chagas Disease Surveillance

Efficacious epidemiological surveillance for *T. cruzi* among human and animal populations alike is hindered by challenges intrinsic to the parasite's endemic circulation in tropical to subtropical climates and in remote regions (Moncayo et al. 2009). One solution to problems related to field collection and storage of body fluid samples relies on the use of blood filter papers. Filter paper has been employed to collect and preserve whole blood samples for immunoassays and taxonomic identifications for at least 120 years (Nuttall 1901). The cost-effectiveness and ease with which filter papers can be used to store blood products and other body fluid specimens without pre-preparation or temperature control constraints makes them a convenient collection method for diagnostic sampling in the field, particularly in cases of remote work in warm climates and with geographically-isolated populations (Bereczky et al. 2005, Lim 2018). The ability of filter papers to preserve extractable DNA in whole blood specimens for multiple years at room temperature further facilitates their application in biobanking and retrospective analyses (Lim 2018). However, previous field studies reported the limitation of sample volume available for extractions and the reduced integrity, stability, and purity of extracted DNA as potential factors contributing to the loss of sensitivity of PCR-based investigations using blood filter papers (Bereczky et al. 2005, Farnert et al. 1999, Lim 2018). In order to address these challenges and to maximize the efficacy of

filter paper in molecular epidemiologic surveillance, cheap, reliable, and sensitive methods for DNA extraction are needed.

1.5. Dermatophytosis and Skunks

Dermatophytosis, cutaneous infections of fungal origin, have been extensively researched in the fields of domestic and livestock veterinary care (Moriello and DeBoer 2012, Hurst 2016) as well as in the realm of public health (Hayette and Sacheli 2015). Despite the capacity for fungal emerging infectious diseases (EID) to cause severe population declines and extinctions in wildlife species and to present costly threats to public and domestic animal health, the trends and diversity of zoonotic dermatophytosis are poorly investigated in wildlife hosts, such as skunks (Aly 1994, Foley et al. 2011, Martel et al. 2013, Scheele et al. 2019).

Skunk species all share a high risk of exposure to infectious mycological skin pathogens as a result of: 1. their direct contact with fungal contaminants in soil through digging and ground foraging habits, excavation of denning sites, and low clearance of their bodies above the ground during their characteristic locomotion, 2. exposure to infectious dermatophytes via direct contact with other wildlife hosts through predation (primarily on rodents and other small mammals), 3. indirect contact with infected wildlife hosts whose burrows they exploit, and 4. transmission across the domestic-wildlife interface through direct and indirect exposure to domestic pets and/or livestock with which their ranges commonly overlap (Verts 1967, Elbroch and Rinehart 2011). Predictably, “ringworm” infections are commonly attributed to North American mephitids anecdotally by wildlife biologists and wildlife rehabilitators (B.E. Gulas-

Wroblewski, unpubl. data). However, only the nonpathogenic *Microsporum cookei* (Ajello), *Trichophyton mentagrophytes* (Blanchard), and *Chrysosporium* sp. indet. (Corda) have been isolated from North American striped skunks by previous studies (Ajello 1959, McKeever et al. 1958b, Knudtson and Robertstad 1970). Any investigation of the type, clinical manifestations, and patterns of infection of pathogenic fungi in skunks is critical to develop more informed decisions on care and prevention strategies for those managing these taxa as well as veterinary and public health agents seeking to combat zoonotic mycological infectious diseases.

1.6. Eastern Spotted Skunks: Conservation Medicine Within a One Health Framework

Populations of *S. putorius* have been experiencing a steady decline across their North American range since at least the 1940s and have subsequently been listed as vulnerable by the International Union for Conservation of Nature (Kaplan and Mead 1991, Gompper and Hackett 2005, Gompper and Jachowski 2016). The level of threat to and management of ESS varies from state to state in the United States, and the subspecies *S. putorius interrupta* (Rafinesque) (Plains Spotted Skunk) are currently under review for protection by the Endangered Species Act (USFWS 2012). A host of hypothetical drivers for ESS population declines have been presented in the literature, and infectious diseases undeniably play a key role in the population dynamics of the species, but the actual factors contributing to their historic and ongoing range-wide losses have yet to be incontrovertibly defined (Choate et al. 1974, Schwartz and Schwartz 2001, Gompper and Hackett 2005, Gompper and Jachowski 2016).

Previous investigations on infectious diseases of ESS have been limited in their geographic, temporal, and/or taxonomic extent, focusing on skunk populations within a restricted region or timeframe (e.g., Lesmeister et al. 2008) and/or surveying a small number of potential pathogenic agents (e.g., Higdon and Gompper 2020). Consequently, discussions of the ecological, environmental, demographic, and behavioral context of pathogen prevalence and virulence in *S. putorius* have been constrained by the context of the population and/or pathogenic agents under review. Although many of the pathogens reported in ESS individuals are zoonotic, few studies expand on the public health implications of these infections (Emmons 1950, McKeever et al. 1958c, Gorman et al. 1962) and even fewer explicitly analyze them from a One Health perspective (Emmons et al. 1949). Therefore, a comprehensive and critical review of infectious diseases within *S. putorius* across their range, the effects of disease on current and future population trends demonstrated by the species, and any potential role ESS play within zoonotic disease transmission dynamics is needed to devise effective conservation management and public health strategies.

1.7. Research Objectives

It is imperative to structure any exploration of skunk disease ecology within a One Health framework, incorporating environmental, domestic animal, wildlife, and public health. Indeed, infectious disease management strategies with the greatest chance of real-world success are those employing One Health approaches (Cunningham et al. 2017). The research conducted herein was directed towards assessing zoonotic pathogens of mephitids of Texas within a One Health framework in order to evaluate the

practical conservation relevance and public health importance of skunk disease ecology. In order to assess the role that skunk species play within the disease dynamics of a zoonotic pathogens, I employed epidemiological investigations of *Trypanosoma cruzi* (the causative agent of zoonotic Chagas disease) and of fungal pathogens responsible for cutaneous infections in Texas mephitids. The results were then incorporated into a comprehensive, One Health-framed review of the disease ecology of *S. putorius* and its conservation implications for this imperiled mephitid. This thesis seeks to contribute key data for informing population viability analyses and epidemiologic models in addition to providing a One Health evaluation of eastern spotted skunks that can serve as a baseline for future disease ecology studies.

The specific objectives of this research endeavor were to: 1. maximize the efficacy of DNA extraction from whole blood stored on blood filter paper in order to generate an optimized procedure for conducting field surveillance of *T. cruzi* in Texas skunks, 2. employ the optimized DNA extraction methodology to conduct a molecular epidemiological investigation of *T. cruzi*, which quantified the *T. cruzi* infection rate of skunk species throughout Texas, USA in relation to their geographic distribution, taxonomy, sex, and age, 3. perform an epidemiological investigation of superficial cutaneous infections in a population of *S. putorius interrupta* live-trapped during an ESS survey in southeastern Texas, U.S.A., and 4. incorporate my epidemiological findings pertaining to ESS into a comprehensive and systematic review of the disease ecology of *S. putorius*.

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2. OPTIMIZATION OF DNA EXTRACTION FROM WHOLE BLOOD ON FILTER PAPER FOR *TRYPANOSOMA CRUZI* DETECTION

2.1. Overview

Blood filter papers cost-effectively store body fluid specimens under challenging field conditions, extending the reach of zoonotic pathogen surveillance and research. We describe an optimized procedure for the extraction of DNA from whole blood (WB) stored on Type I Advantec® Nobuto strips. Based on polymerase chain reaction detection of parasite DNA, Qiagen's DNeasy Blood & Tissue Kit outperformed Zymo Research kits in the recovery of DNA from Nobuto-stored, *Trypanosoma cruzi*-spiked canine WB samples. Modified extraction procedures for the Qiagen kit were further evaluated for recovery of β-actin from skunk WB archived on Nobuto strips. Optimal performance was demonstrated by an extraction protocol with a 90°C incubation step and extended incubation post-addition of proteinase K, a method subsequently employed to identify a *T. cruzi* infection in one of the skunks. Using this optimized extraction method can efficaciously increase the accuracy and precision of future molecular epidemiologic investigations targeting neglected tropical diseases.

2.2. Introduction

The parasitic protozoan *Trypanosoma cruzi* is the causative agent of Chagas disease, which is maintained in domestic, peridomestic, and sylvatic transmission cycles by a diversity of triatomine vectors and mammalian hosts (Bern et al. 2019). This neglected tropical parasite infects an estimated 6-7 million people across the Americas,

making the zoonosis one of the most significant in terms of disease burden and public health importance within the New World (WHO 2020). Developing optimized protocols for *T. cruzi* DNA extraction from blood filter papers greatly expands the efficiency and effectiveness of field investigations into the molecular epidemiology of Chagas disease.

Filter paper has been employed to collect and preserve whole blood (WB) samples for immunoassays and taxonomic identifications for at least 120 years (Nuttall 1901). The cost-effectiveness and ease with which filter papers can be used to store blood products and other body fluid specimens without pre-preparation or temperature control constraints makes them a convenient collection method for diagnostic sampling in the field, particularly in cases of remote work in warm climates and with geographically-isolated populations (Bereczky et al. 2005, Lim 2018). The ability of filter papers to preserve extractable DNA in WB specimens for multiple years at room temperature further facilitates their application in biobanking and retrospective analyses (Lim 2018). However, previous field studies reported the limitation of sample volume available for extractions and the reduced integrity, stability, and purity of extracted DNA as potential factors contributing to the loss of sensitivity of polymerase chain reaction (PCR)-based investigations using blood filter papers (Breczky et al. 2005, Farnert et al. 1999, Lim 2018). In order to address these challenges and to maximize the efficacy of filter paper in epidemiologic surveillance, cheap, reliable, and sensitive methods for DNA extraction are needed. To this end, we evaluated the performance of three commercial extraction kits with adjusted DNA extraction protocols for the PCR recovery of *T. cruzi* from canine WB preserved on filter paper. We then validated the optimized

procedure via PCR recovery of β-actin from WB specimens collected from skunks (Mammalia: Mephitidae) and archived on filter papers.

2.3. Materials, Methods, and Results

All WB samples were collected on Type I Advantec® (Tokyo, Japan) Nobuto blood sampling filter paper due to this product's relatively low cost and wide availability through online markets. Canine WB from a female German Shepherd domestic dog (*Canis lupus familiaris*) was sourced as excess from WB collected for an unrelated diagnostic procedure and donated by Fur & Feather Veterinary Hospital (Houston, TX). Skunk WB samples were collected from an American hog-nosed skunk (*Conepatus leuconotus*), a striped skunk (*Mephitis mephitis*), and a western spotted skunk (*Spilogale gracilis*) as part of a biobanking project conducted by Angelo State Natural History Collections in the Department of Biology, Angelo State University, San Angelo, TX (Table 2.1). This study was exempt from ethical approval from the Institutional Animal Care and Use Committee (IACUC) at Texas A&M University because our methodology did not constitute "use of animals" as defined by IACUC. For each evaluation of sample DNA content, five microliters of extracted DNA were run in duplicate in a 20-µL reaction with TaqMan Fast Advanced Master Mix (Thermo Fisher Scientific, Waltham, MA) on a ViiA 7 Real Time PCR System (Thermo Fisher Scientific). Duplicate extraction negative controls, extraction positive controls, and no template controls were included in each quantitative (q) PCR analysis. Mean DNA recovery estimates were calculated from the average Ct (cycle threshold) values from the triplicates of each sample tested. Ct is the number of amplification cycles required for the fluorescent

signal used in RT-PCR to exceed the background level. As such, Ct values are relative measures of the concentration of DNA in a sample being analyzed: lower Ct values represent higher concentrations of DNA, whereas higher Ct values indicate lower concentrations of DNA in the sample.

Table 2.1. Collection information pertaining to skunk whole blood samples used in DNA extraction optimization testing. All samples were collected from Tom Green County, Texas, USA.

Angelo State Natural History Collections specimen number	Species	Collection date
ASK 11844	*Spilogale gracilis*	January 7, 2017
ASK 11857	*Mephitis mephitis*	February 18, 2014
ASK 11860	*Conepatus leuconotus*	August 27, 2016

2.3.1. Optimization with *T. cruzi*-spiked WB samples

Fresh WB from a *T. cruzi*-negative German Shepherd domestic dog was spiked with cell culture-originated *T. cruzi* parasites. WB was prepared in 1 mL aliquots accordingly: one unspiked control and duplicates for each of the *T. cruzi* spiking loads, medium (MED; expected Ct= 29-30) and high (HI; expected Ct= 24-25) spiked with 20 µL of the respective dilution of parasite. Medium and high spiking loads were confirmed through controls combining 20 µL of the respective dilution of parasite with 1 mL of phosphate-buffered saline solution. Spiked and control samples were processed in duplicate either directly as WB (50 µL) or post-application to Nobuto blood filter strips.

In the latter case, 50 µL of each treatment of WB was applied to Nobuto strips and thoroughly dried in a biosafety cabinet for at least 30 minutes to replicate field preparation of filter paper specimens. Each Nobuto strip was cut into four, equally-sized pieces that were combined with ATL lysis buffer in a 1.5 mL tube for further processing. One aliquot was processed directly without spiking in order to confirm the negative *T. cruzi* status of the canine patient.

DNA extractions can be completed employing a set of standard reagents; however, many scientific companies produce kits to facilitate the timely and easy recovery of DNA from biological specimens. We chose three of the most frequently-used of these extraction kits to test optimized *T. cruzi* DNA extraction methods with the spiked canine WB samples. We followed manufacturer instructions for DNA extraction from WB for the Quick-DNA/RNA Pathogen Miniprep and ZR-DuetTM DNA/RNA MiniPrep Plus kits (Zymo Research, Irvine, CA). Two alternate DNA extraction optimizations were assessed for the DNeasy Blood & Tissue Kit (Qiagen, Germantown, MD). Incubation for an extended period of time and/or at elevated temperatures during the blood cells lysis and removal steps of DNA extraction from WB can increase the purity of the DNA recovered (Qamar et al. 2017). By adjusting these conditions within the optimized extraction protocols, we attempted to increase the quantity of recovered *T. cruzi* DNA detectable on qPCR. In extraction optimization method A for the Qiagen kit, samples were mixed with 150 µL or 180 µL of ATL for direct WB or Nobuto strips, respectively, and incubated at 90°C for 15 minutes. Following this incubation period, 20 µL of proteinase K was added and the sample was incubated at 56°C for 60 minutes.

Next, we added 200 µL of AL buffer, incubated the sample for 10 minutes at 56°C, vortexed and spun the sample at maximum speed for 1 minute, collected the supernatant, and proceeded to follow manufacturer instructions. Extraction optimization method B resembled extraction optimization method A with the exclusion of the initial 15 minute 90°C incubation period and the reduction of the 56°C incubation period post-addition of proteinase K from 60 minutes to 15 minutes.

The DNA accumulated from each sample via each method of extraction was then analyzed in duplicate via qPCR with negative and positive controls. Detection of *T. cruzi* was performed using primers Cruzi 1 and Cruzi 2 and the probe Cruzi 3 described by Piron et al. (2007), which amplifies a fragment of 166 base pairs (bp) in the satellite DNA of all T. cruzi lineages (Table 2.2). The assay exhibits a specificity of 100%, a 95% detection limit positioned at 2.07 parasites/mL, and a 50% detection limit positioned at 0.80 parasites/mL (Piron et al. 2007).

Table 2.2. Primer and probe sets used in qPCR analysis for detection of *Trypanosoma cruzi* DNA and β-actin. Cruzi TaqMan assay developed by Piron et al. (2007). β-actin TaqMan assay developed by Piorkowski et al. (2014).

TaqMan assay	Primer	Sequence (5'-3')	Probe	Sequence (5'-3')
Cruzi	Cruzi 1	ASTCGGCTGATCGTTTTCGA	Cruzi 3	CACACACTGGACACCAA
	Cruzi 2	AATTCCTCCAGCAGCGGATA		
β-actin	Act.f	GTSTGGATYGGHGGHTCBATC	Act.p	ACCTTCCAGCAGATGTGGATC
	Act.r	GAYTCRTCRTAYTCCTSCTTG		

T. cruzi DNA was successfully extracted and recovered via qPCR for spiked WB samples processed with each extraction method from both the Zymo Research and Qiagen kits, though each procedure varied in the recovery of target DNA as measured by

Ct values of the qPCR output (Figure 2.1, Table 2.3). DNA extraction optimization methods employing the Qiagen DNeasy Blood & Tissue Kit outperformed those relying on Zymo Research kits for both WB and WB stored on Nobuto strips (Table 2.3). At medium spiking loads, the difference between the mean Ct value recovered for WB versus WB-saturated Nobuto strips was substantially lower for extraction optimization method A (0.25) compared to the variances evidenced with extraction optimization method B (1.77), the Quick-DNA/RNA Pathogen Miniprep kit (1.1), and the ZR-DuetTM DNA/RNA MiniPrep Plus kit (3.95). Similarly, differences between the mean Ct values generated by high spiking loads for WB and WB stored on Nobuto blood filter paper were lower for the Qiagen DNeasy Blood & Tissue Kit methods (0.65 for extraction optimization method A and 1.74 for extraction optimization method B) than for either of the Zymo Research kits (2.31 for ZR-DuetTM DNA/RNA MiniPrep Plus kit and 5.22 for the Quick-DNA/RNA Pathogen Miniprep kit) (Table 2.3).

Figure 2.1. ***Trypanosoma cruzi*** **DNA recovery from spiked canine whole blood specimens. Bars indicate one standard deviation. As detailed in the text for use with Qiagen DNeasy Blood & Tissue Kit: QIA A = extraction optimization method A; QIA B = extraction optimization method B. ZR Pathogen = Zymo Research Quick-DNA/RNA Pathogen Miniprep; ZR Duet = Zymo Research ZR-DuetTM DNA/RNA MiniPrep Plus kit. Ct=cycle threshold; Nobuto = whole blood samples processed from Nobuto blood filter papers; Blood = whole blood samples processed directly; MED = medium spiking load; HI = high spiking load.**

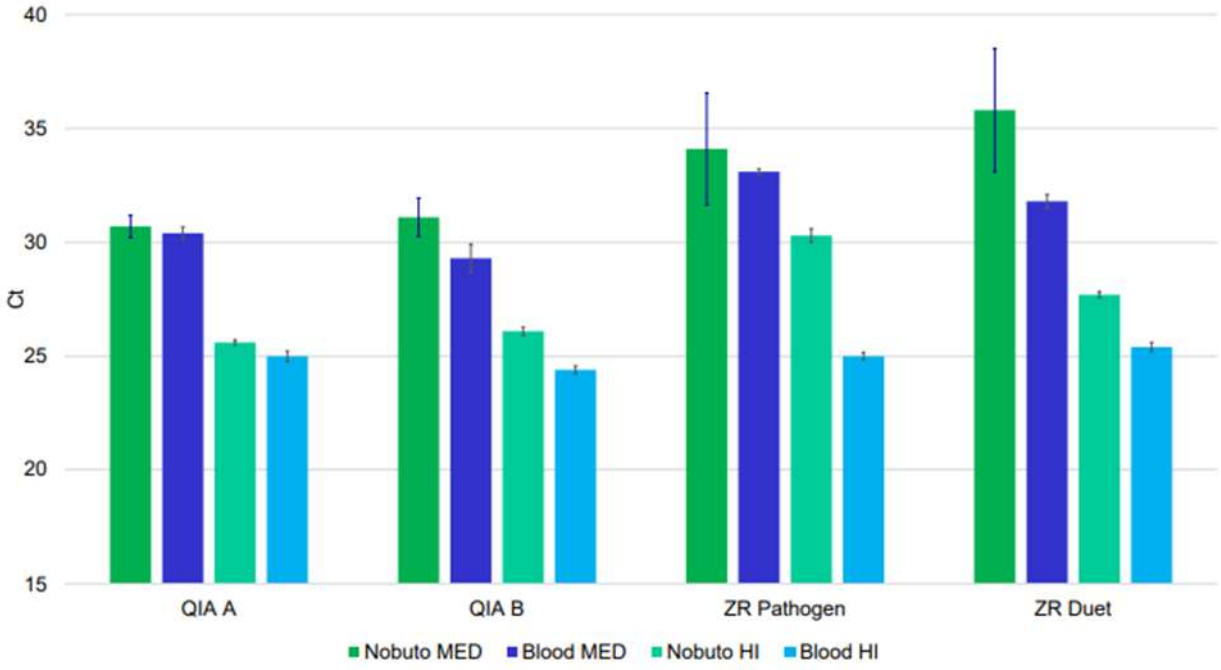

Table 2.3. Quantitative PCR results for *Trypanosoma cruzi* assays performed on spiked canine whole blood specimens. DNA extraction optimization methods detailed in text. Ct = cycle threshold; WB Direct = whole blood samples processed directly; WB Nobuto = whole blood samples processed from Nobuto blood filter papers; MED = medium spiking load; HI = high spiking load.

DNA Extraction Method	WB Direct				WB Nobuto			
	MED		HI		MED		HI	
	Mean Ct Values	Standard Deviation	Mean Ct Values	Standard Deviation	Mean Ct Values	Standard Deviation	Mean Ct Values	Standard Deviation
Qiagen Extraction Optimization Method A	30.4	0.28	25.0	0.23	30.7	0.49	25.6	0.11
Qiagen Extraction Optimization Method B	29.3	0.62	24.4	0.17	31.1	0.84	26.1	0.18
Zymo Research Quick-DNA/RNA Pathogen Miniprep	33.1	0.12	25.0	0.16	34.1	2.64	30.3	0.30
Zymo Research ZR-Duet™ DNA/RNA MiniPrep Plus kit	31.8	0.30	25.4	0.21	35.7	2.70	27.7	0.13

2.3.2. Optimization with skunk WB samples

Once we established the enhanced capacity of the Qiagen DNeasy Blood & Tissue Kit to recover *T. cruzi* DNA from WB specimens stored on Nobuto strips, we repeated extraction optimization methods A and B with an additional alternate extraction procedure on samples of skunk WB archived on Nobuto blood filter paper (Table 2.1). Since the *T. cruzi* status of these individuals was unknown, we employed a qPCR assay developed to detect β-actin-encoding DNA from mammals (Piorkowski et al. 2014) (Table 2.2).

In preparation for DNA extraction, the biobanked skunk Nobuto strips were processed to ensure equivalent quantities of WB were sampled from each. One section approximately 5x5 mm in length was cut from each WB-saturated Nobuto strip and further divided into four, equally-sized pieces that were transferred into a single 1.5 mL tube with ATL lysis buffer for continued processing. DNA was extracted from each strip in accordance with extraction optimization A and B as described above. In addition, we evaluated a third extraction optimization with the Qiagen DNeasy Blood & Tissue Kit for each of these archived WB specimens. Extraction optimization C replicates the steps of extraction optimization B with extension of the 56°C incubation period following the addition of proteinase K from 15 minutes to 16 hours. The DNA extracted from each treatment was then run in duplicate on qPCR with negative and positive controls.

