IMPACT OF ZOONOTIC DISEASES CONTROL POLICIES: AN EVALUATION OF THREE COUNTRIES OF THE FORMER SOVIET UNION

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To my family and beautiful wife
ACKNOWLEDGMENTS

I would like to thank my family and wife for supporting me during this tumultuous time. I owe a debt of gratitude to my chair Dr. Jason K. Blackburn for giving me a chance to work in his lab nearly 10-years ago back at California State University, Fullerton. The opportunities the Spatial Epidemiology and Ecology Research (SEER) lab have given me have been life changing. None of this work would have been possible without the collaboration and friendship I encountered in Georgia, Ukraine and Azerbaijan; it has been a pleasure working with them. The support I received from the Emerging Pathogens Institute was also instrumental in my success and I thank them for all of encouragement over the years. I would also like to thank my committee members: Drs. Jason K Blackburn, J. Glenn Morris, Greggory Glass, Timothy Fik, and Sadie Ryan for giving me the intellectual freedom to peruse my own research ideas.
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Abstract of Dissertation Presented to the Graduate School
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IMPACT OF ZOONOTIC DISEASES CONTROL POLICIES: AN EVALUATION OF THREE
COUNTRIES OF THE FORMER SOVIET UNION

By

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Chair: Jason K. Blackburn
Major: Geography

This dissertation examines the linkages between human and animal health by evaluating the impact of livestock vaccination policies on two widely distributed zoonotic diseases: brucellosis and anthrax. This work addresses major questions about the effectiveness and impacts of livestock disease control on neglected zoonoses and their indirect effect on human health. In addition, this work makes recommendations for future vaccination efforts in countries of the former Soviet Union. First, this work examined the impact of removing compulsory anthrax livestock vaccination on the epidemiology of human anthrax in the country of Georgia. Spatial and statistical analyses were used to identify epidemiological changes following the change in vaccination policy. Second, we used sentinel wild surveillance and environmental risk of models of anthrax to elucidate the spatial distribution of active foci in Ukraine. The true prevalence of anthrax exposure in wild boar (Sus scrofa scrofa) was estimated using Bayesian methods and environmental risk hotspots were identified using a zero-inflated regression model. Lastly, this work examined the impact of Rev1 livestock vaccination on the risk of human brucellosis in Azerbaijan. Interrupted time-series models were used to assess the impact of two vaccination campaigns on human brucellosis risk. Results of the anthrax analysis indicated an
increase in human risk following changes to livestock vaccination policy. The illegal slaughter and sale of infected meat has likely facilitated the spread of infections from foodborne exposure among groups uncharacteristically at risk. In addition, despite recent declines in anthrax outbreaks, our findings confirm the presence of active anthrax foci. Areas of identified as environmentally suitable for anthrax showed, in part, agreement with the serological evidence of exposure in wild boars. Taken as a whole, these findings indicate active foci are likely more widely spread than previously thought and that high-risk areas may still pose a risk for human and livestock anthrax exposure. Results from the interrupted time-series analysis indicate a substantial reduction in human brucellosis risk following livestock vaccination. Countries of the former Soviet Union, sub-Saharan Africa, and Southeast Asia have (re)emerged as foci for transmission of neglected zoonotic diseases. This work has implications for neglected zoonotic disease control programs and supports the growing evidence of the human health benefits of livestock disease control in developed and developing countries.
CHAPTER 1
INTRODUCTION

Introduction to Dissertation

This dissertation examines the impacts of policy on zoonotic diseases by analyzing surveillance data from human, livestock, and wildlife. Specifically, this dissertation focuses on three different phases of vaccination policy: implementation of a livestock vaccination campaign, cessation of a livestock vaccination policy, and pre-implementation of a livestock vaccination policy. By incorporating the use of geographic information systems (GIS) and statistical modeling, this dissertation looks at multi-scale effects of livestock vaccination that can be used to inform ongoing and future disease elimination programs. Two zoonotic diseases, anthrax and brucellosis, are considered neglected because they are often underreported and receive inadequate resources for control and surveillance. This fact makes elimination and management difficult without proper guidance. Both brucellosis and anthrax primarily infect livestock, thereby creating a multifaceted burden to human and veterinary health, in addition to negatively affecting agricultural economics. Since there are effective vaccines against these two zoonoses, human and livestock morbidity and mortality are largely avoidable. However, there is little evidence in the literature regarding the impacts of vaccination on the burden of these neglected zoonotic diseases in low- and middle-income countries, where the burden is greatest. Thus, this dissertation aims to fill a gap in research by examining both the intended and unintended consequences of livestock vaccination policy on the risk of zoonotic diseases, to provide a better understanding of the linkages between human and animal health. Furthermore, it will highlight the importance of evaluating zoonotic disease control policies before altering them. This introductory chapter provides the foundation for the dissertation by providing a describing the disease systems and highlighting the importance of these questions to global public health.
Chapter 2 reports on the analyses of spatial and temporal changes in the geographic patterns and risk of human anthrax following the cessation of compulsory livestock anthrax vaccination in the country of Georgia. This study investigates how switching from a compulsory livestock vaccination policy to a voluntary program has affected the epidemiology and risk factors for human anthrax. We used data on epidemiological case interviews of anthrax patients spanning 14 years, which included 7 years before the policy change and 7 years after the policy change. The findings from this chapter add to the growing body of literature that support the human health benefits of livestock disease control and suggest evaluating the cost-effectiveness of vaccination policies before changing disease control strategies. Chapter 3 examines the use of risk models and sentinel wildlife surveillance of wild boars (*Sus scrofa scrofa*) to elucidate the environmental suitability of anthrax and identify active anthrax foci in Ukraine. This chapter aims to combine evidence from passive livestock surveillance and convenience serological sampling of wildlife to aid in livestock vaccination policy. Given the Ukrainian government is considering ending compulsory livestock anthrax vaccination, the findings from this chapter will aid in better understanding anthrax ecology and provide possible alternative anthrax control strategies. Chapter 4 then investigates the impact of Rev1 small ruminant vaccination on the risk of human brucellosis in Azerbaijan (a middle-income country). Livestock vaccination is the most effective form of brucellosis control, however, there is skepticism regarding the effectiveness of such strategies in low- and middle-middle income countries. This chapter seeks to understand the effectiveness of Rev1 vaccination and to identify what indirect benefits livestock brucellosis vaccination has on human risk. We applied seasonal time-series models to passive surveillance data consisting of monthly human brucellosis counts using a quasi-experimental approach. These findings have implications for global brucellosis control programs and supports the growing
evidence of the human health benefits of livestock disease control in developed and developing countries. Lastly, chapter 5 highlights the potential future directions of these studies and provides conclusions to the research.

**Neglected Zoonotic Diseases**

Zoonotic diseases (those transferrable from animals to humans) comprise a significant threat to human and animal health worldwide [1]. More than half of all human pathogens are zoonotic and approximately 75% re(emerging) disease also have an animal origin [2]. Factors related to zoonotic disease (re)emergence include: land cover change, political turmoil, and population increases [3]. In recent years, diseases such Ebola virus and Zika virus have highlighted the importance of rapidly detecting and controlling zoonotic diseases [1,4–6]. Yet, the ability to detect and respond to emerging threats has been called into question given the inability to detect and control ancient endemic zoonotic diseases [7]. Despite the importance of this group of diseases in causing human morbidity and mortality, control is often neglected due to a perceived lack of importance [8]. Many zoonotic diseases are not considered human health priorities and are often assumedly relegated to animal health issues. Thus, the control of many zoonotic diseases is highly dependent targeting animal reservoir [9]. However, little is known about the impacts of livestock vaccination policies on human zoonotic disease risk in low and middle-income countries. Given the availability of efficacious vaccines, control is plausible and human cases are largely unnecessary. A better understanding of the impacts of animal control policies on human health will promote more effective interventions while improving the health and wellbeing of the population [9,10].

The global economic costs of zoonotic diseases are staggering, with an estimated direct cost of $≈20 billion during the last decade with $>$200 billion in indirect loses [10]. The brunt of
these losses are often disproportionately felt by poor animal livestock holders who typically experience the highest rates of disease morbidity and mortality due to per domestic living arrangements [11,12]. Worldwide, there are estimated to be between 500 and 900 million livestock holders living in poverty [11]. The United Nations Sustainable Development Goals propose, in part, to reduce poverty and hunger by 2030. However, diseases shared between livestock and humans (zoonotic) represent a growing barrier to achieving these goals due their multifarious impacts on health, food, and economic potential. Thus, controlling zoonoses, particularly those that are neglected, remains a crucial step in improving the health and wellbeing of the most vulnerable populations worldwide [13–15]. In response to this increasing interconnectedness between human and animal health, the World Health Organization has recommended more integration between human and veterinary health services. One suggested path towards alleviating this barrier is by improving the health of companion animals and livestock through preventive disease control measures such as vaccination [16–18].

Previous research has suggested that targeting animals with zoonotic disease vaccinations would indirectly benefit human health [9]. These benefits may also go beyond improvements in health; evidence has shown that livestock control programs can have positive economic impacts on the agricultural sector with healthier livestock and better output per head of livestock and improved access to global markets [9,10,17,19]. Vaccination campaigns of dogs for rabies in the capital of Chad, N’Djemena were estimated to reduce the burden of human disease for years if approximately 70% vaccination coverage could be achieved [17]. Similarly, a mathematical modeling study in Mongolia suggested that a 10-year brucellosis vaccination campaign of small and large ruminants would have economic and human health benefits, with an estimated > 49,000 disability adjusted life years, (DALYS) averted [19].
Anthrax and brucellosis are two of the most widely spread zoonotic diseases in the world [20,21]. Since, humans are essentially dead end hosts for the causative agents of these diseases targeting the animal reservoir or host is crucial for successful control. Thus, evaluating and aiding in the development of policies to control zoonotic disease will improve human and animal health as well as benefit agricultural economics. Addressing these aims will fill critical knowledge gaps in anthrax and brucellosis outbreak dynamics and human disease control. Findings from this dissertation will provide critical information for ongoing efforts to strengthening public health preparedness for these zoonoses in the former Soviet Union and elsewhere. A better understanding of the impacts of animal control policies on human health will promote more effective interventions while improving the health and wellbeing of the population [9,10]. In particular, it will better define vulnerability within communities and identify risk within or between communities that will have important implications for improving disease surveillance and intervention strategies (e.g., vaccination campaigns, carcass decontamination and access to improved human care)

**Anthrax**

Anthrax is a globally distributed bacterial zoonotic disease (transferable to humans and animals) found on nearly every continent (except Antarctica) [22]. In the early 19th century employment in abattoirs and industrial tanneries were considered a high risk for anthrax; however improvements in occupational safety and the widespread use of an effective livestock vaccine have reduced transmission [23,24]. The highest risk of disease is associated with groups or occupations that are involve handling or caring for livestock [25,26]. Globally, poor rural livestock keepers, dependent upon subsistence farming or small scale agricultural production, experience a disproportionate burden of anthrax are, due to the fact that their animals are at
greater risk of infection because a lack of veterinary services and poor knowledge regarding the symptoms or treatment of livestock illnesses [26,27]. Heavy reliance on animals for income has also been associated with an increased risk of transmission through the consumption or sale of tainted meat, in an attempt to recoup economic losses from the death of livestock [25,26,28]. However, the risk to these rural populations is also a direct result of their local and national health systems, which are often weakened by low government spending on healthcare; these populations often lack basic access to veterinary or human health services [18,29]. This often leads to a cycle of neglect through underreporting of cases and little to allocation of resources for management and control [30].

Estimates of the occurrence of human anthrax ranges from 1,000 to 100,000 cases annually worldwide; the true burden of disease is unknown due to underreporting and neglect [25]. Evidence suggests that the global occurrence of human anthrax has undergone a decline due to widespread livestock vaccination as seen in several developed countries such as the United States, United Kingdom, Israel, Sweden, and Cyprus [22,23,31–33]. Massive vaccination of livestock in the former Soviet Union during the 1960’s effectively reduced the number of animal case by nearly 10-fold and resulted in a subsequent reduction in human incidence [24]. However, following the collapse of the Soviet Union reports showed a dramatic rise in the incidence of human anthrax across central Asia, the Caucasus, and elsewhere [22]. Studies of human anthrax transmission have revealed regional hotspots of risk in sub-Saharan Africa, the Caucasus (including Turkey), and central and Southeast Asia [18,34–37]. Recently, the country of Georgia has emerged as one area of concern since the incidence of human anthrax has surpassed historic level and now has one of the highest reported incidences of disease in the world [38]. Human anthrax trends from other regions are less clear with primarily sporadic
reports. Although ascertaining the level of underreporting is difficult it is possible to identify the presence of underreporting such as when human anthrax cases are documented without an accompanying livestock case [26].

