

DISSERTATION

A SYSTEMIC REVIEW OF BRUCELLOSIS IN THE KAKHETI REGION OF THE
COUNTRY OF GEORGIA: AN EVALUATION OF THE DISEASE ECOLOGY, RISK
FACTORS AND SUGGESTIONS FOR DISEASE CONTROL

Submitted by

Karyn Alicia Havas

Department of Clinical Sciences

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Colorado State University

Fort Collins, Colorado

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Doctoral Committee:

Advisor: Mo D. Salman

Ashley E. Hill
Robert J. Callan
Shana C. Gillette
Ann L. Magennis

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ABSTRACT

A SYSTEMIC REVIEW OF BRUCELLOSIS IN THE KAKHETI REGION OF THE COUNTRY OF GEORGIA: AN EVALUATION OF THE DISEASE ECOLOGY, RISK FACTORS AND SUGGESTIONS FOR DISEASE CONTROL

Human brucellosis is a neglected disease of poverty often found in highly agrarian, livestock dependent societies (World Health Organization, 2006). It is a purely zoonotic disease in that animals infect humans but there is not human-to-human transmission (Corbel, 2006). The highest human incidence of brucellosis in the country of Georgia is in the eastern region of Kakheti (Navdarashvili et al., 2005), which is also home to the majority of the country's sheep and a significant portion of the country's cattle population (Kvinikadze et al., 2009). In humans, brucellosis is acquired from animals either through direct contact with infected and shedding animals or their afterbirth or via consumption of contaminated dairy products made from the raw milk of a shedding animal. In Georgia, *B. melitensis* is the predominant species cultured from ill humans and has been cultured from sheep as well (Malania et al., 2009; Onashvili et al., 2009). It is likely that this *Brucella* spp. is also present in the cattle population.

The overall aim of this project was to conduct a systemic analysis of the ecology and cause of brucellosis in the Kakheti region of Georgia so as to be able to provide recommendations for disease control. A systemic analysis is an all-encompassing look at

a situation in order to understand its medical, political, economic, social, environmental and cultural aspects. This project studied the risk factors of brucellosis in Georgia and the dairy production and animal management systems associated with the human-animal interaction and how they differ based on the area of Kakheti and ethnic group. The human-animal interface data and the risk factors as well as available population level data from Georgia were used to create an agent-based model. This model simulated the impact of animal level disease interventions on the flock and herd prevalence and human brucellosis incidence.

In the spring of 2010, a rapid assessment of Georgian animal management and pasturing practices as well as the dairy production and distribution practices was done in order to understand the human-animal interface. This study identified the distinction between male and female roles; the use of sheep and cattle; the management of sheep and cattle including any seasonal trends; the pasturing practices; dairy production methodologies and product distribution; and the identification of ethnic group differences in the use and management of the livestock and their associated dairy products.

In order to identify the risk factors associated with brucellosis in the human population a non-matched, hospital-based case-control study was done using incident cases and controls from the Institute of Parasitology and Infectious Diseases in Tbilisi, Georgia in 2010. Findings indicated at a significance level of 10% that sheep ownership (OR: 19.3; 95% CI: 4, 94), living in Kakheti (OR: 278.1; 95% CI: 5, 15454), being older than 44 years of age (OR: 9.3; 95% CI: 0.7, 129) and making dairy products from cow milk (OR: 12.4; 95% CI: 1, 173) were all high-risk groups. The potential reason that the age group of 44 years of age and older had a greater odds of disease as compared to

students and young children, the referent, could be due to a larger role in animal care, or a potential bias from the control group, which had more young people in comparison to the cases. All types of occupations – animal and non-animal related – had increased odds of disease due to the fact that, irrespective of the type of work, individuals cared for animals at the home. The sample population was hospital-based from a highly centralized health care system. Diagnosis for cases and controls required travel to Tbilisi, the capitol city, and thus external validity may be questionable. Also, different strains of *Brucella* spp. have different levels of virulence in human hosts and different levels of dose based on the method of exposure. Therefore, *B. melitensis* and exposure from direct contact may be over-represented among the population as compared to the lesser virulent *B. abortus* and methods of exposure which have a lower dose associated with them, such as from contaminated dairy product consumption.

Finally the agent-based model (ABM) was built to evaluate the impact of animal level interventions on herd and flock prevalence and human disease incidence. It further analyzed the disease impact among shepherds, herders, cow and sheep milkers and cow milk dairy producers. A sheep milker also makes the dairy products and thus incorporates that risk. An agent-based model was used because patterns of human-livestock interactions in the Kakheti region elucidated by rapid assessment could be simulated using ABM whereas without regional or national human-livestock effective contact rates and prevalence statistics, population-based modeling was not possible. The results indicated that the lower the proportion of individuals involved in agriculture, the less impact animal-based interventions had on the human incidence rate; that at least five

years were needed to control the disease (bring the animal prevalence to <2%); and that disease in cattle had the greatest influence on human disease incidence.

This dissertation project provided an in-depth encompassing look at the transmission of brucellosis between livestock and humans in the Kakheti region of Georgia. Further, it provides a broader understanding of the disease ecology in this region due to the fact that it incorporates a study to understand the complexity of the human-livestock interface that is the source of disease transmission from livestock to humans.

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Chapter 1: Introduction and Research Objectives

The microbe is nothing, the terrain everything.

- Louis Pasteur

Brucellosis in the country of Georgia

Brucellosis in Georgia is both a human and animal disease. Currently, human disease data is passively collected. Individuals who are ill with symptoms consistent with a brucellosis infection are referred to the Institute of Parasitology and Tropical Medicine in Tbilisi, Georgia, the capitol city. Once there, they are diagnosed, treated and counted as a case in the national surveillance system. This highly centralized health care limits surveillance because individuals need to have the time and resources to make the trip to the capitol.

Nonetheless, brucellosis is a common event in Georgia. The World Organization for Animal Health's (OIE) World Animal Health Information Database (WAHID) reveals that in 2010 198 human cases were reported (4.2 per 100,000) and brucellosis was ranked first in incidence amongst all zoonotic disease. In 2009 brucellosis was the second most common zoonosis with 162 cases overall and 3.5 per 100,000 individuals, and leishmaniasis was the first most common with 170 cases overall. This was an increase from the 2007 incidence when brucellosis was the country's fifth most important disease where the overall incidence was 3.3 per 100,000 per year or 152 cases per year (World Animal Health Information Database 1.4, 2011b). Another global summary of

brucellosis puts Georgia's human disease incidence at 2.8 cases per 100,000 persons (Pappas et al., 2006). Globally this disease is woefully under-reported because of its vague clinical symptoms, difficult laboratory diagnosis and lack of familiarity by the medical professionals (Corbel, 2006). Therefore, it is likely that the disease is under-reported in Georgia as well based on global under-reporting, passive surveillance and the need to travel to the Tbilisi for disease diagnosis and treatment.

Despite the surveillance limitations, the incidence data from the Georgian National Center for Disease Control and Public Health highlights the uneven distribution of human brucellosis in Georgia. A vast majority of human disease occurs in the eastern and southeastern-most province of Kakheti and Kvemo Kartli (Figure 1.1) (Navdarashvili et al., 2005)



Figure 1.1: The administrative regions of Georgia (The Parliament of Georgia)

The very presence of disease in the human population speaks to the presence of disease in the animal population. Brucellosis is transmitted from animals to people and

rarely if ever from person-to-person. Current testing standards in Georgia require public veterinarians to test 40,000 head of cattle nation-wide, but they are not required to test sheep. This 40,000 cattle sample is a convenience or purposive sample, but it is not a random sample (personal communication, Brucellosis Coordinators, Georgian Ministry of Agriculture, unpublished data). Tests are done at the farmer's request or in areas where disease has been previously recognized. A Rose Bengal Agglutination test (RBT) is used which has a high sensitivity and low specificity (Nielson, 2002; World Organization for Animal Health, 2008a).

In 2010, 111 outbreaks of brucellosis were reported to the OIE WAHID by the country of Georgia as being caused by *B. abortus*. These outbreaks occurred across the country, but of those reported by regions the majority occurred in Kakheti, 15 of the 64 outbreaks (Figure 1.1) (World Animal Health Information Database 1.4, 2011a). The infections are classified as *B. abortus*, but a limited number of cultures and typing have been done in the country, so this classification is not based on laboratory typing. Data from the Gurjaani Ministry of Agriculture laboratory reports a sero-positive prevalence amongst tested cattle at 5.6% of the 8,971 cattle tested from Kakheti in 2009 (personal communication, Gurjaani Ministry of Agriculture Laboratory Director, unpublished data). Although cattle are the primary reservoir for *B. abortus* they can also be infected by *B. melitensis*. According to the Georgian regulations all positive animals were slaughtered under the supervision of the Ministry of Agriculture.

As for testing in sheep, a negative brucellosis RBT is required prior to export. Since there is a growing export market from Georgian livestock to southwest Asian-Arabic countries there are a significant number of sheep being tested. However, testing is

not uniform and antiquated Soviet standards or the cattle RBT method are used and not the modified RBT used for small ruminants as recommended by the World Organization for Animal Health (World Organization for Animal Health, 2008b). For these reasons, sheep disease prevalence and distribution is inadequately understood and likely underestimated. Of the 20,525 sheep tested in 2009, only 0.2% were positive, and although a RBT test was used it was conducted incorrectly. The majority of these tested sheep were for export. It should be noted that, animal cultures from small ruminants have returned *B. melitensis*, proving its presence in the country (personal communication, Marine Ramishvili, National Center for Disease Control, unpublished data) (Onashvili et al., 2009). Overall the livestock disease prevalence is not reliably determined, so all that can be said is that brucellosis is present in the livestock in Georgia.

Characteristics of the country of Georgia

Like all countries, modern day Georgia is defined by its history. Georgian history is a repetitive story of repetitive conquest and survival. Ionian Greeks conquered the area, followed by the Romans, Persians, Arabs and then the Seljuk Turks (Hewitt, 1995). In the 11th and 12th centuries a period of enlightenment characterized the region under the Georgian leadership of Tsar David the Builder and Princess Tamara. This ended abruptly when the Mongols invaded, followed by the Persians and the Ottoman Turks (Central Intelligence Agency, 2011). Finally, the Persians, Turks and Russians fought over the area until Russia cemented its claim to Georgia in the late 1800s (Hewitt, 1995). The Russian stronghold on Georgia effectively lasted through most of 20th century. Communist rule made up the last 75 years of this time period. Despite the homogenizing aims of the final political regime in Georgia, the result of this dynamic history is a

heterogenous population with numerous ethnic groups co-existing under one independent political state.

The Post-Soviet Georgian State

Georgia is a former Soviet state and is still adjusting to democratic independence 20 years after the collapse of the Soviet Union. This is a country still in transition: eastern borders with Azerbaijan are internationally recognized, but locally vague; the republics of Ossetia and Abkhazia desire independent status; and there is instability and strife in solidifying this country into one nation. The result is growing pains that have brought about myriad of troubles.

In current day Georgia there is an eclectic population of eighteen different ethnic groups. In the south there are the Ajars, Mingrelians and Laz with the Ajars being the Muslim component of this group. The Meskhetians once lived in the south, but were deported to Central Asia by Joseph Stalin in 1944. There are also small populations of Armenians, Greeks and Azerbaijanis that are concentrated in south central, southeast and eastern Georgia. Central Georgia is primarily made up of Georgians formed from earlier cultures of Gurians, Imeretians, Kartalians and Kakhetians. The highlands are home to the Khevsurs, Pshavs and Tushetis. Lastly, the Batsby or Kists are also highlanders who do not speak Georgian and are Muslim. The Udis, a Christian group previously of Dagestan, have relocated to a village in eastern Georgia to flee religious persecution in Azerbaijan. The Svans also live in the west and are only distantly related to Georgians. The South Ossetians and Abkhaz make up the autonomous republics within Georgia's borders. These two republics cause the most military disputes due to their desire to be recognized as their own countries—in conflict with the desire of the Georgian

government. Lastly, there are also groups of Assyrians, Ukrainians, Greeks and Jews that remain in Georgia to this day (Colarusso, 1997).

Religious identities are also important and tolerated in Georgia. Georgia was the second country, behind Armenia to adopt Christianity as the state religion in the 3rd century AD (Pelkmans, 2006). Yet today, in the city of Tbilisi there is a Jewish synagogue, an Islamic mosque and a Christian church within a few city blocks from one another. Although the majority of Georgians are Orthodox Georgian Christians, religion is not a significant source of strife in the country. Tolerance is a value amongst the Georgian people, and this has developed because of and helped them live through different conquests that occurred throughout their history (Pelkmans, 2006).

Feasting, toasting and drinking are important social customs in Georgia. Vodka and wine are the alcoholic beverages of the region and are expected at any feast, especially when hosting a guest. Drinking is almost always accompanied with numerous toasts about country, family, ancestors or any topic. The toastmaster leads the drinking and the speeches and is called the Tamada. Drink is a center point of Georgian culture. In business, to have a client, colleague or any other guest without alcohol demeans one's position. For men, it questions their ability to provide and to be a man (Pelkmans, 2006).

Despite 20 years passing since the collapse of the Soviet Union, the government still struggles to identify itself as a tool and workhorse for the people of Georgia. The unparalleled economic collapse characterized by a 78% drop in the gross domestic profit coincided with the establishment of Georgia in the 1990s and left little income on which people relied to meet their basic needs. As a result of this economic crisis and government mistrust, the government was unsuccessful in collecting taxes throughout the

1990s. This resulted in a lack of resources to create effective infrastructure. Despite the Rose Revolution in 2004, which was in response to falsified parliamentary elections and brought into power through public election the new and still standing president, Mikheil Saakashvili, the Georgian government still struggles to meet its populations' needs (Gotsadze et al., 2005; The Administration of the President of Georgia, 2010).