Each of the extraction optimization methods for the Qiagen kit recovered β-actin DNA as indicated by qPCR analysis (Figure 2.2, Table 2.4). Extraction optimization A outperformed the other methods for all three samples, recovering an average of 60% more DNA than the other two protocols. In the case of relatively higher concentrations of DNA (samples from the western spotted and striped skunks), extraction optimization B extracted more DNA than did method C, whereas the latter method excelled with the comparatively lower amount of β-actin DNA present in the American hog-nosed skunk sample (Figure 2.2). Subsequent evaluation of these WB samples for the presence of *T. cruzi* DNA was performed using extraction optimization A, identifying parasitic

infection in the western spotted skunk (Ct value of 27.8) and not detecting T. cruzi DNA from either the American hog-nosed or striped skunk (Chapter 3).

Figure 2.2. β-actin DNA recovered from skunk whole blood archived on Nobuto blood filter paper strips. DNA extracted according to protocols outlined in the text for Qiagen DNeasy Blood & Tissue Kit: Method A = extraction optimization method A; Method B = extraction optimization method B; Method C = extraction optimization method C. Ct=cycle threshold.

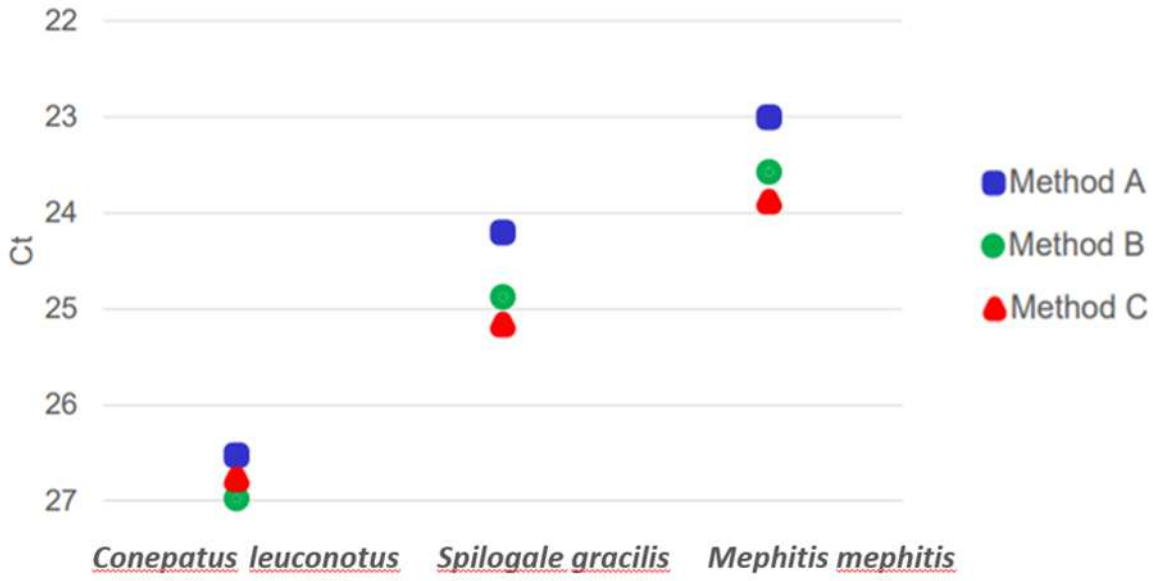

Table 2.4. Quantitative PCR results for β-actin assays performed on skunk whole blood samples archived on Nobuto blood filter strips and processed using three optimized Qiagen DNeasy Blood & Tissue Kit DNA extraction methods as detailed in text. Ct=cycle threshold.

Samples	Mean Ct Values			A/B Fold Difference	A/C fold Difference
	Extraction optimization method A	Extraction optimization method B	Extraction optimization method C		
Conepatus leuconotus, ASK 11860	26.5	27.0	26.8	1.4	1.2
Spilogale gracilis, ASK 11844	24.3	24.9	25.2	1.6	1.9
Mephitis mephitis, ASK 11857	23.0	23.6	23.9	1.5	1.8

2.4. Discussion and Conclusions

Optimization of DNA extraction from WB preserved on blood filter papers can extend the reach, efficacy, and reliability of infectious disease research and surveillance, particularly for investigations in which field constraints typically limit the capacity for body fluid collection and preservation. Developing these procedures for use with commercially-available and user-friendly kits and Nobuto strips further enhances their cost-efficiency and simplicity of application to molecular epidemiological studies. We validate one such optimized DNA extraction method; the Qiagen DNeasy Blood & Tissue Kit with the addition of an initial 90°C incubation step and extended, post-proteinase K 56°C incubation step provided the most accurate and precise recovery of *T. cruzi* DNA. Future work should adopt and continuously adapt this protocol to maximize the sensitivity of these techniques across a range of storage and extraction products, body fluid specimens, target pathogens, and host species sampled.

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3. CHAGAS DISEASE (*TRYPANOSOMA CRUZI*) IN TEXAS SKUNKS (MAMMALIA: MEPHITIDAE)

3.1. Overview

Chagas disease is one of the world's most neglected tropical diseases, infecting over six million people across the Americas. The hemoparasite *Trypanosoma cruzi* is the etiological agent for the disease, circulating in domestic, peridomestic, and sylvatic transmission cycles that are maintained by triatomine vectors and a diversity of wild and synanthropic hosts. One such wildlife host previously reported to play a role in the transmission of Chagas disease within the southern United States is the striped skunk (*Mephitis mephitis*). Public health and wildlife management interventions targeting the adverse effects of *T. cruzi* rely on an understanding of the dynamics driving the ecology of this zoonotic pathogen. To this end, we conducted a PCR-based surveillance of *T. cruzi* in 235 wild skunks, representing four species, across 76 counties and ten ecoregions in Texas, USA, along with an evaluation of risk factors associated with infection. We recovered an overall *T. cruzi* prevalence of 17.9% for all mephitid taxa aggregated, ranging between 6.7% for eastern spotted skunks (*Spilogale putorius*) and 42.9% for western spotted skunks (*S. gracilis*). We report the first cases of *T. cruzi* infection in eastern spotted and American hog-nosed skunks, of important note for conservation medicine since populations of both species are declining within Texas. Although not statistically-significant, we also detected trends for juveniles to exhibit greater infection risk than adults and for differential sex biases in *T. cruzi* prevalence

between taxa, which align with variations in species-specific home range sizes and seasonal activity patterns. No statistically-significant geographic or taxonomic risk factors were identified. Our study contributes key data for population viability analyses and epidemiologic models in addition to providing a baseline for future *T. cruzi* surveillance among skunks and other wildlife species.

3.2. Introduction

The zoonotic hemoflagellate parasite *Trypanosoma cruzi* is the etiologic agent of Chagas disease (American trypanosomiasis), which infects six to seven million people across the Americas (WHO 2020). Although *T. cruzi* has historically inflicted the greatest social and economic costs on poor, rural populations throughout Latin America, a growing number of autochthonous cases of Chagas disease have been reported in the United States in recent years (Bern 2015; Garcia et al. 2015, 2017; Beatty and Klotz 2020). In light of the underreporting of locally-acquired infections and the globalized spread of infection via human migrations, the actual disease burden of the continental United States alone is estimated to be as high as 300,000 cases (Schmunis and Yadon 2010, Bern 2015, Montgomery et al. 2016). To mitigate the proliferation of Chagas disease within North America, effective vector control and disease surveillance strategies must be developed from a holistic understanding of the complex adaptive system that underlays *T. cruzi*'s transmission cycle (Silveira and Vinhaes 1999, Levin 2005, Jansen et al. 2015, Moo-Millan et al. 2019).

The domestic, peridomestic, and sylvatic transmission cycles of *T. cruzi* are maintained in the southern United States by eleven triatomine vectors and a diversity of

wild, domestic, and exotic mammalian hosts, many of which exhibit acute, latent, and chronic Chagas disease symptoms analogous to those evidenced in human patients (Ryan et al. 1985, Kjos et al. 2008, Bern et al. 2011, Bern 2015, Malik et al. 2015, Gunter et al. 2017, Hodo et al. 2018). Among the wildlife taxa reported to play a role in Chagas disease ecology within North America are striped skunks (*Mephitis mephitis*) (McKeever et al. 1958; Ryan et al. 1985; Brown et al. 2010; Charles et al. 2013; Matamoros 2016; Galaviz-Silva et al. 2017; Kramm et al. 2017, 2019; Hodo et al. 2018). Owing to their habitat use and adaptive behavioral traits, striped skunks and other New World mephitids (Mammalia: Mephitidae) are exposed to multiple routes of potential *T. cruzi* infection, though ingestion of infectious triatomines is widely supported as the primary means of exposure (Davis et al. 1980, Ceballos et al. 2006, Roellig et al. 2009, Ribeiro et al. 1987, Charles et al. 2013, Jansen et al. 2015). Moreover, striped skunks are reported to exhibit adverse clinical manifestations of Chagas disease such that *T. cruzi* infection among imperiled North American mephitid species is anticipated to cause some degree of morbidity or mortality, making *T. cruzi* of marked conservation medicine concern (Davis et al. 1980, Ryan et al. 1985, Gompper and Jachowski 2016, Helgen et al. 2016).

To date, only McKeever et al. (1958) have attempted to conduct *T. cruzi* surveillance across a significant number of skunks within an extensive geographic area (Table 1). The risk for *T. cruzi* transmission is especially high in Texas, which encompasses impoverished and vulnerable communities with a history of neglected tropical diseases (Hanford et al. 2007, Sarkar et al. 2010, Hotez et al. 2012a,b). Texas is

also the only state to overlap the range of all five skunk species endemic to the United States, which are distributed across a wide diversity of its eleven ecoregions and variety of anthropogenically-influenced habitats (Gould et al. 1960, Schmidly 1994). Therefore, we aimed to investigate skunk involvement in Chagas disease ecology by: 1. evaluating the prevalence of circulating *T. cruzi* infections in Texas mephitids through polymerase chain reaction (PCR)-based surveillance and 2. exploring factors contributing to their infection risk (i.e., sex, taxonomy, age, and location). Risk factors that we identify can inform ecological niche models to increase the precision and accuracy of predictions related to vector-host interactions and the transmission dynamics of Chagas disease across geographic and temporal scales, which can ultimately be employed in public health strategy development (Peterson 2006, Peterson et al. 2002; Gurgel-Gonçalves et al. 2012). Moreover, an understanding of skunk disease ecology based on this field survey of *T. cruzi* infections can assist in wildlife conservation and management planning, such as creating baseline data for population viability analyses incorporating parasite-induced morbidity and mortality estimates (Wilber et al. 2020a).

Table 3.1. Previous reports of *Trypanosoma cruzi* prevalence in North American striped skunks (*Mephitis mephitis*). Study locations and the methodology employed for pathogen identification are also noted. Counties are included for locations within Texas, USA.

Location	Method(s) of diagnosis	Prevalence of *T. cruzi* infection (number positive individuals/total number tested)	References
Georgia and Florida, USA	Culture grown from kidney tissue	3/306	McKeever et al. (1958)
California, USA	Indirect hemagglutination and histology	1/1	Ryan et al. (1985)
Arizona and Georgia, USA	Indirect immunofluorescent antibody (IFAT)	Arizona: 3/34 Georgia: 1/3	Brown et al. (2010)
Uvalde county, Texas, USA	Hemoculture, PCR, IFAT and Chagas Stat-Pak assay	3/4 (hemoculture, PCR) 4/4 (serology)	Charles et al. (2013)
El Paso county, Texas, USA	PCR	3/24	Matamoros (2016)
Nuevo León, Mexico	PCR, blood smears, and histopathology	11/34	Galaviz-Silva et al. (2017)
Bexar County, Texas, USA	PCR	9/33	Kramm et al. (2017)
Bastrop County, Texas, USA	PCR	2/3	Hodo et al. (2018)
Bexar County, Texas, USA	PCR	3/13	Soria (2018)
Bexar County, Texas, USA	Chembio Assay Test, PCR	1/1 (both serology and PCR)	Kramm et al. (2019)

3.3. Materials and Methods

3.3.1. Ethical approval

This study was exempt from ethical approval from the Institutional Animal Care and Use Committee (IACUC) at Texas A&M University because our methodology did not constitute “use of animals” as defined by IACUC.

3.3.2. Sample collection

Whole blood (WB) specimens were opportunistically collected from living and deceased mephitids within Texas, USA and stored on Type I Nobuto blood sampling

filter paper (ADVANTEC, Tokyo, Japan) prior to deoxyribonucleic acid (DNA) extraction. Road-killed carcasses were sampled between March 2017 and January 2019. Additional samples were provided in the form of excess WB collected primarily for independent purposes (e.g., genetic analyses, diagnostic procedures, health evaluations) by: 1. biologists researching skunk species in Texas with animal use protocols approved by the IACUC of their home institutions, 2. wildlife rehabilitators licensed by Texas Parks and Wildlife Department, and 3. personnel at Angelo State University for Angelo State Natural History Collections' skunk biobanking project. Information related to the following factors were recorded for each individual sampled: 1. species, 2. sex, 3. age (non-sexually-mature juveniles less than one year of age; sexually-mature adults one year of age or older as determined by diagnostic morphological indicators per Crabb [1944] and Verts [1967]), 4. county of origin, and 5. collection date.

3.3.3. DNA extraction and PCR

When compared to serologic and culturing methods, PCR is the optimal means for detecting circulating *T. cruzi* during the acute phase of infection, even when immune response is low or absent (Gürtler et al. 1993; Ferreira and Borges 2002; Picka et al. 2007; Braz 2008; Jiménez-Coello et al. 2008, 2012; Kramm et al. 2019). PCR can also identify *T. cruzi* with high specificity in the more chronic phases of infection if parasites have been liberated and are present in peripheral blood and/or if reinfection has occurred (Braz 2008, Jiménez-Coello et al. 2008).

DNA was extracted from WB samples stored on Nobuto strips using the DNeasy Blood & Tissue Kit (QIAGEN, MD, USA) with the optimized DNA extraction

methodology outlined in Chapter 2. Extracted DNA was added to a 20 µl reaction with TaqMan Fast Advanced Master Mix (Thermo Fisher Scientific, MA, USA), then analyzed on a ViiA 7 Real Time (RT) PCR System (Thermo Fisher Scientific). To detect T. cruzi DNA, Piron et al. (2007)'s Cruzi 1/2/3 assay was used, while the Actin f/r/p assay described by Piorkowski et al. (2014) was employed to detect β-actin (Table 2.2). An extraction negative control, no template control (5 µl of laboratory grade H2O), and a positive control (5 µl of *T. cruzi*/mouse DNA sample) was included in each RT-PCR run. DNA samples were tested in triplicate for *T. cruzi* DNA detection and singly for β-actin detection.

Samples with Ct values below 40 were interpreted as positive in the Cruzi 1/2/3 assay, and those with Ct values below 38 were identified as positive in the Act.f/r/p assay. If all samples for an individual were negative based on these measures, but at least one sample was within 1 Ct value of the cut-off point, the WB specimen was re-extracted, re-tested, and reanalyzed per the protocols detailed above. Individuals were defined as positive for *T. cruzi* if at least one of their three DNA samples was positive in the Cruzi 1/2/3 assay. A skunk was identified as negative for *T. cruzi* if all three DNA samples were negative in the Cruzi 1/2/3 assay but the sample was positive in the Act.f/r/p assay.

3.3.4. Statistical analysis

County information for each sampled skunk was used to delineate the ecoregion and level of Chagas disease hotspot in which the individual was located. Since the ecoregions of Texas cross county lines, counties sampled for skunks were assigned to

ten ecoregions following the Texas Parks and Wildlife Department's deer management ecoregion assessments (https://apps.tpwd.state.tx.us/static/dmpEcoregionTable1.pdf), which represent a modification of Gould's ecoregions of Texas (Gould et al. 1960). Each county was also appointed a value between 0 and 3 to represent the degree to which positive cases of Chagas disease were previously recorded in the jurisdiction. A value of one was added to a county's "Chagas disease hotspot" sum for the presence of each of the following: *T. cruzi*-positive triatomine vectors (2013-2018), *T. cruzi*-positive canine sentinels (2013-2015), and autochthonous human Chagas disease cases (2013-2018) as reported by the Texas Department of State Health Services (TDSHS 2019).

Pearson's Chi-squared tests (α =0.05) were used to evaluate *T. cruzi* prevalence within each species and with all mephitid species pooled for deviations between: 1. species, 2. sexes, 3. ages, 4. ecoregions, and 5. Chagas disease hotspots. When 20% or more of the cells in the Chi-squared tests had expected counts below 5, likelihood ratio values (α =0.05) were assessed. Odds ratios were calculated for sex and age based on all skunk species and within each mephitid taxon. Power analyses were also conducted in order to assess the statistical significance of our final sampling. All statistical analyses were performed on STATA 16.1 software (StataCorp, TX, USA).

3.4. Results

3.4.1. Prevalence of *T. cruzi* in Texas skunks

WB samples were collected and successfully extracted for DNA from a total of 235 individual skunks between March 2004 and June 2019: 42 *C. leuconotus*, 171 *M. mephitis*, seven *S. gracilis*, and 15 *S. putorius*. No hooded skunk (*M. macroura*)

specimens were available for analysis. Overall, 42 mephitids (17.9%) tested positive via PCR for *T. cruzi* infection. Of these positive individuals, 30 were striped skunks, eight were American hog-nosed skunks, three were western spotted skunks, and one was an eastern spotted skunk. Within skunk species, the resulting prevalence of *T. cruzi* ranged from 6.7% (*S. putorius*) to 42.9% (*S. gracilis*) (Figure 3.1). The variation in *T. cruzi* infection between species was not statistically-significant, a pattern that was consistent when both species of *Spilogale* were pooled for analysis and across paired species comparisons (Table 3.2).

Figure 3.1. Prevalence values (%) for circulating *Trypanosoma cruzi* infections in Texas skunk species. "All species" represents the prevalence value for all skunk species pooled. "*Spilogale* spp." represents the prevalence value for all spotted skunk species pooled. Prevalence values reported as number of *T. cruzi*- positive individuals/total number of individuals tested are as follows: all species pooled = 42/235; *Conepatus leuconotus* = 8/42; *Mephitis mephitis* = 30/171; *Spilogale gracilis* = 3/7; *S. putorius* = 1/15; *Spilogale* spp. pooled = 4/22.

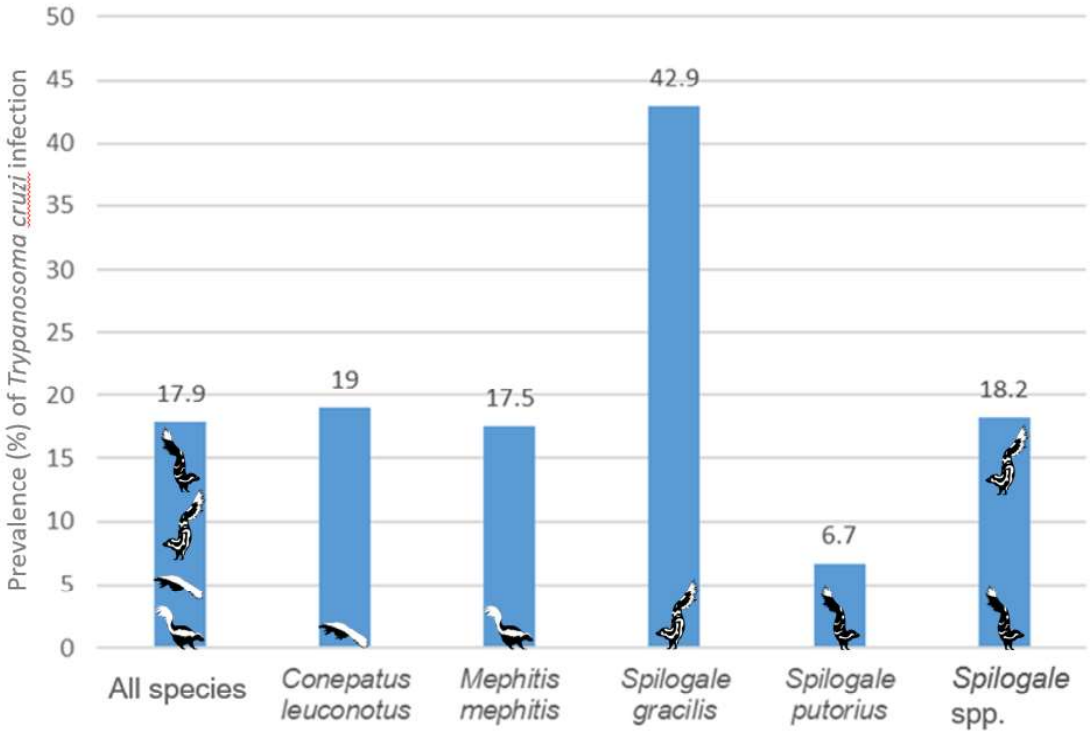


Table 3.2. Pearson's chi-squared or likelihood ratio values for variables evaluated as risk factors for Trypanosoma cruzi infection in all skunk species pooled and within *Conepatus leuconotus* and *Mephitis mephitis*. Variables analyzed include: age (<1 year vs. ≥1 year); ecoregion per Gould's ecoregions of Texas (Gould et al. 1960); Chagas disease hotspot calculated per description in section 3.3.4.; sex (female vs. male); species (prevalence between species); and genus (prevalence between genera with *Spilogale* spp. pooled). *n*=total number of individuals tested; J = total number of juveniles <1 year tested; A = total number of adults ≥1 year tested; F = total number of females tested; M = total number of males tested.

Taxon	Variable	*n*	Pearson's Chi Squared	*P*-value
All skunks	Age	235 (J=9; A=226)	1.50	0.22
	Ecoregion	234	12.12*	0.21*
	Chagas disease hotspot	234	2.62	0.45
	Sex	120 (F=41; M=79)	0.13	0.72
	Species	235	4.01*	0.26*
	Genus	235	0.05	0.97
Conepatus leuconotus	Age	42 (J=2; A=40)	1.31	0.25
	Ecoregion	41	0.25	0.62
	Chagas disease hotspot	41	3.09*	0.21*
	Sex	33 (F=8; M=25)	0.88	0.35
Mephitis mephitis	Age	171 (J=6; A=165)	1.07	0.30
	Ecoregion	171	8.64*	0.47*
	Chagas disease hotspot	171	6.75*	0.08*
	Sex	66 (F=32; M=34)	1.03	0.31

*20% or more of cells have expected count less than 5, so likelihood ratio values reported instead of Pearson's chi-squared values.

3.4.2. Age variation in *T. cruzi* infections

All sampled skunks were definitively classed according to age (juveniles <1 year old; adults ≥1 year old) with 226 adults and nine juveniles identified in total. Six *M. mephitis*, two *C. leuconotus*, and a single *S. putorius* were juveniles, of which 33.3%, 50%, and 0%, respectively, tested positive for *T. cruzi* infection. In comparison, adult striped skunks, American hog-nosed skunks, and eastern spotted skunks exhibited *T. cruzi* infection prevalence of 17%, 17.5%, and 7.1%, respectively. With all mephitid species aggregated for analysis, juvenile skunks were approximately 2.4 times more likely to test positive for *T. cruzi* than were adults, though without statistically-

significant support (*P*-value = 0.2; Table 3.3). Similarly, Chi-squared tests and likelihood ratio values failed to recover any significant relationship between age and *T. cruzi* infection in skunks overall or across species (Table 3.2).