The lack of human reporting can be in part attributed to the fact that the most common form of human anthrax (cutaneous ≈ 95% of cases) is estimated to be self-limiting in approximately 25% of cases [39]. In addition, human case reports are often skewed due to misdiagnosis or a lack of confirmatory tests with the administration of antibiotics, and self-treatment. In the country of Georgia, self-medication is common due to the high out of pocket costs associated with medical care [40,41]. Similarly, in Ghana it was found that approximately 10% of individuals that became sick after consuming meat from a presumably anthrax infected animal would seek treatment at a natural healer rather than a hospital [42]. On the other hand, animal reports are often skewed by a lack of veterinary services to coordinate a proper outbreak response and monitoring [22,25,43]. However, underreporting is also due to an absence of reports from livestock holders; this particularly true of small ruminants (sheep and goats), which are often deemed low value animals. Humans may also skew reporting through the slaughter and consumption of infected livestock. In agro-pastoralist regions, where animals may constitute a significant economic investment, consumption and or sale of tainted animal products is a common way of recouping part of the economic loss suffered from livestock succumbing to anthrax [22,25,34,42].

The causative agent of the disease, *Bacillus anthracis*, is a Gram-positive spore forming bacterium with the remarkable ability to persist in the environment for long periods of time, perhaps decades [22,44]. Humans are typically secondarily infected as a result of direct contact with contaminated animals or their by-products during activities such as the slaughtering or
butchering of livestock [29,34,45,46]. In rare instances, human anthrax has been reported from the sowing or harvesting crops (soil) and insect bites [34,47]. Although transmission from occupational exposure in abattoirs and other industrial animal processing facilities was common in the early 19th century, this route of exposure has been largely eliminated due to improvements in safety [23]. Increases in the number of human infections in urban areas from the sale and purchase of tainted meat documented in the country of Georgia have highlighted the importance of livestock control [28]. In general, with the exception of the situation in Georgia and the transmission of anthrax through injection drug use in Europe, risk factors for anthrax have remained unchanged. There are however, behavioral differences that vary by geographic region, but these still tend to include the handling or consumption of sick or dead animals. In some regions ritualistic slaughter or communal sharing of meat has facilitated transmission [46].

There are several pathways to controlling the burden of anthrax in humans and livestock, although livestock vaccination is considered the most effective [39]. Louis Pasteur’s anthrax vaccine (1881) was one of the first bacterial vaccines ever produced [48]. Despite this fact, anthrax remains a serious public health threat in many regions of the world. Pasteur developed the vaccine by creating an attenuated strain of *Ba* by heating it above 42° C causing it to lose, one of its two plasmids, the pX01 toxin producing plasmid [49]. Vaccination of livestock against anthrax using Pasteur’s whole vaccine until the 1920’s and 1930’s involved a series of two inoculations, however due to instability in the attenuation process the safety and efficacy of the vaccine was questionable [49,50]. Pasteur’s strain remained in wide use until the discovery of an attenuated *Ba* strain by Max Sterne in the late 1930’s [51]. Sterne’s new vaccine strain was considered more stable and lacked the pX02 plasmid that codes for capsule, which allows *Ba* to evade immune system phagocytosis [50–52]. The Sterne strain 34F2 has since become the
standard for livestock vaccination worldwide (Centers for disease Control and Prevention; www.cdc.gov). However, other strains have been used in Europe such as the Sterne 55 strain and other locally produced versions in several countries [25,48,51,52]

The standard dose for anthrax livestock vaccination is 1 ml solution of 2-10 x10^6 spores for large ruminants injected at the front shoulder and a 1 ml1-5 x10^6 spores for small ruminants injected into the neck. However, equal doses can be used on small and large ruminants in most circumstances (OIE; http://www.oie.int/fileadmin/Home/fr/Health_standards/tahm/2.01.01_ANTHRAX.pdf). In general, vaccine is administered once a year, 2-4 weeks prior to animals being to put out to pasture (Centers for disease Control and Prevention; www.cdc.gov) however in high transmission areas or during outbreaks a booster vaccine can be given [25,51].

Other factors that can contribute to control include enhancing knowledge and awareness of the disease, improving livestock/animal surveillance, and providing access to livestock vaccine. In Azerbaijan, the initiation of a livestock control campaign resulted in a dramatic reduction in both human and livestock cases [18]. Given the knowledge of the population at risk and the risk factors for transmission, it may be possible to target specific areas for interventions. Research in Chad showed that coupling livestock vaccination with human disease control efforts along transhumance routes improved vaccination coverage [53]. In the country of Georgia, public health officials have tried to increase the knowledge and awareness of the disease by using targeted public service announcements and fliers in agricultural producing areas (unpublished data). Also, based on the reliance of animals for small livestock holders, indemnity plans that reimburse all or part of losing in animal may help to curb the illegal slaughter and sale of infected meat [28]. Knowledge of at risk populations and areas is one of the first steps to
providing public health management although other challenges such as access to remote regions exist.

**Brucellosis**

Brucellosis has been referred to as a continually remerging disease due to the discovery of new *Brucella* spp. strains, changing geographic patterns, and new preferential hosts [54]. Its origins are ancient, with evidence of human brucellosis infections in skeletal remains dating back to 79 A.D in Italy [54–56]. Similar to anthrax, globally, the risk of brucellosis is highest in agrarian populations that practice subsistence farming and small scale agriculture, exacting a heavy economic burden to agricultural production [21]. Unlike anthrax, there is a greater risk of foodborne transmission even among populations not involved in agricultural production. Poor knowledge of brucellosis and its clinical symptoms in livestock and humans often leads to underreporting or misdiagnoses further exacerbating efforts to control the disease [57]. This often leads to cycle of neglect and missed opportunities for the implementation of interventions. For example, the World Bank’s efforts to quantify the burden of 107 diseases in the Global Burden of Disease study did not include any neglected tropical diseases [26]. Elimination programs have successfully reduced the incidence of disease in developed countries, but brucellosis remains a global health concern in low- and middle-income countries [21]. Since humans are essentially dead-end hosts for brucellosis transmission, targeting livestock with control measures is the most effective method of control.

Despite the availability of effective vaccines, brucellosis is one the most common and widely spread zoonotic diseases in the world, with an estimated 500,00 cases reported annually worldwide; this estimate likely represents an underestimation of the true risk [21,54,55]. Countries in the Middle East, Central Asia, and sub-Saharan Africa are foci for transmission
Globally, the epidemiology of human brucellosis can vary widely due to variability in the occurrence of the primary hosts, species of *Brucella spp.* involved, and routes of infection [58]. Human transmission typically involves consumption of unpasteurized dairy products or contact with infected animals or their by-products [58]. In livestock, transmission is maintained, naturally, both through vertical (birthing) and horizontal (contact with infect materials such as placenta) transmission [59]. Poor animal husbandry practices and mixing of herds on seasonal pastures have been implicated as factors contributing to continued livestock transmission [55,59,60]. Human and livestock infected with brucellosis can also develop chronic sequelae and may experience recurrent fever and malaise several years after infection [61]. Patterns of human brucellosis transmission have also been documented to shift between occupational and foodborne transmission from unpasteurized dairy products [62–64]. For example, in California, the epidemiology of brucellosis shifted from an occupational disease to primarily foodborne transmission among ethnic minorities following improvements to workplace safety [64].

The causative agents of brucellosis are a group of Gram negative bacteria in the genus *Brucella spp.* first isolated by Sir David Bruce in 1887 and originally named *Micrococcus melitensis* [56]; the bacteria he isolated was eventually eponymously renamed *Brucella melitensis*. Within the genus *Brucella* there are ten recognized species that are generally host/animal specific; the most pathogenic to humans are: *B. melitensis* (sheep and goats), *B. abortus* (cattle), and *B. suis* (swine) [58]. Worldwide, *B. melitensis* is the source of the majority of human infections [21]. Geographic variation in host distributions and animal caretaking may influence the dominance of one particular species. For example, in Azerbaijan approximately 85% of isolates taking from humans are *B. melitensis*, which is consistent with the extensive sheep and goat production in that country. On the other hand, in areas where cattle
farming is dominant, *B. abortus* may be the most frequently isolated species associated with human infection [55,65]. Identifying, the primary host and dominant species associated with brucellosis infection is an important part of initiating a successful control program.

There are several methods for controlling brucellosis in livestock and humans including: test and slaughter of infected animals, limiting movements and mixing of untested animals, and livestock vaccination [60,66]. Other safety and preventive measures such as pasteurization of dairy products are considered more downstream forms of control, although still important aspects of ensuring a reduction in the burden of human disease [66]. Livestock vaccination remains the most effective method of controlling brucellosis in both humans and animals [19]. Currently, the most widely used livestock vaccines target large ruminants (cattle) protecting against *B. abortus* using the RB51 and S19 live attenuated vaccines, and small ruminants (sheep and goats) protecting against *B. melitensis* using the Rev1 live attenuated vaccine [60], which can be administered subcutaneously or via the recommended ocular route of administration [60].

The lipopolysaccharide molecule was identified has the primary immunogenic component of *Brucella spp.* [67,68]. The first brucellosis vaccine was the S19 live attenuated strain discovered by John Buck in 1923 and is used to vaccinate cattle against *B. abortus* [68]. As with other live attenuated strains there exists a possibility of infection and animals may be able to shed the vaccine strain for up to 30 days [67]. Several brucellosis vaccine strains are also potentially abortogenic; these vaccines will also cause vaccinated animals to test positive on serological diagnostic tests [69].

This dissertation focuses on the application of the Rev1 vaccine in small ruminants. The live attenuated Rev1 vaccine was discovered in 1957 and has been a crucial component of small ruminant brucellosis control worldwide [70]. This strain of *B. melitensis* was isolated from
culture grown on selective media that was deficient in streptomycin [68]. There are two primary routes of administration of Rev1: ocular and subcutaneous [71]. Both elicit similar immunogenic responses in the target hosts and are thought to have the same effectiveness [67,69]. However, it is safer to administer the vaccine via the ocular route due to the greater number of abortions induced by the subcutaneous administration [67]. The standard dose for ocular and subcutaneous administration is the same (1x10^9 cfu) [67–69]. Despite the greater safety of the ocular administration, experts recommended not to vaccinate pregnant females [69].

**Former Soviet Union**

This dissertation examines data from three countries of the former Soviet Union: Georgia, Ukraine, and Azerbaijan. Once part of the largest public health system in the world, countries of the former Soviet Union have experienced dramatic cuts to public and veterinary health [72]. In addition, the loss of centralized subsidies for agricultural have resulted in a precipitous drop in livestock populations [73]. Subsequently, land and agricultural use patterns have shifted from large state run collective farms to small private holdings [73]. This has had a dramatic impact on the ability to monitor and control diseases because of a lack of centralization and veterinary health infrastructure [72]. Even after nearly 25 years after independence, these countries are faced with ongoing barriers to controlling neglected zoonotic diseases.
CHAPTER 2

EPIDEMIOLOGY OF HUMAN ANTHRAX IN GEORGIA, 2000-2013

Background

Anthrax is a zoonotic disease that is found on nearly every continent [1,2]. The causative agent, *Bacillus anthracis*, is a soil-borne Gram-positive bacterium with the remarkable ability to survive in the environment for long periods of time, perhaps years, and infect a wide range of hosts [2,3]. Herbivorous animals are primarily infected [2–4]. Human infections are typically a result of contact with infected animals or their by-products (e.g. meat or hides) during activities such as livestock slaughtering [1,4]. Clinical manifestations of human anthrax occur in four forms: cutaneous (95% of all reported cases worldwide), gastrointestinal, inhalation, and the recently recognized injection drug use [5].

Targeting livestock with annual vaccination is the most effective method to control anthrax in both humans and animals [1,6]. In the former Soviet Union (FSU), livestock anthrax vaccination combined with improvements in occupational safety produced a nearly 10-fold reduction in animal cases with a concomitant decline in human incidence [7]. Similar decreases were observed in Europe and the United States following mass vaccination of livestock [8]. However, despite the effectiveness of vaccination, anthrax persists in areas with weakened health infrastructures and long-term vaccination strategies may be needed in endemic areas [1,9]. Countries of the FSU, sub-Saharan Africa, and southeast Asia have (re)emerged as foci for transmission [10].