The Georgian healthcare system best illustrates the impact of the financially weak government. The Georgian health care system policy was meant to provide a large component of public services to serve the poorer sectors of society via district health care systems. Yet, the district doctors abandoned their public jobs for private ones and pharmaceutical sales were unregulated and cost-prohibitive for most. The result was that only 32.5% of those ill sought medical services and 67.5% self-treat, with only 62.8% finishing treatment in either groups. Those most often refusing treatment were the middle-aged of the poorest quintile, as cost of medical services and drugs took a larger percentage of their income than it did the rich. The current goal is to reverse this trend by enforcing tax laws and rebuilding the public sector (Gotsadze et al., 2005).

This scenario is repeated throughout Georgian social services. According to current development workers in the country, the Georgian government is eager to improve and is open to ideas. The ministers are young as President Mikheil Saakashvili refuses to hire anyone over the age of 40, for fear that the old Communist tendencies would return to public office. The result is an eager, more tolerant and open leadership that faces many challenges willingly (personal communication with Dr. M.D. Salman).

Finally, it is important to note the role of women in Georgia. Women enjoy a position of respect within the familial structure in this culture. Marriage results in a

patrilocal living situation where the new couple settles in the husband's community, but women have a great deal of control over the development of relationships. They are expected to defend themselves when necessary and are in charge of the home. Women are not to be ignored or their opinions taken lightly. Nonetheless, there are gender specific roles in this society. Men are often the primary fighters, hunters and livestock shepherds, while women tend gardens, maintain the home and prepare the food (Colarusso, 1997).

Agriculture in Georgia

Agriculture is a critical component of Georgian livelihood. Viticulture, tea, barley, wheat, citrus, nuts and other grains are grown throughout much of the region. Many farmers grow crops and still maintain a few head of livestock as well (Vulnerability Analysis and Mapping Unit, 2004; Corbel, 2006). Thirteen percent of the gross domestic profit is from agriculture and 55% of the labor force works within that sector (Central Intelligence Agency, 2011).

A history of livestock agriculture is also strong. Livestock provide meat, milk, labor, income and clothing, especially to the highlanders. The United Nations Environment Program distribution maps of cattle and sheep show the highest densities in the plains to the east of the Black Sea and along the southern borders. According to the 2009 Agricultural Statistical Yearbook, Georgia has 1,104,700 head of cattle and 602,300 head of sheep (Figures 1.2 & 1.3) (Kvinikadze et al., 2009). For an area slightly smaller than the state of South Carolina, this is a substantial number of animals.

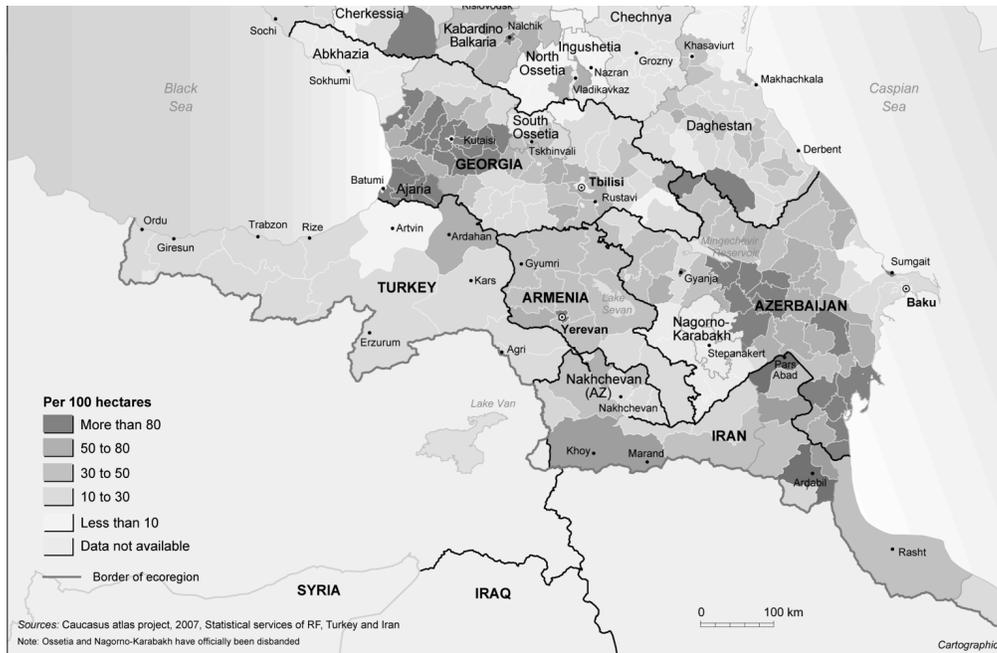


Figure 1.2: Distribution of cattle in the Caucasus (United Nations Environment Programme, 2008)

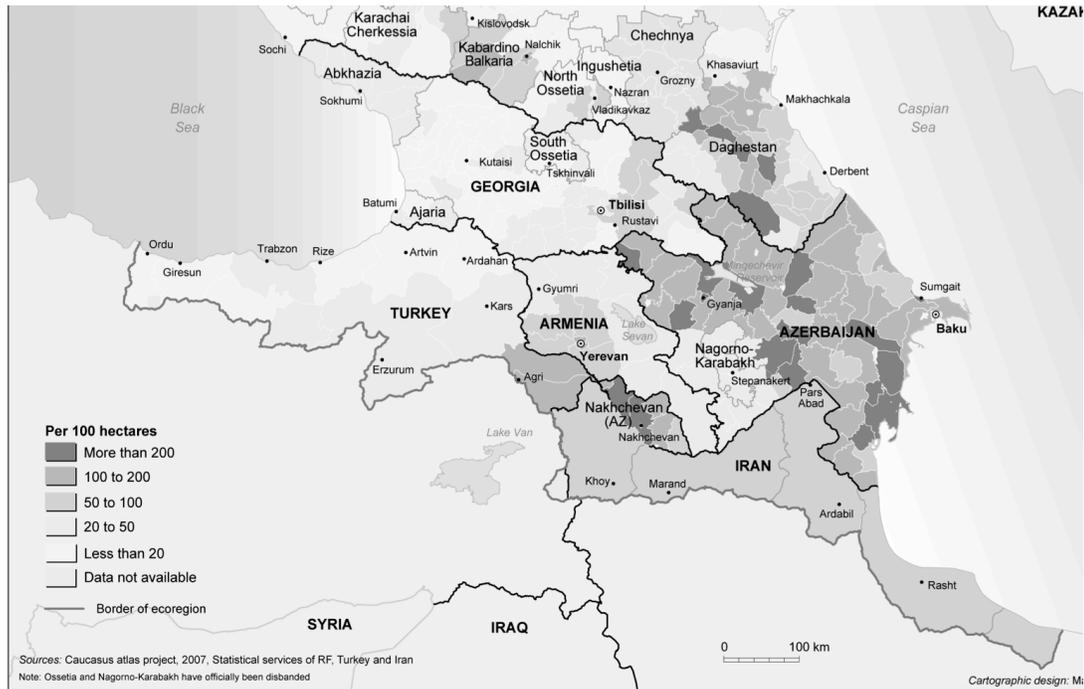


Figure 1.3: Distribution of sheep and goats in the Caucasus (United Nations Environment Programme, 2008)

Historically Georgia is an agrarian society with a reliance on livestock for survival and agriculture employment is still one of the most common forms of making a

living even today. Grazing is the primary method of providing food for these animals. The highlands were the grazing area in the spring and the lowlands or peri-town regions were the pastures during the winter months when snows and cold pushed the people and livestock out of the highlands. Other than this broad generalization, up to this point little has been documented on when these migrations occur and specifically what groups were shepherds.

There are other industries in Georgia as well. There are manganese, iron and copper deposits as well as some minor oil and coal deposits. There are small advances being made annually in metallurgy, machinery, aircraft and chemicals as well as in banking services and construction. This allows for a versatile and diversified industry within the country (Central Intelligence Agency, 2011).

Despite these resources, poverty levels are greater than 30% and unemployment of 13.6% and 16.4% in 2006 and 2009 respectively are noted (Central Intelligence Agency, 2011). The World Food Program describes the degrees of rural wealth as follows: "Wealth among the rural population of Georgia is a function of land and livestock holdings, household labor capacity and access to secondary cash opportunities, i.e., diversified income sources." In other words, some households have additional income from non-agricultural ventures. It still remains that the poor receive only 85% of the Food and Agricultural Organization's (FAO) recommended caloric intake of 2100 kilocalories and the extreme poor less than 75% (Vulnerability Analysis and Mapping Unit, 2004). The International Food Policy Research Institute rates hunger in Georgia in 2010 as moderate based on the Global Hunger Index (Fan et al., 2010).

Millennium Development Goals and Current Standards

An effective way to assess Georgia's economy and current state of poverty is through achievement or progress towards the Millennium Development Goals (MDG) in the Caucasus and Central Asia. Using the United Nations Development Program (UNDP) standards the Georgian situation can be characterized. Poverty is an income of less than 1.25 United States dollars (USD) a day. According to the UNDP International Human Development Indicators, in 2005, 13.4% of Georgia's population lived underneath the poverty line (United Nations Development Programme, 2011). In addition, an overview from 2007 further describes the many steps Georgia still has to take. In 2003, completion of primary education was at 87.4% and secondary education was at 27.2% in 2003, below current standards. As of 2002, 17% and 24% of the population did not have access to improved sanitation and improved drinking water respectively, which remain major sources of disease (Stryck, 2007).

Georgia is a diverse and developing country. It has overcome centuries of invasions and disruptions to become the independent state seen today. The populace is highly dependent upon agriculture as a way of life and income. The country struggles to meet the medical and physical needs of both its people and its livestock. One of these problems is the bacterial disease brucellosis.

Research Objectives and Scope

Brucellosis is a neglected disease of poverty that can greatly contribute to a population's disability adjusted life years (DALYs). Neglected diseases of poverty are endemic diseases that persist in livestock reliant or poor agrarian societies that lead to a decrease in livestock production or disease in the human population, both of which

exacerbate the economic and health situation in these marginalized communities (World Health Organization, 2006). Disability-adjusted life years are the years of productive life lost due to an infection with a disease. It has been estimated that brucellosis infections in can last for 3.11 years on average and a country can save \$19 per DALY averted through a livestock disease control program (Roth et al., 2003). Brucellosis exists in an endemic state in the Caucasus region and has gone relatively unchecked since the collapse of the Soviet Union in 1991. The country of Georgia is highly agrarian, with a majority of the rural population procuring food locally or making it themselves. Livestock ownership is also common for most households and a measure of wealth, allowing a large human-animal interface throughout the country.

The Kakheti region has the highest incidence rate of brucellosis by far in the country (218 per 100,000 in 2008), and has the most sheep (269,400) and a significant number of cattle (82,800; 6th amongst the 11 regions in Georgia) (Kvinikadze et al., 2009; Navdarashvili et al., 2005). Currently, successful human brucellosis cultures have yielded *B. melitensis* as the cause of infection in all of the cases but one, which was *B. abortus* (Malania et al., 2009; Onashvili et al., 2009). *Brucella melitensis* is normally found in sheep and goats, but it can also infect cattle as incidental hosts. Cattle can also shed *B. melitensis* in their vaginal discharges, afterbirth and milk (Coetzer and Tustin, 2004; Radostits et al., 2007). So within Kakheti, the agent of interest, *B. melitensis*, and its reservoirs are commonly found.

The scope of the project for this research is sheep and cattle populations, animal workers and owners and associated dairy products in the Kakheti region. Since Kakheti is the region of Georgia most impacted by brucellosis it was the appropriate area to learn

about animal management and dairy production here since practices may vary between regions. If disease is controlled in Kakheti, it could more than halve the incidence of disease in the country. Nonetheless, Kakheti is an ethnically and geographically diverse area and requires looking beyond the purely biological background of brucellosis in livestock and people. It is necessary to look at the whole system and to understand the economic, social and political drivers that impact brucellosis transmission among animals and from animal to humans. Therefore, the purpose of this study is to understand the differences and similarities of the following concepts among ethnic groups and municipalities in Kakheti:

- The disease ecology of brucellosis
- The human-animal interface
- The dairy production practices and product distribution
- The animal management, movement and pasturing practices
- The topics that required further education
- The interventions that could be successful or could be rejected by the local community

The format of this dissertation is based upon requirements set forth by the Colorado State University's Graduate School. All citations were formatted after the style used by *Preventive Veterinary Medicine*.

Chapter 2: Brucellosis

If you become an expert in brucellosis you will always have a job.

-Mo D. Salman

(Paraphrased from an advisory meeting in October 2008)

Bacteriological characteristics and taxonomy

Brucella species are gram-negative, facultative intracellular cocco-bacilli.

Controversy exists about whether there is one species, *B. melitensis*, with six biovars or six species (Meyer, 1990; Moreno et al., 2002; Ficht, 2010). The current scientific community refers to six separate species, and this paper will do so as well. The non-zoonotic species are *B. ovis* (sheep and goats) and *B. neotomae* (desert wood rats), and the zoonotic species are *B. melitensis* (sheep and goats), *B. abortus* (cattle), *B. suis* (swine, reindeers and rodents), and *B. canis* (canines). *B. canis* can cause human infection, but rarely causes clinical signs and symptoms of illness. It is not considered a significant public health risk (Corbel, 2006; Ficht, 2010). In addition, disease in marine mammals has resulted in the proposition of new species called *B. maris*. Yet, phylogenetic differences have further led to dividing *B. maris* into *B. pinnipediae* (seals and otters) and *B. cetaceae* (porpoise and whale) (Moreno et al., 2002; Corbel, 2006; Mantur et al., 2006).