3.4.3. Sex variation in *T. cruzi* infections

Extensive degradation and/or damage of road-killed and otherwise deceased skunks precluded the determination of sex for 115 sampled individuals (49% of the total tested). However, 41 females and 79 males were incontrovertibly identified. No females were sampled for *S. gracilis* and only one was sampled for *S. putorius*. Overall, there was no statistically-significant difference in the prevalence of *T. cruzi* infections in female skunks (24.4%) and male skunks (21.5%; Tables 3.2, 3.3). When evaluated at the species level, female striped skunks exhibited higher *T. cruzi* prevalence (28.1%) than males (17.6%), whereas prevalence were greater for male American hog-nosed skunks (28%) than for females (12.5%) (Tables 3.2, 3.3).

Table 3.3. Prevalence and odds ratio values for variables associated with *Trypanosoma cruzi* infection in pooled and individual species of Texas skunk. *n*=total number of individuals tested.

Taxon	Risk factor	*n*	Positive	Prevalence (%)	Odds ratio	95% Confidence interval	*P*-value
Skunks	Sex						
	Female	41	10	24.4	1.18	0.43-3.1	0.44
	Male	79	17	21.5			
	Age (years)						
	<1	9	3	33.3	2.4	0.37-11.76	0.2
	≥1	226	39	17.3			
Conepatus leuconotus	Sex						
	Female	8	1	12.5			
	Male	25	7	28.0	0.37	.01-3.95	0.35
	Age (years)						
	<1	2	1	50	4.71	0.05-380.9	0.35
	≥1	40	7	17.5			
Mephitis mephitis	Sex						
	Female	32	9	28.1	1.83	0.49-7.17	0.24
	Male	34	6	17.6			
	Age (years)						
	<1	6	2	33.3	2.45	0.21-17.92	0.28
	≥1	165	28	17.0			
Spilogale gracilis	Sex						
	Female	0					
	Male	7	3	42.9			
	Age (years)						
	<1	0					
	≥1	7	3	42.9			
S. putorius	Sex						
	Female	1	0	0			
	Male	13	1	7.7			
	Age (years)						
	<1	1	0	0			
	≥1	14	1	7.1			

3.4.4. Geographic variation in *T. cruzi* infections

Only one individual, an adult male *C. leuconotus* that tested negative for *T. cruzi*, was unable to be identified to the county-level. At least one skunk was collected from 76 counties, which represent all ten of Gould's ecoregions of Texas (Figure 3.2; Table 3.4). American hog-nosed skunks were sampled in 13 counties, eastern spotted skunks in 5 counties, western spotted skunks in 4 counties, and striped skunks in 66 counties. *T. cruzi* infections were identified in mephitids collected in 24 counties (31.6% of the total counties surveyed) ranging from 7.7% (Val Verde county) to 100% (Coryell, Guadalupe, and Webb counties) prevalence with an average of 44% and a median of 37% (Figure 3.2; Table 3.5). When counties were clustered by ecoregion, *T. cruzi*-positive skunks were recorded for six of the ten regions with prevalence ranging from 7.1% (Rolling Plains) to 27.3% (Blackland Prairies) with a median of 20% (Table 3.4). We did not recover any statistically-significant relationship between skunk infections and county or ecoregion (Table 3.2). However, a weak statistical association between *T. cruzi* prevalence for striped skunks and location within a Chagas disease hotspot (likelihood ratio value of 6.75 with a P-value of 0.08) was recovered by this analysis (Table 3.2).

Figure 3.2. Geographic distribution of *Trypanosoma cruzi* prevalence values for skunks across Texas. Values were calculated by aggregating skunk species within each county. Only counties in which at least one skunk was sampled are shown.

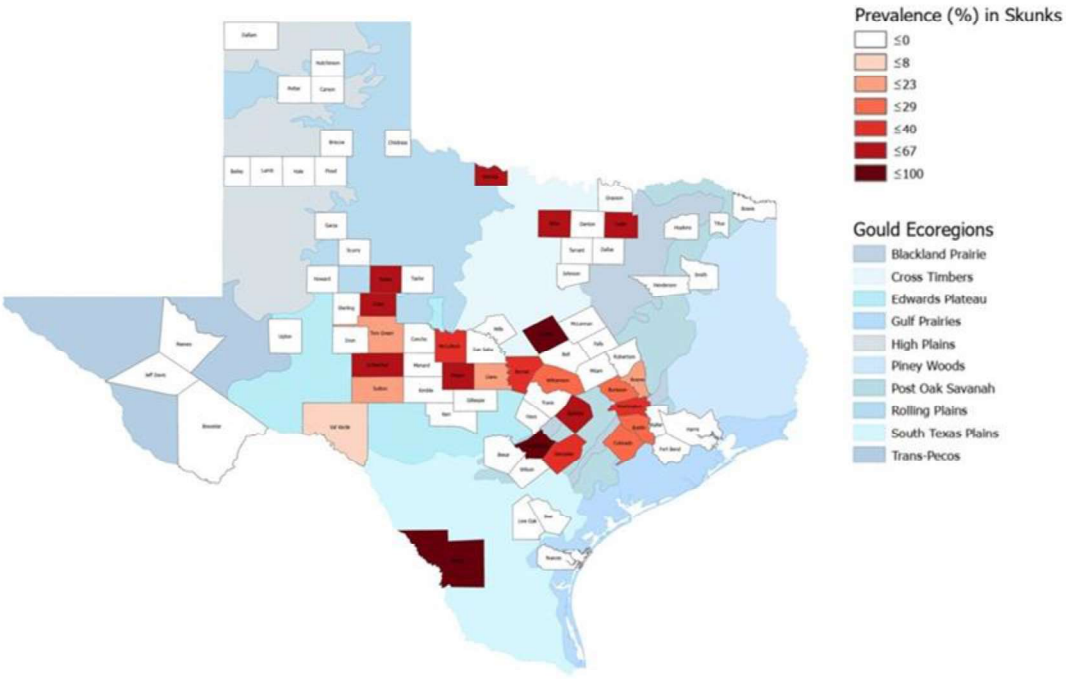


Table 3.4. *Trypanosoma cruzi* prevalence values for each Gould ecoregion of Texas (Gould et al. 1960). Skunk species were aggregated within each ecoregion. *n*=total number of individuals tested.

Ecoregion	*n*	Positive	Prevalence (%)
Blackland Prairies	11	3	27.3
Cross Timbers	20	4	20
Edwards Plateau	103	20	19.4
Gulf Prairies	1	0	0
High Plains	7	0	0
Piney Woods	11	0	0
Post Oak Savannah	57	13	22.8
Rolling Plains	14	1	7.1
South Texas Plains	6	1	16.7
Trans Pecos	4	0	0

Table 3.5. *Trypanosoma cruzi* prevalence in all skunk species by county as reported by this study. Included for comparison are the previous records of *T. cruzi*-positive triatomine, animal host, and autochthonous human cases used to calculate our "Chagas disease hotspot" values. *n*=total number of individuals tested. Previous positive cases from 2013-2018 are as reported by TDSHS (2019): LAH = Locally acquired human cases (2013-2018); T = positive triatomines (2013-2018); A = positive animals (2013-2015).

County	*n*	Positive	Prevalence in Skunks (%)	Previous Positive Cases
Austin	7	2	28.6	A
Bastrop	2	1	50	A, T
Brazos	12	2	16.7	A
Burleson	7	2	28.6	A, T
Burnet	5	2	40	T
Coke	3	2	66.7	
Collin	5	3	60	T
Colorado	8	2	25	A
Coryell	1	1	100	LAH, T
Gonzales	3	1	33.3	A
Guadalupe	1	1	100	A, T
Llano	5	1	20	
Mason	2	1	50	
McCulloch	3	1	33.3	
Nolan	2	1	50	
Schleicher	2	1	50	
Sutton	5	1	20	
Tom Green	40	9	22.5	T
Val Verde	13	1	7.7	A
Washington	6	2	33.3	
Webb	1	1	100	
Wichita	2	1	50	T
Williamson	7	2	28.6	A, T
Wise	2	1	50	T

3.5. Discussion

Our sampling of 235 individual skunks from four species across Texas is the largest and most geographically-extensive *T. cruzi* surveillance of mephitid taxa to date. All prior records of *T. cruzi* infections in North American skunks have been reported from *M. mephitis*, with prevalence ranging between 0 and 100% and a median incidence of infection of 32%, 38%, and 100% based on PCR, culture, and serology, respectively

(Table 3.1). Our value of 18% PCR positivity based on 171 striped skunks is substantially lower than the pooled average (48%) for the total 112 striped skunks tested prior to our investigation, demonstrating the imprecision of low sampling sizes for *T. cruzi* surveillance in this species (Table 3.1).

Previous evaluations of *T. cruzi* in members of the genus *Spilogale* have been limited and recovered negative results for two Mexican southern spotted skunks (*S. angustifrons*) and seven eastern spotted skunks (McKeever et al. 1958, Zavala-Velázquez et al. 1996). As such, ours are the first records of *T. cruzi* infections in the genus *Spilogale* as well as for the species *S. gracilis* and *S. putorius*. We also report the first incidence of *T. cruzi* for American hog-nosed skunks, consonant with the prevalence of infections in South American Molina's hog-nosed skunks (*C. chinga*) and Central American striped hog-nosed skunks (*C. semistriatus*) (Zeledón et al. 1975, Montamat et al. 1991, Pietrokovsky et al. 1991, Wisnivesky-Colli et al. 1992, de Luca D'Oro et al. 1993, Ceballos et al. 2006, Cardinal et al. 2008). The discovery of *T. cruzi* in eastern spotted and American hog-nosed skunks is of conservation concern since both species have been exhibiting population declines across their ranges within the United States (Meaney et al. 2006, Gompper and Jachowski 2016). Any adverse effects of Chagas disease analogous to those described for striped skunks may threaten already-imperiled *S. putorius* and *C. leuconotus* populations with significant parasite-induced morbidity and mortality (Davis 1980 et al., Ryan et al. 1985).

Our detection of circulating *T. cruzi* infections in four species representing all three New World mephitid genera provided a unique opportunity to evaluate risk factors

associated with *T. cruzi* infection in skunks; however, sampling bias and small sample sizes hindered our capacity to assess variations between age classes with statistical confidence (Tables 3.2, 3.3). The non-statistically-significant trend of higher *T. cruzi* risk for juveniles of all skunk species pooled, *M. mephitis*, and *C. leuconotus* aligns with previous findings for a smaller sample of striped skunks in El Paso county, Texas (1/2 *T. cruzi*-positive juveniles versus 2/22 positive adults) as well as for laboratory rats (*Rattus norvegicus*) and domestic dogs and suburban common opossums in Mexico (Pérez et al. 2011, Matamoros 2016, Arce-Fonseca et al. 2017, Galaviz-Silva et al. 2017) (Table 3.3). However, power analyses posit that minimum effect sizes of 42%, 66%, and 39% difference would have been necessary to detect any statistically-significant trends between juveniles and adults based on the sample sizes analyzed for all skunk species pooled, *C. leuconotus*, and *M. mephitis*, respectively. Increased infection risk in juveniles may be a function of detrimental immunoendocrine response, which promotes elevated parasitemias in younger animals (Pérez et al. 2011). *T. cruzi* infection in neonates can also be exacerbated by vertical transmission of parasites as evidenced in bats, laboratory rodents, domestic dogs, and humans (Andrade 1982, Moreno et al. 2003, Sánchez Negrette et al. 2005, Añez et al. 2009, Rodríguez-Morales et al. 2011, Alkmim-Oliveira et al. 2013, Howard et al. 2015). Since other studies have reported more severe disease manifestations and elevated mortality in *T. cruzi*-positive younger individuals when compared to adults, if the described trend towards higher infection risk in juvenile skunks is supported by future research (e.g., a minimum of 199 each of juveniles and adults would need to be tested to verify a deviation of 10% in the prevalence between

the two groups), the finding would hold significance for the population ecology of these species, especially for those of conservation concern (Moreno et al. 2003, Kjos et al. 2008, Rodríguez-Morales et al. 2011).

In contrast, older individuals exhibiting a greater risk for T. cruzi infection come from populations of domestic dogs (Canis lupus familiaris) in Panama and Mexico, Southern Plains woodrats (Neotoma micropus), domestic cats (Felis catus), urban common opossums (Didelphis marsupialis), and Virginia opossums (D. virginiana) in Mexico, and raccoons (Procyon lotor) in Louisiana, USA, all attributed to increased exposure to vectors and pathogens accumulated over time (Ruiz-Piña and Cruz-Reyes 2002, Jiménez et al. 2008, 2010, 2015; Saldaña et al. 2015; Galaviz-Silva et al. 2017, Majeau et al. 2020).

Other studies on *T. cruzi* prevalence in mephitids have reported no difference was found between juvenile and adult *T. cruzi* infection incidence for striped skunks based on 4 samples (1 juvenile, 3 adults) in Uvalde County, Texas or striped skunks based on 34 individuals (6 juvenile, 28 adult) in Neuva León, Mexico (Charles et al. 2013, Galaviz-Silva et al. 2017). Age-bias in *T. cruzi* infections was also absent in populations of domestic dogs in Panama and raccoons and rodent species in south Texas (Pineda et al. 2011, Charles et al. 2013). Therefore, our inability to find a statistically-significant correlation between age and *T. cruzi* infection risk among Texas skunks may represent the absence of any relationship between these conditions.

Our surveillance also did not recover any statistically-significant difference in *T. cruzi* circulating infections between male and female skunk taxa overall, with the

prevalence for both sexes varying by only 2.9% when all species were aggregated (Tables 3.2, 3.3). Similarly, previous studies found no sex bias in *T. cruzi* prevalence among 13 striped skunks in Bexar county, Texas, four striped skunks from Uvalde county, Texas, and 34 striped skunks in Mexico (Charles et al. 2013, Galaviz-Silva et al. 2017, Soria 2018). The independence of host sex and *T. cruzi* infection dynamics has been supported by other studies in relation to parasite loads in laboratory mice, susceptibility in laboratory rats, and prevalence in populations of white-eared opossums (*Didelphis albiventris*) in Argentina, Virginia opossums in south Texas, and domestic dogs in Panama (Wisnivesky-Colli et al. 1992, Pérez et al. 2011, Pineda et al. 2011, Soares et al. 2012, Zecca et al. 2020a).

Within species, sex biases in *T. cruzi* infection risk can stem from differential parasite exposure, susceptibility, virulence, or severity of clinical symptoms in mammalian hosts related to variations in female and male immunoendocrine functions (e.g. the immunosuppressive effect of androgens), behaviors, diets, habitat use, and/or morphology (Zuk and McKean 1996, do Prado et al. 1999, Rabinovich et al. 2001, Schuster and Schaub 2001, Ruiz-Piña and Cruz-Reyes 2002, Yabsley and Noblet 2002, Santos et al. 2007, Lourenço et al. 2008, Brown et al. 2010, Pinto et al. 2010, Tartalini et al. 2011, Galaviz-Silva et al. 2017, Majeau et al. 2020). Small sample size completely precluded our investigation of sex bias in *T. cruzi* infections among spotted skunk species. Based on our sampling of female and male skunks across Texas, power analyses indicate that minimum effect size differences of 29%, 45%, and 25% would have to exist between sexes in *T. cruzi* prevalence in all skunk species pooled, *C.*

leuconotus, and *M. mephitis*, respectively, for our analyses to have recovered statistically-significant trends in our data. Although we were unable to statistically validate trends related to sex bias in *T. cruzi* infection in Texas skunks, discernible, albeit non-statistically-significant, variations in parasite prevalence between male and female American hog-nosed and striped skunks were suggested by our data (Tables 3.2, 3.3).

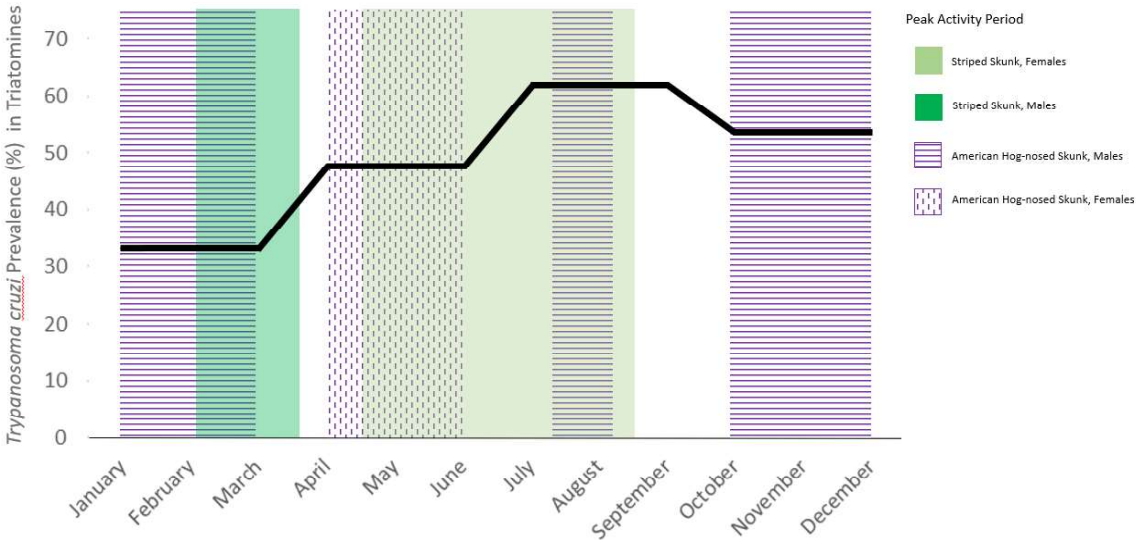
If the sex biases in these taxa's *T. cruzi* prevalence suggested by our data are real, the higher *T. cruzi* prevalence evidenced in male *C. leuconotus* appears to contrast with the elevated risk noted for female *M. mephitis*, an interspecific deviation that might reflect differences in sexually-divergent home range sizes and seasonal activity patterns among these skunk taxa. Male striped skunks are relatively tolerant of overlapping home ranges of neighboring males in Texas, and, when male and female home ranges are studied concordantly, the size of males' approximate those of females' (Storm 1972, Rosatte and Gunson 1984, Greenwood et al. 1985, Vander Lee 1995, Hansen 1997, Bixler and Gittleman 2000, Weissinger et al. 2009). Despite the extensive intra- and intersexual overlap of home ranges in American hog-nosed, the home ranges of males in Texas averaged three times that for females (Brashear et al. 2015). A widely-accepted epidemiological relationship holds that larger home range sizes of mammals correlate to use by that host species of a larger diversity of habitats and to hosts' exposure to a greater number of other potential hosts as well as vectors of infectious pathogens (Nunn et al. 2003, Lindenfors et al. 2007, Bordes et al. 2009). Therefore, greater disease risk is exhibited by mammals with larger home range sizes (Nunn et al. 2003; Lindenfors et al.

2007). In following, the larger areas traversed by male *C. leuconotus* may elevate their risk of exposure to *T. cruzi* in comparison to females with more restricted home ranges, while female and male *M. mephitis* may bear relatively equal exposure risks based on home range spans.

A complimentary explanation for the sex bias we describe in these taxa's *T. cruzi* prevalence might be tied to the interspecific variation in their sexually-divergent seasonal behavioral patterns. The highest total daily movement (in terms of both activity level and distance covered) corresponds with the period(s) of greatest energetic requirements in skunks and varies between sexes (Zhang et al. 2019). For female mephitids, total activity levels and energetic costs are greatest during the stages of lactation and young at heel, while males experience these during the mate-searching and intrasexual competitions of the breeding season (Larivière and Messier 1997, Ellsworth 2016, Zhang et al. 2019). This span of time corresponds to the period when individuals are traveling greater distances are, thus, potentially at a higher risk of exposure to *T. cruzi* (Nunn et al. 2003, Lindenfors et al. 2007, Bordes et al. 2009). In Texas, the peak breeding season for striped skunks ranges from late February through the end of March, while females are lactating with dependent young primarily between May through August and into early fall in the case of late-season litters (Patton 1974, Schmidly 1994). Inferences based on specimen collection dates, testes size measurements, and the timing of mating behavior indicate male American hog-nosed skunks participate in two breeding seasons to exploit the reproductive strategy of delayed implantation in females of the species, exhibiting peak activity January through March, August, and October

through December (Dragoo and Honeycutt 1999, Brashear et al. 2015, Ellsworth 2016). The majority of female *C. leuconotus* in Texas are lactating with young from April through late June (Bailey 1905, Taylor and Davis 1947, Patton 1974, Schmidly 1994, Ellsworth 2016). Prevalence of *T. cruzi* infections in triatomine vectors across the southern United States exhibit seasonal variations (Wozniak et al. 2015, Curtis-Robles et al. 2018a). When these seasonal behavioral patterns are aligned with *T. cruzi*-prevalence reported for Texas triatomines by Curtis-Robles et al. (2018a), the periods of greatest exposure to triatomid vectors for striped skunk females and American hog-nosed males coincide with peak parasite prevalence within triatomines. In contrast, these time periods for male striped skunks and female American hog-nosed skunks overlie seasons of lower triatomine *T. cruzi*-positivity (Figure 3.3).

Figure 3.3. Temporal intersection of *Trypanosoma cruzi* prevalence of triatomine vectors and sexually-divergent peak activity periods of American hog-nosed (*Conepatus leuconotus*) and striped skunks (*Mephitis mephitis*) in Texas. See text for detailed description of skunk activity patterns. Prevalence values of triatomine vectors per Curtis-Robles et al. (2018a).



The majority of mephitids we sampled were striped skunks (73%), and, although a substantial representation of American hog-nosed skunks was analyzed (18%), very few spotted skunks were tested. Western spotted skunks and eastern spotted skunks demonstrated the highest and lowest *T. cruzi* prevalence of all mephitid taxa. When the results for both these *Spilogale* is combined, the total value more closely approximates those for *M. mephitis* and for *C. leuconotus* (Figure 3.1). Since no female western spotted skunks were sampled, it is possible that the disparate value of *S. gracilis* reflects gender-biased differences in *T. cruzi* exposure and/or susceptibility rather than interspecific variations. The breeding season of *S. gracilis* is restricted to the autumn months, mainly September and October in Texas, a time span that overlaps the two periods of highest *T. cruzi* prevalence in triatomines as reported by Curtis-Robles et al. (2018a) (Mead 1968, Greensides and Mead 1973, Schmidly 1994). Therefore, the unique mating season of western spotted skunks may expose males to greater risk of *T. cruzi*, consistent with the high prevalence values we recovered for the species in our survey. Future research expanding the sample size of both male and female mephitids tested should focus on the possible influence of sex biases in *T. cruzi* infection among various skunk taxa.