The country of Georgia has experienced repeated outbreaks of human anthrax with a recent increase in human incidence (2010-2012) [11]. Reports of an anthrax-like disease in humans dates to the 17th century [12] and >500 locations have been registered as foci (permanent locations of anthrax risk) in Georgia since 1881 [13]. During Soviet governance,
anthrax was a mandatory reportable infectious disease in both humans and livestock. Following the dissolution of the Soviet Union in 1991, and Georgian independence in December of the same year, anthrax reporting remained mandatory. To combat the spread of anthrax, from 1995 through 2006 the government carried out annual compulsory livestock vaccination. In 2007, Georgia ended this policy, placing the responsibility of vaccination on private livestock owners [14]. A case control study of a 2012 anthrax outbreak revealed that nearly 60% of livestock workers/owners surveyed did not vaccinate their animals [14]. Yet, little is known about how the alteration in livestock immunization policy and the concomitant decline in the number of anthrax vaccine doses administered impacted the epidemiology of human anthrax. In the context of >90% private livestock ownership in Georgia, and the high risk associated with agricultural production, identifying changes in anthrax epidemiology are crucial for implementing control strategies and limiting its spread.

Our objective was to assess how the change in livestock vaccination policy impacted the epidemiologic characteristics of human anthrax in Georgia from 2000-2013 by identifying changes in risk factors and rates of self-reported sources of infection.

**Methods**

We obtained passive surveillance data on epidemiological surveys of human anthrax case patients and the annual number of livestock vaccine doses administered from the National Centers for Disease Control and Public Health (NCDC) during 2000-2013. We estimated national incidence rates per 100,000, using population data from the Georgian national census (Georgian State Statistical Committee, GeoStat). To describe the trend in human anthrax incidence and identify trend breakpoints if present, we used segmented regression (JoinPoint 4.4.0.0, https://surveillance.cancer.gov/joinpoint/) [87]. Breakpoints in a trend are characterized by an inflection point in the line segment indicating an increasing or decreasing rate of change.
We defined the dependent variable as the annual crude incidence rate and independent variable was the year. To adjust for heteroscedastic errors, we used a weighted least squares approach with weights applied to each observation following Kim et al. [87]: \( w = y^2/v \) where \( w \) is the weight, \( y \) is the dependent variable and \( v \) is the square of the standard error of the dependent variable at each observation. We allowed for between 0 and 2 breakpoints in the regression line. For each possible regression line segment provided by the best fit model, the rate of change is given by the average annual percent change (AAPC):

\[
AAPC = \left\{ \exp \left( \frac{\sum w_i b_i}{\sum w_i} \right) - 1 \right\} \times 100
\]  

where \( b_i \) is the slope coefficient of each line segment in the final regression model and \( w_i \) is the length of each line segment in years.

Risk ratios and incidences per 1 million were estimated for age, sex, ethnicity, region, self-reported source of exposure, season, and occupation. Season was defined as: winter (December, January, and February); spring (March, April, and May); summer (June, July, and August); fall (September, October, and November). We derived the region binary dummy variable with ethnic enclaves defined as district municipalities with \( \geq 30\% \) of the population reporting as non-Georgian. Statistical analyses (Figure S1) and accompanying 95% confidence intervals of estimates were performed in R v3.3.1 (R Core Development Team).

Human anthrax data were aggregated to district municipality, and we mapped crude average annual human incidence per 100,000 persons (total cases/population) for each district during 2000-2006 and 2007-2013. The ethnic composition (percent of non-Georgian population) of each district was derived from the Georgian census (http://www.geostat.ge/).

We used classifications of four main ethnic groups defined in the census data: Georgian, Azerbaijani, Armenian, and other (Russian, Ukrainian, Greek, and Yazidi). We calculated
ethnicity adjusted incidence rates per municipality before and after the policy change using the indirect standardization method with the expected number of human cases during 2000-2013 as the internal standard [88,89].

To test for changes in the spatial dependence in human anthrax incidence among district municipalities between the two time periods, we used the global Moran's I statistic (OpenGeoDa 1.0.1, GeoDa Center, ASU, Arizona). This statistic is a measure of spatial autocorrelation or similarity among spatial units with values close to +1.0 indicating clustering while values close to -1.0 indicate dispersion and is written following Moran [90]:

\[
I = \frac{N}{\sum \sum W_{ij}} \frac{\sum \sum W_{ij}(X_i - \bar{X})}{\sum(X_i - \bar{X})^2}
\]  

(2-2)

where \( N \) is the number of districts, \( \bar{X} \) is the average district incidence, \( X_i \) and \( X_j \) are the incidence in district \( i \) and \( j \), and \( W_{ij} \) is the spatial weight matrix defining the spatial relationships between districts. To characterize the spatial relationship among district municipalities, we used a queen contiguity matrix with row standardization [91]. We tested the null hypothesis of no spatial association in incidence rates of human anthrax among district municipalities before and after the change in policy for both crude and ethnicity adjusted rates.

**Results**

**Temporal Trends**

From 2000 to 2013, 736 human anthrax cases (annual range: 15-143) were reported in Georgia (Figure 2-1). During this 14-year period, the trend in rates was characterized by a breakpoint in the regression line in the year 2010 (95% CI: 2008, 2011) indicating an increasing rate of reporting post-policy change with an AAPC=10.2% (95% CI: 9.3, 10.9; p=0.02) (Figure 2-1). The annual human incidence per 100,000 increased from 0.6 cases (95% CI: 0.4, 0.8) in 2000 to 3.7 cases (95% CI: 3.1, 4.4) in 2013. Following the policy change in 2007, there was a
precipitous decline in the average annual number of livestock anthrax vaccine doses administered: 2 million doses (95% CI: 1.2, 2.8) during 2000-2006 compared to 201 thousand doses (95% CI: 32, 436) during 2007-2013 (Figure 2-1).

Persons age 40-64 years had higher rates of human anthrax nearly every year except in 2010 when they were surpassed by rates in persons age 65 years and older (Figure 2-2). Prior to 2007, annual incidence rates were not consistently higher among any ethnic group. In 2007, when the compulsory livestock vaccination program supported by the government ended, incidence rates increased among all ethnic groups (Georgians, Azerbaijanis, and Armenians). Rates began to diverge in 2010 with a rapid increase in the risk of human anthrax among ethnic Azerbaijanis; rates among ethnic Azerbaijanis ranged from 0 to 25.3 cases per 100,000 (Figure 2-3).

**Risk Before and After Policy Change**

When comparing periods before and after the policy change, males were two times more likely to have reported human anthrax compared to females during 2000-2006; by 2007-2013 males were at least four times more likely to have reported compared to females (Table 2-1). Persons age 40-64 were at higher risk of infection compared to all other age groups during 2000-2006 and remained so following the policy change. From 2000-2006, Azerbaijanis accounted for 8% of cases, increasing to 30% during 2007-2013; the relative risk compared to Georgians increased from 1.3 to 6.1 (Table 2-1). Azerbaijanis and Armenian ethnicities comprised approximately 8% of the total Georgia population while accounting for 35% (187) of all anthrax cases during 2007-2013. Notably for 2012-13, these ethnicities accounted for 48% of all cases (Figure 2-3). Regionally, ethnic enclaves were two and a half time more likely to report anthrax cases compared to other district municipalities pre-policy change, increasing to six times more likely during 2007-2013. Seasonal patterns of reporting indicated that cases were essentially split
between summer (38%) and fall (41%) during 2000-2006; by 2007-2013 it was 1.5 times more likely for a case to be reported during the summer than the fall.

The self-reported source of infection was disclosed for 709 (709/736, 96%) cases during 2000-2013; most cases reported slaughtering/butchering livestock before (108;61%) and after (300;56%) the policy change, 2000-2006 and 2007-2013 respectively (Table 2-2). Of the cases that reported slaughtering/butchering, 46 (23%) confirmed the animal was sick or dead during 2000-2006 compared to 49 (9%) during 2007-2013. Prior to the policy change, purchasing meat was reported as source of infection in 21 (11%) cases compared to 114 (21%) cases after the policy change.

Occupation was available for 415 (56%) cases (Table 2-2). Working with animals or handling animal by-products during 2000-2006 was documented in 8 (4%) cases during 2000-2006 compared to 149 (28%) cases during 2007-2013. After the policy change in 2007, there was an increase in the number of housewives that reported anthrax: from 8 (4%) cases to 61 (11%). (Table 2-2).

Geographic Patterns of Anthrax Cases

The spatial distribution of ethnicity-adjusted and crude rates changed with the vaccine program (Figure 2-4). Ethnic composition of the district population ranged from 0.2 to 90.4%. There was no discernable pattern in the distribution of rates during 2000-2006 indicated by the absence of clustering for both the crude (Moran’s $I = -0.03$, z-score= -0.23, p=0.8) and adjusted (Moran’s $I = 0.06$, z-score= -0.94, p=0.4) rates (Figure 2-4A and C). Following the change in policy, areas of high rates coincided with ethnic enclaves with high rates in the west along the Black Sea and in the southeast near the border with Azerbaijan and Armenia. During this later period (2007-2013), the spatial distribution of cases shifted from being randomly distributed to spatially clustered for both the crude (Moran’s $I = 0.3$, z-score= 4.0, p<0.001) and adjusted
(Moran’s \( I = 0.19 \), z-score= 2.5, \( p=0.01 \)) rates (Figure 2-4B and D). The significance of the Moran’s \( I \) test in both the adjusted and crude rates indicates that the presence of clustering was not due to the underlying spatial distribution of the ethnic population.

**Discussion**

During Soviet governance (1950-1980), the incidence of human anthrax declined due to widespread livestock vaccination and improved occupational safety, mirrored by reductions globally [20,79–81,92]. Following alterations to the number of anthrax livestock vaccinations administered, our findings show a recent dramatic spike in human anthrax among several demographic groups and a shift in reported exposures. The random pattern of reporting experienced during the early part of the last decade has been replaced by the clustering of incidence. The largest increase in reporting has primarily occurred among ethnic Azerbaijanis; this demographic is more often employed in high risk occupations such as shepherding, with ethnic enclaves situated near intensive agricultural production facilities [93]. The epidemiology of brucellosis in Georgia has also shifted to a disease primarily of Azerbaijani ethnicity, supporting the hypothesis of increased ethnically-biased occupational exposures [93]. In Georgia, Azerbaijanis are less likely to vaccinate livestock due to a belief that anthrax livestock vaccination permanently disrupts milk output (*pers. comm.* Zviadi Asanishvili; National Food Agency [NFA], Tbilisi, Georgia). In the context of the already low levels of vaccination (<50%) documented among private livestock owners during the 2012 outbreak [86], this further suggests likely suboptimal levels of voluntary vaccine adoption among livestock herders. The change in policy, from compulsory anthrax livestock immunizations to voluntary participation, has likely facilitated the ongoing outbreaks of anthrax, highlighting the human health benefits of livestock vaccination.
Consistent with previous studies [74,94–96], we found that the majority of anthrax cases were associated with slaughtering or butchering livestock. Concomitantly, we found an increased proportion of cases that reported foodborne transmission from meat purchases, following the change in policy. Historically, approximately 93% of human anthrax cases occurred in the rural population, with the vast majority (>90%) of those involving occupations that handle animals on government collective farms [92]. In contrast, our findings suggest that the spread of infection has likely been facilitated by illegal slaughter and sale of infected meat, as documented in previous epidemiological trace-back investigations [86,97]. This is also consistent with recent research noting an increased risk in urban populations by a suspected route of infectious spillover from infected animal by-products at informal meat markets [97]. The use of these markets, not regulated by the NFA, to recoup economic losses from livestock mortality, parallels the sale and distribution of anthrax contaminated meat documented elsewhere [98].

Interestingly, by 2007-2013, the risk of anthrax had increased among males, and those aged 40-64 years old. While gender differences in anthrax risk are not typically observed [74,94,96], our findings suggest the high risk among males is related to occupational risks as observed elsewhere [99]. The high risk among the 40-64 age group is consistent with a cohort effect, as these individuals would have been the typical age of livestock tenders (15-40 years of age) twenty-five years ago during the extensive agricultural production of Soviet Union, and it plausible that they have maintained this agrarian lifestyle.

The spatial patterns of human anthrax also changed over time. Risk increased among ethnic enclaves during 2007-2013 to >6 times that of all other district municipalities, with evidence of spatial clustering not uniquely driven by the underlying distribution of the ethnic population. Previous studies also documented high rates of zoonotic disease among ethnic
enclaves in the United States [63] and Germany [100]. Following decollectivization in the 1990’s, livestock ownership was privatized across the FSU. In Georgia, agriculture was grouped into two categories: subsistence farming and private enterprises (http://www.geostat.ge/). One plausible explanation for this clustering is that enterprise agriculture (higher livestock density) is more common among ethnic enclaves in the southeast, compared to the west where subsistence agriculture predominated and the ethnic composition is low ($\leq10\%$) (Figure S1). These changes, in conjunction with the alteration to livestock immunization, have exacerbated the risk associated with areas of agricultural production. These may also overlap with ecological zones, such as alkaline soils, which can support $B.\ anthracis$ (11, 17, 36).