There are key morphologic differences between species of brucellosis. The most virulent zoonotic species, *B. melitensis*, *B. abortus* and *B. suis*, all have a smooth lipopolysaccharide (S-LPS) on their outer cell membrane. The S-LPS contains an *O*-

polysaccharide (OPS) that is chemically defined as a homopolymer of 4,6-dideoxy-4-formamide- α -D-mannose, linked via 1,2-glycosidic linkages (Nielson, 2002). *B. ovis* and *B. canis* lack the OPS on their LPS and have a rough-lipopolysaccharide (R-LPS) on their outer cell membrane rather than the S-LPS. The lack of the OPS makes the R-LPS more immunogenic when in the host. It is believed that the S-LPS is able to evade innate immunity and to be a less potent inducer of inflammatory cytokines. These activities protect the bacterium against the initial immune response and enhance its ability to survive in the host. Once inside a phagocytic cell, the S-LPS species are able to prevent the infected cell from antigen presentation to T helper cells via the major histocompatibility complex II (MHC II). The S-LPS enables the bacteria to hinder apoptosis by the infected cell as well. These evasion techniques add to the S-LPS *Brucella* spp. virulence in comparison to the R-LPS strains. The latter are unable to inhibit the host immune response and are greatly impacted by the innate immune system, and are thus prevented from having a more severe effect on the host (Corbel, 2006; Franco et al., 2007).

Another key difference between species is DNA cleavage by phages specific to *Brucella* spp. There are forty known phages that can cause full lysis or lysis of the genome of one or more *Brucella* spp. The phages used for species typing are: Tbilisi (Tb), Weybridge (Wb), Izatnagar₁ (Iz₁) and R/C. The latter causes lysis in species with R-LPS, *B. ovis* and *B. canis*, and the former three phages differentiate the species with S-LPS, *B. melitensis*, *B. abortus* and *B. suis*. *B. melitensis* is susceptible to lysis with the Izatnagar₁ and has variable lysis with Weybridge; *B. abortus* is susceptible to lysis by all and *B. suis* biovar three is susceptible to partial lysis or full lysis by Weybridge and

Izatnagar¹. (Garin-Bastuji et al., 1998; Scientific Committee on Animal Health and Animal Welfare, 2001; Corbel, 2006; World Organization for Animal Health, 2008a).

In addition, there is not only variation among the *Brucella* spp., but there is variation within the species. *B. abortus* has seven distinct biovars, *B. melitensis* has three and *B. suis* has five. *B. ovis*, *B. canis* and *B. neotomae* have no biovars. The different biovars have different biochemical and growth features. They have different abilities to grow in the presence of carbon dioxide, to produce hydrogen sulfide, to agglutinate with mono-specific A and M antisera and to grow in the presence of 20 µg/ml of urease, thionin and basic fuchsin. It is via these biochemical characteristics that laboratories use to differentiate the biovars (Meyer, 1990; Moreno et al., 2002; Corbel, 2006).

Epidemiology and pathophysiology

The person, place and the time as well as the host, agent and environmental interactions of brucellosis amongst livestock, wildlife and humans are variable across the globe based on a myriad of factors. Evidence of a risk factor and a successful intervention in one location does not mean that the same risk factor exists or intervention will be successful if applied to another. This section will examine the pathogenesis of infection, the methods of spread among livestock and the methods of zoonotic spread from livestock to humans.

Disease spread among livestock

Endemic brucellosis in livestock is maintained via ingestion of contaminated food or grasses, inhalation of aerosolized bacteria in crowded and dry conditions, direct contact of the organism with broken skin or mucous membranes from the contaminated environment or from a shedding animal, venereal transmission and latent infection of

neonates (Corbel, 2006). In the adult populations, naïvely infected pregnant animals often abort or give birth to weak offspring. The aborted tissues, fetus, fetal fluids and placenta are laden with bacteria that contaminate the environment (Garin-Bastuji et al., 1998; Corbel, 2006; Olsen and Tatum, 2010). Infected animals shed the bacteria in vaginal discharges and milk, further contaminating the environment and posing a disease threat to suckling young and other livestock. Infected males can shed the bacteria in their semen as well. In *B. canis* and *B. ovis* the venereal transmission route is an important factor in the maintenance of disease (Blasco, 1990; Carmichael, 1990; Greene and Carmichael, 2006), but in *B. abortus* artificial insemination plays a larger role (Olsen and Tatum, 2010). Finally, cattle can give birth to latently infected calves that show no serological evidence of disease, but these latently infected calves will shed the bacteria after their first parturition (Crawford et al., 1990; Olsen and Tatum, 2010). A small percentage of ewes do the same, but latent infections are believed to be associated with disease transmission via suckling rather than in utero infection (Alton, 1990a; Garin-Bastuji et al., 1998).

There is individual animal as well as herd and flock level factors that promote disease spread. Sexual maturity of the animal has a role in disease transmission in that animals that are not sexually mature are less likely to be infected. Pregnant animals are most susceptible to brucellosis infection. The method of spread among animals differs based on management practices. Pastoral management of animals leads to infection via ingestion of the organism. *Brucella* spp. survive in cool and damp environments but not sunlight, dry weather and high temperatures. It is important to note that *Brucella* spp. do not multiply outside the host, but survive in the environment. In housed animals,

conjunctival exposure is the most important route, and this route has a smaller infectious dose than the dose needed for livestock maintained on the pasture. No matter the route of infection the amount of *Brucella* spp. excreted into the environment plays an important role in transmission. The use of maternity pens to isolate animals during and post-parturition is critical because these animals shed the most bacteria. Isolation of post-parturient animals reduces the spread of infection to the rest of the herd or flock. Vaccination can also reduce the number of animals shedding the organism. Finally, the initial introduction of disease into a herd or flock is often due to replacement animals coming from an infected flock or flock of unknown disease status, shared pastures or fence line contact, all of which spread disease to uninfected flocks and herds (Crawford et al., 1990).

Disease spread from livestock to humans

Brucellosis in humans is considered a food borne disease or a disease related to occupational exposures. The routes of infection for humans are similar to those for animals: ingestion, inhalation, or through direct contact of the organism with a break in the skin. The key feature of brucellosis as a zoonosis is that it is a pure zoonosis: a disease transferred only from animals to people. Human-to-human transmission has occurred, but is exceedingly rare.

The *Brucella* spp. and their biovars have different zoonotic potential. *B. ovis* and *B. suis* biovars two, four and five have essentially no zoonotic potential. *B. canis*, *B. abortus* biovar five and *B. neotomae* have very low zoonotic risk, but all other biovars of *B. melitensis*, *B. abortus* and *B. suis* can cause illness in humans (Moreno et al., 2002; Corbel, 2006).

Food borne illness is contracted through the consumption of raw milk or raw milk dairy products. Meat products are not considered high risk and the actual risk is likely negligible (International Commission of Microbiological Specifications for Foods, 1996). The one exception is with pork meat products. Bacteremia in swine results in disseminated infection rather than an infection localized to the reticuloendothelial system and reproductive tract; as a result there can be a substantial number of bacteria in the muscle tissue (Alton, 1990b). In addition, the consumption of organ meats poses a risk (Corbel, 2006).

Dairy products can vary in their bacterial load at the time of consumption. The rate of bacterial death is based on the pH, salinity, water content, temperature and fat content of a product. *Brucella* spp. die off at higher storage temperatures; refrigeration protects *Brucella* organisms. At even higher temperatures the survival of *Brucella* spp. is even more threatened. A log reduction of bacteria in a milk sample occurs with a time-temperature treatment of 65.6 C (150 F) for 0.1 to 0.2 minutes. *Brucella* dies off when the pH \leq 4 (International Commission of Microbiological Specifications for Foods, 1996; Corbel, 2006). At a pH range of 5-6, *Brucella* spp. do not grow, but they also do not die. Water content also plays a role in bacterial load. The lower the water content, the shorter the survival time. *Brucella* spp. are killed in as quickly as six days in hard cheeses but take at least 57 days in soft cheeses. Fat content diminishes the bactericidal effect of increasing the temperature from refrigeration temperatures to room temperature. This suggests, along with the lasting survival of organisms in butter (International Commission of Microbiological Specifications for Foods, 1996; Corbel, 2006), that a higher fat content can be protective. The salt concentration of a product also has an

effect on the survival of the bacteria. In butter with a 2.3% salt concentration, *Brucella* spp. survives for only six months, in comparison to the 13 months it could survive in unsalted butter. *Brucella* spp. can survive for 45 days at temperatures between 11 C (52 F) and 14 C (57 F) when stored in 27% brine. Ideally, pasteurization of the raw milk would be performed prior to dairy product production. This is an effective control step to reduce contamination, but if not done, then raw milk dairy products remain a viable source of infection (International Commission of Microbiological Specifications for Foods, 1996).

Pathogenesis in Livestock and Humans

The pathogenesis of brucellosis is similar among livestock species and humans. First, the bacteria invade the mucosa or break in the skin. This is typically the oral mucosa, as ingestion is the primary method of horizontal spread of disease. *Brucella* organisms are ingested by phagocytes in the submucosa during the inflammatory response (Enright, 1990). Once ingested, some of the *Brucella* organisms are able to evade or hinder the phagolysosomal action of the neutrophils and macrophages by redirecting the intracellular trafficking of the phagolysosomal action (Dornand et al., 2002; Gorvel and Moreno, 2002; Franco et al., 2007). The virulence factor VirB is thought to play a significant role in these intracellular survival events. VirB is a secretory pump that selectively pumps proteins and macromolecules across membranes and is critical in pathogenesis and virulence of brucellosis infections. It helps ensure the survival of the bacteria in the phagolysosome (Franco et al., 2007). Typically, the phagolysosome destroys the engulfed and ingested bacteria. *Brucella* spp. evasion of the host's innate immune system's bactericidal effects is most frequently seen in the

neutrophil, but 15% to 30% of infected macrophages have bacteria that successfully redirect the cellular processes and prevent phagolysosomal action. If the organisms survive, then they are able to replicate within the phagocyte without disrupting cellular metabolism or causing cell lysis. Intracellular bacteria also inhibit host cell apoptosis (Dornand et al., 2002; Gorvel and Moreno, 2002; Franco et al., 2007). This intracellular replication is only seen with smooth strains of *Brucella* spp.—*B. melitensis*, *B. suis*, and *B. abortus* (Dornand et al., 2002). Finally, these infected macrophages are localized in the regional lymph nodes. The most infected lymph node is typically the most proximal lymph node to the site of inoculation and these lymph nodes are enlarged due to hyperplasia and infiltration by inflammatory cells (Enright, 1990). All of these actions occur during the incubation period, which is highly variable, ranging from two weeks to seven months. The method of escape from the phagocytic cells is yet undiscovered but overall ends in cell lysis (Gorvel and Moreno, 2002).

The persistence of the bacteria in the phagocytic cells allows for bacterial replication in these cells. Replication leads to release of the bacteria from the cells, thus resulting in a bacteremic phase (Enright, 1990; Ragan, 2002). The bacteremia allows for colonization of the bacteria in multiple tissues, but in livestock the bacteria most frequently colonize in the lymphoid tissues, mammary gland and reproductive tract (Enright, 1990; Ragan, 2002). In swine, the bacteremic phase can lead to disseminated colonization of the bacteria throughout the body (Alton, 1990b). In humans, bacteremia results in clinical disease symptoms and colonization of multiple tissues as well (Franco et al., 2007). Human bacteremia can impact any organ of the body and often results in clinical signs where symptoms from a specific organ predominate. A fever and

osteoarticular symptoms are the most common (Corbel, 2006; Franco et al., 2007). This is the point where the disease is “localized” (Corbel, 2006).

The localization of *Brucella* spp. in the reproductive tract leads to colonization of the chorionic trophoblast of the placenta in pregnant livestock. This affinity for the chorionic trophoblast is due to the presence of a steroid called erythritol, a substance present in allantoic fluids that stimulates the replication of *Brucella* spp. (Smith et al., 1962; Corbel, 2006). The stimulation of *Brucella* spp. seen in the presence of erythritol is due to the preferential use of erythritol by *Brucella* spp. as an energy and carbon source, even in the presence of glucose and other metabolites. The reason for the preferential use of erythritol is due to its ease of uptake by the bacteria, as compared to glucose. This makes erythritol more readily available to the bacteria for energy consumption (Anderson and Smith, 1965). The resulting placentitis caused by replicating bacteria results in ulceration of the chorioallantoic membrane while sparing the endometrium of the uterus. The resulting pathology leads to late gestation abortions in naïvely infected livestock (Enright, 1990; Radostits et al., 2007). Erythritol is not present in the human uterus and abortions associated with human infections are normally seen in the first trimester (Corbel, 2006). The presence of erythritol in the testes of male species leads to a localization of the *Brucella* spp. in their reproductive tracts with a resulting epididymitis and orchitis (Enright, 1990).