Divergence in *T. cruzi* prevalence among closely-related wildlife species may be further influenced by taxon-specific variations in distribution within ecoregions, anthropogenically-impacted habitats, and/or areas of elevated pathogen and vector density as related to disease metacommunity dynamics (Jansen et al. 2015, Johnson et al. 2015, Almeida et al. 2016, Lilioso et al. 2017, Lima-Cordón et al. 2018, Correa et al.

2020, Wilber et al. 2020b). Studies from across the range of *T. cruzi* endemicity have demonstrated that areas marked by elevated human activity, habitat fragmentation, and native biodiversity loss drive alterations in host-parasite interactions, which result in greater transmission potential for *T. cruzi* (Valente 1999; Vaz et al. 2007; Roque et al. 2008; das Chagas Xavier et al. 2007, 2012; Gottdenker et al. 2012, Pellecer et al. 2013). The correlation between *T. cruzi* risk in wildlife populations and high densities of *T. cruzi*-positive triatomines and/or synanthropic hosts, such as rodents, domestic dogs, and domestic cats, has also been well-established (Zeledón et al. 1975, das Chagas Xavier et al. 2012). This correlation is evidenced in the case of the higher *T. cruzi* prevalence of striped skunks in Chagas disease hotspots in comparison to those in counties for which no previous records of *T. cruzi*-positive triatomines, human residents, and domestic dogs exist (likelihood ratio value of 6.75 with a *P*-value of 0.08; Table 3.2). Although the paucity of samples for other skunk species within Chagas disease hotspots precludes comparative investigation of this association in other mephitid taxa, *M. mephitis* have been described as the most anthropogenically-tolerant of all North American skunk species (Verts 1967, Schmidly 1994). Therefore, there is a greater probability for striped skunks to be involved in the same transmission cycles that include previously-sampled triatomines, domestic dogs, and humans of public health interest.

Within Texas, there is significant variation in the distribution of triatomines and *T. cruzi*-prevalence within these vectors across regions, with the highest risk of *T. cruzi* infection for rodent species, domestic dogs, and humans reported for south Texas (Kjos et al. 2009; Sarkar et al. 2010, Aleman et al. 2017, Curtis-Robles et al. 2018a,b).

However, our survey did not recover any support for a relationship between the prevalence of *T. cruzi* in skunks and ecoregions or regions with *T. cruzi*-positive vectors and hosts, or counties within south Texas (Tables 3, S1, S2; Figure 2). Sampling bias may have hindered our ability to detect geographically-influenced parasite prevalence patterns in skunks. For instance, we only tested one individual from the Gulf Prairies ecoregion and four skunks from the Trans Pecos ecoregion (Table 3.4). Despite this limitation, we detected a widespread distribution of moderate to high *T. cruzi* infections in skunks across the state. Our detection of parasite prevalence between 50% and 60% in the northern counties of Collin, Wichita, and Wise stand in stark contrast to the more southern concentration of cases described by previous studies within Texas (Sarkar et al. 2010, Aleman et al. 2017, Curtis-Robles et al. 2018 a,b) (Table 3.5, Figure 3.2).

3.6. Conclusions

We performed the first wide-scale and species-diverse survey for circulating *T. cruzi* infections in skunks across Texas, reporting the first positive cases for eastern spotted, western spotted, and American hog-nosed skunks. Although sampling biases and small samples sizes precluded our incontrovertible assessment of the risk factors associated with *T. cruzi* prevalence in mephitids, several trends were discernable related to age and sex biases and location within Chagas disease hotspots, which can inform population and host-parasite models supporting conservation, wildlife management, and public health strategies. Future research should extend the geographic scope and number of individuals sampled, particularly in the case of spotted skunks, to further investigate the risk factors for *T. cruzi* in skunks and the roles these mesocarnivores play within

Chagas disease transmission cycles. The present study highlights the value of surveying multiple taxa of closely-related wildlife species across a wide expanse of habitats to investigate the complex adaptive systems that underpin the ecology of vector-borne zoonotic diseases.

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4. ITCHING FOR RECOGNITION: DERMATOPHYTOSIS IDENTIFIED IN AN EASTERN SPOTTED SKUNK (*SPILOGALE PUTORIUS*) POPULATION IN TEXAS

4.1. Overview

The diversity, clinical manifestations, and impact on overall health of pathogenic fungi in *Spilogale putorius* (Eastern Spotted Skunk; ESS) have been poorly investigated. Herein, we describe the first reported cases of fungal dermatitis in ESS from a population in southeastern Texas. In 2016, two of three ESS live-trapped in Harris County, Texas exhibited symptoms consistent with dermatophytosis. Fungus was isolated from one of two cultures grown from infected ESS skin scraping samples and was morphologically identified as *Microsporum canis*. Reviewing local wildlife rehabilitation records, we were able to confirm the presence of dermatophytosis within local mephitid populations. We examine the implications of the discovery of a zoonotic fungal pathogen in ESS in light of its importance in wildlife medicine as well as its ramifications for domestic animal and public health.

4.2. Introduction

Historically, *Spilogale putorius* (L.) (Eastern Spotted Skunk, ESS) was a relatively common mesocarnivore throughout its North American range, but a steady decline in ESS populations since the 1940s led to its recent listing as vulnerable by the International Union for Conservation of Nature (Gompper and Hackett 2005, Gompper and Jachowski 2016, Kaplan and Mead 1991). Populations of *S. putorius interrupta* (Rafinesque) (Plains Spotted Skunk) are particularly imperiled and are currently under

review by the United States Fish and Wildlife Service for listing under the Endangered Species Act (USFWS 2012). Although the specific threats driving ESS population declines have yet to be concretely identified, infectious disease is a likely contributing factor to significant range-wide morbidity and mortality for the species (Choate et al. 1974, Gompper and Hackett 2005, Gompper and Jachowski 2016, Schwartz and Schwartz 2001). However, the disease ecology of ESS and their pathogens has been poorly investigated, hampering any attempts to assess the adverse impact of infectious diseases on ESS population dynamics (Chapter 5). Incorporation of pathogen sampling into field surveys and collection protocols for ESS research is expanding our collective understanding of infectious disease prevalence and risk for these skunks. Herein, we describe our collaborative work on investigating superficial cutaneous infections in several Plains Spotted Skunks live-trapped during an ESS survey in southeastern Texas, U.S.A.

4.3. Materials and Methods

From 30 October 2016 to 5 November 2016, we conducted a field survey for ESS on Katy Prairie Conservancy (KPC) property located in Harris County, Texas. Using collapsible Tomahawk Live Traps (15 x 15 x 48 cm; Tomahawk Live Trap LLC, Hazelhurst, WI), we successfully captured and then anesthetized (10 mg/kg ketamine) two adult and one subadult male *S. p. interrupta*. We photographed the coat coloration of each individual and assessed them for sex, reproductive status, weight, and total body, tail, and hind foot length in addition to attaching an ear tag identification (size 1005-3, National Band and Tag Co., Newport, KY) and removing a 2 mm ear clip from the distal

tip of the pinna for genetic analysis (Shaffer et al. 2018). We performed a physical health evaluation for each skunk during which time ectoparasites, fungal swabs, fecal specimens, and body fluid samples (nasopharyngeal mucus, urine, and whole blood) were collected whenever possible. We followed the handling procedures outlined by the American Society of Mammalogists' guidelines for the use of wild animals in research (Sikes et al. 2011), and all procedures were approved by the Angelo State University Institutional Animal Care and Use Committee (IACUC Approval No: 15-15). We collected fungal swabs by vigorously rubbing sterile dry applicator collection devices (Puritan, Guilford, ME) across any areas of suspected dermatitis on each skunk such that scales and hair shafts were visible on the swab post-sampling. Within 10 h of collection, we inoculated dermatophyte test medium (SABDEX with C&G/DTM biplates; Hardy Diagnostics, Santa Maria, CA) with the fungal swabs at room temperature. We stored culture biplates at room temperature and checked them daily for up to 21 days.

4.4. Results

Two of the three ESS examined (individuals identified herein according to Angelo State Natural History Collections record numbers for their archived tissue samples: ASK 12480 and ASK 12490) exhibited multifocal and diffuse scaling and erythema of the skin with patches of minor alopecia and hair breakage consistent with dermatophytosis. In both skunks, scaling and patchy alopecia were concentrated in the muzzle area, particularly at the juncture with the rhinarium, as well as the ventral region of the tail. ASK 12480 experienced erythema diffused across the dorsal and ventral trunk and additional hair loss and crusted skin along both pinnae, the dorsal aspect of the right

shoulder, and the dorsal aspect of the left hip. ASK 12490 presented with less extensive erythema, but significant scaling and hair loss on the dorsal aspect of the digits of both thoracic limbs, without nailbed or claw involvement. Fungal swabs were collected from each region of apparent dermal infection and inoculated into a single biplate per individual. Only one of the two cultures demonstrated fungal growth after 21 days. On day 12, the biplate inoculated with ASK 12480's pooled fungal swabs began to develop dermatophyte colonies, which were marked by white, feathery top surface growth over a sulphur-yellow undersurface. On microscopic inspection, we observed spindle-shaped fungal macroconidia consisting of five to seven cells with thick walls and terminal knobs. We identified the dermatophytic pathogens as *Microsporum canis* (E. Bodin) on the basis of these diagnostically-distinct morphological features (Moriello and DeBoer 2012).

4.5. Discussion and Conclusions

The identification of *M. canis* in *S. p. interrupta* is the first record of a cutaneous fungal pathogen in ESS and of a pathogenic keratinophilic fungus in any mephitid species. The nonpathogenic *M. cookei* (Ajello) was isolated from two out of 239 *Mephitis mephitis* (Schreber) (Striped Skunk) cultured for cutaneous fungi in southwestern Georgia and northwestern Florida, while another study recovered the same fungus from four of 487 *M. mephitis* samples collected in the same area (Ajello 1959, McKeever et al. 1958). Random sampling of four, asymptomatic *M. mephitis* in South Dakota identified the fungal pathogens *Trichophyton mentagrophytes* (Blanchard) in one

individual and *Chrysosporium* sp. indet. (Corda) in another (Knudtson and Robertstad 1970).

Although there is a paucity of dermatophytosis reports for mephitids in peer-reviewed literature, "ringworm" infections are commonly attributed to skunks anecdotally by wildlife biologists and wildlife rehabilitators (B.E. Gulas-Wroblewski, unpubl. data). In order to assess the prevalence of fungal dermatitis in mephitid species in the KPC region, we reviewed admission records from 2007 to 2015 for Dove Key Ranch Wildlife Rehabilitation, Inc. (DKRWR), the primary skunk rescue and rehabilitation facility serving Harris and its neighboring counties in southeastern Texas. We excluded all admissions from outside the Houston Metropolitan Statistical Area and the counties directly bordering this region (OMB 2020). Dermatophytosis in mephitid patients was diagnosed by an experienced, state- and federally-licensed wildlife rehabilitator and/or wildlife veterinarian on the basis of clinical symptoms (Figure 4.1). We found only three ESS admitted during this timeframe, one of which, a female juvenile, was diagnosed with a cutaneous mycosis on initial examination. A total of 36 out of 167 (22%) Striped Skunks exhibited signs consistent with fungal dermatitis. When we aggregated *M. mephitis* cases into family units (e.g., siblings in the same litter, a female skunk and her offspring), 16 out of 89 family units (18%) were positive for dermatophytosis. Percentages of infected *M. mephitis* individuals varied year to year, with a median of 10% and ranging from 0% in 2007 and 2014 to 62.5% in 2012. Female Striped Skunks accounted for the majority of pathogenic fungal diagnoses (24 out of 36), but our low sample size prevents us from evaluating the statistical-significance of this

trend. Within this context, our discovery of fungal dermatophytosis in the ESS population on the KPC property aligns with the regional prevalence of the condition within ESS and Striped Skunks.

Figure 4.1. Representative example of clinical manifestation of dermatophytosis in juvenile *Mephitis mephitis* undergoing rehabilitation. Note the erythema, dermal scaling, and hair loss on right forearm.

Fungal emerging infectious diseases (EID) can cause severe population declines and extinctions in wildlife species. *Batrachochytrium dendrobatidis* (Longcore) (Amphibian Chytrid Fungus) and *B. salamandrivorans* (Martel) (Bsal) continue to devastate global amphibian populations, while *Pseudogymnoascus destructans* (Blehert & Gargas) (etiological agent of White-nose Syndrome) is pushing some North American cave bat species towards extinction (Foley et al. 2011, Martel et al. 2013, Scheele et al. 2019). However, we anticipate the pathogenic fungus we describe to produce negligible effects on survivorship and fecundity in ESS populations. *Microsporum canis* induces a low-grade immune response in healthy animals, typically causing symptoms in the very young or old, individuals with pre-existing diseases, and/or those who are otherwise

immunocompromised. Furthermore, the majority of these types of mycoses fail to escalate into conditions serious enough to significantly impair normal behavior and functioning (Chermette et al. 2008, Moriello and DeBoer 2012). With the exception of substantial alopecia and its potential to compromise ESS thermal regulation, secondary infections facilitated by *M. canis* penetration of the dermal layer are the largest threat to infected ESS health. In fact, the only mephitid patients admitted to DKRWR with ringworm that were listed as "severe" dermatitis were five *M. mephitis* littermates suffering from simultaneous *Sarcoptes scabiei* (L.) (etiological agent of sarcoptic mange) and indeterminate bacterial skin infections. As such, we consider it unlikely that these pathogenic fungi independently impart a significant adverse impact on *S. putorius interrupta* population dynamics; however, symptomatic *M. canis* infections hold the potential to exacerbate other health factors contributing to morbidity within populations.

Dermatophytes are transmitted via direct contact with an infected animal, contact with infectious hair and/or skin scale deposited in the environment or transported by arthropod vectors, or contact with contaminated fomites in the environment. Infectious material can persist in the environment for over a year (Hubka et al. 2018, Moriello and DeBoer 2012). *Microsporum canis* commonly causes dermatophytosis in *Felis catus* (L.) (Domestic Cat), *Canis lupus familiaris* (L.) (Domestic Dog), and *Equus ferus caballus* (L.) (Domestic Horse), but is also pathogenic in a number of wildlife species and is responsible for tinea corporis and tinea capitis in humans (Baker et al. 1971, Bentubo et al. 2006, Chermette et al. 2008, Hubka et al. 2018, Malmasi et al. 2009, Marsicano et al. 2010, Mattei et al. 2014, Mota et al. 2017, Pereira et al. 2018, Sharma et al. 2007, Sykes

and Ramsay 2007, Takatori et al. 1981). Cats are the domestic animal most often implicated in the spread of *M. canis* within the peridomestic realm, while infections in free-ranging wildlife are often attributed to transmission across the wildlife-domestic boundary facilitated by anthropogenic habitat modification (Baker et al. 1971, Marsicano et al. 2010, Pereira et al. 2018, Seeliger et al. 1963). The KPC transect sampled hosts a wide variety of native wildlife as well as Domestic Dogs, livestock, and humans associated with conservation management, cattle ranching, oil and gas production, research, hunting, and other recreational activities that take place on the property. Although we did not find Domestic Cats to be widely dispersed on the KPC, the ranges of feral and pet cats on adjacent properties may overlap to some degree with those of the ESS live-trapped in our survey. Additional camera trap surveys on KPC from 2019 – 2020 indicate 3.5% occupancy (2/57) with only three total detections of Domestic Cats (J.C. Perkins, unpubl. data). As such, we can only speculate on the origin of the cutaneous fungal infections exhibited by the Plains Spotted Skunks within the KPC.

The presence of zoonotic *M. canis* in ASK 12480 holds important One Health implications. In the specific case of KPC management, humans, domestic animals, and wildlife co-habiting the property may be involved in the mycotic transmission cycle and should be continuously monitored for dermatophytosis. Whenever possible, infected individuals should receive antifungal therapies and be restricted from access to or quarantined within the KPC property to mitigate environmental contamination and the subsequent transmission of dermatophytes in the area. In light of ESS's ability to

develop symptomatic cutaneous fungal infections, we strongly advise ESS biologists to strictly adhere to personal protective equipment (PPE) protocols and to thoroughly decontaminate live traps and any other equipment that come into contact with animals and/or environmental fomites to prevent the transmission of pathogenic fungi among wild individuals and between humans and Prairie Spotted Skunks. Similarly, wildlife rehabilitators and veterinarians should test all admitted ESS and other mephitid patients for fungal dermatitis, instigate full treatment procedures for all asymptomatic and symptomatic infections, and maintain high standards in their quarantine, PPE usage, and decontamination practices. Captive-bred ESS can be legally kept as pets in several states within the continental U.S.A. and are occasionally used for wildlife educational programming. We urge handlers of captive individuals to be cognizant of the potential for ESS and other mephitid species to develop and transmit symptomatic, zoonotic dermatophytosis within domestic and peridomestic settings and to take all necessary precautions in their care and management.

Despite the small sample size, our successful culturing of one out of two media plates falls within the upper range of positive culture results recovered from fungal swabs conducted on symptomatic domestic animals (0-50% positive cultures; Moriello and DeBoer 2012). Although we were able to definitively diagnose *M. canis* as a causative agent for ASK 12480's mycosis, this finding does not preclude the possible involvement of other fungal taxa in the cutaneous infection nor does it confirm that *M. canis* was the causative agent of the cutaneous infection of the other ESS captured at KPC (ASK 12490). We suggest future research be directed towards wider geographic

and temporal surveillance of ESS populations for pathogenic fungi, employing combined culturing and molecular identification techniques to increase the specificity and sensitivity of diagnoses. Molecular epidemiological investigations into the disease ecology of the dermatophytes affecting *S. putorius* are also needed to elucidate the role of ESS in the transmission of zoonotic fungi, most notably the source(s) of skunk infection and the direct and indirect effects of dermatophytosis on morbidity and mortality within populations. Assessing the diversity of ESS mycoses within a One Health framework will assist us in developing effective strategies to mitigate the spread of these fungal diseases within and between threatened skunk populations, other wildlife, domestic animals, and humans. Our description of the first confirmed pathogenic cutaneous fungus in ESS is a first step towards building a more comprehensive understanding of the disease ecology of this threatened skunk taxon and its ramifications for veterinary and public health.

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5. REVIEWING INFECTIOUS DISEASES OF EASTERN SPOTTED SKUNKS (*SPILOGALE PUTORIUS*) WITHIN A ONE HEALTH FRAMEWORK

5.1. Overview

As a result of their omnivorous diet, den site habits, foraging activities, mating behaviors, and other pertinent habitat use and natural history traits, *Spilogale putorius* (L.) (Eastern Spotted Skunk; ESS) are exposed to a wide variety of pathogenic agents. Understanding the diversity of infectious diseases affecting ESS in conjunction with the role that ESS play in the disease ecology of these pathogens is crucial not only for devising efficacious management strategies for the species across all relevant geographic and temporal scales, but also in formulating comprehensive veterinary and public health strategies for combatting zoonotic diseases. Herein is reviewed the reported infectious diseases of ESS with reference to potential pathogens of interest based on the more extensively-sampled mephitid analogs. The implications of these pathogens for current and future population health of the species are addressed with reference to the changing dynamics of ESS disease ecology influenced by increasing habitat fragmentation, exposure to pathogens across the domestic-wildlife interface, fluctuating climatic patterns, quickly-evolving recombinant strains of infectious disease and antimicrobial-resistant pathogens, and the profusion of immunosuppressant chemicals throughout the environment. Employing a One Health framework to evaluate the role that ESS play in the disease ecology of zoonoses further highlights the interrelationships of ESS health to the health of the environment, humans, domestic animals, and other wildlife. The

potential for ESS to serve as hosts, "dilutors," and/or sentinels for zoonoses amplifies their importance in public health initiatives and supplies an opportunity to leverage their disease-mitigating benefits for conservation appeals aligned with ecosystem service provisioning.

5.2. Introduction

Populations of the mesocarnivore *Spilogale putorius* (L.) (Eastern Spotted Skunk, ESS) have been experiencing a steady decline across their North American range since at least the 1940s and have subsequently been listed as vulnerable by the International Union for Conservation of Nature (Gompper and Hackett 2005, Gompper and Jachowski 2016, Kaplan and Mead 1991). The level of threat to and management of ESS varies from state to state in the United States, and the subspecies *S. putorius interrupta* (Rafinesque) (Plains Spotted Skunk) are currently under review for protection by the Endangered Species Act (USFWS 2012). A host of possible drivers for ESS population declines have been presented in the literature, and infectious diseases likely play a key role in the population dynamics of the species, but the actual factors contributing to their historic and ongoing range-wide declines have yet to be incontrovertibly defined (Choate et al. 1974, Gompper and Hackett 2005, Gompper and Jachowski 2016, Schwartz and Schwartz 2001).

Infectious diseases contribute most markedly to the decline and extinction of wildlife species in cases where spillover or emerging novel pathogens affect naïve populations and/or endemic disease dynamics are altered by environmental or demographic shifts (Daszak et al. 2000). In order to assess the potential or real effects

any pathogenic agent imposes on a population, subspecies, or species, an understanding of the abiotic and biotic drivers contributing to the pathogen's interactions with the taxon must be evaluated across geographic and temporal intervals. In particular, interrelationships between host behavior and demography, determinants of pathogen virulence, and disease transmission cycles regulate population and species level disease outcomes (Daszak et al. 2000). The introduction of novel pathogens and epidemiological changes in pre-existing disease dynamics of ESS can be significantly influenced by anthropogenic processes, especially for *S. putorius* populations at the wildlife-domestic interface. Likewise, these ESS may operate within peridomestic disease transmission cycles, bridging sylvatic systems and those of the domestic realm with varying consequences for human and animal health (Daszak et al. 2000, Northover et al. 2018, Silk et al. 2019). Consequently, it is imperative to structure any exploration of ESS disease ecology within a One Health framework, incorporating environmental, domestic animal, wildlife, and public health. Indeed, infectious disease management strategies with the greatest chance of real-world success are those employing One Health approaches (Cunningham et al. 2017).

Previous investigations on infectious diseases of ESS have been limited in their geographic, temporal, and/or taxonomic extent, focusing on skunk populations within a restricted region or timeframe (e.g., Lesmeister et al. 2008a) and/or surveying a small number of potential pathogenic agents (e.g., Higdon and Gompper 2020a). Accordingly, any accompanying discussions of the ecological, environmental, demographic, and behavioral context of pathogen prevalence and virulence in *S. putorius* have been

constrained by the context of the population and/or pathogenic agents under review. Although many of the pathogens reported in ESS individuals are zoonotic, few studies expand on the public health implications of these infections (Emmons 1950, McKeever et al. 1958, Gorman et al. 1962) and even fewer explicitly analyze them from a One Health perspective (Emmons et al. 1949; Chapter 4). In this chapter, I provide a broad overview of the natural history and behavioral traits of *S. putorius* that influence individuals' exposure and susceptibility to various infectious diseases, which serves as an introduction to a review of the pathogenic agents recorded in ESS. Since there is a paucity of research into the bacteria, fungi, parasites, and viruses impacting *S. putorius* populations, I also draw from more extensively-studied mephitid taxa, such as *Mephitis mephitis* (Schreber) (Striped Skunk) and *Spilogale gracilis* (Merriam) (Western Spotted Skunk), as analogs to identify pathogens of interest for future ESS disease surveillance. I then outline some of the key abiotic and biotic factors that will likely shape ESS disease dynamics into the future along with the integrated One Health ramifications for these processes. Finally, I offer a summary of the practical applications *S. putorius* disease ecology holds for best practices of wildlife professionals. As such, this review is intended to serve as both a base for future research into mephitid disease ecology and a functional guide for ESS practitioners.