The data we used in this were obtained through passive surveillance and are subject to systematic error. Our epidemiological questionnaire-based surveys relied on the ability of case patients to accurately recall information and may be prone to recall bias. A large proportion of occupational responses from the epidemiological questionnaires were missing, limiting our inference regarding differences in this category. There may be also an unwillingness to admit to illegally slaughtering sick livestock, and thus the underreporting of livestock anthrax cases, has likely skewed the true sources for the occurrence of the disease. Conversely, areas with better access to healthcare facilities may be overrepresented in our sample. Future studies should focus on collecting better livestock case data and vaccination records for use in cost-effectiveness studies.

The change in livestock vaccination policy has dramatically affected the epidemiology of human anthrax in Georgia. In addition to the increasing rates of transmission, the epidemiology of the disease has shifted such that ethnic enclaves, and in particular, Azerbaijanis, are now at highest risk. This may be a result of this population being more likely to work with unvaccinated
animals during agricultural production. The continued illegal slaughter and sale of infected meat has likely increased the frequency of infections among occupations uncharacteristically at risk for anthrax. Consumers should be warned to only purchase meat from licensed vendors. Indemnity programs that provide compensation for vaccine and livestock losses should be considered as an approach for reducing exposure and enhancing reporting. The cessation of livestock anthrax vaccination in Georgia in 2007 highlights the need for weighing the costs and benefits of ending policies that have substantial human health impacts. Public health programs that focus on ‘One Health’ preventative measures would be beneficial, as they can integrate awareness of disease among high risk populations such as agricultural workers of Azerbaijani ethnicity. Food safety regulations, and livestock vaccination campaigns needed to address the evolving epidemiology of anthrax.
Figure 2-1. Annual number human anthrax cases in Georgia, 2000-2013 (grey bars). Total annual number of livestock anthrax vaccine doses administered (100,000 doses) in Georgia, 2000-2013 (solid black line). The modeled incidence per 100,000 trend in humans (black dotted line) displaying the breakpoint in the rate of change at 2010 (95% CI: 2008, 2011).
Figure 2-2. Incidence per 100,000 of human anthrax by age group during 2000-2013 in Georgia.
Figure 2-3. Incidence of human anthrax cases/100,000 by ethnicity, and by gender in Georgia during 2000-2013.
Figure 2-4. Spatial distribution on crude human anthrax incidence rates in Georgia during A) 2000-2006 B) 2007-2013 and ethnicity-adjusted incidence rates during C) 2000-2006 and D) 2007-2013.
Table 2-1. Demographic characteristics of human anthrax and relative risk estimates before (2000-2006) and after (2007-2013) a change in vaccine policy.

<table>
<thead>
<tr>
<th>Demographic Characteristics</th>
<th>No. cases (95% CI)</th>
<th>Average annual incidence per 1,000,000 (95% CI)†</th>
<th>Relative risk (95% CI)†</th>
<th>No. Cases (% of Cases)</th>
<th>Average annual incidence per 1,000,000 (95% CI)†</th>
<th>Relative risk (95% CI)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>200 (100)</td>
<td>6.5 (5.7, 7.5)</td>
<td>—</td>
<td>536 (100)</td>
<td>17.7 (16.3, 19.3)</td>
<td>—</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>70 (35)</td>
<td>4.3 (3.4, 5.5)</td>
<td>Reference</td>
<td>113 (21)</td>
<td>7.1 (5.9, 8.6)</td>
<td>Reference</td>
</tr>
<tr>
<td>Male</td>
<td>130 (65)</td>
<td>9 (7.5, 10.7)</td>
<td>2.1 (1.6, 2.8)</td>
<td>423 (79)</td>
<td>27.5 (25, 30.2)</td>
<td>3.9 (3.1, 4.8)*</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-19</td>
<td>9 (5)</td>
<td>1.1 (0.5, 2)</td>
<td>0.1 (0.04, 0.2)</td>
<td>26 (5)</td>
<td>3.5 (2.3, 5.1)</td>
<td>0.1 (0.1, 0.2)</td>
</tr>
<tr>
<td>20-39</td>
<td>53 (26)</td>
<td>6 (4.5, 7.9)</td>
<td>0.5 (0.4, 0.7)</td>
<td>177 (33)</td>
<td>20 (17.1, 23.1)</td>
<td>0.6 (0.5, 0.8)</td>
</tr>
<tr>
<td>40-64</td>
<td>108 (54)</td>
<td>11.8 (9.7, 14.3)</td>
<td>Reference</td>
<td>292 (54)</td>
<td>31.3 (27.8, 35.1)</td>
<td>Reference</td>
</tr>
<tr>
<td>≥ 65</td>
<td>30 (15)</td>
<td>0.6 (0.4, 0.9)</td>
<td>0.6 (0.4, 0.9)</td>
<td>41 (8)</td>
<td>9.6 (6.9, 13)</td>
<td>0.5 (0.3, 0.7) *</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Georgian</td>
<td>153 (76)</td>
<td>6 (5.1, 7)</td>
<td>Reference</td>
<td>338 (63)</td>
<td>13.3 (12.2, 15.1)</td>
<td>Reference</td>
</tr>
<tr>
<td>Azerbaijani</td>
<td>15 (8)</td>
<td>7.7 (4.3, 12.6)</td>
<td>1.3 (0.7, 2.2)</td>
<td>163 (30)</td>
<td>83.3 (71, 97.1)</td>
<td>6.1 (5.1, 7.4)*</td>
</tr>
<tr>
<td>Armenian</td>
<td>14 (7)</td>
<td>8 (4.4, 13.7)</td>
<td>1.4 (0.8, 2.3)</td>
<td>24 (4)</td>
<td>15.8 (10, 23.6)</td>
<td>1.2 (0.8, 1.8)</td>
</tr>
<tr>
<td>Other</td>
<td>4 (2)</td>
<td>3.3 (0.4, 12)</td>
<td>0.6 (0.1, 1.9)</td>
<td>9 (2)</td>
<td>16.2 (7.4, 3)</td>
<td>1.2 (0.6, 2.3)</td>
</tr>
<tr>
<td>Unknown</td>
<td>14 (7)</td>
<td>—</td>
<td>—</td>
<td>2 (0.4)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Region</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethnic enclaves‡</td>
<td>61 (31)</td>
<td>14.3 (10.9, 18.4)</td>
<td>2.4 (1.8, 3.3)</td>
<td>281 (52)</td>
<td>65.8 (58.3, 74)</td>
<td>6.1 (5.1, 7.2)*</td>
</tr>
<tr>
<td>Other</td>
<td>139 (70)</td>
<td>5.9 (5.7)</td>
<td>Reference</td>
<td>255 (48)</td>
<td>10.9 (9.6, 12.3)</td>
<td>Reference</td>
</tr>
<tr>
<td>Season</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>10 (5)</td>
<td>0.3 (0.2, 0.6)</td>
<td>0.1 (0.1, 0.2)</td>
<td>32 (6)</td>
<td>1.1 (0.7, 1.5)</td>
<td>0.2 (0.1, 0.3)</td>
</tr>
<tr>
<td>Spring</td>
<td>32 (16)</td>
<td>1.0 (0.7, 1.5)</td>
<td>0.4 (0.3, 0.6)</td>
<td>79 (15)</td>
<td>2.7 (2.1, 3.3)</td>
<td>0.5 (0.4, 0.6)</td>
</tr>
<tr>
<td>Summer</td>
<td>75 (38)</td>
<td>2.5 (1.9, 3.1)</td>
<td>0.9 (0.7, 1.2)</td>
<td>258 (48)</td>
<td>8.7 (7.7, 9.9)</td>
<td>1.5 (1.3, 1.9) *</td>
</tr>
<tr>
<td>Fall</td>
<td>83 (41)</td>
<td>2.7 (2.2, 3.4)</td>
<td>Reference</td>
<td>167 (31)</td>
<td>5.6 (4.8, 6.6)</td>
<td>Reference</td>
</tr>
</tbody>
</table>

† 95% Confidence Intervals (CI); * Significant difference between relative risk in 2000-2006 and 2007-2013, based on the lack of overlap between the 95% CI; ‡ Ethnic enclaves represent municipalities with ≥ 30% of the population comprised of ethnic groups.
### Table 2-2. Reported risk factors for human anthrax before (2000-2006) and after (2007-2013) a change in vaccine policy in Georgia.

<table>
<thead>
<tr>
<th>Anthrax risk factors</th>
<th>2000-2006</th>
<th></th>
<th>2007-2013</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. cases</td>
<td>% of Cases</td>
<td>No. Cases</td>
<td>% of Cases</td>
</tr>
<tr>
<td>Self-reported source</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slaughter or butcher livestock</td>
<td>108</td>
<td>54 (47, 61)</td>
<td>313</td>
<td>58 (54, 63)</td>
</tr>
<tr>
<td>Yes/animal was sick or dead‡</td>
<td>46</td>
<td>23 (17, 29)</td>
<td>49</td>
<td>9 (7, 12)*</td>
</tr>
<tr>
<td>Process, cook, or handle meat</td>
<td>51</td>
<td>26 (19, 32)</td>
<td>160</td>
<td>30 (26, 34)</td>
</tr>
<tr>
<td>Yes/animal was sick or dead‡</td>
<td>5</td>
<td>2 (0, 3)</td>
<td>4</td>
<td>1 (0.01, 2)</td>
</tr>
<tr>
<td>Yes/purchased meat¥</td>
<td>21</td>
<td>11 (6, 15)</td>
<td>114</td>
<td>21 (18, 25)*</td>
</tr>
<tr>
<td>Soil</td>
<td>30</td>
<td>15 (10, 20)</td>
<td>47</td>
<td>9 (6, 11)</td>
</tr>
<tr>
<td>Unknown</td>
<td>11</td>
<td>6 (2, 9)</td>
<td>16</td>
<td>3 (2, 4)</td>
</tr>
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<td>Occupational risks</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Handle animals/animal by-products</td>
<td>8</td>
<td>4 (1, 7)</td>
<td>149</td>
<td>28 (24, 32)*</td>
</tr>
<tr>
<td>Housewife</td>
<td>8</td>
<td>4 (1, 7)</td>
<td>61</td>
<td>11 (9, 14)</td>
</tr>
<tr>
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<td>14</td>
<td>3 (1, 4)</td>
</tr>
<tr>
<td>Unemployed</td>
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<td>6 (3, 9)</td>
<td>76</td>
<td>14 (11, 17)*</td>
</tr>
<tr>
<td>Other</td>
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<td>7 (3, 10)</td>
<td>74</td>
<td>14 (11, 17)*</td>
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<td>157</td>
<td>79 (73, 84)</td>
<td>162</td>
<td>30 (26, 34)*</td>
</tr>
</tbody>
</table>

† 95% Confidence Intervals (CI); * Significant difference between relative risk in 2000-2006 and 2007-2013, based on the lack of overlap between the 95% CI. ‡ Number of case patients that answered yes to butchering/slaughtering livestock or processing/cooking/handling meat and that the animal was also sick or dead. ¥ Number of case patients that processing/cooking/handling meat and answered to purchasing the meat.
CHAPTER 3
RETHINKING LIVESTOCK VACCINATION: USING WILDLIFE SURVEILLANCE FROM WILD BOAR (SUS SCROFA) AND LIVESTOCK RISK MODELS TO AID IN ANTHRAX CONTROL STRATEGIES IN UKRIANE

Background

Anthrax, is a widely spread zoonotic disease found on nearly every continent [1]. To control anthrax, annual livestock vaccination is recommended in endemic areas [2,3]. For example, ~160 million doses of vaccine were administered in the FSU during a single year (1960) resulting in nearly a 9-fold reduction in human anthrax cases [4]. However, underreporting and poor surveillance of anthrax may hamper decision-making [1]. In the absence of disease reporting, policy makers may scale down interventions or terminate vaccination campaigns in order to cut costs. Further exacerbating this issue is the ability of the causative agent of anthrax, *Bacillus anthracis*, to survive in the environment for long periods (perhaps decades) [5]. Under proper environmental conditions such as alkaline soil pH, outbreaks may reappear after extended periods of quiescence [3,5,6].