The late term abortion is likely due to a variety of reproductive physiological pathways. The placentitis can result in impaired oxygen and nutrient delivery to the fetus causing fetal stress. The fetal stress results in rising fetal adrenal cortical production of cortisol. These increasing cortisol levels initiate the production of estrogen and PGF₂.

over progesterone by the endometrium prematurely. Specifically, it is the production of PGF₂, leads to the initiation of premature parturition (Enright, 1990)

It is important to note that the pathologic features of disease vary slightly between *Brucella* spp. The R-LPS species, *B. ovis* and *B. canis*, tend to have a greater impact on the male reproductive tract. The organism causes an epididymitis and orchitis that can result in sperm stasis and spermatocoele formation. These changes result in infertility. Of course, in pregnant animals, placentitis can still develop and late-stage abortion can occur (Greene and Carmichael, 2006; Radostits et al., 2007). Both *B. suis* and *B. canis* can have a more systemic spread of disease. *B. suis* results in localization of the bacteria in myriad of tissues including the uterus, udder, lymph nodes, bone marrow, and musculoskeletal system (Radostits et al., 2007; Alton, 1990b). *B. canis* is commonly found in intervertebral disks, the eye, the kidneys and in the meninges (Greene and Carmichael, 2006). *B. abortus* and *B. melitensis* are very similar in their pathogenesis, and more closely follow what is described above with an affinity for the reproductive tract and mammary tissues (Radostits et al., 2007).

Clinical Disease

Disease in livestock

There is a great deal of variability of disease seen among species. Reproductive issues are seen in all infections, but the severity and the sex-association of the most severe reproductive issues vary based on the *Brucella* spp. causing the infection. Most clinical disease manifests as fertility-related issues in cattle and small ruminants. Yet, in small ruminants, the less virulent *B. ovis* presents differently than *B. melitensis*.

Small ruminants are the reservoir for both *B. ovis* and *B. melitensis*. In *B. ovis* infections, which are primarily infections of sheep, the rams are more affected than the ewes. Rams develop orchitis and epididymitis due to infection and associated inflammation of the epididymes and testes. Semen quality and the viable spermatozoa in a sample of ejaculate decrease significantly. In ewes, a placentitis can occur that results in the birth of weak lambs, stillbirths or abortions (Blasco, 1990; Radostits et al., 2007).

As for infection with *B. melitensis*, the impact of disease is greater amongst the ewes than the rams. The most common sign of disease is late term abortion in naïvely infected pregnant animals. In endemic flocks, abortions are less common and most infected ewes give birth to weak offspring as sheep rarely abort twice (Olsen and Stoffregen, 2005). Retained placentas are commonly found among ewes in endemic flocks as well. Rams experience orchitis and epididymitis. In addition, animals with polyarthritis can also be seen in endemic flocks (Alton, 1990a; Garin-Bastuji et al., 1998; Corbel, 2006; Radostits et al., 2007).

Cattle infected with *B. abortus* and *B. melitensis* have the same pathogenesis of disease and, thus, the same clinical signs. Late term abortion or the birth of weak non-viable calves are both characteristics of disease in naïve animals but not in endemic herds. Abortion is less common with an infection caused by *B. melitensis* than in *B. abortus* infections (Alton, 1990a; Corbel, 2006; Olsen and Tatum, 2010). In fact, when *B. melitensis* is the causative agent of disease, cattle may not abort at all, but they will shed bacteria in their milk. When this occurs, human illness may be the only sign of disease in the cattle (Radostits et al., 2007). Similarly to sheep, infected cattle can

develop arthritis and hygromas as well (Corbel, 2006). Bulls develop orchitis, epididymitis and seminal vesiculitis (Crawford et al., 1990).

Brucellosis in swine is more insidious than in other species. Disease can be asymptomatic in swine herds. Yet, sows are prone to abortion when infected with *Brucella* spp., but abortion can occur at any point in the pregnancy. In addition, mastitis and associated abscesses can also develop. In boars, the seminal vesicles are most commonly infected, but the prostate and testes are also commonly infected. All swine are prone to synovitis, bursitis, tendonitis as well as bone, spleen and liver involvement associated with brucellosis (Alton, 1990b).

Dogs are similar to swine in that diverse clinical signs can develop. Bitches can abort between 45 and 60 days of gestation, and severe orchitis, epididymitis and infertility are common in sires (Carmichael, 1990; Greene and Carmichael, 2006). All infected canines can develop diskospondylitis, glomerulonephritis, anterior uveitis, meningitis and splenomegaly (Greene and Carmichael, 2006).

Other species that show clinical signs of disease include camels and horses. In horses a condition called Fistulous Withers develops as a result of infection with *B. abortus* (Crawford et al., 1990; Olsen and Tatum, 2010). Camels are also prone to infection from *B. abortus* and *B. melitensis* (Crawford et al., 1990; McDermott and Arimi, 2002; Refai, 2002; Corbel, 2006). Infections in camels can result in abortion in females and epididymitis and orchitis in males (Teshome et al., 2003).

Disease in humans

Humans are an incidental host of brucellosis, and the pathogenesis from initial infection to phagocytic cell uptake is identical to animal hosts. The lysis of the

phagocytic cells that releases the *Brucella* organisms also results in the release of cellular debris and pyrogenic endotoxins that cause an episode of fever. Since this cell lysis and release of *Brucella* organisms and pyrogenic endotoxins can occur repeatedly from different infected phagocytic cells, so can the fever—this is the source of the undulant fever seen in human infections (International Commission of Microbiological Specifications for Foods, 1996). The bacteremia that results leads to bacterial colonization in numerous sites of the body. Therefore, the acute disease symptoms are vague: malaise, joint pain, headache, inappetance, hepatomegaly, splenomegaly and fever (International Commission of Microbiological Specifications for Foods, 1996; Corbel, 2006; Mantur et al., 2006; Franco et al., 2007). In addition, pregnant women can abort including abortion during early trimesters (Corbel, 2006).

Chronic disease develops when acute disease is left untreated. Chronic infections of organs, especially the spleen and liver, result in the formation of granulomas around infected phagocytes and cells (International Commission of Microbiological Specifications for Foods, 1996; Franco et al., 2007). As a result, complications associated with granuloma formation are seen. These complications include endocarditis, osteoarticular disease, meningitis, hepatic dysfunction and impacts on any body system where granulomas are affecting function (Corbel, 2006; Mantur et al., 2006; Franco et al., 2007).

Diagnostic Testing

Serologic tests in livestock

Testing modalities for brucellosis include antigen and antibody detection as well as agent isolation through culture. These methods have different advantages and

disadvantages in the laboratory and in their use in disease control and eradication programs.

Serologic testing is critical to brucellosis disease control and eradication programs. Culture requires a bio-safety level three laboratory (World Organization for Animal Health, 2008a), which makes the ability to culture samples prohibitive. Instead, serologic screening and confirmatory tests are used to identify infected animals. The World Organization for Animal Health (OIE) prescribes the use of a buffered *Brucella* antigen test called the buffered plate antigen test and the Rose Bengal Test (RBT) as approved screening tests and the complement fixation test as the confirmatory test. For bovine brucellosis the prescribed test by the OIE are buffered brucellosis antigen tests, the complement fixation test, the indirect and competitive enzyme-linked immunosorbent assay (ELISA) and the fluorescence polarization assay. In sheep and goats the prescribed tests are the buffered brucellosis antigen test and the complement fixation tests. The brucellin skin test and the fluorescence polarization assay are considered alternative tests. Swine brucellosis uses the indirect ELISA as a prescribed test and the buffered brucellosis antigen test and fluorescence polarization assay as alternative tests (World Organization for Animal Health, 2008c).

The two most commonly used acidified antigen agglutination tests are the Rose Bengal Test (RBT) and the Buffered Plate Antigen Test (BPAT). The RBT and BPAT use acidified antigens to reduce the binding of IgM antibodies and to encourage the IgG₁ binding. Cross-reactions of the IgM antibodies can occur with *Yersinia enterocolitica* O:9 (World Organization for Animal Health, 2008a, b). The standard antigen used to test for *B. abortus*, *B. melitensis* and *B. suis* is from a laboratory *B. abortus* strain. This can be

done because the *O*-polysaccharide on the smooth-lipopolysaccharide (S-LPS) surface of *B. abortus*, *B. melitensis* and *B. suis* are antigenetically similar because their epitopes are similar. *B. ovis* and *B. canis* tests utilize antigens from each specific species (Nielson, 2002). More specifically, serologic tests use antigens from the laboratory *B. abortus* strains 1119-3 and 99 stained with a dye to make it visible (World Organization for Animal Health, 2008a). When the prepared antigen and serum with antibodies are mixed, an antigen-antibody complex is formed and the agglutination is visible due to the dyed-antigen. Thus agglutination indicates that the tested animal is positive for brucellosis. The buffered plate antigen test is more sensitive than the RBT (Nielson, 2002; Gall and Nielson, 2004; World Organization for Animal Health, 2008a, b), but requires more precise equipment, making it more difficult to do in the field.

There is a difference in the use of the RBT with sheep and goats as compared to cattle. With sheep and goats 75µl of serum and 25µl of reagent are used as compared to the 30µl of serum and reagent used in the bovine RBT. The adjustment is done in sheep and goats to increase the sensitivity, especially when used without a confirmatory test (World Organization for Animal Health, 2008a, b). Overall the buffered *Brucella* antigen tests have a higher sensitivity but also have a less reliable specificity; this results in a reduced number of false negatives and a significant number of false positive. Therefore a confirmatory test with higher specificity is needed (Nielson, 2002; World Organization for Animal Health, 2008b).

Indirect enzyme linked immunosorbent assays (iELISA) tests are also approved for use by the OIE as a brucellosis screening test because of its high sensitivity. Currently, it is a prescribed test in bovine brucellosis diagnosis and an alternative test for

swine brucellosis. The reason that it is not approved for international trade in small ruminant testing is due to a lack of *B. melitensis* reagent standardization and its inability to differentiate between antibodies produced in response to vaccination versus natural infection (World Organization for Animal Health, 2008b). The iELISA used in bovine brucellosis also suffers from cross-reactions and the inability to distinguish between vaccine antibody and natural infection antibody. Yet, it does have standardized antigens. The iELISA uses a standard *B. abortus* antigen (S-LPS), a serum sample and an anti-bovine IgG₁ antibody that has a horseradish peroxidase attached to it. If the anti-S-LPS antibody is in the serum sample, then the anti-bovine IgG₁ with an attached peroxidase causes a color change in the assay, which indicates a positive test (Nielson, 2002; Corbel, 2006; World Organization for Animal Health, 2008a). Finally, since swine brucellosis does not have an effective vaccine, the iELISA does not have to distinguish between vaccine-associated and natural infection antibodies. Therefore the iELISA is a useful diagnostic test in swine.

An alternative test for confirmation of *B. melitensis* in small ruminants is the brucellin skin test. This test evaluates the cell-mediated immunity of the tested animal against brucellosis. It has a high sensitivity and a higher specificity than agglutination tests since it does not cross-react with other bacteria (World Organization for Animal Health, 2008b). It is effective for use in non-vaccinated animals. A rough strain of *B. melitensis* (B115) is used to avoid the presence of an S-LPS antigen that would react with antibodies and create a local area of inflammation (World Organization for Animal Health, 2008a). The brucellin derived from the lysed B115 cells are injected

intradermally into the lower eyelid and read 48 hours later. A positive test is indicated by a thickening of greater than 2 mm (World Organization for Animal Health, 2008b).

A commonly used confirmatory test is the complement fixation test (CFT). This is the approved confirmatory test for international trade as prescribed by the OIE. The CFT uses the complement activation system's ability to lyse cells, and in this case erythrocytes. If the serum sample contains antibody against S-LPS it will bind the reagent S-LPS antigen on the *B. abortus* cells. This antibody-antigen complex then activates the available complement in the test reagent. So, when sheep red blood cells sensitized with rabbit anti-sheep antibodies are added to the mixture, the complement in the reagent has already been activated and the blood cells do not lyse. If the test serum did not have antibodies, the S-LPS antigen would remain unbound and the sensitized red blood cells would have activated the complement causing their lysis. So, a test result showing no red blood cell lysis is a positive test (World Organization for Animal Health, 2008a, b). The high sensitivity and specificity (Nielson et al., 2008) of this test makes it a very good confirmatory test, but it is intensive to run, requiring many reagents. None of the serologic tests are capable of distinguishing S19 and Rev 1 vaccination related antibodies present in cattle and small ruminants respectively from antibodies due to natural infection (Gall and Nielson, 2004). This is a considerable hindrance in control and eradication programs when the livestock's disease status is not known prior to vaccination and when they are not permanently marked to indicate them as a vaccinated animal.

The fluorescence polarization assay (FPA) helps address the issue of sero-conversion in vaccinated animals. The concept behind this test utilizes the physics

principle that the rate of rotation of an object is inversely proportional to the size of the object. The rate of rotation differs for antigen alone and for antigen bound to an antibody and this can differentiate between animals that have sero-converted and those that have not. A standardized antigen that is 22 kilo-Daltons in size and a component of the S-LPS, the *O*-polysaccharide is used. The FPA is highly sensitive and specific, is quick to run and can be done in the field (Nielson, 2002; Nielson et al., 2008). It is also capable of determining a field strain and vaccine strain positive (Gall and Nielson, 2004).

Another test that is able to distinguish between sero-conversion due to natural infection and vaccination by Rev 1 or S19 for small ruminants and cattle respectively is the competitive ELISA (cELISA). The cELISA uses the concept that vaccine-induced antibodies have a lesser affinity for the antigen than antibodies that arise from natural infection. Theoretically, this lesser affinity develops due to the shorter exposure time that the humoral immune system has to develop antibodies in a vaccination exposure as compared to a natural infection. The increased amount of time of pathogen exposure to the humoral immune system in a natural infection creates an antigen-antibody complex with a greater affinity (Nielson, 2002; Gall and Nielson, 2004). Thus when using a cELISA, the vaccine antibodies can be inhibited in binding by a competing antibody, but the natural antibodies cannot be inhibited. It is a highly sensitive and specific test as well (Nielson et al., 2008). The cELISA is a prescribed test for cattle trade and an alternative test for swine trade (Nielson, 2002; World Organization for Animal Health, 2008a; World Organization for Animal Health, 2009).