5.3. Discussion

5.3.1. ESS Natural History and Behavior of Epidemiological Relevance

The habitat use, omnivorous diet, and other behaviors exhibited by ESS increase their exposure and susceptibility to a wide diversity of pathogens. As omnivores, *S.*

putorius consume a variety of invertebrates and small vertebrates, which can serve as direct vectors of infectious disease and/or as hosts for ectoparasites that may transmit pathogenic agents (Crabb 1941, 1948; Howell 1906; Kinlaw 1995; Manaro 1961, McCullough and Fritzell 1984; Pellett 1913; Polder 1968; Selko 1937). By capturing and ingesting a *Peromyscus leucopus* (Rafinesque) (White-footed Mouse), an ESS can inadvertently consume infective *Skrjabingylus chitwoodorum* (Hill) (Sinus Roundworm) while simultaneously gaining a Lyme disease-carrying *Ixodes scapularis* (Say) (Deer Tick) previously hosted by the rodent. In the pursuit of prey, fungi, and vegetative matter, ESS express a range of foraging tactics, such as digging in substrate, hunting, consuming carrion, raiding the caches of musteloids, or opportunistically exploiting agricultural and peridomestic resources, all of which expose individuals to pathogenic agents, such as fungi in soil or bacteria in carrion (Crabb 1941, 1948; Kinlaw 1995; Polder 1968; Van Gelder 1953). The subterranean (i.e., burrow systems) and arboreal hunting and foraging behaviors of ESS expand their exposure to hosts, vectors, and pathogens circulating in habitats at multiple vertical strata. These actions increase the likelihood of *S. putorius* serving as a connector between disease transmission cycles otherwise constrained by vertical limits within an ecosystem and acting as a host for a wider range of ectoparasites, such as ticks and mosquitoes that typically exhibit highly stratified vertical distribution patterns (Arsnoe et al. 2015, Edman and Spielman 1988, Jansen et al. 2015, Ulyshen 2011).

ESS are further exposed to infectious pathogens in their use of den sites. Crabb (1948) notes the key components of *S. putorius* dens as being weather-proof, dark inside,

and predator-deterrent, and members of this species utilize a wide variety of sites to fulfill these requirements: rock crevices, human structures, hollow logs and trees, deserted or coopted burrows of other animals, self-constructed burrows, debris piles, and protective vegetative cover (Crabb 1948, Frank and Lips 1989, Kinlaw et al. 1995, Lesmeister et al. 2008b, Manaro 1961, McCullough and Fritzell 1984, Polder 1968, Sprayberry and Edelman 2018). Similar habitat preferences are shared by arthropod vectors of disease, notably triatomine carriers of *Trypanosoma cruzi* (Chagas) (etiologic agent of Chagas disease) and tick-borne relapsing fever-bearing *Ornithodoros* spp. (Sonenshine 1991, Wozniak et al. 2015). In addition, ESS exploiting den sites recently inhabited or co-inhabited by other animals risk direct or indirect exposure to infectious diseases carried by these wildlife or transmitted by shared ectoparasites (Houseknecht 1969). In fact, western spotted skunks inhabiting *Spermophilus beecheyi* (Richardson) (Beechey Ground Squirrel) burrows demonstrated a greater density of "ground squirrel fleas" *Oropsylla montanus* (Baker) (Rock Squirrel Flea) and *Hoplopsyllus anomalus* (Baker) (Rodent Flea) than did sympatric striped skunks that used alternate den sites (Mead 1963). The prolonged persistence of certain pathogenic agents in the environment, especially in the favorable microclimatic conditions provided by many of these resting sites, further elevates the risk of infection from use of contaminated den sites. For instance, infectious spores of ringworm-inducing *Microsporum canis* can persist in moist soil for 12 months, and eggs of *Baylisascaris* spp. roundworms can survive in the natural environment for seven to twelve years (Hubka et al. 2018, Kazacos 2016, Moriello and DeBoer 2012).

Den sites host much of the intraspecific social interaction of ESS. *S. putorius* individuals are predominantly solitary, though aggregations of up to seven skunks within the same den have been recorded by Crabb (1948). Although these groupings may represent an adult female and her subadult offspring, co-habitation of burrows by multiple, presumably-unrelated, adult striped skunks, including an instance of a male residing with twenty adult females reported by Seton (1926), are not uncommon (Allen 1939, Allen and Shapton 1942, Houseknecht 1969, Seton 1926, Verts 1967). In colder northern regions of ESS distribution, communal winter denning may be a thermoregulatory adaptation (Allen 1939, Allen and Shapton 1942, Verts 1967). Concomitant or temporally-segregated use of dens and other habitat features by ESS individuals introduces a more elevated risk of infection by cross-shared pathogens than does similar overlap between *S. putorius* and other species, which are more likely to exhibit differential susceptibility to pathogenic agents (Houseknecht 1969).

Aside from den use and maternal-offspring sociality, ESS are most likely to interact during breeding season. At this time, male *S. putorius* exhibit "questing" behavior in the search for females in estrous, expanding their home range size and experiencing physiological stress from both elevated physical activity and reduced foraging (Crabb 1948, Lesmeister et al. 2009, McCullough and Fritzell 1984). As a result, ESS males are more prone to both exposure and susceptibility to infectious diseases. Concurrently, frequency-dependent disease transmission, which holds great potential for significant adverse impact on small or declining populations, is elevated for *S. putorius* during the breeding season (Silk et al. 2019, Harris et al. this issue). The

"quite violent" (p. 388, Mead 1968) mating behavior of *S. putorius* involves the larger male ESS biting and holding the smaller female by her "scruff" prior to and during intromission. This behavior frequently results in the penetration of the female's dermis by the male's canines and may include aggressive defensive behavior by the female that causes injury to the male. Accordingly, infectious pathogens transmitted via blood, saliva, and other bodily fluids pose a greater threat to breeding ESS expressing mating behaviors. Transmission of rabies, which can be shed in *M. mephitis* saliva for up to six days prior to clinical symptoms onset, is of special concern during the ESS breeding season (Charlatan et al. 1991).

The defensive behavior of ESS is an additional trait that increases their risk of exposure to rabies. *S. putorius* rely on their aposematic coloration, elaborate warning displays, and infamous anal scent gland secretions to deter potential predators, and commonly display defiant behavior to threatening animals uncharacteristic of other, non-mephitid mesocarnivores of a similar size (Johnson 1921, Manaro 1961). As a result, ESS are more likely to be the recipient of aggressive interactions with mammals exhibiting the furious form of rabies infection.

ESS's potential exposure to pathogenic agents is exacerbated by anthropogenic processes, especially for populations that cross the wildlife-domestic interface. Although *S. putorius* often inhabit sylvatic habitats (reviewed by ESSCSG 2019) and exhibit a lower tolerance for human development than striped skunks, ESS frequently to occasionally exploit agricultural environs and peridomestic settings across their North American range (Barbour and Davis 1974, Boulerice and Zinke 2017, Buskirk 2016,

Crabb 1948, Davis 1945, Diggins et al. 2015, Emmons et al. 1949, Higdon and Gompper 2020, Lesmeister et al. 2008b, McCullough and Fritzell 1984). A litter of *S. putorius interrupta* (Rafinesque) (Plains Spotted Skunk) was even recovered from within the city limits of Houston, TX (B.E. Gulas-Wroblewski, unpubl. data). Current ESS populations experience a greater degree of urbanization-mediated habitat fragmentation and transgression by anthropogenic influence, but midwestern *S. putorius* in agricultural areas were historically associated with human activity (Crabb, 1948). Consequently, the direct and indirect disease processes associated with exposure to the peridomestic realm have significantly influenced ESS disease ecology since at least the time of agricultural expansion across the United States.

S. putorius's use of edge habitats, agricultural, and peridomestic habitats subjects them to increased contact with a wider variety of hosts and vectors of infectious pathogens, notably humans, domestic animals, and the greater density of wildlife that typically aggregates in these environs (Daszak et al. 2000, Faust et al. 2018). In these settings, spill-over events pose a great risk for wildlife populations, especially those like ESS that are declining or consist of small numbers of individuals. Novel pathogenic agents introduced from the domestic or peridomestic realm to naïve ESS populations can decrease population size to the level at which demographic stochasticity can drive the population to extinction. Frequency-dependent diseases, such as vector-borne pathogens, pose the greatest risk for spillover-induced epidemics in wildlife, most prevalent in areas with intermediate edge effects, similar to those frequented by *S. putorius* (Faust et al. 2018). Furthermore, domestic reservoir hosts of disease can lower a

pathogenic agent's threshold density, even in the case of endemic diseases, causing significant declines of local wildlife populations (Daszak et al. 2000). The introduction of novel or additional pathogenic agents to a system disrupts the pre-existing dynamics of the pathogens already interacting in the individual. Such alterations to the pathogen community can compound, mitigate, or otherwise affect the manifestations of disease within the skunk (Daszak et al. 2000, Northover et al. 2018).

Concurrent with these direct effects on ESS disease risk, populations of *S. putorius* face elevated susceptibility to infectious pathogens linked to the indirect influence of the physiological stressors associated with existence at the wildlife-domestic interface. Relatively poor nutrition, exposure to chemical toxicants, and stress all contribute to immunosuppression, which influences susceptibility to opportunistic infections (Daszak et al. 2000, Northover et al. 2018, Serieys et al. 2018). As evidenced by *Toxoplasma gondii* infections in Illinois *Marmota monax* (L.) (Groundhog) and *Salmonella* in Ontario's *Procyon lotor* (L.) (Raccoon), wildlife species residing in peridomestic habitats, analogous to those used by some ESS populations, demonstrate higher rates of disease than those in more pristine, wild environments (Jardine et al. 2011, Leher et al. 2010).

Alternatively, certain populations of sylvatic ESS may be at an equal or higher risk of disease spillover from the domestic realm than are those inhabiting agricultural and peridomestic settings. Watts and Alexander (2012) identified the link between dispersal of *Canis latrans* (Say) (Coyote) from urban Calgary to more rural locations such that parasite richness was elevated in rural coyotes, which hosted a combination of

urban- and rural-derived parasites. Similar dynamics acting across North America could facilitate the infectious disease contamination of sylvatic ESS populations via far-ranging, urban-tolerant species like coyotes. This pattern may be exaggerated by the decreased density of coyotes present in agricultural areas relative to urban and sylvatic habitats in certain regions of ESS's range (Lombardi et al. 2017). Conversely, far-ranging *S. putorius*, especially males during breeding season, may themselves serve as bridges of disease transmission between domestic, peridomestic, and sylvatic wildlife populations.

Clinical manifestations of infectious pathogens can further alter ESS behavior to increase exposure and susceptibility of individuals and populations to diseases. Parasite manipulation of wildlife hosts to enhance transmissibility has been extensively investigated (reviewed by Heil 2016). The neurologic symptoms associated with viral pathogens evidenced in ESS (i.e., rabies, canine distemper virus) can accelerate the spread of these diseases through *S. putorius* populations (Charlatan et al. 1991, Diters and Nielsen 1978, Harris et al. this issue). Any alterations to ESS adaptive behavioral responses by pathogen-induced neurologic abnormalities can concomitantly increase affected individuals' exposure to secondary infections and/or impair their ability to avoid alternate causes of morbidity and mortality. In striped skunks, rabies infections were correlated with increased rates of mortality via vehicle strikes, and *Skrjabingylus*-related ataxia has been linked to ESS euthanasia and public health testing for rabies surveillance (Burkel et al. 1970, Higdon and Gompper 2020, Hughes et al. 2018). In addition, activation of the immune system by exposure to pathogens can itself alter endocrine

system function and induce physiological stress that elevates an individual's susceptibility to infectious diseases, increases the virulence of pathogens, and/or reactivates pre-existing infections (Diters and Nielsen 1978, Hing et al. 2016).

5.3.2. Infectious Diseases of ESS

Despite the intriguing potential ESS hold for disease ecology research, relatively few investigations have been launched into the infectious pathogens that the species hosts, especially when compared to the work devoted to other mephitid species. In the following sections, I review the pathogenic agents that have been reported in *S. putorius* with reference to the potential role of ESS play in disease transmission cycles and assess any attendant threats to their populations. When relevant, I also highlight pathogens of interest for future investigations, proposed on the basis of infectious diseases recorded in more extensively-sampled western spotted, hooded, hog-nosed, and striped skunks.

5.3.2.1. Bacteria

5.3.2.1.1. Tularemia

Francisella tularensis (McCoy and Chapin) is a highly-pathogenic, gram-negative intracellular bacterium responsible for tularemia (rabbit fever, deer-fly fever). In a survey of small wild mammals in southwestern Georgia and northwestern Florida, two out of eight agglutination tests for antibodies to *F. tularensis* performed for ESS reacted, though only one revealed a significant titer of 1:80. The resulting 12.5% positivity rate reported for the species is lower than that recovered for *M. mephitis* in the same study area (65 out of 311; 21%), though the difference may reflect the lower

sampling size for *S. putorius* rather than an accurate variance in cross-species *F. tularensis* prevalence (McKeever et al. 1958).

This zoonotic bacterium exhibits an extremely low infectious dose and can cause severe disease in both humans and animals, resulting in its designation as a notifiable disease to the Centers for Disease Control and Prevention (CDC), a World Health Organization (WHO) risk 3 category pathogen, and a category A select agent with potential for use in bioterrorism within the United States (Farlow et al. 2005, Nelson et al. 2013). *F. tularensis* can be transmitted via ticks (Ixodidae, particularly *Dermacentor variabilis*, *D. andersoni*, *Amblyomma americanum*) and biting flies (Tabanidae), ingestion contaminated food or water, direct contact with infected tissue, or inhalation of contaminated aerosols (Dennis et al. 2001, Farlow et al. 2005, Jellison 1974). Of note are reports of potential tularemia tick vectors in wild-sampled ESS (Table 1). The majority of human cases are reported from central and western US (CDC 2019). Within this region, *S. putorius* populates, and is thus at risk of infection in, Arkansas, Kansas, Oklahoma, Missouri, and South Dakota, all of which exhibited high numbers of cases between 2008-2018 (CDC 2019).

Tularemia is enzootic in rodents and lagomorphs, but the roles filled by ESS, skunks, and mesocarnivores in general are unknown for its sylvatic transmission cycles (Origgi et al. 2013). Clinical symptoms of *F. tularensis* in mammals present differently based on the species involved, the specific strain of bacterial infection, and other epidemiological factors. Non-human animals exhibit disease manifestations ranging from asymptomatic presentations to anorexia with low-grade fever to severe systemic

disease or sudden death (Baldwin et al. 1991, Henson 1978, Origgi et al. 2013). The nearest physiological analog to ESS in which tularemia has been clinically investigated is the mustelid *Neovison vison* (Schreber) (Mink). Henson (1978) evaluated an outbreak on an Idaho mink farm that led to the death of 60 of 5,000 animals. Although some presented with anorexia and/or dehydration prior to death, most were in good body and fur condition before succumbing to "sudden death." Necropsies revealed necrotic nodules in infected minks' lungs, livers, spleens, and mesenteric lymph nodes (Henson 1978). If similar disease dynamics prevail in ESS, tularemia holds the potential for contributing to reduced survivability within exposed populations.

5.3.2.1.2. Leptospirosis

Leptospira interrogans is a spirochete bacterium, which acts as the etiologic agent of leptospirosis. Pathogenic *L. interrogans* serovar *ballum* was successfully cultured from two out of seven samples of kidney tissue from ESS in southeastern Georgia (Gorman et al. 1962). *Leptospira* spp. infect over 160 domestic and wild animal species worldwide, causing subclinical to fatal disease in humans and animals alike (Babudieri 1958, Ko et al. 2009, van de Maele et al. 2008, WHO 2011). Transmission routes include contact with contaminated urine, water, food, soil, or fomites; consumption of contaminated rodents and other infected hosts; venereal or placental transfer; or, rarely, bites from infected animals (Ellis 2015, Lane et al. 2016, Shophet and Marshall 1980). These spirochete bacteria remain viable for several months in damp soil, though freezing, dehydration, and U.V. exposure decrease their survival rates (Greene et al. 2012, Levett 2001). *L. interrogans* is more prevalent in wet, tropical

climates and in areas with stagnant or slow-moving water. Within the continental US, Texas and California boast the greatest number of human and domestic animal leptospirosis cases (Guerra 2013). ESS inhabiting areas with moist substrate, extensive water sources, and warmer climates and/or that prey on the small mammals likely to serve as reservoir hosts for *L. interrogans* are more likely to participate in the leptospirosis transmission cycle.

Asymptomatic kidney infections facilitate the continuous shedding of *L. interrogans* by wildlife hosts. Striped skunks are widely recognized as an important reservoir host for this infectious pathogen in both peridomestic and sylvatic transmission cycles (Britton et al. 2017, Ellis 2015, Shearer et al. 2014, Straub et al. 2020). In fact, *Leptospira* spp. can persist in the kidneys of experimentally infected striped skunks for more than 6 months and in those of naturally infected individuals for over two years (Roth et al. 1963, Tabel and Karstad 1967). The findings of Gorman et al. (1962) demonstrate the capacity for asymptomatic ESS to maintain *L. interrogans* infections in their kidneys, supporting their probable role as reservoir for this pathogen rather than symptomatic host. As such, *Leptospira* infections in *S. putorius* hold more important implications for domestic veterinary and public health than they do for ESS conservation medicine.

5.3.2.1.3. Other bacterial pathogens of interest

ESS are parasitized by a wide variety of ectoparasites, which transmit a diversity of pathogenic bacteria (Bitam et al. 2010, Eisen and Gage 2012, Fedele et al. 2020, Moro et al. 2005, Norman et al. 1999, Parola and Raoult 2001) (Table 5.1). Moreover,

ectoparasite-vectored bacterial diseases have been described for both western and striped skunks, including Lyme disease (caused by *Borrelia* spp.), murine typhus (caused by *Rickettsia typhi*), the plague (caused by *Yersinia pestis*), Q fever (caused by *Coxiella burneti*), Rocky Mountain spotted fever (caused by *Rickettsia rickettsii*), and tickborne relapsing fever (caused by *Borrelia turicatae*) (Alexander et al. 1972, Brinkerhoff et al. 2009, Clark et al. 2012, Holbrook and Frerichs 1970, LoGiudice et al. 2003, Magnarelli et al. 1983, Morlan et al. 1950, Riemann et al. 1978, Salkeld and Stapp 2006, Smith et al. 1984, Wormser and Pritt 2015, Verts 1967; B.E. Gulas-Wroblewski, unpubl data). Additional bacterial pathogens with potential individual- and population-level effects on *S. putorius* are *Staphylococcus* spp., *Listeria* spp., and *Brucella abortus*, which have been documented in western and/or striped skunks (Aarestrup 2001, Bolin et al. 1955, Moore and Schnurrenberger 1981, Osebold et al. 1957, Verts 1967).

Table 5.1. Ectoparasites reported in *Spilogale putorius* (L.) (Eastern Spotted Skunk). When relevant, common names and zoonotic pathogens for which they serve as vectors are listed. * Also responsible for alpha-gal meat allergy in humans (Crispell et al. 2019).

Ectoparasites	Species	Common name	Zoonotic pathogens transmitted	References
Fleas	*Ctenocephalides felis*	Cat flea	*Bartonella* spp., *Dipylidium caninum*, *Rickettsia* spp.; possibly *Borrelia burgdorferi*	Morlan 1952, Schwartz 1952
	Ctenophtalmus pseudagyrtes		*Bartonella vinsonii* subsp. *arupensis*	Timm 1980
	Echidnophaga gallinacea	Hen flea, stickfast flea, sticktight flea		Hopkins and Rothschild 1953, Layne 1971, Morlan 1952
	Orchopeas howardi	Squirrel flea	*Rickettsia prowazeki*	Morlan 1952
	Orchopeas leucopus	Rodent flea	*Babesia microti*, *Bartonella vinsonii* subsp. *arupensis*, *Rickettsia* sp. indet.	Timm 1980
	Polygenis gwyni			Layne 1971, Morlan 1952, Schwartz 1952
	Pulex irritans	House flea, human flea	*Dipylidium caninum*	Morlan 1952
	Xenopsylla cheopis	Oriental rat flea, tropical rat flea	*Hymenolepis diminuta, H. nana, Rickettsia typhi*, *Yersinia pestis*	Morlan 1952

Table 5.1. Continued.

Ectoparasites	Species	Common name	Zoonotic pathogens transmitted	References
Lice	*Neotrichodectes* (*Trichodectes*) *osborni*			Emerson and Price 1981, Hopkins 1960, Morlan 1952, Price et al. 2003, Reeves et al. 2007, Werneck 1948, Wiseman 1959
	Trichodectes mephitidis			Kellogg 1914
Mites	*Androlaelaps casalis*		*Coxiella burnetii*	Whitaker and Wilson 1974
	Androlaelaps fahrenholzi		*Coxiella burnetii*, *Rickettsia prowazeki*	Whitaker and Wilson 1974, Whitaker et al. 2007
	Androlaelaps [*Haemolaelaps*] *geomys*			Morlan 1952, Whitaker and Wilson 1974
	Androlaelaps [*Haemolaelaps*] *glasgowi*		Hantavirus, *Rickettsia* spp.	Morlan 1952
	Androlaelaps [*Haemolaelaps*] *megaventralis*			Morlan 1952
	Echinonyssus staffordi			Whitaker and Wilson 1974, Whitaker et al. 2007
	Eucheyletia bishoppi			Whitaker et al. 2007
	Eulaelaps stabularis		*Coxiella burnetii*, *Elleipsisoma thomsoni*, *Rickettsia felis*	Morlan 1952, Whitaker and Wilson 1974, Whitaker et al. 2007
	Haemogamasus reidi		*Rickettsia prowazeki*	Whitaker et al. 2007
	Hirstionyssus staffordi			Morlan 1952
	Ornithonyssus bacoti	Tropical rat mite	*Borrelia burgdorferi* sensu lato, *Coxiella burnetii*, Eastern equine encephalitis virus, *Francisella tularensis*, Hantavirus, *Rickettsia* spp., *Trypanosoma cruzi*	Morlan 1952, Whitaker and Wilson 1974
	Pygmephorus designatus			Whitaker et al. 2007
	Xenoryctes latiporus			Whitaker et al. 2007
Ticks	*Amblyomma americanum*[*]	Lone star tick, northeastern water tick, turkey tick	*Borrelia* spp., *Coxiella burnetti, Ehrlichia chaffeensis E. ewingii, Francisella tularensis*, Heartland bandavirus, *Rickettsia amblyommii*	Morlan 1952
	Amblyomma auricularium	Exotic armadillo tick	*Rickettsia amblyommii*	Mertins et al. 2017
	Dermacentor variabilis	American dog tick, wood tick	*Francisella tularensis*, *Rickettsia rickettsii*	Morlan 1952, Kinlaw et al. 1995, Wilson and Kale 1972
	Ixodes bishoppi			Morlan 1952
	Ixodes cookei	Groundhog tick	Powassan virus	Cooney and Hays 1972, Morlan 1952, Kinlaw et al. 1995, Wilson and Kale 1972,
	Ixodes minor	Bird tick	*Borrelia burgdorferi* sensu lato	Clark et al. 2001

Table 5.1. Continued.