Anthrax is an endemic disease in Ukraine with a long history of transmission dating back more than a century [7]. In the early 1900’s, the Soviet Union (including Ukraine) reported approximately 40,000-60,000 livestock cases annually [4]. Following mandated mass livestock anthrax vaccination campaigns, the occurrence of the disease decreased substantially [4]. The Veterinary Service of Ukraine (VSU) undertook the control and documentation of anthrax following the dissolution of the Soviet Union and independence in 1991. Since then, the occurrence of Anthrax in Ukraine has declined substantially; only a single case was reported during the last 6-years [7]. Given this dramatic reduction, the government in Ukraine has been considering ending its compulsory livestock vaccination campaigns as a cost cutting measure. However, recent serological evidence from wild boar in Ukraine suggests ongoing anthrax
exposure and research has indicated that wildlife may be involved in propagating anthrax exposure [5,8,9]. This has brought attention as to whether or not passive surveillance alone is a good indicator of anthrax activity.

To overcome the limitations imposed by passive surveillance reporting, previous research has shown that convenience sampling of sentinel surveillance from wildlife hunting bags and environmental risk models of anthrax may aid in policy and help identify priorities for targeted interventions such as vaccination [10,8]. Thus, using sentinel wildlife surveillance from hunting bags taken during a Ukrainian government supported program to control wild boar populations may provide clarity regarding the status of anthrax foci (active vs. not active). Our objective was to 1) examine serological data from wild boar to assess the exposure and geographic extent of anthrax foci in Ukraine 2) develop risk models of environmentally suitable areas 3) assess the geographic identify the concordance of anthrax risk models with wildlife exposure in wild boars to help aid in vaccination policy decision making. This study will lead to a better understanding anthrax ecology.

Methods

Data Sources

We obtained serological samples of wild boar (Sus scrofa scrofa) collected from hunting bags during the 2013-14 fall/winter hunting season (November 2013 – February 2014) across districts in Ukraine. In order to detect anthrax exposure, the Institute of Veterinary Medicine (IVM) tested the samples using a 96 well ELISA to detect Protective Antigen (PA83) IgG (Alpha Diagnostics, Cat. No. 900-115-83G) according to the manufacturer protocols.

In addition, we also obtained disease registry data on the number of livestock anthrax outbreaks per district in Ukraine during 1979-2015 reported to VSU. Outbreaks were defined as ≥ 1 confirmed case of anthrax in livestock via clinical diagnosis and bacteriology. To estimate
risk, we used livestock population data for the year 2005 from the Gridded Livestock of the World (GLW) v2.0. The GLW database provides a global estimate of livestock populations at a spatial resolution of $\approx 1 \text{ km} \times 1 \text{ km}$ adjusted to match national totals. Since historical livestock population estimates were only available at the regional scale, we estimated the district level population going back to 1980 in order to include livestock population as an offset in our regression models. To estimate past livestock population estimates, we used 2005 GLW data adjusted for annual livestock population growth during 1980-2015. The adjusted GLW data were then back fit to match regional (n=27) livestock population census estimates in Ukraine (State Statistics Service of Ukraine; http://ukrstat.org/en/work/contakt_e.html). We then derived adjusted quinquennial GLW population estimates for each district. We used only cattle population to estimate risk since a majority of outbreaks involved cattle ($\approx 75\%$).

In Ukraine, there are currently three different live anti-anthrax vaccines; vaccine strain use is differentiated by livestock type: strain SB (reg. # BB-00088-02-09), strain K-79Z (reg. # BB-00368-02-11) and strain Sterne 34F2 (reg. # BB-00397-02-13). We obtained the number of livestock anthrax vaccine doses administered by livestock type (cattle, small ruminants, swine, horses) documented by VSU during 1996-2015.

Data Analysis

Anthrax prevalence in wild boar

We based our estimates of anthrax exposure in wild boar on an imperfect diagnostic test with an unknown specificity and sensitivity. The apparent prevalence $p_i$ of each district is then given by the ratio of the number of positive serological samples $y_i$ to the sample size $n_i$ in each district; $p_i = y_i / n_i$. We assume that the number of positive anthrax exposure test in each district is binomial distributed:

$$Y_i \sim Binomial (n_i, \pi_i) \quad (3-1)$$
where \( n_i \) is the number of serological samples from each district and \( \pi_i \) represents the unknown true disease prevalence. Given that the probability of a positive serological test of anthrax exposure in wild boar is unknown and shows overdispersion, we chose a beta-binomial model. The beta-binomial model assumes that the mean parameter \( \pi \) is beta distributed. The beta distribution is a two-parameter distribution within the interval \([0, 1]\) and shape parameters \( \text{Beta}(\alpha, \beta) \) with a mean of \( \frac{\alpha}{\alpha + \beta} \) and a variance \( \frac{\alpha \beta}{(\alpha + \beta)^2 (\alpha + \beta + 1)} \). Based on Bayes theorem, the posterior mean distribution is proportional to the product of the binomial likelihood and the beta distribution prior; since the beta distribution is a conjugate prior to the binomial distribution the posterior predicted distribution will also beta distributed. We used uninformative priors on beta selecting Jeffreys prior \((0.5, 0.5)\) [11]. Due to our large number of sampling units over the entire country of Ukraine, we used a computationally more efficient Bayesian framework implemented through the integrated nested Laplace approximation (INLA) [12]. The INLA approach is described in detail elsewhere [12]. Briefly, INLA is a deterministic algorithm that aids in the spatial and spatio-temporal analysis of large datasets that may otherwise be computationally inefficient to implement using a traditional Bayesian Markov Chain Monte Carlo (MCMC) implementation.

To estimate the true prevalence, we adopted the framework that Staubach et al. (2002) used for wildlife surveillance that incorporates an unstructured and a spatially structured random effect in a beta-binomial model. The model takes a form of the Besag, York, and Mollie (BYM) model [13], but with a binomial logistic rather than a Poisson log-normal model:

\[
\log \frac{\pi_i}{1 - \pi_i} = \mu + u_i + v_i
\]

Where \( \mu \) is an intercept term, \( u_i \) is a spatially structured random effect with an intrinsic Conditional Autoregressive (iCAR) prior, with the conditional distribution of \( u_i \) considered to be
normal with the mean equal to the average of the neighboring spatial units and an unknown variance $\sigma_u^2$. The choice of the log-gamma priors on $\sigma_u^2$ allows for a strong smoothing component while preserving geographic variation [14]. We used a log-gamma prior on $\sigma_u^2$ of (0.5, 0.005). The unstructured random effect, $v_i$, is modeled as normal identically and independently distributed (iid), with a mean equal to zero and variance $\sigma_v^2$ [15]. We used a prior a log-gamma prior on the unstructured random effect variance $\sigma_v^2$ of (0.1, 0.001). Based on the large number of zeros within the sampled boar data, we adopted a zero-inflated beta-binomial model that allows for structural and sampling zeros with the incorporation aforementioned random effects. The resulting model output is a spatial smoothed prediction of the estimate true anthrax exposure prevalence in wild boars at the district level.

**Livestock anthrax risk**

To model the environmental risk of anthrax in Ukraine by district, we constructed models model using a generalized linear models (GLM). The dependent variable was the number of livestock anthrax outbreaks per district during 1979-2013 with an offset consisting of the log of the livestock population during 1980-2013. We first chose a GLM with a Poisson distribution because the outcome variable, anthrax outbreaks per district, was composed of non-negative integer values; however, due to over dispersion (mean/variance ratio > 1) in the number of outbreaks per district we explored other probability distributions. After identifying the poor fit of the negative binomial distribution based on the $\chi^2$ goodness-of-fit test, we used a zero-inflated negative binomial model to handle the large number of zeros in the outcome variable (n=287; 57%). A zero-inflated model is a mixture model composed of one process generating counts and another process generating zeros [16]. We chose this model since the process generating zero anthrax outbreaks at the district level was likely dictated by two factors: zero outbreaks because
of unfavorable environmental conditions and zero outbreaks because they occurred but were not reported. We included biologically relevant independent variables in the model drawn from the literature [10,17,18], consisting of averaged climatic and environmental indicators for each district in Ukraine using data we obtained from: WorldClim [19], Harmonized World Soils Database (HWSD) [20,21], and Trypanosomiasis and Land Use in Africa (TALA) [22] (Table 1). To select the most parsimonious model, we used the ‘gmult’ package the in R v3.3.1 (R Core Development Team) to construct a list of candidate models based on a possible combination of all biologically relevant variables from Table 3-1 in the count and zero-inflation portion of the zero-inflated negative binomial model. We then used the Akaike Information Criterion (AIC) to quantify and compare candidate models, selecting the model with the lowest AIC score. To adjust for spatial autocorrelation in the model residuals, we incorporated an autocovariate component in the model [23]. We estimated the autocovariate component following Dorman et al. [23] with the inverse distant weighted average of the outcome variable (anthrax outbreaks) within a predefined distance around a given district \(i\);

\[
A_i = \sum_{j \in k} w_{ij} y_j
\]  

(3-3)

where \(k_i\) is the set of neighboring districts included in a defined distance, \(y_i\) is the outcome variable value at each district \(j\) and \(w_{ij}\) is the inverse-distance weight given to site \(j\)’s influence over site \(i\). We will explore candidate models using an information theoretic approach to select models with a combination of biologically plausible explanatory environmental factors in the count and logistic components of the model. We tested three different distance based autocovariate thresholds (59.6 km, 119.2 km, 178.8km) choosing the threshold that contributed significantly to the models at the \(p=0.10\) level of statistical significance and resulting in the lowest AIC value. The minimum autocovariate threshold was based on the minimum distance
between the district centroids. A training (n=381) and testing (n=127) split of the data were used to test the fit of the model; a $\chi^2$ analysis was used to determine if the distribution of the predicted and observed outcome variable were different for the testing and training data. The predictions from the zero-inflated model represent the average number of outbreaks per district. The final model was used to create a predicted anthrax outbreak risk surface by district in Ukraine: low risk (0-1 outbreaks), medium risk (1-2 outbreaks), and high risk (≥ 3 outbreaks).

We compared the predicted seroprevalence from wild boar to the predicted risk in livestock to assess the concordance of historical anthrax risk with ongoing wildlife exposure. We carried out all statistical analysis in R v3.3.1 (R Core Development Team). Model predictions and serological results were mapped using ArcGIS v10.3.1 (ESRI, Redlands, CA).

**Results**

**Anthrax Prevalence in Wild Boar**

The geographic distribution of serological samples obtained from wild boars is shown in Figure 3-1A. Of the 984 wild boar serological samples taken from hunting bags in Ukraine, 45 were positive for anthrax exposure. Of the 210 districts were samples were collected, 31 had a positive sample. The overall test prevalence was 4.6% (95% CI: 3.4, 6.1). District test prevalence ranged from 0-100%, with geographic variation in prevalence indicated by higher rates of exposure in the southern and western part of Ukraine (Figure 3-1A). After adjusting for unstructured and spatial structured random effects in the Bayesian model, the results are shown in figure 3-1B. The spatial structure of anthrax prevalence in boars is now more apparent with a higher level of exposure in the south and the west. In general, low levels of anthrax exposure are predicted in the southeast in and around the Crimean peninsula.

**Livestock Anthrax Risk**
From 1979 to 2015 there were 475 livestock anthrax outbreaks in Ukraine. The frequency of outbreaks by district illustrates that a majority (57%) of the 508 districts did not experience an outbreak during the last 27 years (Figure 3-2). The geographic distribution of outbreaks at the district level is shown in Figure 3-3. Outbreaks per district ranged from 0 to 10; a higher number of outbreaks were concentrated in the central and eastern portion of the country.

From 1996 to 2015, the VSU vaccinated approximately 171.6 million cattle, 32.2 million sheep and goats, 1.4 million swine, and 8.9 million horses (Figure 3-4). Vaccination numbers declined markedly over time corresponding to decreases in livestock populations (Figure 3-5); in 2015, approximately 5.1 million doses of vaccine were administered nationally compared to 24.6 million doses in 1996.

The final model from the zero-inflated negative binomial regression was selected based on the lowest AIC from a list of competing models. Results of final model are shown in Table 3-2. A \( \chi^2 \) analysis indicated that there was no difference between the observed and predicted distribution of counts (Figure 3-6). Model estimates indicated that soil pH and cattle density were important predictors in the zero-inflation portion of the model; there was a negative association between soil pH indicating that as in zero outbreak districts decreased. The autocovariate term (59.6 km) was included in both the zero-inflation and count portion of the model. Areas of high anthrax risk were primarily predicted in the southcentral part of the country with pockets of high risk areas in the northeast (Figure 3-7).