Serologic tests in humans

Human serologic diagnostics are very similar to animal diagnostics. These tests also utilize the S-LPS antigen to detect antibody in the patient. Recall that the R-LPS species, rarely cause zoonotic disease (*B. canis*) or are not zoonotic (*B. ovis*). Yet again, serologic diagnosis is hampered by cross-reactions with other gram negative bacteria, particularly *Yersinia enterocolitica* O:9, *Escherichia coli* O157, *Francisella tularensis*, *Salmonella urbana* O:30 and *Vibrio cholerae* (Corbel, 2006; Franco et al., 2007). In the absence of culture facilities, and because cultures can result in false negatives, serology is often used. Typically there is a screening and confirmatory test. Like in animals, the Rose Bengal test is often a screening test with a sensitivity > 99% (Corbel, 2006; Franco et al., 2007) in acute cases, but it lacks sensitivity in chronic and relapsing patients (Araj, 2010). There are multiple other agglutination tests that are also commonly used for screening along with the RBT and all of them require a confirmatory test (Corbel, 2006; Franco et al., 2007).

In addition to the RBT there are tube agglutination, serum agglutination and a Coomb's tests that are commonly used. Slide and tube agglutination tests (SAT and TAT) use a cut-off point in the titers to detect a positive case. This cut-point can be increased to increase specificity and decreased to increase sensitivity (Franco et al., 2007). The serum agglutination and tube agglutination tests use standardized S-LPS antigen to assess for antibody presence in the serum sample. If agglutination occurs, the serum contained antibodies to the S-LPS and the individual is test positive for brucellosis (Corbel, 2006; Franco et al., 2007). Agglutination occurs with IgG, IgM and IgA antibodies (Araj, 2010). Yet, in chronic or relapsing brucellosis low antibody titers are

common (Corbel, 2006; Franco et al., 2007) often resulting in low sensitivities with these tests (Araj, 1999). This makes setting a titer cut-point complicated. To decrease the cross-reactions of IgM antibodies of other gram-negative pathogens with the S-LPS, 2-mercaptoethanol can be added to the SAT or TAT. This is a phosphate buffer that inhibits IgM binding with other gram-negative bacteria (Corbel, 2006; Mantur et al., 2006; Franco et al., 2007). The complement fixation test can also be used, but it is a complex test that is demanding in terms of resources. It has a high sensitivity and specificity and where possible can be a very good confirmatory test (Corbel, 2006).

The indirect Coombs test is used as a confirmatory test of the serum agglutination tests when chronic or relapsing disease is suspected. In these situations the SAT and TAT have low sensitivities and yield a higher number of false negatives. There are often un-agglutinated S-LPS antibodies in the serum despite the presence of the antigen with S-LPS. This phenomenon is known as prozoning. The negative SAT or TAT dilutions are taken and the cells recovered via centrifugation and the pellet is then re-suspended. An anti-human IgG antibody is added to the suspension and agglutination with the human S-LPS antibodies is assessed (Araj, 2010). Another anti-globulin test is the Brucellacapt. This test is quicker than the Coomb's test, can test for IgA and still has the same specificity and sensitivity. It utilizes a well plate coated with anti-human IgG and anti-human IgA antibodies to which patient serum is added. After that a dye-tagged killed *B. melitensis* is added and the plate is incubated and read for human IgG that is also bound to *B. melitensis* (Araj, 1999).

An indirect fluorescing antibody is another test that can be used. A commercially available *B. abortus* or *B. melitensis* antigen is incubated with patient serum and a

fluorescent tagged anti-human IgG, IgM or IgA antibody. Positive patients will have a test slide that fluoresces. Results are similar to the ELISA in regards to sensitivity and specificity, but there is greater variation seen than with the ELISA based on the antigen source (Araj, 2010).

The indirect ELISA is sensitive and specific and a very useful test for multiple reasons. The iELISA has the capability to differentiate between IgM, IgA and IgG (Corbel, 2006; Araj, 2010). The other tests detect these antibodies, but they are not able to differentiate between them. Thus, it is possible to determine whether the infection is chronic (IgG) or acute (IgM). Further, the iELISA does not use whole cell antigens, but it uses cytosolic S-LPS fragments, thus decreasing the cross-reaction with other gram-negative bacteria that is seen with the other serologic tests. This test is excellent for use in chronic or relapsing patients and for sero-surveys (Araj, 1999; Corbel, 2006; Franco et al., 2007; Araj, 2010). Despite the benefits, the specificity can be questionable. Once again a cut-off point is determined based on local data and an increase in the cut-off point enhances specificity at the expense of sensitivity (Franco et al., 2007). This test is very useful, but must be used in light of that limitation. Other limitations are a lack of standardized reagents, subjective cut-off points and inadequate tests with which to run comparisons to determine iELISA's actual sensitivity and specificity (Corbel, 2006).

There are many options for diagnosis of brucellosis in human medicine. Typical combinations used are; the SAT or TAT with the Coomb's test, RBT with SAT using 2-ME and any agglutination followed by an ELISA (Corbel, 2006; Mantur et al., 2006). Based on the discussion above it is evident that in acute cases, the screening and confirmatory tests need to be positive—testing in series, and in chronic cases the opposite

is true due to lower sensitivities in the screening tests, both tests need to be negative to declare the patient negative—testing in parallel.

Culture of *Brucella* spp.

Culturing the organism remains the diagnostic gold standard and the only method to definitively state that an individual has been infected with a *Brucella* spp. However, the culture can be dangerous due to the zoonotic nature of the organism, and, therefore, requires specially equipped laboratories and training. The laboratories need to be bio-safety level three facilities. Further constraints are the time it takes to declare a culture negative and the high false negative rate associated with cultures. *Brucella* spp. are slow growing organisms and can take up to 45 days to grow in culture conditions (Corbel, 2006). In addition, they are intracellular pathogens, so once the bacteremia has subsided, the blood culture will be negative in infected patients. Cultures from acutely ill patients have sensitivity ranges from 40% to 90%, but in chronic cases the sensitivity is worse, ranging from 5% to 20% (Araj, 2010). In animals, it is recommended to culture milk, vaginal discharges, afterbirth and aborted materials including the aborted fetus rather than the blood. In humans, *Brucella* spp. can be cultured from purulent discharge, cerebrospinal fluid, bone marrow, tissue samples, ascites, pleural fluid and joint fluid in addition to blood (Corbel, 2006; Franco et al., 2007). In cases that have been treated with antibiotics, the bone marrow provides a result 15% to 20% more often than blood cultures (Franco et al., 2007; Araj, 2010). If blood is used, it is recommended to lyse the leukocyte fraction prior to attempting to culture in order to increase the likelihood of growth.

The recommended method is to use a biphasic Castañeda's media. This media contains both a liquid and solid fraction. This method of using a biphasic medium reduces laboratory handling and thus laboratory-acquired infections. In addition, the culture should be grown in an atmosphere of 5% carbon dioxide (Corbel, 2006). There are automatic culture systems (Bactec) that are replacing Castañeda's media that result in a faster rate of culture growth as well (Corbel, 2006). Using Castañeda's media, growth can be seen no earlier than the fourth day, but when using the automatic methods growth can be detected by the third day (Corbel, 2006). In addition, the Bactec culture can be declared negative after 14 days while it takes 45 days with traditional methods (Araj, 2010). The automated methods also have an increased overall sensitivity (Franco et al., 2007).

Using these blood cultures, species typing can occur as described under the bacteriologic characteristics section. A culture can definitely be used to diagnose the patient with brucellosis and also return the species and biovar that is infecting them. This information is useful for control strategies in the animal populations.

Polymerase Chain Reaction

To date, polymerase chain reactions (PCR) have been developed for the diagnosis of brucellosis in humans and animals, but they lack validation and improvement of specificity and sensitivity in comparison to other tests. Therefore, they have not become a recommended testing method by the World Organization for Animal Health or the World Health Organization.

PCR assays are able to identify the presence of *Brucella* spp. by identifying the presence of highly conserved gene loci, but PCRs targeting these gene loci cannot

differentiate between the species of *Brucella*. This loci is the location of the 16s rRNA (Bricker, 2002; Araj, 2010) genes and a gene called *BCSP31*. *BCSP31* encodes an antigenic and periplasmic protein whose function is unknown, but is present in all *Brucella* spp. (Araj, 1999; Bricker, 2002). To differentiate and implicate a *Brucella* spp., gene loci that are variable between the different species are needed. Recall that there is disagreement as to whether or not there are six species of *Brucella* or one species with six variants. Needless to say, the genetic material between species is very homogeneous (Bricker, 2002). This has provided a challenge to the development of a PCR assay to differentiate *Brucella* spp.

PCRs used to diagnose brucellosis without determining the causal species have been published in the literature since 1990. The early assays resulted in the common use of the 16S rRNA gene loci. Primers to this gene segment do not cross-react with other bacteria, except for *Brucella*'s closest relative, *Ochrobactrum anthropi*, a rare cause of opportunistic infection in immuno-compromised individuals (Bricker, 2002). The use of *BCSP31* has also been developed. It is highly conserved amongst *Brucella* spp., and it does not cross-react as commonly with *O. anthropi*. *BSCP31* is highly sensitive and specific and has been chosen as the PCR primer of choice in many laboratories (Bricker, 2002).

Nonetheless, species-identification is desirable. It can assist with development of a control program, regulatory controls and identifying the risk factors associated with contracting disease. The AMOS-PCR, named for its ability to identify *B. abortus* biovars 1, 2 and 4, *B. melitensis*, *B. ovis*, and *B. suis* biovar 1 (Ewalt and Bricker, 2000), uses a highly specific primer that reduces the false positive rate (Bricker, 2002). The AMOS-

PCR uses IS elements in the genome (Araj, 1999; Bricker, 2002). IS elements are commonly conserved in number and placement in the genome and each species tends to have a unique chromosomal location for its IS element. An additional benefit to AMOS-PCR is that it can differentiate S19 and RB51 vaccine positive versus naturally infected positive animals, a definite benefit to any animal control program (Ewalt and Bricker, 2000; Bricker, 2002). The United States Department of Agriculture's National Veterinary Services Laboratory found that the AMOS-PCR is highly accurate in its diagnosis (Ewalt and Bricker, 2000; Bricker, 2002). The drawbacks to this test is that its use requires strict adherence to the established protocols for it to be successful (Bricker, 2002). The World Organization for Animal Health recognizes its usefulness, but does not find it to be ready to be implemented as a primary diagnostic modality (World Organization for Animal Health, 2008a, b).

An alternate PCR method to differentiate species and their biovars uses the outer membrane protein 2 locus (*omp2*). The *omp2* is subdivided into *omp2a* and *omp2b*. Of the two, only the *omp2b* is expressed, but both genes can be used for PCR purposes (Bricker, 2002; Corbel, 2006). The *omp2* is a highly conserved genetic locus but different species and biovars have polymorphisms within the nucleotide sequence. The use of restriction endonucleases on the amplified DNA allows for the analysis of the restriction fragment length polymorphisms (RFLPs) within these gene segments. These RFLPs differ between species and biovars based on the restriction endonucleases used and the polymorphisms in the nucleotide sequence that are unique to specific *Brucella* spp. and biovars. This has become a common method for evaluating new *Brucella*

species and biovars as they are discovered as well as having diagnostic value (Bricker, 2002).

Samples submitted for PCR differ between animals and humans due to the different tissues used. Animal samples include milk, aborted fetuses and associated tissues, nasal swabs, blood and semen. The DNA needs to be extracted and purified and excess DNA removed for the PCR to be successful. Human PCR is primarily run using the *BCSP31* primer on a blood sample. It has been noted that whole blood samples have a much lower sensitivity (61%) than use of the serum alone (94%). This is due to the inhibitory effects of heme (from hemoglobin) on the polymerase activity of the assay (Bricker, 2002). There are also concerns with laboratory contamination of blood with bacterial DNA and amplified replicons (Corbel, 2006). Another use of the PCR can be genus identification of the animal and human cultured colonies rather than using biochemical tests (Corbel, 2006).

The literature agrees that there is potential for use of PCR as a diagnostic tool. It is sensitive, specific and rapid (Araj, 1999). Compared to culture, which can take weeks, the PCR can return results in 24 hours (Ewalt and Bricker, 2000). However, the tests still require increased standardization to allow for consistency between laboratories and thus reliability of results (Araj, 2010). The complexity of doing a PCR is also a limiting factor in many laboratories (Gall and Nielson, 2004). In addition, validation against serology and culture, and improvement in peripheral blood sample analysis are needed (Araj, 1999; Gall and Nielson, 2004; Corbel, 2006).

Disease Control and Eradication

Reducing brucellosis zoonosis requires reducing the exposure of humans to the disease agent. There are two primary exposure patterns of importance that can be targeted for intervention. The first is occupational-associated exposure. These individuals contract disease through direct contact with infected and shedding animals, the contaminated environment or through inhalation associated with aerosolization of the bacteria in dense animal holding areas. The second route of exposure is through contaminated food such as fresh and soft cheese made from raw milk, raw milk and other dairy products made from raw milk with a high water content, low salinity, low curing time in high salinity and a pH > 5. Ingestion of the bacteria in these products can lead to illness as well (Corbel, 2006).

Occupational disease is controlled through the animal population. There is no human vaccine available to directly protect these workers (Shurig et al., 2002), but there are effective *B. melitensis* vaccines for small ruminants and *B. abortus* vaccines for cattle. Unfortunately, there is not a *B. suis* vaccine commonly used (Radostits et al., 2007). The small ruminant *B. melitensis* vaccine is the Rev 1 modified live vaccine and the *B. abortus* cattle vaccines are the S19 and RB51 modified live vaccines. Self-inoculation can cause disease in humans and vaccination of pregnant animals can result in abortion. The Rev 1 and S19 vaccines interfere with serologic tests, complicating disease control and eradication programs.