Ectoparasites	Species	Common name	Zoonotic pathogens transmitted	References
	Ixodes scapularis[*]	Bear tick, black-legged tick (east coast USA), deer tick	*Anaplasma* spp., *Babesia* spp., *Borrelia* spp., Powassan virus, *Theileria microti*	Ellis 1955

5.3.2.2. Fungi

5.3.2.2.1. Histoplasmosis

The pathogenic fungus *Histoplasma capsulatum* was cultured from spleen and/or liver tissue collected from five *S. putorius* individuals sampled in Georgia (Emmons et al. 1949). Recovery of *H. capsulatum* from ESS is not surprising, considering this species' North American range and habitat use patterns. The Mississippi, Missouri, Ohio, and Potomac river valleys have historically been major endemic areas of *H. capsulatum* with greater numbers of reported human histoplasmosis cases spanning the region from Kansas, Illinois, Indiana, and Ohio southwards to Mississippi, Louisiana, and Texas. More recently, this area of high prevalence has been shifting northwards into the upper Missouri River basin (Acha and Szyfres 1980, Kwon-Chung et al. 1992, Maiga et al. 2018).

Birds and bats serve as the primary wildlife reservoirs, with *H. capsulatum* exposure typically associated with direct contact with bat guano and/or avian droppings or soil contaminated with these infectious agents. Accordingly, areas directly surrounding poultry operations and caves demonstrate relatively high concentrations of H. capsulatum (Emmons 1950, Kwon-Chung et al. 1992). ESS populations risk high exposure to this pathogenic fungus when denning, resting, and/or foraging in rocky

crevices and outcrops, abandoned structures, logs and tree hollows, and other areas potentially co-habitated by bat colonies or roosting birds (Barbour and Davis 1974, Boulerice and Zinke 2017, Buskirk 2016, Crabb 1948, Crooks 1994, Davis 1945, Diggins et al. 2015, Higdon and Gompper 2020, Lesmeister et al. 2008b, McCullough and Fritzell 1984). The predilection of some individuals for domestic poultry further increases their exposure to *H. capsulatum* (Crabb 1948, Van Gelder 1953). The presence of *S. putorius* remains in modern to Pleistocene cave deposits in Indiana (where they were present until at least 1920; Lyon 1936) and Missouri points to an historic interaction between ESS and this fungal pathogen (Richards 1984).

H. capsulatum usually induces subclinical infections in humans and other animals, but symptomatic presentations involving pulmonary disease and subsequent lymphatic and hematogenous systemic diffusion typify infections in immunocompromised individuals and/or those associated with large infectious doses (Kwon-Chung et al. 1992). Woolf et al. (1985) described significant weight loss, anemia, and gross lesions on lungs associated with histoplasmosis in a wild striped skunk, all of which are consistent with typical clinical symptoms exhibited by *Felis catus* (L.) (Domestic Cat) and *Canis lupus familiaris* (L.) (Domestic Dog) (Brömel and Sykes 2005). Although *H. capsulatum* may cause similar symptoms in *S. putorius*, mycoses are more likely to adversely affect previously-immunocompromised individuals with population-level effects evident only when other factors, such as toxicological immune suppression, act in concert on the ESS population. The role of mephitids in the transmission cycle of histoplasmosis has yet to be defined, so the collection of two *H.*

capsulatum-positive ESS from "farm premises" is of interest from a One Health perspective (Emmons et al. 1949).

5.3.2.2.2. Dermatophytosis

Symptomatic cutaneous fungal infections were exhibited by two out of three *S. putorius interrupta* live-trapped in southeast Texas, one of which produced a culture positive for *Microsporum canis* (Chapter 4). Review of wildlife rehabilitation records for the region revealed that one out of three (33%) ESS and 21.6% of striped skunks admitted displayed symptoms consistent with dermatophytosis (Chapter 4). Although *M. canis* may induce individual-level effects related to its potential to cause alopecia and facilitate secondary infections, it is unlikely this fungal pathogen will independently impact *S. putorius* populations to any significant degree. However, the zoonotic nature of *M. canis* and its common infection of domestic animals and humans highlights the importance of evaluating the disease ecology of ESS mycoses within a One Health framework (Chapter 4).

5.3.2.2.3. Other fungal pathogens of interest

The diversity, clinical manifestations, and influence on overall health of pathogenic fungi have been poorly investigated in *S. putorius*. The discovery of dermatophytosis in ESS underlines the need to more comprehensively survey this species for zoonotic pathogenic fungi associated with dermatitis, such as *Epidermophyton* spp. and *Trichophyton* spp., especially in populations at the domestic-wildlife interface (Moriello and DeBoer 2012). Symptomatic aspergillosis was reported in multiple striped skunks and should also be considered in pathogenic fungal

surveillance of *S. putorius* populations (Durant and Doll 1939, Verts 1967). Fungal emerging infectious diseases (EID) have driven population declines and extinctions in a wide range of wildlife species, including bats, amphibians, and snakes (Foley et al. 2011, Lorch et al. 2016, Martel et al. 2013, Scheele et al. 2019). Continuous monitoring of ESS populations for potential exposure and susceptibility to various mycoses will be necessary to prevent future epizootics related to fungal EIDs.

5.3.2.3. Parasites

Polyparasitism is the norm for wildlife, and ESS are no exception (Northover et al. 2018). When combined with their social, denning, and foraging behaviors, the habitat use and dietary preferences of ESS expose them to a wide variety of ectoparasites and endoparasites. Within an individual, these parasites participate in a variety of interspecific and intraspecific competitive, mutualistic, parasitic, commensalistic, amensalistic, or neutral interactions, all of which affect parasite virulence, host fitness, and host exposure and susceptibility to other pathogens (Northover et al. 2018). Intuitively, one would expect that the more parasites infecting a skunk, the greater the severity of disease and attendant adverse effects would be for the individual. However, the biodiversity of parasite assemblages often serves a protective function for the host. Competitive, parasitic, and amensalistic interrelationships of parasites can suppress the pathogenicity of one or more pathogens, create an inhospitable environment that reduces the population density of damaging parasites, and/or block the colonization of the host by additional and/or more virulent parasites (Northover et al. 2018, Trevelline et al. 2020). Such is the case with *Bettongia penicillata* (Gray) (Woylie, Brush-tailed

Bettong) in which *Trypanosoma vegrandis* prevents infection by the more pathogenic *T. copemani* (Thompson et al. 2014). In human populations, generalist helminth hookworms competitively suppress the density of specialist *Plasmodium virax* (causative agent of malaria), while generalist *P. falciparum* controls the population of the intestinal helminth *Nestor americanus* by limiting red blood cell availability (Budischak et al. 2018). Consequently, it is critical to not only describe the diversity of parasites infecting ESS populations, but also to evaluate their ecological interactions with one another and the consequences of these dynamics for *S. putorius* health.

5.3.2.3.1. Ectoparasites

ESS host a variety of fleas, lice, mites, and ticks, some of which are zoonotic and many of which likely represent spillover infections across the domestic-wildlife interface (Table 5.1). The direct effects of ectoparasites on wildlife range from mild anemia, localized inflammatory reactions or infection at the bite site, or alopecia to more severe anaphylactic allergic reactions and/or tissue damage. Resultant hair loss can impact thermoregulatory efficiency, while dermatitis can facilitate more pathogenic, secondary infections. In humans, the saliva of certain tick species, two of which are hosted by ESS, can cause an alpha-gal meat allergy, though this condition has not been explored in wildlife taxa (Crispell et al. 2019) (Table 5.1). The most serious health threat posed by ectoparasites to *S. putorius* individuals and populations is their transmission of infectious diseases (Bitam et al. 2010, Eisen and Gage 2012, Fedele et al. 2020, Moro et al. 2005, Norman et al. 1999, Parola and Raoult 2001) (Table 5.1).

5.3.2.3.2. Endoparasites

The helminth and protozoal endoparasites reported from S. putorius individuals are listed in Table 5.2. The transmission dynamics, zoonotic nature, and clinical manifestations of each parasite varies based on the species, host factors, and abiotic and biotic drivers characterizing the infection.

Table 5.2. Endoparasites reported in *Spilogale putorius* (L.) (Eastern Spotted Skunk). When relevant, common names and zoonotic potential are noted. *Experimental infection. [Z]Zoonotic parasite.

Endoparasites	Species	Common name and/or disease caused	References
Protozoans	*Eimeria mephitidis*	Coccidia	Lesmeister et al. 2008a
	Isospora sengeri	Coccidia	Lesmeister et al. 2008a, Levine and Ivens 1964
	Isospora spilogales	Coccidia	Lesmeister et al. 2008a, Levine and Ivens 1964
	Sarcocystis sp. indet.		Lesmeister et al. 2008a
	Trypanosoma cruzi[Z]	American trypanosomiasis, Chagas disease	Chapter 3
Helminths	*Acanthocephala* sp. indet.		Tiner 1946
	Baylisascaris columnaris[Z]	Skunk roundworm	Lesmeister et al. 2008a
	Capillaria aerophila[Z]	"Lungworm"; bronchial capillariasis, pulmonary capillariasis, thominxosis	Lesmeister et al. 2008a
	Capillaria hepatica[Z]	Hepatic capillariasis	Layne and Winegarner 1971
	Capillaria [*Aonchotheca*] *putorii*	Cat stomach worm	Lesmeister et al. 2008a
	Capillaria procyonis		Lesmeister et al. 2008a
	Centrorhynchus conspectus		Holloway 1958
	Crenosoma sp. indet.	"Lungworm"	Lesmeister et al. 2008a
	Macracanthorhynchus ingens[Z]		Richardson 2014
	Mesocestoides sp. indet.[Z]	"Tapeworms"	Tiner 1946
	Molineus sp. indet.		Lesmeister et al. 2008a, Tiner 1946
	*Physaloptera maxillaris**		Tiner 1946
	Physaloptera sp. indet.		Lesmeister et al. 2008a
	Placoconus lotoris	"Hookworms"	Lesmeister et al. 2008a
	Skrjabingylus chitwoodorum	Sinus roundworm	Ewing and Hibbs 1966, Higdon and Gompper 2020, Hill 1939, Kirkland and Kirkland 1983, Lesmeister et al. 2008a, Tiner 1946, Tumlison and Tumlison 2019
	Trichinella spiralis[Z]	Pork worm; trichinosis	Solomon and Warner 1969, Zimmermann et al. 1962

S. chitwoodorum is the endoparasite that has received the most attention regarding its impact on the historic and current population declines of *S. putorius*

(Higdon and Gompper 2020). The skunk sinus worm was first identified in striped skunks and ESS in Oklahoma (Hill 1939). Subsequent studies have reported prevalence estimates for *S. chitwoodorum* in North American ESS ranging from approximately 21-85% (Higdon and Gompper 2020, Kirkland and Kirkland 1983, Kirkland and Maldonado 1988, Lesmeister et al. 2008a).

Terrestrial gastropods serve as intermediate hosts and multiple species of small vertebrates (e.g., rodents, amphibians, shrews) act as paratenic hosts for *Skrjabingylus* spp. Skunk hosts become infected upon ingesting invertebrates or small vertebrates containing infective third-stage larvae (Lankester and Anderson 1971, Santi and Parker 2012). The transmission cycle is maintained when first-stage sinus worm larvae are shed in the infected skunk's feces and later penetrate foraging gastropods in which the larvae undergo two molts prior to reaching an infective third-stage form (Hansson 1967). As a result, ESS exhibit higher prevalence of *S. chitwoodorum* infections in regions with higher precipitation, which benefits both the obligate intermediate gastropod hosts and the environmental persistence of sinus worm larvae in feces (Fuller and Kuehn 1984, Hansson 1974, Higdon and Gompper 2020, Kirkland 1975, Kirkland and Kirkland 1983).

Once infective larvae enter the gastrointestinal system of a *S. putorius*, they may take as little as six days to migrate to the individual's sinus cavities, though significant *Skrjabingylus*-inflicted damage to the soft tissue and cranium usually develops over several months to a year, depending on the intensity of the infection (Higdon and Gompper 2020, Santi and Parker 2012). Sinus worm infections can compromise

immune system functioning, while simultaneously creating lesions that facilitate the development of secondary infections. Cranial damage is accompanied by inflammation, which can cause the frontal sinuses to expand in striped skunks, ultimately exerting pressure on the infected skunk's brain (Maldonado and Kirkland 1986). The larva migrans themselves might contribute to neurological problems for their host, especially if larvae stray off-course during migration (Santi and Parker 2012). Experimentally-infected and wild, naturally-infected striped skunks display neurologic symptoms similar to those associated with rabies (Ewing and Hibbs 1966, Lankester 1970, Lankester and Anderson 1971). Since ESS exhibit comparable damage to their soft tissues and osteology, infected *S. putorius* undoubtedly experience adverse neurologic behavior as a result of sinus worm infections. The prevalence of *S. chitwoodorum* in *S. putorius* samples submitted for rabies testing by public health agencies has been offered as supporting evidence for the neurologic symptoms induced in ESS by sinus worm infection (Higdon and Gompper 2020, Hughes et al. 2018). In following, *Skrjabingylus* infections may not only directly impact the survivorship of ESS but may also indirectly elevate individuals' exposure and susceptibility to other sources of morbidity and mortality.

S. chitwoodorum holds the potential for significant impacts on *S. putorius* at the population, subspecies, or species level and has been hypothesized as a major contributor to the decline of the plains spotted skunk (Higdon and Gompper 2020). However, Higdon and Gompper (2020) noted *S. chitwoodorum* prevalence was lower and damage was less severe in midwestern and eastern genetic clades of ESS when

compared to those for western and southwestern ESS, a pattern opposite to that predicted by a scenario in which plains spotted skunk declines are attributable to sinus worm infection. In fact, midwestern *S. putorius* have been co-evolving with *Skrjabingylus* for at least two thousand years, suggesting that any significant impact of sinus worms on ESS populations reflects a disruption of pre-existing disease dynamics. Evidence for this long host-parasite interrelationship is provided by a complete skull of *S. putorius* recovered from Freeman's Pit, Monroe County, Indiana (refer to Figure 2, p. 661, Richards 1985). Radiocarbon dated to 2,315 ± 65 years B.P., the skull demonstrates clear signs of *Skrjabingylus* infection (level 2 damage, following Kirkland and Kirkland [1983]) (Richards 1985). Therefore, any impact of sinus worm infections on *S. putorius* populations appears to emerge only in concert with other, recently-introduced, driving factors.

5.3.2.3.3. Other parasites of interest

Efforts should continue to explore the diversity of parasites hosted by ESS and to elucidate their direct and indirect effects on *S. putorius* within different abiotic and biotic contexts. Whenever relevant, ectoparasites should be collected not only for identification, but also for testing to investigate the infectious diseases for which they may act as carriers and/or vectors. Zoonotic pathogenic agents will be of interest to veterinary and human medical professionals alike, and parasites functioning as protective mechanisms for *S. putorius* health should be identified and conserved across individuals and populations to promote viability of the species.

Toxoplasma gondii exposure has caused clinical illness and contributed to significant population declines in wild musteloids, and serologic evidence exists for *T. gondii* exposure in western spotted and striped skunks (Burns et al. 2003, Conrad et al. 2005, Diters and Nielsen 1978, Franti et al. 1976, Gabriel et al. 2008, Møller and Nielsen 1964, Riemann et al. 1978, Suzán and Ceballos 2005, Tizard et al. 1976). The adverse impact of *Dirofilaria immitis* (etiologic agent of heartworm disease) and other filarial pathogens on wildlife populations has gone largely uninvestigated, but microfilarial infections have been recorded in striped skunks and *D. immitis* was detected in a striped skunk and a *S. g. amphiala* (Dickey) (Island Spotted Skunk) (Bakker et al. 2006, Chandler 1947, Saito and Little 1997, Webster and Beuregard 1964; B. Gulas-Wroblewski, unpubl. data). Moreover, the key geographic areas of canine heartworm prevalence overlap with the current range of *S. p. interrupta* (Bowman et al. 2016). *Conepatus chinga* (Molina) (Molina's Hog-nosed Skunk) have been diagnosed with *Leishmania*, while *Spilogale pygmaea* (Thomas) (Pygmy Spotted Skunk) and ESS have both been proposed as reservoirs for the protozoan parasite in Mexico (Buitrago et al. 2011, Stephens et al. 2009). Furthermore, the North American distribution of canine and human leishmaniosis corresponds with the range of the plains spotted skunk (Baneth and Solano-Gallego 2012). Each of these three zoonotic parasites could potentially play a role in ESS population dynamics, and understanding this species' participation in their transmission cycles is critical for veterinary and public health strategies directed towards the mitigation of these pathogenic agents.

The preservation of imperiled parasites has grown into a major movement within the conservation community, and parasites are listed on the IUCN Red List along with their threatened and endangered host species. In fact, taxon-specific parasites are often more endangered than their vertebrate hosts (Carlson et al. 2020, Kwak 2018). In light of ESS decline across its North American range, the parasites of S. putorius themselves should be assessed for threatened status and comprehensively integrated into any conservation measures enacted for ESS populations. The protocols adopted for the concurrent protection of the highly-endangered *Lynx pardinus* (Temminck) (Iberian Lynx) and their lice, *Felicola isidoroi*, can serve as a model for any ESS parasite preservation initiatives that may prove necessary in the future (Pérez et al. 2013).

5.3.2.4. Viruses

5.3.2.4.1. Canine distemper virus

Canine distemper virus (CDV) is widespread in North American mephitids and other wildlife and has contributed to the declines of terrestrial carnivores worldwide (Carpenter et al. 1976, Deem et al. 2000, Diters and Nielsen 1978, Feng et al. 2016, Gehrt 2005, Goss 1948, Helmboldt and Jungherr 1955, Martinez-Gutierrez and Ruiz-Saenz 2016, Møller and Nielsen 1964, Rendon-Marin et al. 2020, Roscoe 1993, Verts 1967, Woolf et al. 1986). Harris et al. (in rev.) report on the first cases of canine distemper virus (CDV) in an ESS population in western North Carolina. At least 50% of the authors' study population succumbed to this morbillivirus. The low connectivity of regional *S. putorius* populations exacerbates the impact of this high mortality rate on the population and elevates the risk of localized ESS extirpation (Harris et al., in rev.).

While acknowledging the potential influence of infectious disease on the rapid decline experienced by ESS in the 1940s and 1950s, Gompper and Hackett (2005) dismiss CDV as a plausible explanation. The authors argue that the *S. putorius* population crash failed to demonstrate the periodicity and spatial dynamics typifying CDV or rabies epidemics (Gompper and Hackett 2005). However, several devastating outbreaks of CDV in domestic dogs and wildlife populations were reported across the United States in the 1950s (Panzera et al. 2015). More recent investigations of CDV disease dynamics in Africa have recorded asynchronicity in the cyclicity commonly associated with these viral outbreaks. The deviation from traditional disease patterns occurs when the virus spills over into wildlife from and is maintained by domestic dog populations, a characteristic likely exhibited by the CDV epizootics of mid-20th century U.S.A. (Viana et al. 2015). Consequently, it is impossible to discount CDV as a potential causative agent in the precipitous decline of ESS populations during the 1940s and 1950s. Irrespective of this virus' connection to an historic population crash, CDV is undoubtedly responsible for a significant degree of morbidity and mortality across *S. putorius* today and may contribute substantially to future extinctions of regional populations (Harris et al., in rev.).

5.3.2.4.2. Rabies

ESS play an integral role in the enzootic and epizoonotic cycles of the rabies virus across North America, a disease ecology that has been thoroughly reviewed by Krebs et al. (2000, 2001). In fact, spotted skunks were christened the "phobi-cat" or "phobey cat" by 18th century explorers and pioneers crossing the American plains, who

associated *S. putorius* with rabies and the attendant symptom of hydrophobia (Beran 1994, Fagan 1950).

As referenced above, Gompper and Hackett (2005) excluded rabies from the viral candidates likely to have contributed to ESS declines in the 1940s-1950s. Nevertheless, Steele (1988) reports on the history of rabies in skunk populations as follows:

> *Skunks were known to transmit rabies in the mid19th century, and outbreaks of skunk-transmitted disease were reported by the U.S. Army as a source of rabies among horses and soldiers in the western frontier forces in the 1870s. However, this situation did not evolve into an epizootic until the 1940s and 1950s, when the great plains epizootic erupted (1950-1951). The University of Minnesota held a conference on skunk rabies in the spring of 1952. Reports at the meeting told of tens of thousands of skunks that were coming out of winter hibernation and dying. Wildlife officers said they piled up frozen dead skunks in cords- a measurement used for woodpiles. (p. 587, Steele 1988)*

Certainly, the "great plains epizootic" imposed heavy losses on ESS populations, which may have depressed population numbers sufficiently to subject them to a chronic decline influenced by demographic stochastic factors. Moreover, the absence of the predicted periodicity of rabies outbreaks during this time period and the following decades can be attributed to the migration and mixing of a diversity of rabies strains across the range of

S. putorius. Several genetically distinct variants of the rabies virus circulate within the U.S. and are maintained in the wild by terrestrial carnivore species, including skunks, bats, and raccoons (Bacon 1985). Although variants arise in and are strongly associated with specific reservoir species, they can spill over and adapt to alternate reservoir species during epizootics (Bacon 1985). Fox rabies was first recognized in eastern North America during the colonial period, but did not begin increasing in prevalence until the 1940s after which the strain grew to epizootic importance and accounted for the majority of rural rabies cases from Canada south to Florida and west to Texas (Fagan 1950, Sikes 1962, Steele 1988). In the decades to come, human-directed movement of infected domestic dogs and wildlife reservoirs of raccoon rabies and other strains of the virus combined with the natural migration of fox rabies to disrupt historic disease dynamics across the midwestern U.S. and eastern seaboard (Dobson 2000, Steele 1988). The introduction of a diversity of rabies viral variants to a pool of potential wildlife and domestic animal hosts generated the ideal conditions for the establishment of continuous epizootics within animal populations. Overall elevated susceptibility to rabies infection combined with a lowered rate of post-infection immunity in a continuously-interacting aggregation of mammalian hosts such that no break existed between the exposure of one population and that of another and no immune population survived to prevent the development of the next outbreak (Blancou 1988).

Although the degree to which historic and current population dynamics of ESS have been influenced by rabies infections is unclear, the potential for the disease to cause temporary or permanent regional extirpations of the species across its range increases as

populations decline in number and extenuating factors elevate individuals' exposure and susceptibility to the virus.