**Discussion**

Livestock vaccination is one of the most effective methods of controlling anthrax in both humans and animals [2–4]. After years of declining livestock anthrax rates, in response to >50 million doses of vaccine administered, the government in Ukraine has been exploring the option of terminating compulsory annual vaccination campaigns. Although ending such programs will
have immediate cost-savings, the risk of prematurely ending vaccination can have negative consequences [24]. To aid policy decision making, we combined sentinel surveillance of anthrax exposure in wild boars and modeled the environmental risk to identify the distribution of active anthrax foci and high-risk areas. Our findings indicate the presence of environmentally suitable areas at high-risk for anthrax in addition to recent wildlife exposure from active anthrax foci. In keeping with previous modeling studies, we identified heterogeneity in risk characterized by environmental factors such as alkaline soil pH [25,26]. Wild boar exposure, in general, coincided with high-risk areas identified by environmental risk models, although the geographic distribution of active foci appear more widely spread than previously thought. Despite the lack of passive livestock surveillance during the last 6-years, our findings support the hypothesis of ongoing anthrax transmission from active anthrax foci that pose a risk to livestock and humans and may necessitate continued vaccination in high-risk areas [27].

In general, the effectiveness of anthrax livestock vaccination appears high, with reductions in the disease worldwide following vaccination campaigns [2,3]. However, support for ending livestock anthrax vaccination in endemic areas is mixed. In an area of the Middle East a 10-year livestock anthrax vaccination campaign was carried out on livestock after which vaccination was ended and no new cases were reported [28]. Conversely, the cessation of livestock vaccination in Azerbaijan and in the country of Georgia were associated with a dramatic rise in the incidence of both human and livestock anthrax [24,29]. It is unclear whether the persistence of the bacterium *B. anthracis* in the soil over extended periods (>5 years) is able to propagate continued exposure; limited evidence from the literature supports the hypothesis of long-term infectivity [6]. *B. anthracis* in the environment may also lose its virulence over time allowing for the return to fallow fields [30]. Given the underreporting of anthrax, it is uncertain
whether a lack of passive surveillance is strong enough evidence of an interruption in transmission.

Measurable anthrax titers are typically short-lived in animals that are exposed and recover [31]. Our findings showed that district level anthrax true prevalence in wild boars ranged from ~0 to 37% supporting the hypothesis of recent infections from active foci. Suids are considered more resistant to anthrax infections compared to other species [3]. Thus, they may serve as good sentinels for exposure due to their foraging behavior and scavenging of infected carcasses [32]. The daily home ranges of wild boar (~20-30 km²) suggest the exposure occurred within the district they were captured although transmission could have taken place in a neighboring district [33].

Anthrax is a neglected disease underreported in livestock, wildlife, and humans worldwide [1]. Thus, our data likely represents an underestimation of the true occurrence of the disease. We used convenience sampling of hunting bags to obtain serological surveillance of wild boar, which may bias our samples to areas with higher boar density or better accessibility to hunters. The use of an imperfect diagnostic test with an unknown sensitivity and specificity suggests that the true prevalence of the exposure may be higher or lower than our estimates. Although suids are less susceptible to anthrax infection our sampling design may have missed severe cases of disease leading to mortality as previously documented [34]. Furthermore, we modeled the environmental aggregated over a long period (27 years) because outbreaks during 1979-2015 were relatively rare. Although our zero-inflated model could have incorporated a component in the model to address temporal correlation within and among districts, the initial evaluation of the temporal structure in the data revealed no significant trends by district. To adjust for livestock population changes, we estimated historical population estimates and used it
as an offset in the model. In this manner, our predictions were adjusted for spatial variation in livestock population. Further studies are needed to better understand the dynamics of anthrax infection from the environment to livestock and the cost-effectiveness of vaccination.

In conclusion, our findings suggest ongoing anthrax transmission from active foci across broad geographic areas identified as environmentally suitable for anthrax exposure. Our study highlights the advantage of incorporating sentinel wildlife surveillance into estimating risk of an underreported neglected zoonosis and provide guidance on the possibilities of scaling down national control strategies. These findings can be used to inform policy decisions regarding vaccination. Policies aimed at altering control strategies should weigh the cost and benefits.
Figure 3-1. Prevalence estimates of anthrax exposure taken from wild boar (*Sus scrofa scrofa*) serological samples across districts (n=210) in Ukraine. The test-prevalence is shown A) and full Bayesian model predictions are shown in B).
Figure 3-2. Observed frequency of anthrax outbreaks per district in Ukraine 1979-2015.
Figure 3-3. Number of anthrax outbreaks in Ukraine at the district level during 1979-2015.
Figure 3-4. Doses of livestock anthrax vaccine administered in Ukraine during 1996 to 2015.

Figure 3-5. Livestock populations in Ukraine during 1990 to 2015.
Figure 3-6. Frequency distribution of observed and predicted anthrax outbreaks (counts) in Ukraine using a zero-inflated negative binomial regression. Inset A shows the distribution of the observed and predicted frequency of counts from the training set (n=381) and inset B shows the observed and predicted counts from the testing set (n=127). Chi-square test indicated that there was no difference between the observed and predicted counts.
Figure 3-7. Anthrax outbreak risk predicted by the zero-inflated negative binomial regression model. Risk areas were grouped into low (0 outbreaks), medium (1-2 outbreaks), and high (>2 outbreaks) risk.
Table 3-1. Environmental and climatic variables used in the zero-inflated negative binomial regression model.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
<th>Database</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevation</td>
<td>Elevation (m)</td>
<td>WorldClim</td>
<td>187 m</td>
</tr>
<tr>
<td>Bio1</td>
<td>Annual mean temperature (mm)</td>
<td>WorldClim</td>
<td>11.7° C</td>
</tr>
<tr>
<td>Bio7</td>
<td>Annual temperature range (° C)</td>
<td>WorldClim</td>
<td>33.3° C</td>
</tr>
<tr>
<td>Bio8</td>
<td>Temperature of the wettest quarter (° C)</td>
<td>WorldClim</td>
<td>17.8° C</td>
</tr>
<tr>
<td>Bio9</td>
<td>Temperature of the driest quarter (° C)</td>
<td>WorldClim</td>
<td>0.2° C</td>
</tr>
<tr>
<td>Bio10</td>
<td>Temperature of the warmest quarter (° C)</td>
<td>WorldClim</td>
<td>19.2° C</td>
</tr>
<tr>
<td>Bio11</td>
<td>Temperature of the coldest quarter (° C)</td>
<td>WorldClim</td>
<td>-3.5° C</td>
</tr>
<tr>
<td>Bio12</td>
<td>Annual precipitation (mm)</td>
<td>WorldClim</td>
<td>573 mm</td>
</tr>
<tr>
<td>Bio16</td>
<td>Precipitation of the wettest quarter (mm)</td>
<td>WorldClim</td>
<td>204 mm</td>
</tr>
<tr>
<td>Bio17</td>
<td>Precipitation of the driest quarter (mm)</td>
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<td>104 mm</td>
</tr>
<tr>
<td>Bio18</td>
<td>Precipitation of the warmest quarter (mm)</td>
<td>WorldClim</td>
<td>201 mm</td>
</tr>
<tr>
<td>Bio19</td>
<td>Precipitation of the coldest quarter (mm)</td>
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<td>120 mm</td>
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<td>Soil pH</td>
<td>Minimum soil pH</td>
<td>HWSD</td>
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<td>Wd0114a0</td>
<td>Mean normalized difference vegetation index (NDVI)</td>
<td>TALA</td>
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<td>Wd0114a01</td>
<td>Annual amplitude NDVI</td>
<td>TALA</td>
<td>0.27</td>
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Table 3-2. Results of the zero-inflated negative binomial model.

<table>
<thead>
<tr>
<th>Negative Binomial Model</th>
<th>Estimate</th>
<th>Std. Error</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
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<td>-0.023</td>
<td>0.010</td>
<td>0.023</td>
</tr>
<tr>
<td>Bio 10</td>
<td>0.017</td>
<td>0.009</td>
<td>0.055</td>
</tr>
<tr>
<td>Autocovariate term</td>
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<td>0.075</td>
<td>&lt;0.01</td>
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<tr>
<td>Cattle density</td>
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<td>0.028</td>
<td>0.029</td>
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**Zero-inflation Model**

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>Std. Error</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle density</td>
<td>-0.5864</td>
<td>0.2672</td>
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<td>Soil pH</td>
<td>-1.3608</td>
<td>0.6787</td>
<td>0.036</td>
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<tr>
<td>Autocovariate term</td>
<td>-2.6318</td>
<td>1.4979</td>
<td>0.078</td>
</tr>
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</table>
CHAPTER 4
IMPACT OF REV1 LIVESTOCK VACCINATION ON THE RISK OF HUMAN BRUCELLOSIS: AN INTERRUPTED TIME-SERIES ANALYSIS

Background

The United Nations Sustainable Development Goals propose, to reduce poverty and hunger by 2030. Diseases shared between livestock and humans (zoonotic) represent a growing barrier to achieving these goals due their multifaceted impacts on health, food, and economic potential. Thus, controlling zoonoses, particularly those that are neglected, remains a crucial step in improving the health and wellbeing of an estimated 500-900 million poor rural livestock-keepers worldwide [13–15]. In response to this increasing interconnectedness between human and animal health, the World Health Organization (WHO) has recommended more integration between human and veterinary health services. One such strategy is to improve the health of companion animals and livestock through preventive disease control measures such as vaccination [16–18], which has been shown to have human and livestock health benefits in addition to positive economic impacts [9,10,17,19].

Brucellosis is one of the most common and widely spread zoonotic diseases in the world, exacting a tremendous health and economic burden, with an estimated 500,000 new cases annually [57,133,134]. Although low and middle-income countries experience a disproportionate burden of brucellosis, it has reemerged as an economic and public health threat in developed countries as well [100,135]. Human infections typically occur through two main sources of transmission: foodborne (the consumption of unpasteurized dairy products) and occupational (contact with infected animal materials) [136]. Brucellosis is caused by a group of bacteria in the genus *Brucella* spp. that are, in general, host specific with the most pathogenic to humans: *B. melitensis* (sheep and goats), *B. abortus* (cattle), and *B. suis* (swine). Vaccination of livestock is the most effective method of control in both humans and animals. Modeling studies have
indicated that reducing brucellosis in livestock would have an overall net benefit to human, livestock, and agricultural economies [9,19], although there is sparse evidence of sustained brucellosis control in low and middle-income countries. Currently, the most widely used livestock vaccines target large ruminants (cattle), protecting against *B. abortus* using the RB51 and S19 live attenuated vaccines, and small ruminants (sheep and goats), protecting against *B. melitensis* using the Rev1 live attenuated vaccine [60], which can be administered subcutaneously (Rev1S) or ocular (Rev1O).

In Azerbaijan, brucellosis was first documented in the 1920’s in the northeastern region and subsequently spread to more than two-thirds of the country by the 1950’s [137]. Brucellosis is now endemic in the country and ranks among some of the highest reported rates globally [133]. From the late 1980’s up to the early 1990’s, human brucellosis incidence in Azerbaijan increased by ≈25% annually [138]. Previous reports indicated that males experienced a disproportionate burden of risk compared to females with the highest incidence of disease in age group 15 to 19 years (18 cases per 100,000). Among the isolates recovered from human cases, *B. melitensis* was the most frequently documented species (>85%) [138]. In 2002, the Azerbaijani government initiated a pilot vaccination campaign targeting small ruminants with Rev1 vaccination via a subcutaneous administration (Rev1S); the program ended in 2005 due to a perceived lack of benefits. In fall of 2007, the Azerbaijani government, with support from the World Bank, reinstituted a national livestock control program using Rev1 via an ocular administration (Rev1O) aimed at reducing the burden of brucellosis in both domestic livestock and humans. Whole flock livestock vaccination of all non-pregnant female small ruminants was carried out in 2008 and 2011, followed by vaccination of replacement animals and those >6 months of age during 2009-2010 and 2012-2013.
Despite evidence of successful brucellosis livestock control programs in developed countries, there is skepticism regarding the safety and effectiveness of Rev1 livestock vaccination campaigns in endemic low- and middle-income countries [139]. Highlighting this issue is a recent multinational initiative aimed at incentivizing ($30 million USD award) the development of new brucellosis vaccine for small ruminants [139]. Given the substantial global economic and public health burden of brucellosis, identifying the effectiveness of interventions is crucial. The objective of our study was to evaluate the impact of small ruminant Rev1 vaccination on human brucellosis incidence in Azerbaijan (middle-income country).