There are a few prerequisite programs that need to be in place prior to any intervention, disease control program or eradication program. For success in eradication, local stakeholders need to be engaged and in support of the effort and the collaboration

between the farmers, their customers and government entities at local, state and national levels (Blasco and Molina-Flores, 2011). Such programs take a large investment of time, resources and money. Further, region-specific seroprevalence needs to be determined and interventions need to be tailored to the epidemiologic unit of interest or region. It is a mistake to assume uniformity of disease prevalence or epidemiology within a country (Blasco and Molina-Flores, 2011).

Vaccination in disease control programs

The Rev 1 vaccine is a modified live *B. melitensis* vaccine that confers protection to 65% of the animals vaccinated and the vaccine immunity can last from three to five years (Olsen and Stoffregen, 2005; Zinnstag et al., 2005). Drs. Sander Elberg and Mendel Herzberg developed the Rev 1 vaccine in the 1950s. *B. melitensis* was modified to be streptomycin resistant and was attenuated in a mouse model. It differs from the field strains in its streptomycin resistance (Blasco and Molina-Flores, 2011), but it is susceptible to penicillin. In addition, it is susceptible to high concentrations (20 µg/ml) of fuchsin and thionine and its urease production is slightly decreased which is in contrast with the field strain (Banai, 2002).

The route of administration and the population targeted for Rev 1 has impacts on the success of the program. Spain attempted to vaccinate only the replacement animals (lambs kept as the next generation for the flock) between three and seven months of age, and saw little reduction in prevalence of disease, primarily due to the fact that large portions of the populations remained unvaccinated. Israel was able to vaccinate just replacement flocks, but they had a concomitant test and slaughter program of infected adults (Banai, 2002; Olsen and Stoffregen, 2005). The purpose of vaccinating only the

replacement animals was to prevent abortions in the pregnant animals that can occur upon vaccination. The use of reduced dose vaccination ($1 \times 10^{4-6}$ CFUs) rather than full dose (1×10^9 CFUs) can be protective against abortion, but it does not result in adequate immunity (Blasco, 1997; Olsen and Stoffregen, 2005). Despite the potential abortions associated with vaccination, mass vaccination is the most effective method for control of the disease (Garin-Bastuji et al., 1998; Olsen and Stoffregen, 2005; Blasco and Molina-Flores, 2011). Ideally vaccination with Rev 1 in small ruminants occurs in animals aged three to four months, in adults prior to breeding season or while lactating, but in females not in mid-gestation (Blasco and Molina-Flores, 2011). The use of conjunctival inoculation will also reduce the interference with serology (Olsen and Stoffregen, 2005) and can protect against but not prevent abortion (Blasco, 1997; Garin-Bastuji et al., 1998; Blasco and Molina-Flores, 2011). Rather than a systemic reaction that occurs with subcutaneous injections, the conjunctival injection results in the antibody response being located in the cranial lymph nodes (Olsen and Stoffregen, 2005). Thus, the conjunctival route of administration is the recommended method (Blasco, 1997; Blasco and Molina-Flores, 2011).

Use of the Rev 1 vaccine has been suggested for *B. melitensis* infections in cattle as well. Mongolia is the only country to date that has used Rev 1 to control *B. melitensis* in cattle. This vaccination strategy was pursued when the Mongolian Ministry of Agriculture recognized that the S19 did not confer adequate protection against *B. melitensis* in cattle. The result of vaccination of cattle with the Rev 1 vaccine was animals that were immunologically resistant to both *B. melitensis* and *B. abortus* (Banai, 2002; Blasco and Molina-Flores, 2011). Current data are limited to Mongolia and

further studies need to be done (World Health Organization, 1997; Olsen and Stoffregen, 2005).

Brucella abortus can also be controlled in cattle populations using vaccination. There are two vaccines in use today: the S19 and RB51. The S19 vaccine was developed in the 1930s and 1940s in the United States. The attenuation of a virulent S19 strain of *B. abortus* occurred when it was accidentally left at room temperature for one year. Like the Rev 1 vaccine for *B. melitensis*, the S19 vaccine uses the S-LPS antigens to initiate an immune response. This is the same antigen used in serologic tests (Shurig et al., 2002). In fact it is not biochemically different from *B. abortus* biovar 1 (Blasco and Molina-Flores, 2011). Because of its close relationship to pathogenic strains of *B. abortus* the S19 vaccine induces a serological response that interferes with testing. Initially the immune response to vaccination is the production of IgM antibodies, but is followed shortly by IgG₁ and IgG₂ antibody production. Sero-conversion back to negative status can occur. This sero-conversion back to sero-negative post-vaccination is slow in older, pregnant cattle that were vaccinated with larger doses of vaccine, but calves vaccinated between three and eight months of age will be sero-negative within nine months of the inoculation (Nicoletti, 1990).

Another method to reduce the serological response and testing interference of the S19 vaccination in calves is to use the reduced dose vaccination (3 to 10 x 10⁹ CFUs) rather than full dose (2.5 to 12 x 10¹⁰ CFUs). This vaccination still protects 65% to 75% of animals for their lifetime. In pregnant adults, S19 can induce abortions, but only does so in 1% to 2.5% of pregnant animals (Shurig et al., 2002). Reduced dose vaccination of

0.3 to 3×10^9 CFUs can be used to infer immunity and protect against abortion (Olsen and Stoffregen, 2005).

The RB51 vaccine was developed after the S19. The *Brucella abortus* strain 45/20 vaccine showed that a rough strain-based vaccine could confer protection against the zoonotic smooth-strain *Brucella* spp. infection. This led to the finding of the *B. abortus* strain 2388 with rifampicin-resistance. The 2388 strain produces very little *O*-polysaccharide, the antigen against which host antibodies are made when an individual is infected with zoonotic S-LPS species of *B. abortus*, *B. melitensis* and *B. suis*. Therefore, vaccinated animals do not produce *O*-chain antibodies and thus the vaccination does not result in interference with serological testing (Shurig et al., 2002; Vemulapalli et al., 2002). The benefits of the vaccination is that it does not have abortifacient effects, it induces similar immunity to S19 and it does not interfere with serology when a 1 to 3.4×10^{10} CFU dose is given subcutaneously in calves and 10^9 dose in pregnant cows (Shurig et al., 2002). RB51 confers immunity through induction of the host's cell-mediated immunity using Th1 cells rather than a humoral immunity (Vemulapalli et al., 2002). There is some evidence that when using S-LPS based ELISA diagnostic tests in RB51 vaccinated animals a false positive can result. This false positive is due to exposure of core epitopes of the agent for which the RB51 vaccinated animal has antibodies. Agglutination-based serological tests use whole cell antigen and ELISA does not; it uses epitopes, thus accounting for the potential false positive results (Blasco and Molina-Flores, 2011). Nonetheless, RB51 has become the only vaccine used in the United States for eradication of *B. abortus* (Shurig et al., 2002; Vemulapalli et al., 2002).

Vaccination is a control strategy to reduce the spread of disease among animals and to humans. It is an effective method to reduce the disease prevalence to levels where an eradication program can be successfully initiated. If vaccination strategies are not used to reduce the prevalence, test and slaughter methods used in eradication programs can be cost prohibitive. An important note about the Rev 1, RB51 and S19 vaccines is that they can cause disease in humans who accidentally inoculate themselves. Therefore, caution and training is critical among the individuals who carry out the vaccination plans (Corbel, 2006; Blasco and Molina-Flores, 2011).

Food safety in disease control programs

Reducing disease at the animal level benefits occupational workers and those who consume dairy products. Further, direct food safety techniques can be applied to dairy products. From all meats, except those contaminated with *B. suis*, the risk of infection via consumption is rare (International Commission of Microbiological Specifications for Foods, 1996). Nonetheless, the Food and Agricultural Organization recommends discouraging the consumption of raw meat and blood (Corbel). *B. suis* is the only species that significantly invades the muscle tissue during the bacteremic phase and can be a source of infection when raw or undercooked sausages or pork are consumed (Alton, 1990b). The majority of risk comes from the contamination of dairy products.

Brucella spp. are readily killed by pasteurization or heating of raw milk. This would preclude the need for further discussion, but pasteurization is not a process that is available in all parts of the world where brucellosis is found. Heating of the milk is also a simple and quick method. The milk must be heated to 80 C to 85 C (176 F to 185 F) for several minutes or be boiled (Corbel, 2006). There is resistance to even heating the milk

in many locations as individuals have concerns of the quality of dairy product that will result from milk that has been heat-treated. Other methods to reduce the risk of *Brucella* spp. contamination in dairy products and raw milk are needed.

The probability of *Brucella* spp. survival differs based on the storage temperature, salinity, water content and pH of the product. Soft cheeses carry a higher risk of contamination than hard cheeses due to the increased water content. Acidified products, such as yogurt, sour creams and butter, carry a lesser risk of *Brucella* spp. survival, but death of all organisms can only be assured at a pH < 3.5 (International Commission of Microbiological Specifications for Foods, 1996; Corbel, 2006). Salinity varies by the product. *Brucella* spp. survive up to six months in 2.3% salted butter, but they can survive up to 13 months in unsalted butter. Organisms can survive in sheep cheese kept in 27% brine for up to 45 days. Fat content also has an effect; the higher the fat content the more protected the *Brucella* spp. in the product. Finally, storing the product at room temperature causes greater bacterial death than when it is stored at refrigerated temperatures. In addition, these different factors interact. High fat and low temperature storage will cause longer survival of bacteria and low pH and high temperature storage (pH < 5 and 38 C or 100 F) will cause bacterial death with 24 hours (International Commission of Microbiological Specifications for Foods, 1996). Consequently there are no definitive methods to account for dairy product safety beyond using pasteurized or heated milk. As a result, the Food and Agriculture Organization recommends cheeses be aged for six months prior to consumption or sale if made from raw milk (Corbel, 2006).

Due to the complicated nature of preventing contamination by *Brucella* spp. in dairy products in areas where pasteurized or heated milk is unavailable or resisted,

reducing the presence of disease in the animal population best controls the disease in humans. Dairy products from endemic areas should be considered suspect for consumption due to the organism's persistent survival for variable lengths of time in these products (International Commission of Microbiological Specifications for Foods, 1996).

Components of an eradication program

Eradication of disease is the ultimate goal of any brucellosis disease control program, but it is expensive to achieve, requires a collaborative effort by the stakeholders and long term funding by the government (Corbel, 2006; Blasco and Molina-Flores, 2011). Yet, the long term benefit of eradication and being defined as disease free are the ability to trade internationally (Godfroid et al., 2002; Blasco and Molina-Flores, 2011), decreased testing and the suspension of vaccination (Blasco and Molina-Flores, 2011). There are many component parts to eradication: test and slaughter, continued vaccination of replacement stock, animal movement control, availability of disease-free replacements of the slaughtered animals and an adequate surveillance system (Corbel, 2006). Surveillance will be discussed in detail in its own subsection.

Test and slaughter programs utilize screening and confirmatory tests to identify sero-positive animals, which are then slaughtered at designated facilities. In order to be a cost-effective disease control measure test and slaughter is best implemented in areas where there is a less than two percent prevalence of disease in the flocks and herds (Alton, 1990a; Corbel, 2006; Hegazy et al., 2009). Test and slaughter of adult animals is often done in combination with vaccination of replacement animals. Test and slaughter can be difficult to carry out and garner local support for due to the economic demand

created by the indemnities paid to the farmers for slaughtered animals and the slaughtering of farmer's animals when they test positive. This is especially true in developing countries (Garin-Bastuji et al., 1998; Blasco and Molina-Flores, 2011). Slaughter can be done at established abattoir facilities in order reduce infection to abattoir workers and to make the meat available for consumption since, once cooked, it poses very little if any threat.

The complication of test and slaughter after mass vaccination comes with the serologic interference that comes with S19 and Rev 1 vaccination use (Nicoletti, 1990; Garin-Bastuji et al., 1998; Olsen and Stoffregen, 2005). Unless these animals are identified as having been vaccinated, testing and slaughter are much more complicated. There are methods of handling these situations. Recall that in cattle vaccinated between three and seven months, many revert to being sero-negative nine months after vaccination (Nicoletti, 1990). So the timing of the test and slaughter program after the last vaccination is important to consider. The cELISA test has also been shown to be effective in differentiating cattle vaccinated with the S19 vaccine as well and can be used as a confirmatory test (Gall and Nielson, 2004; World Organization for Animal Health, 2008a). The validation of cELISA in sheep has not yet met the criteria to make it a recommended test (World Organization for Animal Health, 2008b) and sheep vaccinated with the Rev 1 vaccines do not show the same reversion to a sero-negative status that calves do. Thus, there are further complications in a flock test and slaughter program.

There are a few solutions to the complications that exist with positive serology due to sheep vaccinations during a test and slaughter program. The specificity of testing protocols are improved when the conjunctival vaccination method is used, and it is best

to use this route of vaccination when coupling a vaccination campaign with test and slaughter either sequentially or simultaneously. In sheep, waiting for a period of two years prior to conducting serologic testing for test and slaughter campaigns can reduce the false positive rate among the adult animals. During this interim period it is important to continue vaccinating the replacement stock. There is one other testing option that has a higher specificity in sheep vaccinated with Rev 1: the native hapten gel precipitation test (Blasco and Molina-Flores, 2011). Beginning a test and slaughter program six to 12 months after the vaccination campaign ends can be done without sacrificing specificity by using this test (Blasco and Molina-Flores, 2011).