5.3.2.4.3. Other viruses of interest

Gompper and Hackett (2005) identify parvoviruses as the most likely candidates for a disease-related decline in ESS, a hypothesis well-supported by the effects of parvoviruses on other mephitid and wildlife species. The authors posit the 1940s global outbreak of mink enteritis virus, first documented on mink farms in the United States, as a potential source of spillover to *S. putorius* (Gompper and Hackett 2005). A similar transmission of Aleutian disease parvovirus (ADV, carnivore amdoparvovirus 1) to a raccoon population transpired on a mink farm in Utah (Oie et al. 1996). In the 1980s, canine parvovirus mutated from the feline panleukopenia virus and quickly expanded into a global pandemic, causing up to 90% mortality in some wildlife populations (Parrish 1994, Steinel et al. 2001). Mink viral enteritis, feline panleukopenia, canine parvovirus, and ADV can infect and are responsible for varying degrees of morbidity and mortality in western spotted and striped skunks throughout North America (Allender et al. 2008, Bakker et al. 2006, Barker et al. 1983, Britton et al., 2015, Farid, 2013, Gehrt 2005, Giannitti et al. 2017, Glueckert et al. 2019, LaDouceur et al. 2015, Nituch et al., 2015, Oie et al. 1996, Pennick et al. 2007, Suzán and Ceballos 2005, Woolf and Gremillion-Smith 1986). Most recently, a skunk-specific amdoparvovirus (SKAV or carnivore amdoparvovirus 4) was identified as the causative agent of an epizootic impacting striped skunks throughout California (Canuti et al. 2017, Glueckert et al. 2019).

Other non-zoonotic viruses that are symptomatic in *M. mephitis* and may, therefore, play a role in S. putorius disease ecology include infectious canine hepatitis and canine herpesvirus (Alexander et al. 1972, Karstad et al. 1975, Diters and Nielsen 1978).

Several zoonotic viruses may prove to be of combined conservation and One Health interest in ESS populations. West Nile virus (WNV) infects striped skunks, while exposure to a non-WNV flavivirus was reported for striped skunks and *M. macroura* (Lichtenstein) (Hooded Skunk) (Anderson et al. 2001, Bentler et al. 2007). Powassan virus, rotaviruses, and low pathogenic avian influenza have all been reported in striped skunks (Evans 1984, Johnson 1987, Root et al. 2014). In two cases, striped skunks have been the recipients of reverse zoonotic exchanges of viruses from humans: once in a rhinitis infection and another in a fatal case of Herpes simplex (Britton et al. 2019, Charlton et al. 1977).

5.3.3. Future Trends in ESS Disease Ecology

The dynamic interplay of each of these pathogens in the current and future population ecology of *S. putorius* is constantly evolving, transformed by the effects of burgeoning habitat fragmentation and alteration, pathogen spillover across the domestic-wildlife interface, anthropogenic climatic change, the development of recombinant strains of infectious disease and antimicrobial-resistant pathogens, and the profusion of immunosuppressant chemicals throughout the environment (Cunningham et al. 2017, Daszak et al. 2000). In this section, I will explore the possible ramifications of these anthropogenic effects for ESS disease ecology.

5.3.3.1. Globalization and development

As modernization and urbanization drive demographic and habitat use changes across North America, ESS experience the direct and indirect impacts of these alterations. The physiological stress induced by habitat fragmentation and loss, exposure to increased anthropogenic activity, and contact with chemical toxicants employed in domestic and agricultural settings increases the susceptibility of *S. putorius* to infectious diseases (Daszak et al. 2000, Murray et al. 2019, Serieys et al. 2018). Simultaneously, ESS are exposed to a greater variety of infectious diseases across the domestic-wildlife interface as discussed above. ESS exposure to human activities themselves can instigate disease. For example, wind-blown dust from construction projects has been linked to *Histoplasma capsultum* outbreaks in adjacent human populations (Leznoff et al. 1964).

Anthropogenic habitat alteration can fragment ESS populations, severing connectivity between previously-linked aggregations. As described by Harris et al. (in rev.) in the case of a CDV outbreak, disease-induced mortality can have drastic consequences for isolated populations of *S. putorius*. Alternatively, loss of connectivity between ESS populations may be protective, reducing the spread of pathogenic agents between populations when epizootics erupt in isolated aggregations, as illustrated by feline immunodeficiency virus outbreaks in *Lynx rufus* (Schreber) (Bobcat) in California (Kozakiweicz et al. 2020).

With globalization linking both animal and human populations closer together and reducing the timeframe within which infectious diseases spread across geographic space, *S. putorius* populations experience greater risk from the spillover of highly-

pathogenic diseases of veterinary and zoonotic importance. The rapid and devastating global spread of novel strains of parvoviruses and CDV in domestic animal and wildlife populations are testament to this impending epidemiological threat (Anis et al. 2018, Behdenna et al. 2019, Panzera et al. 2015, Steinel et al. 2001). The spillover of pathogenic agents is not limited to those circulating in other wildlife or domestic animal populations, but include those infecting humans as well, evidenced by the reverse zoonotic transmission of rhinitis and Herpes simplex in striped skunks (Britton et al. 2019, Charlton et al. 1977). Of immediate concern should be SARS-CoV-2, the causative agent of COVID-19. The highly infectious virus is responsible for respiratory disease outbreaks and high mortality in mink farms across the world (Molenaar et al. 2020, Munnink et al. 2020, Bosco-Lauth et al. in rev.). Molecular tracing of 16 of these incidents revealed their origins to be human to mink transmission of the disease with a single case of reverse spillback to a Dutch worker (Munnink et al. 2020). Mustelid species are generally employed as analogs for mephitid taxa in disease investigations due to their similar physiologies and clinical manifestations of pathogenic agents. In following, ESS are predicted to respond to exposure and infection with SARS-CoV-2 in an equivalent presentation to that evidenced in these mink. In fact, striped skunks that have been experimentally infected with the virus are not only susceptible to infection, but are competently shed SARS-CoV-2 in respiratory secretions (Bosco-Lauth et al. in rev.).

Anthropogenic activity and globalized trade networks seed introduced, non-native taxa throughout the ranges of ESS (Bellard et al. 2016). The use of peridomestic

and edge habitats by *S. putorius* populations also elevates their risk of exposure to invasive alien species (IAS), which holds varying consequences in terms of ESS disease ecology. IAS promote the spread of infectious disease directly by introducing novel infectious agents that then spill over into naïve endemic populations, amplifying endemic and novel diseases, maintaining endemic and novel pathogens at artificially-elevated levels, causing outbreaks of pathogenic agents, initiating spillback amplification that increases the relative parasite load of native species, and/or acting as "mixing pots" for especially virulent zoonotic pathogens (Young et al. 2017). IAS also indirectly affect infectious disease dynamics by altering linkages within ecosystems to promote the transmission of disease. In the case of ESS, *Sus scrofa* (L.) (Feral Hog) greatly overlap *S. putorius* ranges throughout the United States, undoubtedly threatening the health of these skunk populations (USDA 2020). Feral hogs host and directly transmit a wide variety of infectious diseases and, through their rooting behavior, indirectly create ideal mosquito breeding habitat, which increases the spread of mosquito-borne pathogens, such as WNV (Hutton et al. 2006, Pejchar and Mooney 2009). Conversely, IAS may serve to mitigate or even eliminate infectious diseases from certain wildlife populations by diluting endemic and/or invasive parasites or pathogens, releasing native taxa from disease pressure, or predating parasites, disease vectors, or hosts of pathogenic agents (Young et al. 2017). Although ESS experience direct and indirect negative effects from S*olenopsis invicta* (Buren) (Red Imported Fire Ant), they may also benefit from these ants' consumption of mites, ticks, and other, pathogen-spreading ectoparasites (Graham et al. 2012, Harris and Burns 1972).

5.3.3.2. Climate change

Anthropogenic climate change manifests in rising incidents of climatic extremes (hurricanes, droughts, flooding events), long-term changes in rainfall and temperature, global and localized sea-level rise, and disruptions to food production systems and water resources, all of which greatly influence human and animal movements and human alteration of habitats (Caminade et al. 2019, Daszak et al. 2000, Cunnigham et al. 2017, Woolhouse et al. 2005). These migrations shift and mix pathogen hosts, reservoirs, dilutors, and/or vectors, dramatically altering disease dynamics at every scale (Caminade et al. 2019, Daszak et al. 2000, Cunnigham et al. 2017, Woolhouse et al. 2005). Alterations in temperature, humidity, and precipitation combine to further mediate disease transmission and vectorial capacity. For instance, increased precipitation, reduced freezing events, warming, droughts, and other climatic shifts create environments more amenable to the pathogens themselves, their spread, their hosts, and their vectors (Caminade et al. 2019, Dye 1986, Lafferty and Mordecai 2016).

The ramifications of these changing climatic patterns have been recently explored for a number of ESS pathogens. As the arthropod vectors for tularemia and tick-borne diseases follow the expansion of their preferred dry, forested habitat northward in the United States, so too do the pathogenic agents they transmit (Caminade et al. 2019; Eisen et al. 2008, 2016; Estrada-Pena et al. 2012; Nakazawa et al. 2007). In other regions, increased flooding episodes, tropical storms, and average amount of rainfall correlates with elevated cases of histoplasmosis, dermatophytosis, and leptospirosis (Friedman and Schwartz 2019, Lau et al. 2010, Maiga et al. 2018). The

northward movement of the freeze line and milder winters in more recent years has also extended the survival of infective dermatophytes, *Histoplasma* spores, and *Skrjabingylus* larvae over winter and shifted disease prevalence for these pathogenic agents to more northerly animal and human populations (Friedman and Schwartz 2019, Hughes et al. 2018, Kirkland and Maldonado 1988). The increased transmission and movement of these infectious agents will impact *S. putorius* differentially throughout their range, but these patterns can serve as general predictors for what diseases may be introduced, become endemic, or increase in virulence for certain ESS populations.

5.3.3.3. Recombinant strains and resistance-evolving organisms

Human-mediated, environmental, ecological, and climatic changes have driven the development of recombinant strains and resistant forms of organisms worldwide. Rising global temperatures promoted the evolution of more heat-tolerant forms of fungal pathogens, while urbanization facilitated the diversification and hybridization *Histoplasma* strains, which spillback into endemic populations via migrating birds and bats (Robert et al. 2015, Rodrigues et al. 2020). The extensive (mis)use of pesticides, fungicides, and antimicrobial drugs has hastened the evolution of chemical-resistant insects, rodents, fungus, and other pathogenic agents (Daszak et al. 2000, Friedman and Schwartz 2019, Hemingway and Ranson 2000, McGee et al. 2020, Naqqash et al. 2016, O'Neill 2016, Weathered and Hammill 2019). These dynamics can increase the exposure and susceptibility of ESS to potentially more-virulent or novel strains of infectious disease, while simultaneously risking adverse effects on pre-existing host-pathogen relationships via genotypic evolution of pathogens, host switching,

hybridization of strain and vectors, and the elimination of natural vector control agents (Daszak et al. 2000, O'Neill 2016, Weathered and Hammill 2019). The spillover of antimicrobial resistant microbes from agricultural operations into adjacent natural areas elevates the risk of exposure to these pathogens in ESS frequenting these areas, with unknown consequences for *S. putorius* and other wildlife health (O'Neill 2016). Pesticide resistance in the rodent and invertebrate prey of ESS increases their ingestion of these chemical toxicants and the linked immunosuppression and disease susceptibility effects (McGee et al. 2020, Serieys et al. 2018).

5.3.3.4. Chemical toxicants

The increased use of synthetic pesticides has been hypothesized as one of the contributors to the historic decline of ESS either through direct losses from primary and/or secondary poisonings or reduced survivability from loss of prey (Gompper & Hackett 2005, Hamilton & Fox 1987, Landholt and Genoways 2000, Nilz and Finck 2008, Schwartz & Schwartz 1981). Although an ecotoxicological study of *S. putorius* has yet to be conducted, a review of pesticides reported in striped skunks supports the likelihood that ESS are exposed to a number of organochlorines, organofluorines, anticholinesterase pesticides, non-anticoagulant rodenticides, and first and second generation anticoagulant rodenticides (Table 5.3). In many cases, *M. mephitis* not only exhibit more exposure to chemical pesticides at higher concentrations in natural environments, but also can withstand greater dosages of these toxicants compared to mustelids and other mesocarnivores (e.g., Savarie et al. 1983; Hill & Dent 1985). For example, Frank et al. (1979) recovered higher residues of p,p'-DDE in skunk tissues

than in those of *Vulpes pop* (L.) (Red Fox) and raccoons. These authors also found traces of TDE in the brains of 18% of red foxes, 5% of raccoons, and 88% of striped skunks sampled (Frank et al. 1979). A controlled feeding study of five *M. mephitis* and two *Mustela erminea* (L.) (Ermine), measuring the toxicity of diphacinone, killed 50% of the mustelids without any effect on the five striped skunks (Pank & Hirata 1976). These findings suggest that ESS exposure to similar toxicants is unlikely to cause direct mortality, instead compromising their immune functioning to elevate disease susceptibility and/or altering ESS behavior to potentially increase their exposure to pathogenic agents. For instance, the neurologic effects induced in skunks by chemical toxicants can reduce their avoidance behavior to vectors of disease (Scott et al. 1959, Spencer 1945; Marsh 1967; U.S. Army 1976; Hegdal et al. 1979, Eastland and Beasom 1987). In addition, inflammatory responses and immune suppression linked to rodenticide exposure increases wildlife's susceptibility to opportunistic infections (Serieys et al. 2018). The association between notedric mange and sublethal first and second generation anticoagulant rodenticide secondary poisoning has been well-established in bobcats and *Puma concolor* (L.) (Mountain Lion) (Riley et al. 2010; Serieys et al. 2015, 2018). Comparable interactions between chemical toxicants and disease dynamics are expected for *S. putorius*, most notably in those populations more heavily exposed to pesticides in agricultural and peridomestic habitats.

Table 5.3. Chemical insecticides and rodenticides reported in wild *Mephitis mephitis* (L.) (Striped Skunk). Tissues collected and natural accumulation of pesticides at lethal and sublethal doses are listed for studies in which these samples were tested. *Animals euthanized for study. †Animals found dead post-chemical application. CF=Content in fat. N/A=Not applicable. U=Unspecified. WWT=Wet weight tissue.

Pesticide	Individuals sampled	Tissue sampled	Amount of pesticide measured in tissue	Geographic location	References
Brodifacoum (3-[3-[4-(4-Bromophenyl)phenyl]-1,2,3,4-tetrahydronaphthalen-1-yl]-2-hydroxychromen-4-one)	1†	Liver tissue	1.05 ppm	Albany County, NY	US EPA 2002
Brodifacoum (3-[3-[4-(4-Bromophenyl)phenyl]-1,2,3,4-tetrahydronaphthalen-1-yl]-2-hydroxychromen-4-one)	1†	Liver tissue	0.3 ppm	Delaware County, NY	US EPA 2002
Bromadiolone (3-[3-[4-(4-Bromophenyl)phenyl]-3-hydroxy-1-phenylpropyl]-2-hydroxychromen-4-one)	3†	Liver tissue	0.02 ppm, 0.28 ppm, 0.08 ppm	Westchester County, NY	Stone et al. 1999
Carbofuran (2,2-Dimethyl-2, 3-dihydro-1-benzofuran-7-ylmethylcarbamate)	1†	Brain tissue	0.85 and 0.79 µmol acetylthiocholine iodide hydrolyzed/min/g WWT	Saskatchewan, Canada	Wobeser et al. 2004
Carbofuran (2,2-Dimethyl-2, 3-dihydro-1-benzofuran-7-ylmethylcarbamate)	4†	N/A	N/A	Canada	Wobeser et al. 2004
Carbofuran (2,2-Dimethyl-2, 3-dihydro-1-benzofuran-7-ylmethylcarbamate)	1†	N/A	N/A	VA	Stinson et al. 1994
Chlordane (1,2,4,5,6,7,8,8-Octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane) + Toxaphene	3†	N/A	N/A	Northcentral WY	Post 1951
Cyclodiene (1,2,3,4,5,5-hexachlorocyclopenta-1,3-diene)	1†	N/A	N/A	Albany, NY	Okoniewski & Novesky 1993
DDE (1,1′-(2,2-dichloroethene-1,1-diyl)bis(4-chlorobenzene))	8*	brain tissue	Mean = 13 ppb; SD = 9.5 ppb WWT	Ontario, Canada	Frank et al. 1979
DDE (1,1′-(2,2-dichloroethene-1,1-diyl)bis(4-chlorobenzene))	24*	liver tissue	Mean = 6.0 ppb; SD = 14.0 ppb WWT	Ontario, Canada	Frank et al. 1979
DDE (1,1′-(2,2-dichloroethene-1,1-diyl)bis(4-chlorobenzene))	26*	Muscle tissue	Mean = 9.3 ppb; SD = 9.1 ppb WWT	Ontario, Canada	Frank et al. 1979
DDE (1,1′-(2,2-dichloroethene-1,1-diyl)bis(4-chlorobenzene)) +TDE (1,1-Bis(p-chlorophenyl)-2,2-Dichloroethane)	8*	brain tissue	Mean = 140 ppb; SD = 130 ppb CF	Ontario, Canada	Frank et al. 1979
DDE (1,1′-(2,2-dichloroethene-1,1-diyl)bis(4-chlorobenzene)) +TDE (1,1-Bis(p-chlorophenyl)-2,2-Dichloroethane)	24*	liver tissue	Mean = 61 ppb; SD = 49 ppb CF	Ontario, Canada	Frank et al. 1979
DDE (1,1′-(2,2-dichloroethene-1,1-diyl)bis(4-chlorobenzene)) +TDE (1,1-Bis(p-chlorophenyl)-2,2-Dichloroethane)	26*	Muscle tissue	Mean = 57 ppb; SD = 58 ppb CF	Ontario, Canada	Frank et al. 1979
DDT (1-chloro-4-[2,2,2-trichloro-1-(4-chlorophenyl)ethyl]benzene)	2†	N/A	N/A	Camp Bullis, TX	George & Stickel 1949

Table 5.3. Continued.

Pesticide	Individuals sampled	Tissue sampled	Amount of pesticide measured in tissue	Geographic location	References
Dieldrin ((1aR,2R,2aS,3S,6R,6aR,7S,7aS)-3,4,5,6,9,9-hexachloro-1a,2,2a,3,6,6a,7,7a-octahydro-2,7:3,6-dimethanonaphtho[2,3-b]oxirene)	8*	brain tissue	Mean = 0.3 ppb; SD = 0.3 ppb WWT	Ontario, Canada	Frank et al. 1979
Dieldrin ((1aR,2R,2aS,3S,6R,6aR,7S,7aS)-3,4,5,6,9,9-hexachloro-1a,2,2a,3,6,6a,7,7a-octahydro-2,7:3,6-dimethanonaphtho[2,3-b]oxirene)	2 (1†)	N/A	N/A	IL	Scott et al. 1959
Heptachlor epoxide (1,4,5,6,7,8,8-Heptachloro-3a,4,7,7a-tetrahydro-4,7-methano-1H-indene)	1†	Liver tissue	3.7 ppm	central LA	DeWitt et al. 1962
Heptachlor epoxide (1,4,5,6,7,8,8-Heptachloro-3a,4,7,7a-tetrahydro-4,7-methano-1H-indene)	1†	Kidney tissue	18.7 ppm	central LA	DeWitt et al. 1962
Methomyl ((E,Z)-methyl N {[9methylamino)carbonyl]oxy} ethanimidothioate)	8†	N/A	N/A	College Station, TX	Connolly et al. 1986
Mirex (1,1a,2,2,3,3a,4,5,5,5a,5b,6-dodecachlorooctahydro-1H-1,3,4-(methanetriyl)cyclobuta[cd]pentalene)	2*	Muscle tissue	6 months post-treatment: 3.5 ppm WWT	AL and GA	Hill & Dent 1985
Mirex (1,1a,2,2,3,3a,4,5,5,5a,5b,6-dodecachlorooctahydro-1H-1,3,4-(methanetriyl)cyclobuta[cd]pentalene)	2*	Muscle tissue	1.5 years post-treatment: 1.34 ppm WWT	AL and GA	Hill & Dent 1985
Mirex (1,1a,2,2,3,3a,4,5,5,5a,5b,6-dodecachlorooctahydro-1H-1,3,4-(methanetriyl)cyclobuta[cd]pentalene)	2*	Muscle tissue	2 years post-treatment: 0.19 ppm WWT	AL and GA	Hill & Dent 1985
Mirex (1,1a,2,2,3,3a,4,5,5,5a,5b,6-dodecachlorooctahydro-1H-1,3,4-(methanetriyl)cyclobuta[cd]pentalene)	1*	Liver tissue	6 months post-treatment: 0.44 ppm WWT	AL and GA	Hill & Dent 1985
Mirex (1,1a,2,2,3,3a,4,5,5,5a,5b,6-dodecachlorooctahydro-1H-1,3,4-(methanetriyl)cyclobuta[cd]pentalene)	2*	Liver tissue	1.5 years post-treatment: 0.61 ppm WWT	AL and GA	Hill & Dent 1985
Mirex (1,1a,2,2,3,3a,4,5,5,5a,5b,6-dodecachlorooctahydro-1H-1,3,4-(methanetriyl)cyclobuta[cd]pentalene)	1*	Liver tissue	2 years post-treatment: 0.13 ppm WWT	AL and GA	Hill & Dent 1985
Compound 1080 (Sodium 2-fluoroacetate)	3†	Stomach tissue	1.5 ppm	south-central CA	Hegdal et al. 1979
Compound 1080 (Sodium 2-fluoroacetate)	U	N/A	N/A	Fort Ord complex, CA	U.S. Army 1976
Compound 1080 (Sodium 2-fluoroacetate)	U	N/A	N/A	USA	Spencer 1945; Marsh 1967
TDE (1,1-Bis(p-chlorophenyl)-2,2-Dichloroethane)	8*	brain tissue	Mean = 0.6 ppb; SD = 0.4 ppb WWT	Ontario, Canada	Frank et al. 1979
Toxaphene	1†	N/A	N/A	NE	McCarraher & Dean 1959
Warfarin	U	N/A	N/A	USA	McCulloch 1952

5.3.4. ESS in a One Health Framework

Depending on the evolutionary and ecological context, *S. putorius* play varying roles in the transmission cycles of different pathogenic agents: host, vector, reservoir, amplification host, or dilutor. As described above, *S. putorius* host a number of zoonoses of great veterinary, conservation medicine, and/or public health importance, most notably rabies, *Leptospira*, the potentially-bioterrorist agent *Francisella tularensis*, and *Baylisascaris columnaris*, the sister taxon of which (*B. procyonis*) has decimated the endangered *Neotoma magister* (Baird) (Allegheny Woodrat), (Babudieri 1958; Farlow et al. 2005; Kazacos 2016; Ko et al. 2009; Farlow et al. 2005; Nelson et al. 2013; Page et al. 2012; Sapp 2016, 2018; van de Maele et al. 2008, WHO 2011). Although the degree to which ESS contribute to the maintenance of these infectious pathogens in sylvatic and peridomestic cycles is unknown, their role as hosts for these diseases can be of benefit to epidemiologists. The previous review of *S. putorius* disease ecology serves as a base for exploring the capacity of ESS to act as sentinels and dilutors of zoonotic diseases within a One Health framework.