**Methods**

We analyzed reported monthly human brucellosis cases from the Republican Anti-Plague Station in Baku, Azerbaijan (1999-2013) nationally and by region: Absheron, Aran, Ganja, and Shaki (Figure 4-1). Incidence per 100,000 population was calculated (total cases/population) for each month using the estimated mid-year population (Azeri State Statistical Committee, azstat.org). We obtained livestock vaccination data from the State Veterinary Service, Baku (1999-2013). To estimate vaccine coverage (total vaccinated/total population) during 1999-2013, we used the entire small ruminant livestock population of Azerbaijan (Azeri State Statistical Committee, azstat.org) because census data distinguishing male and female small ruminants were unavailable. To evaluate the impact of Rev1 livestock vaccination on the risk of human brucellosis, we used an interrupted time-series framework (ITSF) with an A-B-A-B reversal design to account for two vaccination campaigns (Rev1S and Rev1O), where A is the control series and B is the intervention series. Interrupted time series are quasi-experimental approaches commonly used to evaluate the impact of an intervention by assessing changes after the implementation of a policy [140].
To model the seasonality and autocorrelation structure in the monthly incidence of human brucellosis (Figure 4-2), we used methods devised by Box and Jenkins [141]. This approach uses ‘autoregressive’ and ‘moving average’ model parameters to extract a signal from noise in the time-series and applies differencing to achieve stationarity in the series. We adopted this approach with a seasonal autoregressive integrated moving average (SARIMA) model containing parameters \((p,d,q)(P,D,Q)_{12}\), where \(p\), \(d\), and \(q\); and \(P\), \(D\), and \(Q\) are the non-seasonal and seasonal: autoregressive (AR), differenced, and moving average (MA) components, respectively. First, we log transformed the monthly human incidence of brucellosis (dependent variable) to stabilize the variance and adjust for outliers. Second, we tested for stationarity in the log monthly incidence by using the augmented Dickey-Fuller (ADF) test [142], which tests the null hypothesis that there is a unit root (non-stationarity). Third, we assessed the model for the presence of residual autocorrelation by visual inspection of the autocorrelation function plots, and tested for correlation among higher order lags under the null hypothesis of no serial correlation using the Breusch-Godfrey test up to 24 lags [143,144]. Fourth, we selected candidate models using the corrected Akaike information criterion (AICc), a measure of the goodness of fit while penalizing the number of parameters; we chose the model with lowest AICc value [145,146]. We defined four periods in our time-series models: pre-vaccination with 36 months (1999-2001), Rev1\(_S\) (subcutaneous administration) with 36 months (2002-2004), post-vaccination with 30 months (2005-05/2007) and Rev1\(_O\) (ocular administration) with 57 months (2008-09/2012).

Fifth, we examined intervention components also known as ‘transfer functions’ to evaluate the impact of the two Rev1 vaccination campaign (Rev1\(_S\) and Rev1\(_O\)) time periods aforementioned. We tested three hypotheses: 1) a gradual change during the Rev1\(_S\) and Rev1\(_O\)
(no level change) using a variable coded 0 before vaccination and sequentially after, 2) a permanent level change in incidence with a intervention dummy variable representing the impact of Rev1S coded 0 before vaccination and 1 during vaccination, and a dummy variable representing the impact Rev1O coded 0 before vaccination and 1 during vaccination and 3) a gradual and permanent change using a combination of dummy variable terms in hypotheses 1 and 2 [147,148].

Sixth, final models were selected based on the presence of statistically significant covariates at the p=0.10 level of statistical significance and forecasted 15-months into the future. Forecasted estimates were then compared to a data validation set withheld from the model building process that consisted of 15 time points (10/2012-12/2013). We examined the concordance of the forecasted estimates and the validation dataset using the Pearson correlation coefficient (ρ), root mean square error (RMSE) and mean absolute error (MAE). Model predictions were back-transformed to incidence and plotted against observed values. We repeated steps 1-6 for national and regional models to assess the influence of geographic interaction effects (Figure 4-1). Regression coefficient estimates on the log scale were exponentiated and interpreted as the percent change in human brucellosis risk.

To estimate the relative risk reduction (RRR) by case patient age groups, we compared the average annual incidence of brucellosis per 100,000 population by age group (0-14, 15-19, 20-29, 30-59, >60) before (1999-2001) and after vaccination (2009-2012). Chi-square analysis was performed on the seasonality of cases comparing intervention and non-intervention years with seasons defined as: winter (December, January, and February); spring (March, April, May); summer (June, July, August); and fall (September, October, November). All statistical analyses were performed in R v3.3.1 (R core Development Team).
**Results**

National time-series incidence data of human brucellosis is shown in Figure 4-2. From 1999 to 2013, there were 6,703 human brucellosis cases. National incidence exhibited strong seasonality during 1999 to 2001 with peak reporting across the summer and fall, and a spike in winter incidence; by 2003, peaks had dampened substantially with a shift to a primarily spring and summer seasonality (Figure 4-2). The seasonality of human cases shifted, nationally, during the Rev1 vaccination periods compared to the non-intervention periods ($\chi^2 = 78.81$, df =3, $p<0.01$). Regionally, incidence exhibited similar seasonal patterns during pre-vaccination reporting with geographic heterogeneity in the dampening of seasonality following livestock vaccination (Figure 4-3). Livestock vaccination was carried out in campaigns targeting all female small ruminants in 2003, 2008, and 2009. Approximately, 45% of all small ruminants were vaccinated in 2008 (Figure 4-4). On average there were 1.2 million vaccinations administered during the subcutaneous campaign (2002-2004) compared to 2.1 million doses administered during ocular vaccine administration (10/2007-2013).

We examined time-series data for stationarity using the ADF test and failed to reject the null hypothesis of a unit root, therefore, we used seasonal differencing for all models. After examination of the autocorrelation functions, we derived a list of candidate models with a combination of AR and MA components after the Breusch-Godfrey test failed to reject the null hypothesis of no serial correlation. The best fitting models from a list of candidate models nationally and regionally were selected based on the lowest AICc and included the following SARIMA models: (2,0,0)(1,1,1)$_{12}$ nationally; (1,0,1)(1,1,1)$_{12}$ Aran; (1,0,1)(1,1,1)$_{12}$ Absheron; (1,0,1)(0,1,1)$_{12}$ Gazakh; and (1,0,0)(0,1,1)$_{12}$ Shaki (Table 4-1).

We tested three hypotheses regarding the impact of the two Rev1 livestock vaccination campaigns on human brucellosis risk using the SARIMA models (Table 4-2). After controlling
for seasonality and trends in the data, the models indicate regional heterogeneity in the impact of vaccination on human risk. Nationally, the most parsimonious model supported the hypothesis of a gradual declining trend in human brucellosis risk of -6% (-12, -0.3) per year following Rev1s and a permanent level change in risk of -28% (-44, -8) following Rev1o (Table 2). The fit of the modeled data was plotted against the observed data for the national estimates (Figure 4-5). When models were stratified by geographic region, there was evidence that the national model masked regional variability in the impact of the livestock vaccination campaigns. The regions of Absheron and Gazakh mirrored the national hypothesis with declining annual trends of -13% (-25, 0.2) and -11% (-17, -5) following Rev1s, respectively; and permanent level changes in risk of -61% (-78, -29) and -35% (-50, -15) following Rev1o, respectively (Table 4-2). Evidence supporting the hypothesis of an impact of Rev1s was documented in Aran, with level changes in risk of -32% (-39, -24) and -58% (-72, -5) following Rev1s and Rev1o, respectively. Shaki was the only region examined with no evidence of a level in change human risk following either Rev1s or Rev1o (Table 4-2).

Average brucellosis incidence per 100,000 population by age group before and following vaccination differed among age groups (Figure 4-6). The greatest relative risk reduction was documented in the 20 to 29 age group (78%, 95% CI: 74, 83). Case patients age 15 to 19 years had the highest average incidence/100,000 before (24.3 cases, 95% CI: 9.7, 40) and also following (7.2 cases, 95% CI: 2.4, 12.1) the vaccination campaign, compared to other age groups (Figure 4-3).

**Discussion**

We assessed the impact of two different routes of Rev1 livestock vaccination administration (Rev1s – subcutaneous, and Rev1o - ocular) on the risk of human brucellosis in Azerbaijan over a 15-year period. Despite concerns regarding the effectiveness and sustained
control of brucellosis with Rev1 in middle-income countries [139], our findings indicate that livestock vaccination substantially reduced the overall burden of human brucellosis. Our study had three salient findings. First, Rev1\textsubscript{S} vaccination, in general, did not support the hypothesis of permanent reduction (shift to a new endemic equilibrium) in human brucellosis risk, but rather indicated a gradual declining trend. Second, following Rev1\textsubscript{O} vaccinations, there was evidence of an abrupt permanent reduction in human risk indicating a shift to new lower endemic equilibrium, nationally and in three of the four regions examined (Absheron, Aran, Shaki, and Gazakh). Third, the greatest reduction in risk was among a demographic group (20-29 years of age) characteristically at risk for occupational brucellosis transmission.

Previous studies have documented human health impacts of Rev1 vaccination, but sustained control programs have primarily occurred in high-income countries [149–151]. However, the analyses presented here are consistent with mathematical models of livestock control with concomitant reductions in human brucellosis risk [16,19]. Research in Tajikistan indicated that Rev1 livestock vaccination led to an 80% reduction in seropositive animals although over a longer time period than our study [152]. A study in Greece also demonstrated an abrupt decrease in human brucellosis following livestock vaccination of small ruminants with a subsequent increase in human risk following the removal of interventions [151].

Regional heterogeneity in the impact of Rev1 vaccination in our study may be a result of variation in vaccine coverage or because areas with more developed infrastructure elicited greater benefits, illustrated by the >60% reduction in risk in the more urbanized Absheron region (Figure 4-1). Interestingly, the absence of a measurable impact in risk regionally, following, vaccination may influence national policy. This may have, in part, accounted for the cessation of the Rev1\textsubscript{S} vaccination campaign due to a perceived lack of an effect. Other factors such as
differences in agricultural production intensity may have also played a role. Although Rev1O and Rev1S have similar immunogenic properties [67], we identified variation in their impacts on human risk that could be due to lower vaccine coverage during Rev1S campaigns and a shorter duration of Rev1S administration. The lack of evidence for a declining trend in human brucellosis in response to Rev1O in our study suggests further reductions in human risk may require altering control measures; this could include measures such as increasing vaccine coverage among small ruminants, targeting other animal reservoirs (cattle) with vaccination, or focusing on livestock test and slaughter campaigns [67]. We found that demographic groups at highest risk of brucellosis also experienced the greatest risk reduction, supporting the hypothesis of proper food sanitation measures related to dairy products and interrupting occupational transmission by targeting animals reservoirs with vaccination should remain a priority [136,138].

Strengths of our study include the interrupted time-series A-B-A-B reversal design using long-term data. This methodology adjusts for bias by controlling for cyclical seasonal effects, autocorrelation, and secular trends in the data. Our data were collected in a similar manner by the Anti-Plague station during the entire study period and relied on the same diagnostic tests for confirmation of human brucellosis. Other factors may also account for the reduction in brucellosis such as improved food safety measures, livestock brucellosis screening, or increased awareness of the disease. However, a previous study had detected an increasing trend prior to the vaccination campaign [138].

There were several limitations to our study. Passive human surveillance data may bias reporting towards areas with improved healthcare access and is subject to underreporting given the insidious onset of and non-specific clinical symptoms of brucellosis. Diagnostic tests for brucellosis are imperfect, and the true prevalence and risk of the disease is likely underestimated.
The geographic distribution of vaccines was unavailable; we assumed vaccine coverage was proportional to the regional livestock population. Future studies should explore the cost-effectiveness of alternate control strategies such as campaigns that include large ruminant vaccination and molecular characterization of *Brucella* spp. isolates in Azerbaijan [136].

In conclusion, our findings demonstrate the human health benefits of livestock vaccination that reduce spillover of pathogens. Controlling brucellosis requires a One Health approach that emphasizes cooperation between human, veterinary, and environmental health practitioners [153]. The availability and effectiveness of livestock brucellosis vaccines makes human morbidity and mortality from the disease preventable in many regions of the world. Our findings suggest vaccinating livestock against brucellosis elicits indirect human health benefits, contributing to the Sustainable Development Goals of reducing poverty and hunger by improving the health of livestock holders and their flock. Interventions aimed at reducing the burden of zoonotic diseases require governmental support; in the case of brucellosis, it may take several years for the benefits of a control program to manifest, necessitating a sustained, long-term control program, with periodic evaluation.
Figure 4-1. Map of Azerbaijan and the regions used in the interrupted time-series analysis

Figure 4-2. Incidence of human brucellosis per 100,000 nationally in Azerbaijan 1999-2013. The dashed red lines represent the period of subcutaneous Rev1 (Rev1s) vaccination during 2002-2004). The dotted red lines represent the period of ocular Rev1 vaccination (Rev1o) during (October 2007-2013).
Figure 4-3. Incidence of human brucellosis per 100,000 in Azerbaijan 1999-2013 by region (A) Aran, (B) Absheron, (C) Gazahk, and (D) Shki. The dashed red lines represent the period of subcutaneous Rev1 (Rev1s) vaccination during 2002-2004. The dotted red lines represent the period of ocular Rev1 vaccination (Rev1o) during (October 2007-2013).
Figure 4-4. Annual small ruminant livestock vaccination coverage (black line) and millions of vaccine doses administered (blue bar). Shaded grey region shows period of subcutaneous administration of Rev1 (Rev1S) and ocular administration of Rev1 (Rev1O).
Figure 4-5. Observed (black line) incidence of human brucellosis in Azerbaijan (1999-09/2012) compared to the modeled (red line) values from the interrupted time-series analysis with a SARIMA(1,0,1)(1,1,1)_{12}. The validation holdout data is shown in dark grey and the prediction from the forecast is shown in dark blue.