A significant goal of an eradication program is to reach a state where vaccination is not needed and test and slaughter is done of flocks and herds that are identified during regular, active surveillance programs until no more are identified. When vaccination can be suspended and the brucellosis “officially-free” status can be maintained then standards are met for international trade (Blasco and Molina-Flores, 2011).

In addition to vaccination protocols and test and slaughter procedures for an eradication program, certain other animal regulations need to be implemented. These include permanent identification of vaccinated animals, movement controls of animals to prevent contact between infected and susceptible animals and identification of disease-free replacement stock. The term replacement stock here refer to animals brought into the herd or flock from an outside source and not animals born into the herd or flock (Blasco and Molina-Flores, 2011). Movement control is the most difficult to enforce, but also one of the most critical components (Corbel, 2006; Blasco and Molina-Flores, 2011). Exclusion of infected animals from clean areas or uninfected flocks and herds is critical

to maintaining the disease status attained in that area. Clean areas should only be accessed by animals that have permanent individual identification, come from negative herds and flocks and are negative on all serologic tests (Corbel, 2006).

Surveillance strategies

Surveillance methods allow a country to identify sero-positive animals at the end of an eradication program when few positive animals are left and to maintain disease free status with a certain level of assurance once it has been attained. Surveillance requires use of diagnostic tests or disease reporting data to be collected at pre-determined levels so as to allow for the presence or absence of disease to be known at a certain level of assurance.

When a disease is rare or eradicated from domestic species there are increased complications with diagnostic tests. The predictive values of diagnostic tests are affected by the prevalence of disease in the area: the higher the prevalence in an area the higher the positive predictive value, or, in other words, the probability that a test positive is actually positive. As the prevalence decreases, so does the positive predictive value (Blasco and Molina-Flores, 2011).

Common strategies employed in an active surveillance program for brucellosis incorporate numerous sampling methods. The most thorough method would be to randomly test a representative sample of the population of interest. It is important to calculate the sample size with the desired ability to detect an infection at a predetermined prevalence. This type of testing should be done annually at the very least (Blasco and Molina-Flores, 2011).

Testing of animals in areas where they congregate is often used as a convenient source of identifying positive animals without conducting a random sample of the entire population. The United States uses this type of surveillance as the prevalence level in a state falls (Ragan, 2002; Ebel et al., 2008). This sampling strategy is known as first point of concentration testing. The most common is the testing of animals at slaughter facilities and markets. This system requires that animals can be traced back to the herd or flock of origin. The inherent bias to this method of surveillance is that it will more likely identify large infected herds in comparison to smaller herds. This type of testing is also impacted by the economy, consumer demand of the product and the farmer's inventory of livestock, and thus the use of the markets or slaughterhouses (Crawford et al., 1990).

This sampling method of first point of concentration testing is a two-stage sample: herds or flocks that cull animals for slaughter or sale and then a sample from cattle or sheep in those herds and flocks. This explains the bias in that there is a higher probability of detecting those herds that are large enough that they will sell or cull animals, and farmers that cull or sell more animals are more likely to have herds that are detected (Ebel et al., 2008). Finally, if this surveillance is conducted in conjunction with vaccination, the S19 vaccine can mask the presence of disease in a herd when 95% of the flock is vaccinated. Presumably, this same phenomenon would occur with the Rev 1 vaccine. This method of detection is useful when the herd prevalence is high, even if the overall numbers of herds affected is not high (Crawford et al., 1990).

Another active surveillance option for testing among dairy herds is the bulk milk ring test (Ragan, 2002). This test is not an effective test for dairy sheep and goat flocks because the higher milk fat in these animals interferes with the test (MacMillan, 1990;

Nielson, 2002; World Organization for Animal Health, 2008b). In dairy herds, it is a simple and low cost testing procedure that can be done on pooled samples rather than individual animals. It is even effective at identifying an infected herd when the herd prevalence of disease is low. It must be cautioned, however, that as the herd size increases, the bacteria will become more diluted and the size of the pooled sample would need to be adjusted (World Organization for Animal Health, 2008a). In addition, it is important to use this test in a way that all animals are represented in the sample over the course of a year. In many countries testing quarterly ensures all animals are tested in the pooled sample (Crawford et al., 1990).

Passive surveillance can be incorporated into an area or regional surveillance plan by collecting data used for diagnostic purposes from local clinicians or laboratories. However, without the use of active surveillance methods as well, surveillance systems reliant on passive methods of data collection cannot ensure the absence of disease in an area nor allow for early detection of disease (Blasco and Molina-Flores, 2011). Making abortion notifiable in an area can allow for investigations into the cause of abortion and potentially the identification of a brucellosis infected animal. This method of detection relies on compliance by farmers and private veterinarians and the assurance that there will be enough resources to conduct adequate investigations following a report of an abortion (Crawford et al., 1990). One could posit that such a scheme could be tailored to conduct investigations when abortions in an area are occurring at a rate beyond what is expected when brucellosis is not endemic to that area as well.

When disease does occur or a reactor is identified by the surveillance system, it is necessary to investigate the animal, the herd of origin and the area around that herd.

Adjacent herds to the infected one should have each animal tested. If the agricultural industry is dense in the area, the testing can go beyond adjacent herds as well. The status of all potentially infected herds should be determined. In response to a positive herd, the whole herd can be culled, or positive reactors can be culled and follow up testing done until a certain number of negative herd tests are achieved (Crawford et al., 1990; Ragan, 2002). It is best to quarantine a herd or restrict movement of animals to and from the potentially infected herd during this testing period (Crawford et al., 1990).

Surveillance is a critical part of an eradication and preventive program. During the final stages of eradication it is used to identify the remaining positive herds and flocks (Ragan, 2002). In preventive programs, surveillance is used to ensure that flocks and herds are negative and remain negative. When a disease is eradicated and control programs such as vaccination are decreased or completely terminated, the disease free population is naïve and, thus, more susceptible to the associated disease if it was reintroduced. Unless preventive surveillance is done for potential disease threats, reintroduction and significant spread can occur before the disease is recognized and diagnosed. This is especially true for a disease like brucellosis— a chronic disease that has few clinical signs beyond abortion (Ragan, 2002). Therefore surveillance is both necessary to rid an area of a disease and to keep it out once it is gone.

Global distribution of disease

Brucellosis is one of the “neglected diseases of poverty” according to the World Health Organization. Such diseases are endemic zoonotic diseases that are found primarily in impoverished parts of the world that are heavily reliant on livestock agriculture (World Health Organization, 2006; Blasco and Molina-Flores, 2011). These

nations lack the diagnostic and medical tools to diagnose and treat the diseased (Corbel, 2006). Brucellosis is one of the world's most ubiquitous zoonoses, and thus a significant contributor to disability-adjusted life years (DALYs) and financial loss associated with zoonotic disease. DALYs are the years of productive life loss due to disability as compared to individuals without disability (World Health Organization, 2006; Blasco and Molina-Flores, 2011).

Brucellosis is a disease of importance in many parts of the world. This includes Mediterranean countries (south and east Europe), north and east Africa, central and south America, Asia and the Middle East (Corbel, 2006). Just like the situation with most infectious diseases, the occurrence differs greatly between developed and developing countries. The former can have human incidence rate less than 1 per 100,000 people, while the latter have recorded incidence rates as high as 200 per 100,000 people. Often, especially in locations where diagnostics are not readily available, this disease is underreported (Blasco and Molina-Flores, 2011). Therefore, these incidence rates are just estimates of the minimum amount of disease present. An estimate of the global incidence of disease is approximately 500,000 cases per year (Pappas et al., 2006).

Human brucellosis in former Soviet states and the Middle East

The former Soviet Union was expansive. Its former states are present in modern day eastern Europe, the Caucasus and Asia. With the breakdown of the Soviet Union came a breakdown of services that have contributed to the re-emergence of brucellosis in these areas. Former Soviet states are hotspots for brucellosis in today's world.

The Caucasus region accounts for some of the highest incidence rates of brucellosis in Europe. Dagestan reports an annual incidence rate of 100 cases per

1,000,000 persons and the surrounding countries of Azerbaijan (52.6 per 1,000,000), Armenia (31.3 per 1,000,000) and Georgia (27.6 per 1,000,000) also report high levels of disease (Pappas et al., 2006).

There are seven former Soviet states in the top 25 countries with the highest human incidence of brucellosis. Leading the way among the former Soviet States is Mongolia (605.9 per 1,000,000), which is ranked second only to Syria with 1603.4 cases per 1,000,000. In terms of morbidity in Mongolia, brucellosis is one of leading causes of illness in this country's nomadic population (Mocellin and Foggin, 2008). Also included in the top 25 are Kazakhstan (115.8 per 1,000,000), Kyrgyzstan (362.2 per 1,000,000) and Tajikistan (211.9 per 1,000,000), all of which have rising numbers of brucellosis every year as well. Azerbaijan, Georgia, Uzbekistan, Turkmenistan and Armenia also report a significant number of human brucellosis cases annually (Pappas et al., 2006).

The Middle East has long been endemic for brucellosis as have the former Soviet republics. The Middle East is also home to the country with the highest annual incidence of disease, Syria. It should also be noted that Turkey, Iran, Saudi Arabia and Iraq also have a significant burden of disease with 262.2, 238.6, 214.4 and 278.4 cases per 1,000,000 persons annually, respectively (Pappas et al., 2006).

The countries discussed here have the highest incidence rates of disease. They are either similar in geographic location or by their political and social experiences. The country of Georgia is in their midst and also has a significant brucellosis burden. The concern with brucellosis is that re-emergence is occurring, especially of *B. melitensis* in sheep and goats and thus humans (Blasco and Molina-Flores, 2011). Without further efforts to control this disease in these areas, the burden will grow.

Unfortunately, data for animal brucellosis is not very thorough within the former Soviet States and the Middle East. Many countries have conducted various types of surveys to quantify their animal disease burden that highlight the burden of disease within the country.

Former Soviet states are working to re-establish veterinary services and to understand the disease situation in these countries. Numerous countries of the former Soviet bloc have conducted serologic surveys to determine the level of prevalence of brucellosis within livestock. Tajikistan reports a prevalence of 5.8% in sheep, 5.5% in goats and 2.1% in cattle (Jackson et al., 2007). This was not a national survey, but a regional survey, so to be representative of the country's livestock burden more sero-surveillance is necessary. A national livestock sero-survey conducted in Kazakhstan found that 5.4% of cattle, 1.3% of sheep and 0.7% of goats with 2.4% of all cattle, sheep and goats were sero-positive for brucellosis (Lundervold et al., 2004). Mongolia's national livestock surveillance program from 2000 to 2003 found a seroprevalence in sheep between 0.4% and 0.9% and in cattle from 0.9% to 1.5% (Zinnstag et al., 2005). Georgia has reported the presence of *B. melitensis* and *B. abortus* in both animal and human cases (Malania et al., 2009; Onashvili et al., 2009), but a representative serological survey has not yet been conducted. Armenia has reported cattle brucellosis in 28 of the 38 regions in the country or 73.7% of regions (Baghiyan et al., 2009), and antibodies against *Brucella* spp. were found in 1.2% of cattle, 1.5% of sheep and 2.7% of goats (Food and Agriculture Organization, 2010).

For many former Soviet bloc countries there is significant levels of disease in both livestock and humans. Since humans contract disease primarily from animals and

human-to-human spread is exceedingly rare, without quantifying and controlling the disease in livestock, the illness in humans will not disappear. As for the potential brucellosis threat in the Caucasus region, the most commonly reported cause of brucellosis in humans in the Middle East is *B. melitensis* biovar 3, although human cases of *B. abortus* are being reported, suggesting a rise of *B. abortus* in the livestock population (Radostits et al., 2007). It is possible that the same *Brucella* spp. are the source of disease in the Caucasus region as well.

Conclusions

Brucellosis is an infectious disease of the animal and human populations that is caused by a gram-negative and facultative intracellular cocco-bacillus. Animal populations indicate the presence of infection primarily through abortion, but decreased fertility and milk production are other common sequelae. In humans, an acute disease will develop into chronic disease if left untreated. The primary clinical manifestation of chronic disease involves osteoarticular complications. Spread from animals to humans is through occupational exposure and food contamination, and thus control is done at the animal level. Disease control and eradication programs require the use of vaccination, test and slaughter and active surveillance. The complications with diagnostic testing, vaccination interference with available tests and the underreporting of this disease contributes to the reason that brucellosis persists throughout the world. The areas that carry the greatest burden include Mediterranean countries, former Soviet states and the Middle East.

Chapter 3: “One Health” requires more than “One Medicine”

Nothing has such power to broaden the mind as the ability to investigate systematically and truly all that comes under thy observation in life.

-Marcus Aurelius

Karyn A. Havas and Mo D. Salman

Animal Population Health Institute
Department of Veterinary Medicine and Biomedical Sciences
Colorado State University
Fort Collins, Colorado
United States

Summary

Reaching optimal health for animals, humans and the environment is the goal of the One Health initiative, and it is a complex endeavor requiring more than just One Medicine—the combined efforts of human and veterinary medicine. As health is affected by complex factors such as access to multiple resources, economics, political interactions, culture and ecological resources, we must look beyond natural science’s limited triad of host, agent and environment. In order to attain optimal human and animal health, effective programs must include input from experts of multiple disciplines. These may include anthropologists, wildlife conservationists, urban planners, entomologists, ecologists and climatologists just to name a few. In addition, these programs should be based on the societal infrastructure and include collaboration with the local population and government representatives. The necessity and limitation of “One Medicine”, the complex web of disease, and the challenges for the future of “One Health” are discussed.