5.3.4.1. ESS as sentinels for zoonoses

The behavioral and natural history traits detailed above make *S. putorius* ideal sentinels for infectious disease based on their increased exposure to pathogens and co-habitation of both sylvatic and anthropogenic environments (Neo and Tan 2017, Rabinowitz et al. 2010). The ability of ESS to respond to pathogenic agents in a detectable manner (i.e. via serologic, PCR, fecal analyses, and other forms of diagnostic testing), to serve as markers for continuous exposure risk, and to facilitate the

investigation of the ecology of the pathogen(s) of interest maximize their potential for surveillance strategies aimed at the early detection of epidemics or epizootics, on-going monitoring of infectious disease, and/or evaluations of the efficaciousness of various disease control measures (McCluskey 2003). In practice, ESS are already effectively incorporated into rabies surveillance programs conducted by public health agencies across their North American range. The coordinated, opportunistic sampling of study animals, individuals harvested by trappers and hunters, those submitted for rabies testing, individuals trapped by animal control operations, road-kills, and patients of wildlife rehabilitation facilities can effectively counter the rarity and cryptic nature of ESS and expand the scope of epidemiological surveillance. As such, *S. putorius* prove to be excellent potential sentinels for disease not only for other wildlife species, but also for regional domestic animal and human populations (Neo and Tan 2017).

5.3.4.2. ESS as dilutors of zoonoses

Wildlife taxa can "dilute" the amount or diversity of pathogenic agents circulating in a system by reducing the transmission of vector-borne infectious disease in three key ways: 1. acting as an inefficient agent of transfer for the pathogen to a feeding vector, 2. limiting the number and/or efficiency of disease vector(s), and 3. limiting the number and/or efficiency of competent reservoirs for the disease (Keesing et al. 2006, Levi et al. 2016). ESS exhibit each of these disease dilution mechanisms in varying contexts.

The inefficiency of *S. putorius* in the transmission of an infectious agent to a vector can be evidenced in either the failure of transfer at the time of interaction with a

vector or the inability of the vector to engage in the behavior necessary to facilitate the transfer. WNV provides an example of the former scenario. The level of viremia in the whole blood of WNV-infected mammals is too low to infect feeding mosquitoes. Consequently, the viral pathogen is not transferred from the mammal to the mosquito and, thus, cannot be transmitted to other hosts by the hemophilic vector (Austgen et al. 2004, Bunning et al. 2002, Komar 2000). Therefore mosquito-parasitized ESS may become infected with WNV, but the skunks serve as a dead-end host for the virus and fail to refresh the circulating supply of pathogens in the WNV transmission cycle in which they play a part. *S. putorius* may also serve as dilutors for other infectious diseases by virtue of being relatively ineffective hosts for the vectors of the pathogens. Levi et al. (2016) quantified the feeding success of tick larvae on striped skunks as 4-24%, most of the ectoparasites being removed by grooming prior to completion of feeding. An analogous ESS-tick relationship is predicted for ESS, supporting their role as dilutors for tick-borne diseases.

A wildlife host can further reduce infectious pathogen transmission by reducing the number or efficiency of disease vectors or reservoirs. The dilution effect is greatly elevated in the case of reservoirs that serve as "amplification hosts" for infectious pathogens (Keesing et al. 2006, Levi et al. 2016). As reviewed earlier, the omnivorous diet of ESS includes a wide variety of invertebrate and small vertebrate prey, which frequently act as both reservoirs and vectors of pathogenic agents. Contained in this list are terrestrial gastropods (vectors for *Skrjabingylus* and *Angiostrongylus cantonensis*, the zoonotic parasite responsible for rat lungworm), triatomine insects (vectors for

Trypanosoma cruzi, etiologic agent of Chagas disease), and rodents like the white-footed mouse (amplification hosts for *Leptospira* spp., *Borrelia* spp., Hantavirus, and a number of other zoonotic pathogens). The dietary habits of ESS thus limit the number and efficiency of both vectors and competent reservoirs of pathogenic agents, diluting the pool of diseases available to infect other wildlife, domestic animals, and humans in their environment.

Through their multiplicity of diluting effects on vector-borne zoonotic pathogen transmission, *S. putorius* provide the invaluable ecosystem services (i.e., Nature's Contributions to People) of disease prevention and mitigation (Kadykalo et al. 2019, Pascual et al. 2017). In a post-COVID-19 world, where over 60% of all known human diseases and an estimated 75% of all emerging and re-emerging pathogens are zoonotic and infectious disease is listed as among the top five contributors to worldwide wildlife extinction and biodiversity loss, the importance of any diluting roles ESS play in the transmission dynamics of infectious diseases cannot be overstated (Daszak et al. 2000, Smith et al. 2006).

5.3.5. Guidance for ESS Practitioners

The One Health relevance of *S. putorius* disease dynamics and the impact of infectious diseases on the population ecology of ESS translate into a number of practical concerns for researchers, wildlife managers, and other ESS professionals. Within this section, I will review some of the applications of epidemiological knowledge to the practice of wildlife management and research, including recommended disease control measures and developing One Health strategies for the conservation of ESS.

5.3.5.1. Prevention and mitigation of disease transmission

Many of the pathogens hosted by *S. putorius* are zoonotic in nature and pose serious threats to humans as well as other animals. Similarly, the circulation of zoonoses in human, domestic animal, and wildlife populations to which ESS are exposed can either directly or indirectly impact *S. putorius*. The full diversity of infectious pathogens hosted and transmitted by ESS are unknown, complicating attempts to diagnose symptomatic infections and predict asymptomatic ones. Of the described zoonoses evidenced in *S. putorius*, many manifest as subclinical infections or fail to produce any symptoms whatsoever. For instance, *Leptospira* spp. can be maintained and shed from the kidneys of asymptomatic wild striped skunks for more than two years, while infectious levels of rabies virus can be shed in the saliva of *M. mephitis* up to six days before individuals display neurologic and behavioral abnormalities (Charlatan et al. 1991, Roth et al. 1963, Tabel and Karstad 1967). This high level of uncertainty in the degree to which apparently healthy ESS are shedding infectious material and the specific pathogens that are being transmitted highlights the danger of the unknown for skunk handlers. Accordingly, conscientious employment of appropriate personal protective equipment (P.P.E.), quarantine measures, animal handling and care, and equipment decontamination must be followed whenever working with ESS. P.P.E. and other protective measures should be practiced not only to prevent the spread of disease from ESS to others, but also to mitigate the transmission of zoonotic pathogens from human handlers to skunks. Even when asymptomatic, infections in humans, such as Herpes

simplex, hold the potential for being fatal in *S. putorius* (Britton et al. 2019, Charlton et al. 1977).

Strategizing sterilization procedures for all materials that come into contact with *S. putorius* individuals, their body fluids, or other potentially-infectious fomites is a critical component of fieldwork planning that is often overlooked, but which can have major, deleterious consequences for the transmission of diseases between ESS study animals, other wildlife, personnel, and the larger community of humans and animals to which each is inextricably linked. Recommended decontamination procedures specific to zoonotic diseases of interest can be accessed at http://www.cdc.gov. Some products may not be amenable to the required sterilization techniques in which case alternative or disposable items must be substituted. For example, the adhesive eggs of *Baylisascaris* spp. are extremely resistant to drying, freezing, and U.V. exposure, surviving for at least twelve years in natural environments, six months at -15°, and two months at -20°, and developing to an infective stage in 10% formalin and in zinc sulphate solution. Spot application of extreme heat (e.g., via propane torch, boiling water, autoclave, or incinerator) is the only method through which infective *B. columnaris* passed in the feces of ESS can be eliminated from live traps and other equipment (CDC 2018 Kazacos 2016, Moran et al. 1994)

The threat posed by unpredictable and unidentified infectious pathogens in ESS is shared by laboratory personnel handling any biological material collected from these individuals. The situation is aggravated further in the case of veterinary diagnosticians and epidemiologists, when serious concerns arise not only for a biosafety, but also the

possibility of a missed diagnosis of a serious pathogen. Screening for zoonotic infectious agents for which no consistent pathological signs are present can be a dangerous and challenging task for lab workers, as was the case when *F. tularensis* (normally only tested for in rodents, lagomorphs, and primates) was recovered from a seemingly healthy striped skunk (Origgi et al. 2013).

SARS-CoV-2 raises a timely concern regarding protocols for ESS work. The discovery of the ability for striped skunks to become infected with and effectively spread the virus via infected respiratory secretions highlights the risk of working in close contact with mephitid species (Bosco-Lauth et al. in rev.). The capacity for the virus to spill over into and spillback from mustelids underscores the critical responsibility wildlife professionals bear in the prevention of disease transmission to, from, and between the animal and human communities with which we interact (Molenaar et al. 2020, Munnink et al. 2020). SARS-CoV-2 poses a threat not only to the ESS populations it could infect, but also risks establishment within these skunks as natural wildlife reservoirs for future transmission and maintenance of the virus as an endemic disease within North America. Since the disease dynamic is similar for bats, researchers and other personnel working with *S. putorius* should follow the decision framing and risk assessment protocols outlined in Runge et al. (2020). In addition, wildlife management agencies should consult with public health authorities and consider instituting restrictions on the handling of any ESS and other mustelids and mephitids until their potential role in the transmission of COVID-19 can be established. It is also highly recommended that those working with *S. putorius* in the field suspend any

activities necessitating direct contact with ESS; in the very least, gloves and face masks should be worn at all times when handling living or dead skunks. Furthermore, any *S. putorius* presenting clinical symptoms or post-mortem signs (e.g., respiratory or gastrointestinal abnormalities) consistent with SARS-CoV-2 and/or found dead or euthanized with potential previous exposure to SARS-CoV-2 should be submitted for testing per the guidance provided by CDC (2020).

5.3.5.2. Prevention and mitigation of disease susceptibility

Activities related to ESS research and handling not only hold the potential for exposing skunks to infectious diseases, but also risk increasing the susceptibility of individuals to these pathogens. Any prolonged or repeated exposure to stressors (i.e., capture; handling; exposure to anthropogenic stimuli, such as sounds, smell, and sight; confinement in a radio collar) can cause acute and chronic stress in skunks, which depresses their immune system functioning and elevates their susceptibility to infections (Aiello et al. 2014, DuRant et al. 2020, Northover et al. 2018). The short- and long-term impact that these activities inflict on the health of ESS should be fully factored in to all research and management strategies. Practitioners should prioritize non-invasive means of collecting data, such as the use of game cameras, feces and fur as bioindicators of health and for hormonal work, high-throughput sequencing and other molecular techniques for genetic analyses of feces, near infrared spectroscopy for diet analyses, the sampling of ESS already in captivity (i.e. undergoing wildlife rehabilitation, in a zoological collection, participant in captive breeding operation) or deceased by other means (i.e. road mortalities, animal control euthanasia, harvested by hunters or trappers),

or striped skunks as analogs (Bryan 2013, Laguardia et al. 2015, Srabia et al. 2020, Vance 2016). ESS should only be handled when alternate, non-invasive methodologies are absent and the data produced are of substantial enough practical value to offset the threat to individual skunks and the collective ESS population. Conservation welfare and ethics frameworks beyond the minimum requirement set by Institutional Animal Care and Use Committees should guide these planning efforts (Beausoleil 2020, Lindsjö et al. 2016, Soulsbury et al. 2020, Van Patter and Blattner 2020, Varner 2008, Wallach et al. 2018). Whenever fieldwork is conducted with S. putorius, the stress to these animals must be reduced as much as possible; capture and handling time should be limited and sensory exposure of any kind to humans and their equipment should be minimized.

Scat detection dogs prove a special case: these animals should never be used when they have been diagnosed or are suspected to have been exposed to any infectious disease. Similarly, they should be dissuaded from defecating, urinating, or otherwise contaminating any ESS habitat with bodily fluids so as to mitigate the potential for pathogen transmission. The sound, sight, or smell of these potential skunk predators can also induce immunocompromising stress in *S. putorius* individuals, so care must be taken to minimize the potential for direct or indirect (i.e., barking, onsite urination or defecation) interactions between dogs and skunks.

5.3.5.3. Use of prophylactics and veterinary treatments

Practitioners may consider the use of prophylactics in the course of ESS management, especially in cases where infectious diseases cause population-level mortality events (Harris et al., in rev.). However, when an animal is dewormed or

otherwise treated, the individual may lose advantageous pathogens and the acquired immunogenic variation that is maintained by natural host-parasite interactions. In either scenario, the ESS can experience overall elevated exposure and susceptibility to infectious agents, which may prove more threatening than the disease for which it has undergone treatment (Daszak et al. 2000, Faria et al. 2010, Kołodziej-Sobocińska et al. 2018, Northover et al. 2018). Maintaining pre-existing host-parasite interrelationships are important for the overall health of ESS at both the individual level and population level, so prophylactics should only be deployed in cases where a disease is causing clinical symptoms of threat to individuals, the ESS is being cared for in a captive setting in which control of the disease is unfeasible, or the pathogen poses a significant threat to human health. Otherwise, strict quarantine, hygiene, and prophylactic protocols of staff (e.g., pre-exposure rabies vaccinations) should be established to maintain any host-parasite interactions and to minimize subsequent disease risk. Further guidance is available in the IUCN Guidelines for Reintroductions and Other Conservation Translocations (IUCN/SSC 2013).

When the field use of prophylactics is considered necessary, such as in controlling plague within North American *Cynomys* spp. (Rafinesque) (Prairie Dog) populations, wildlife veterinarians and rehabilitators should be consulted throughout the planning and implementation process (Salkeld 2017). In this manner, wildlife managers, biologists, and veterinary specialists can avoid any unintended and adverse outcomes of prophylactic programs. For example, only killed vaccines are safe for use in mephitids and mustelids, a hard lesson learned from the vaccine-induced canine distemper virus

mortalities experienced by *Mustela nigripes* (Audubon and Bachman) (Black-footed Ferrets) and confirmed in skunk rehabilitator practice (Carpenter et al. 1976).

5.3.5.4. Building One Health collaborations

Approaching ESS research and management from a One Health perspective entails building collaborative teams of medical and public health professionals, ecologists, veterinarians, wildlife biologists, wildlife rehabilitators, social scientists, entomologists, ecotoxicologists, and other stakeholders. These integrated and multidisciplinary networks can enhance the efforts directed towards *S. putorius* conservation by generating more holistic and accurate perspectives of their disease ecology as it relates to individual-, population-, and species-level dynamics as well as producing more effective, efficient, and sustainable management policies for the species. These collaborations can also maximize the potential of ESS management and research by: 1. serving as a source for additional data collection and analysis, such as molecular diagnostic identification of zoonotic diseases hosted by ESS by public health and domestic veterinary surveillance initiatives, 2. providing a source for the continuous monitoring of health-related threats, opportunities, and shifts in some of the ecological and environmental drivers acting on other animal and human hosts and vectors of pathogens of importance in the disease dynamics of *S. putorius* populations, and 3. provide access to additional and diverse resources for the investigation of ESS disease ecology and health. For example, receiving a preemptive warning from One Health practitioners regarding emerging epidemics and epizootics would enable conservation and wildlife managers to enact strategic contingencies in advance to account for any

potential challenges imposed by the disease events. The One Health monitoring program initiated during the *Castor fiber* (L.) (Beaver) reintroductions in Scotland serves as an exemplary model for devising a multidisciplinary team for infectious disease monitoring in a species of conservation concern (Goodman et al. 2017).

5.3.5.5. Leveraging One Health for conservation messaging

An additional benefit derived from fostering One Health collaborations lays in their capacity to expand the reach of conservation messaging, particularly when shared themes of public health and veterinary relevance are incorporated. The disease control benefits provided by ESS in their roles as sentinels and dilutors for infectious pathogens can be effectively leveraged for One Health communications for use in wildlife education programs, conservation marketing campaigns, and other efforts to promote tolerance for and conservation of the *S. putorius* (Lu et al. 2017, Wright et al. 2015, Ryan et al. 2019). The detrimental consequences of human-skunk conflict significantly impact ESS from the individual to the population level (Gompper and Jachowski 2016). Applying psychology to social marketing and education tactics strengthens the effectiveness of any outreach directed towards the mitigation of human-generated skunk mortality. Messages related to the preservation of human, companion animal, and livestock health can be very persuasive, especially in the wake of the COVID-19 pandemic, and psychological models have been proposed to assist in the reduction of human-wildlife conflict (Bruskotter and Wilson 2014, Buttke et al. 2015, Lu et al. 2017). The capacity for skunks to dilute vector-borne zoonotic diseases and to serve as effective sentinels for zoonotic disease surveillance can be integrated into the "perceptions of

benefits" component of Bruskotter & Wilson's hazard-acceptance model modified for use with large carnivores (Bruskotter and Wilson 2014). As long as the message is carefully framed to avoid the stigmatization of ESS as disease vectors, One Health campaigns hold great promise for benefiting conservation efforts focused on *S. putorius* (Buttke et al. 2015).

5.4. Conclusions

While the extent to which infectious pathogens have influenced historic and current population dynamics of ESS remains unknown, the interrelated effects of habitat loss and alteration, increased exposure to chemical toxicants, and disease are most likely to blame for drastic population declines and regional extinctions of *S. putorius*. Unfortunately, these pressures are only going to intensify in the future. The natural history and behavioral patterns of *S. putorius* significantly affect their exposure and susceptibility to infectious diseases. Depending on the ecological and environmental context in which these host-pathogen relationships are nurtured, the effects of bacterial, fungal, parasite, and viral agents impact individuals and populations in a variety of ways. Many limitations exist in our understanding not only of the diversity of infectious pathogens that are hosted by ESS, but also on the diversity of effects these pathogens impose on individual to population-level dynamics of *S. putorius* under different and ever-changing abiotic and biotic conditions. Future investigations of ESS disease should approach these gaps in our knowledge as opportunities to exercise a One Health approach in the holistic evaluation of the roles played by S. putorius within the transmission cycles of zoonotic pathogens. By employing a multidisciplinary, fully

integrative One Health approach and prioritizing the welfare of ESS, we can more comprehensively and effectively investigate the disease ecology of this imperiled species as the complexity of anthropogenic threats grow. The collaborative framework provided by the Eastern Spotted Skunk Cooperative Study Group serves as an excellent platform from which to organize and conduct these studies.

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6. CONCLUSIONS

Within the course of this research, I was able to successfully evaluate the disease ecology of Texas skunks (Mammalia: Mephitidae) within a One Health framework, the results of which can be practically applied to public health strategies, conservation planning, and wildlife management for the promotion of both animal and human health. By optimizing the methodology for DNA extraction from whole blood preserved on Nobuto blood filter papers, I not only facilitated my own molecular epidemiological surveillance of *Trypanosoma cruzi* in Texas skunks, but I also developed an improved mechanism by which the reach, efficacy, and reliability of infectious disease research and surveillance can be extended, particularly in the cases of neglected tropical diseases in which field constraints typically limit the capacity for body fluid collection and preservation. Developing these procedures for use with commercially-available and user-friendly kits and Nobuto strips further enhances their cost-efficiency and simplicity of application to molecular epidemiological studies, of critical importance for resource-limited and infrastructure-challenged public health agencies.

The use of this optimized DNA extraction technique supported my capacity to perform the first wide-scale and species-diverse molecular epidemiological survey for circulating *T. cruzi* infections in skunks across Texas. I reported the first positive cases for eastern spotted and American hog-nosed skunks, especially notable for these species as they are both experiencing range-wide population declines. Although sampling biases precluded an incontrovertible assessment of the risk factors associated with *T. cruzi* prevalence among Texas mephitids, several non-statistically-significant trends were

discernable related to age and sex biases, which can inform population and host-parasite models supporting conservation, wildlife management, and public health strategies after confirmation with additional sampling. My findings can serve as a foundation for future research investigating risk factors for *T. cruzi* in skunks and other wildlife or the roles mesocarnivores play within Chagas disease transmission cycles. This study further underscores the value in surveying multiple taxa of closely-related wildlife species across a wide expanse of habitats to investigate the complex adaptive systems that underpin the ecology of vector-borne zoonotic diseases.

Commensurate with the wide-scale *T. cruzi* surveillance among mephitid species in Texas was a more focused study of dermatophytosis in a single population of eastern spotted skunks in southeast Texas. From this investigation, I was able to report the first incidence of the zoonotic fungal pathogen *Microsporum canis* and its clinical symptoms in two *Spilogale putorius interrupta* individuals. In addition, I demonstrated the utility of wildlife rehabilitation records in the study of wildlife disease ecology by comparing these cases to regional trends in dermatophytosis among striped and eastern spotted skunks admitted to licensed wildlife rehabilitators. Assessing the diversity of skunk mycoses within a One Health framework can assist veterinary and public health agents in developing effective strategies to mitigate the spread of these fungal diseases within and between threatened skunk populations, other wildlife, domestic animals, and humans. A such, my description of the first confirmed pathogenic cutaneous fungus in *S. putorius* is a first step towards building a more comprehensive understanding of the disease ecology of this threatened skunk taxon and its ramifications for veterinary and

public health. In addition, the results of this study can contribute to future research directed towards wider geographic and temporal surveillance of skunk populations for pathogenic fungi.

The recovery of *T. cruzi* and *M. canis* in Texas populations of eastern spotted skunks directly factored in to my comprehensive and critical review of *S. putorius* disease ecology. Within this review, I was able to evaluate: 1. the natural history and behavioral traits of *S. putorius* that influence individuals' exposure and susceptibility to various infectious diseases, 2. the pathogenic agents recorded to date in eastern spotted skunks, 3. the potential bacteria, fungi, parasites, and viruses impacting *S. putorius* populations based on more extensively-studied mephitid and mustelid analogs, 4. key abiotic and biotic factors that will likely shape eastern spotted skunk disease dynamics into the future along with the integrated One Health implications for these processes, and 5. the practical applications *S. putorius* disease ecology holds for best practices of wildlife professionals. As such, the compilation serves as a base for future research into mephitid disease ecology, a functional guide for eastern spotted skunk practitioners on One Health-related practices, and a starting point for conservation planning, including strategies reliant on population viability assessments.

The cumulative effects of infectious diseases and anthropogenic processes can impart catastrophic impacts on wild skunk populations throughout the New World. The decline of mephitid taxa compromises not only the economic and sociocultural benefits they offer to people, but also the ecosystem services they provide to a wider swath of animal and human communities alike. The objective of this study was to apply One

Health epidemiological research to conservation efforts directed towards both imperiled and populous skunk species. By delineating some of the driving ecological and environmental factors that regulate host-pathogen interrelationships between North American mephitids and zoonotic diseases, I provided a foundation for the development of conservation medicine and wildlife health initiatives that benefit all entities under the One Health umbrella. In addition, those striving to develop holistic interventions fostering human-skunk coexistence can integrate the epidemiological research described by this dissertation into practical methodology introduced by the practitioners of anthrotherology, conservation psychology, and conservation marketing.

Figure 6.1. THE END