Figure 4-6. Relative risk reduction among age groups in Azerbaijan comparing incidence rates before Rev1 vaccination (grey) to after vaccination (white).
Table 4-1. Candidate time-series models based on the corrected Akaike Information Criterion (AICc) using the Box-Jenkins approach

<table>
<thead>
<tr>
<th>Model</th>
<th>AICc</th>
</tr>
</thead>
<tbody>
<tr>
<td>National</td>
<td></td>
</tr>
<tr>
<td>(1,0,1) x (1,1,1)\textsubscript{12}</td>
<td>129.2</td>
</tr>
<tr>
<td>(2,0,0) x (1,1,1)\textsubscript{12}</td>
<td>127.0</td>
</tr>
<tr>
<td>(1,0,1) x (1,1,0)\textsubscript{12}</td>
<td>133.9</td>
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<tr>
<td>(1,0,1) x (0,1,1)\textsubscript{12}</td>
<td>129.1</td>
</tr>
<tr>
<td>Aran</td>
<td></td>
</tr>
<tr>
<td>(1,0,1) x (1,1,1)\textsubscript{12}</td>
<td>277.4</td>
</tr>
<tr>
<td>(0,0,3) x (1,1,1)\textsubscript{12}</td>
<td>281.2</td>
</tr>
<tr>
<td>(2,0,1) x (1,1,1)\textsubscript{12}</td>
<td>278.4</td>
</tr>
<tr>
<td>(1,0,0) x (1,1,1)\textsubscript{12}</td>
<td>268.6</td>
</tr>
<tr>
<td>Absheron</td>
<td></td>
</tr>
<tr>
<td>(1,1,1) x (1,0,1)\textsubscript{12}</td>
<td>370.4</td>
</tr>
<tr>
<td>(0,1,1) x (1,0,0)\textsubscript{12}</td>
<td>367.9</td>
</tr>
<tr>
<td>(1,0,1) x (2,1,0)\textsubscript{12}</td>
<td>363.1</td>
</tr>
<tr>
<td>(1,0,1) x (0,1,1)\textsubscript{12}</td>
<td>346.5</td>
</tr>
<tr>
<td>Gazakh</td>
<td></td>
</tr>
<tr>
<td>(1,0,2) x (0,0,1)\textsubscript{12}</td>
<td>269.3</td>
</tr>
<tr>
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</tr>
<tr>
<td>(1,0,1) x (0,1,1)\textsubscript{12}</td>
<td>267.3</td>
</tr>
<tr>
<td>Shaki</td>
<td></td>
</tr>
<tr>
<td>(4,0,1)</td>
<td>356.3</td>
</tr>
<tr>
<td>(3,0,0) x (0,1,1)\textsubscript{12}</td>
<td>342.1</td>
</tr>
<tr>
<td>(2,0,0) x (0,1,1)\textsubscript{12}</td>
<td>340.5</td>
</tr>
<tr>
<td>(1,0,0) x (0,1,1)\textsubscript{12}</td>
<td>341.4</td>
</tr>
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Table 4-2. Results of seasonal autoregressive integrated moving average (SARIMA) interrupted time-series models. Geographic regions are defined in Figure 4-1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>National (% change)</th>
<th>Absheron (% change)</th>
<th>Aran (% change)</th>
<th>Gazakh (% change)</th>
<th>Shaki (% change)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rev1S level</td>
<td>__</td>
<td>__</td>
<td>-32 (-39, -24)***</td>
<td>__</td>
<td>__</td>
</tr>
<tr>
<td>Rev1S trend</td>
<td>-6 (-12, -0.3)**</td>
<td>-13 (-25, 0.2)*</td>
<td>__</td>
<td>-11 (-17, -5)***</td>
<td>__</td>
</tr>
<tr>
<td>Rev1O level</td>
<td>-28 (-44, -8)***</td>
<td>-61 (-78, -29)***</td>
<td>-58 (-72, -5)***</td>
<td>-35 (-50, -15)***</td>
<td>__</td>
</tr>
<tr>
<td>Rev1O trend</td>
<td>__</td>
<td>__</td>
<td>__</td>
<td>__</td>
<td>-8 (-27, -1)**</td>
</tr>
<tr>
<td>Validation</td>
<td>RMSE</td>
<td>0.05</td>
<td>0.11</td>
<td>0.10</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>MAE</td>
<td>0.04</td>
<td>0.09</td>
<td>0.09</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>Pearson ρ</td>
<td>0.85***</td>
<td>0.48</td>
<td>0.77***</td>
<td>0.45*</td>
</tr>
</tbody>
</table>

***Significant at p=0.01, **Significant at p=0.05, * Significant at p=0.10
CHAPTER 5
CONCLUSION

This dissertation set out to examine the linkages between human and animal health in respect to two zoonotic diseases: brucellosis and anthrax in the former Soviet Union. Specifically, the studies in the previous chapters investigated unanswered questions regarding the impacts of livestock vaccination policy on human risk and the integration of risk models with sentinel wildlife surveillance to aid policy decisions. This dissertation examined three different phases of vaccination policy: implementation of a livestock vaccination campaign, cessation of a livestock vaccination policy, and pre-implementation of a livestock vaccination policy. Although these studies have helped fill gaps in the literature, during the investigative process new questions, these studies have posed new questions regarding the linkages between human and animal health.

In chapter 2, I examined the effect of changing livestock vaccination policy on the epidemiology of human anthrax in the country of Georgia. Although anthrax is a neglected disease, it still requires a well-devised control strategy and altering livestock vaccination policies appear to have unintended human health consequences. Using a combination of spatial and statistical analysis, I found there were significant correlations between the change in policy and human disease risk. Following, the change to a voluntary livestock vaccination policy in Georgia, there was a dramatic increase in risk, and a shift in burden to ethnic enclaves, as well as foodborne transmission. These findings support the human health benefits of livestock vaccination. The implications of these results is that the human health impacts of livestock vaccination should considered when changing disease control policies.

A major application of spatial analysis and GIS is in analyzing surveillance data to gather empirical evidence used to guide vaccination policy. To this end, Chapter 3 examines the use of
risk models and sentinel wildlife surveillance of wild boars *Sus scrofa scrofa* to elucidate the environmental suitability of anthrax and identify active anthrax foci in Ukraine. Given the fact that Ukraine is considering ending its compulsory vaccination policy it is important to evaluate draw sound conclusions. Although the analysis of the wildlife data involved interpreting results from an imperfect diagnostic test, I used Bayesian methods to estimate the true prevalence of anthrax exposure in wild boars. In the absence of passive surveillance from livestock, there was evidence on ongoing anthrax transmission in wildlife from active foci broadly distributed across the landscape. These foci agreed, in part, with the modeled environmental suitability of anthrax. These findings suggest that the absence of passive surveillance data does not equate to a cessation of anthrax transmission. The use of sentinel surveillance highlights the benefits of using multiple data streams to guide important health policies. Chapter 4 then looked at the impact of implementing a zoonotic disease control program on the risk of human brucellosis in Azerbaijan. Given the skepticism regarding the effectiveness of Rev1 livestock vaccination to control human brucellosis in low- and middle-income countries understanding the effectiveness of this control measure is crucial for elimination campaigns. Following Rev1 vaccination there was a significant reduction in the risk of human brucellosis. Despite skepticism regarding the effectiveness of Rev1 vaccination in middle-income countries, our findings indicate a substantial reduction in human brucellosis risk following livestock vaccination. This research has implications for global brucellosis control programs and supports the growing evidence of the human health benefits of livestock disease control in developed and developing countries.

**Anthrax Control and Ecology**

This dissertation suggests crucial aspects of control for future anthrax elimination and planning. We found that livestock vaccination policy was associated with human risk; however, it is unclear what proportion of animals should be vaccinated to confer optimal protection.
Research has suggested that vaccinating all animals in endemic areas is required although there is no consensus on the length of time required to disrupt transmission [18,24,25,31]. Further work is needed to evaluate strategies related to vaccine coverage in livestock and issues of timing. Recent research by Tamborrini et al. [74] has also found that West African strains of *Ba* were deficient in a specific sugar, anthrose, located on the protein BclA, which may represent a vaccine escape mechanism. More recently, Blackburn et al. [75] also found that *Ba* isolates from Nigeria were also deficient in the sugar anthrose providing further evidence of possible unique West African lineage. It is not currently possible to know whether the strains in Georgia share similar phenotypic characteristics related to this anthrose deficiency. To answer the question of vaccine effectiveness, molecular data need to be collected and field studies carried out.

Cost often limits vaccine accessibility and use. In Ukraine, the government was thinking of abandoning livestock anthrax vaccination because of costs. Anthrax is one of the few ancient neglected tropical zoonotic disease that has not had any efforts to characterize its burden by looking at disability adjusted life years (DALYS) or by examining the cost-effectiveness of vaccination strategies. Research on the cost-effectiveness of brucellosis has highlighted that sharing costs across human, veterinary, and agricultural sectors would have synergistic net societal benefits [16,19]. The current lack of data on the economics of vaccination may leave many countries looking for guidance without any answers. Efforts to understand the burden of anthrax will help prioritize control efforts and funding for elimination campaigns in the future.

The work presented here suggests interesting associations with climatic and environmental characteristics. Focusing on a smaller spatial scale will be an important aspect of understanding the ecology and timing of outbreaks in Ukraine and Georgia. Research by Blackburn and Goodin [76] suggested fine scale vegetation dynamics may drive anthrax
outbreak severity and timing. Under certain environmental and climatic conditions that are poorly understood, outbreaks of anthrax are triggered on the landscape [44]. Anecdotal evidence suggests that the timing of anthrax outbreaks follow a climatic pattern of a wet spring, followed by a hot dry summer and a subsequent summer rain [44]. As the availability of high-resolution remotely sensed data continues to increase, further research will be able to ask more questions regarding associations between anthrax and the environment in Ukraine, Georgia, and elsewhere.

Brucellosis Control

The impact of brucellosis vaccination on human risk described in Chapter 3 leaves further questions about the cost-effectiveness of vaccinating other livestock hosts. In Azerbaijan, approximately 15% of human isolates were identified as *B. abortus*; however, there are currently no efforts to carry out vaccination campaigns in cattle against this species. A mathematical study in Mongolia suggested that vaccinating cattle, sheep, and goats was a crucial aspect of reducing the burden of disease [19]. Since, the risk of brucellosis has appeared to reach a steady-state equilibrium; further research could examine the cost-effectiveness of cattle vaccination and possibly project whether vaccinating this host would elicit further reductions in risk beyond what has already been achieved. Furthermore, the regional differences in the impact of vaccination poses the question of the spatial effects of vaccination. Further, research could examine if there is an indirect benefit to unvaccinated herds in proximity to vaccinated herds, and whether or not that effect decays with distance.

Although this dissertation has posed several more questions, it makes clear that human and animal health are inextricably linked. Future studies should examine the cost-effectiveness of alternative control strategies while taking into account the human health aspects associated with livestock vaccination. The results of this work support the value of using of GIS and statistical approaches to evaluate the impacts livestock vaccination policies.
LIST OF REFERENCES


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BIOGRAPHICAL SKETCH

Ian Thomas Kracalik was born in Hinsdale, IL and shortly thereafter moved to Southern California. He grew up in Oceanside, CA and graduated from EL Camino High School. After attending Mira Costa Jr. College he moved to Arcata, CA to pursue a degree in geography from Humboldt State. Following his graduation with a Bachelor of Arts in geography, he enrolled in the graduate program in geography at California State University where he began working in the Spatial Epidemiology and Ecology Research lab (SEER) lab and received his Master of Arts in geography in 2010. In 2012, he graduated from the University of Florida with a Master of Public Health in epidemiology. He enrolled in PhD program in medical geography at the University of Florida in 2015.