Introduction

The concept of health is complex, incorporating nutrition, sanitation, access to resources, access to food and clean water, ecosystem resource management and climate. The importance of these health determinants is found in the societies where they are lacking and the health has suffered as a result. Poor health often results in a disease state. Disease is a general term for conditions resulting from changes in the physiology, immunology or anatomy of a person or animal that impacts their function or structure. These conditions can be chronic or acute and infectious or non-infectious. For this paper the infectious and non-infectious conditions will be used for discussion.

Physical factors of health impact susceptibility to infectious, metabolic, cardiovascular, mental and other diseases. Malnutrition, overcrowding and sanitation go hand-in-hand with infectious diseases. Poor sanitation leads to an over-exposure of vectors such as rats and mice, which can carry Plague, Lyme disease, Hantavirus or Leptospirosis, among others. Contaminated water is regularly associated with gastrointestinal illness the world over. Overcrowding increases the contact rate between individuals. This is a characteristic in which diseases like Tuberculosis, Influenza and other communicable diseases flourish. Non-infectious diseases are often impacted by genetics, nutrition and ecosystem degradation. The type of foodstuffs that are available and/or affordable can impact an individual's risk of developing heart disease, diabetes, and other non-infectious related health concerns. Inadequate nutrition can also impact immune function making individuals more prone to communicable or infectious disease. Ecosystem resource management is a topic that covers water availability and cleanliness, rangeland and soil fertility to name a few.

Anything that limits the ability of livestock to convert low nutritional density forage to nutritionally dense foodstuffs, such as milk, meat and blood, and to provide draft power and transportation can be associated with a loss of resources that can have devastating effects on health (Osborn, 1996). Kenya experienced a drought that led to such a resource loss and it resulted in starvation and a limited ability of the population to cope with further disturbances (Wakabi, 2006; Gettleman, 2009). Rinderpest devastated the animal population in Africa in the 1890s and the result was severe human suffering (Schwabe, 1984). The environment, animals and humans are all inter-related. These and the supporting physical factors of health are important to consider for sustainability of programs and maintenance of necessary resources.

Physical characteristics are important, but we must not forget social characteristics of illness. These include social power, social standing, employment, poverty, exposure to traumatic experiences, and other variables. Across the globe there is a division of wealth that has further delineated the Global North and South. The South is the source of human labor by which products for the North's consumers are made (Held and McGrew, 2007a). The social standing of the global South in the realm of trade is to serve the North. The higher social standing sits with the consumers. Along with this division based on labor and consumption, there is also a division of wealth. As of 1998, the ratio of wealth between the rich and poor, or North and South is 74 to 1, a significant increase of the 1973 ratio of 4:1 (Steger, 2009). In addition, there is a North-South differential distribution in terms of access to local foodstuffs and technology as well (Europe, 1993; Zhu, 2004; Hazell and Wood, 2008). Poverty also follows this longitudinal geography. This is a macro view of social factors impacting poverty. It is

mirrored by the spatial distribution of disease across the globe, as poverty and disease are often linked (Leon and Walt, 2001). Infant mortality, less than 5 years of age mortality, maternal mortality, and the locations with the shortest life spans and highest disability adjusted life-years all fall into the global South (Mathers et al., 2008). To focus the discussion more, women are often those in society with poor social standing, low social power and high poverty. The push from within the Millennium Development Goals (MDG) to educate women highlights the needs of this group (Tyer-Viola and Cesario, 2010). Access to resources, social standing, and social power provide decision-making opportunities, and is correlated with greater wealth and greater maternal and family health (Link and Phelan, 2002). Social factors play a significant role in the distribution of infectious, nutrition-related and mental disease. They cannot be ignored.

This translates into a multifaceted effect on health that is beyond medical treatment or preventive medicine. To achieve the best possible level of health across the globe, health professionals have embraced two concepts: One Health and One Medicine. One Health is defined as, “the collaborative efforts of multiple disciplines working locally, nationally, and globally, to attain optimal health for people, animals, and our environment.” (One Health Initiative Task Force, 2008). “One Medicine” is the collaboration of human and animal medicine for the purpose of disease recognition and prevention (Kahn, 2006). Calvin Schwabe re-introduced the concept of One Medicine to scientific discussion in the 1980s. He recognized the comparative nature of medicine and the overlap of the same needs between the human and animal branches (Schwabe, 1984). This medical collaboration is necessary to achieve the optimal health of humans, animals, and the environment, but it is not a sufficient solution. The insufficiency arises from the

reality that disease goes beyond individual wellbeing and often requires societal changes (Link and Phelan, 2002). Diagnosis and treatment may help recognize and cure many factors of disease, but it does not address the ecological, sanitary, crowding and social factors that make people more prone to disease.

This paper will discuss the web of factors that determine health or disease in animals and humans; the need of One Medicine in achieving optimal health; and a discussion on the need for inter-disciplinary collaboration on multiple levels.

The Web of Disease

Disease is a complicated process and the infectious component is often summarized using the epidemiological triad of host, agent and environment. It could be argued that the triad could also be used for non-infectious diseases. For example, atherosclerosis is caused by plaque build-up in the body's cardiovascular system. The build-up of plaque is affected by the predisposition or genetics of the person as well as environmental factors such as diet, exercise and access to medical care. For both infectious and non-infectious diseases, tangential factors have a significant impact on the propagation of disease, but are not commonly incorporated into this triad and include a variety of political, economic, and social factors. These additional factors transform the triad into a web with the interplay of the factors resulting in health or disease for the individual (Figure 3.1).

Animals are affected by disease and they impact human health. Human health is often linked directly to animal health via infectious diseases. Zoonoses comprise 75% of the emerging infectious diseases in humans, and of the 27 diseases on the World Health Organization's (WHO) Global Burden of Diseases Contributing to Disability-Adjusted

Life Years, 20 are zoonotic (World Organization for Animal, 2006). The majority of these zoonoses arise from the livestock and wildlife sectors and include Influenza Viruses, Hemolytic-Uremic Syndrome, West Nile Virus, Bovine Spongiform Encephalopathy, and AIDS.

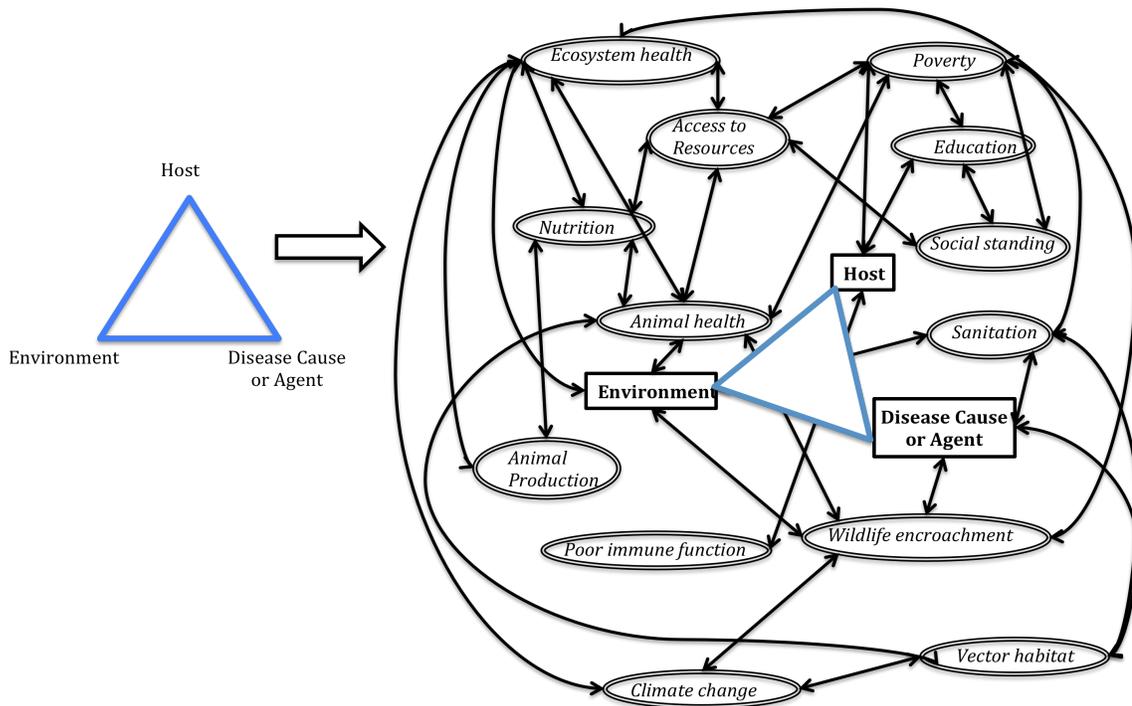


Figure 3.1: Conversion of the epidemiological triad of disease to a multi-disciplinary web of disease

Animals go beyond being a source of disease; they are also a foundation of life and health for most societies. They are an important source of protein via meat, milk and blood. As transportation and draft animals they are the backbones for food production and distribution in many countries (Olmstead, 2009). Their impact goes beyond the physical realm and plays a role in the mental and social realms as well. The loss of animals during a disease outbreak resulting in high mortality or government control, can lead to grief and depression amongst the farmers. For example, the quarantine and slaughter of cows in response to a Foot and Mouth Disease outbreak in the United

Kingdom and in the Netherlands led to mental health concerns amongst the farmers (Mort et al., 2005; Olff et al., 2005). The number of animals is also a sign of wealth and social standing in numerous cultures. These animals provide emergency funds, social prestige and food security, but the hoarding of animals for social standing can also lead to environmental degradation (Doran et al., 1979; Smith et al., 2001). Thus a loss of animal resources can have an acute effect on the nutrition and wealth of a community, creating a cycle of illness, poverty, hunger and malnutrition as well as on community mental wellbeing. In comparison though, an accumulation of animals can overwhelm an ecosystem, which can lead to degraded health states in animals and people as well. The social aspect to disease is complex.

Also included in the web is the ecological environment; many of the pressures related to health are ecological in nature. Drought, floods, natural and man-made disasters, including war and social strife, ecosystem service depletion (such as rangeland overuse), overpopulation, and climate change as well as land privatization resulting in fragmentation and isolation of groups from environmental resources all place negative pressures on animal and human health. These factors can even culminate in the loss of human and animal life through starvation (Osborn, 1996; Wakabi, 2006).

Society and culture also affect the health of a community. Culture is comprised of common beliefs, behaviors, values, and traditions among a group of people, and includes a certain level of understanding of disease and health. This knowledge in its cultural context must be taken into account during any attempt to improve the health status of a community (Polanyi, 1970). Although Western society decided in the 18th century to live by reason based on science, this does not mean that all societies developed

understanding in this way. The belief that Western reason prevails is the greatest source of our failure to succeed in many development goals (Polanyi, 1970). The response from Western science should be to turn to anthropologists and sociologists for guidance and to the local community for insight. Many societies blame witchcraft, voodoo, spirits or a lack of balance as causes of disease. Epilepsy in Haiti is believed to be due to voodoo and spirit possession, Filariasis in Ghana is caused by witchcraft and illness in general in the Mazateca people of Oaxaco, Mexico is caused by sorcery or a humoral imbalance in their environment. Treatment is via traditional healers, medicinal plants or modern medical treatments (Gyapong et al., 1996; Giovannini and Heinrich, 2009; Cavanna et al., 2010). These traditional beliefs need to be considered when addressing a health problem in non-Western cultures.

The impact of international relations and globalization on local politics and economics can also negatively impact human health. This is especially true in poorer countries where the disparity in income as compared to wealthier countries continues to grow (Held and McGrew, 2007b). As a result, these countries cannot compete in the global market and personal income is the main hurdle to providing sufficient food, especially in growing urban areas (Hazell and Wood, 2008). This marginal nutrition increases susceptibility to disease. Thus it is understood that many factors from local to international play a role in the web of disease.

One Medicine – A first step, but an incomplete solution

Early modern medical discoveries, both human and veterinary, have contributed to the improvement of human health via infectious disease control. Jenner's observation that milkmaids did not contract smallpox and Koch's and Pasteur's development of the

Germ Theory via the study of anthrax and tuberculosis are just two examples of how an integration of human and animal medicine increased our knowledge about disease and how to prevent it (Thrusfield, 2007). These early discoveries are still used to save human lives and illustrate the tremendous benefit of such collaborative work. In order to attain optimal health worldwide, it is critical that this cooperation between medicines continues.

Another reason collaboration between human and animal medicine remains paramount is that the eradication of diseases is difficult and often is not attainable. As stated earlier, 75% of emerging infectious diseases of humans are zoonotic and since eradication is a rare option, control of these diseases is necessary. For zoonotic control to be successful it must occur at the source of the infection—the animals. Veterinary medicine must develop methods to contain disease, and human medicine must develop complementary methods to protect and treat human patients. Animals can also be used as sentinels for human disease and vice versa. Open communication and integration of surveillance and monitoring in both humans and animals will be mutually beneficial, so it is critical that physicians and veterinarians cooperate in their efforts.

Preventing, controlling, and treating non-zoonotic and non-infectious disease in both humans and animals is also important. Animal health is related directly to their productivity for their human counterparts. The healthier the animal the more food, draft power and offspring they provide. This allows for more reliable food sources and income to many households in the world. This is reciprocal as well. Animal care and welfare improves as the health of humans improves. Once individuals have more disposable income the human-animal bond is enriched and animal healthcare begins to go beyond disease control efforts. The advancements in animal medicine that speak to the power of

the human-animal bond include heart valve replacement surgeries, veterinary diets, cancer therapies and the longer lifespan of animals in the developed countries of the world.

The introduction of West Nile Virus into North America highlights the need for collaborative medicine. Simultaneously New York City was studying outbreaks of dying birds and dying humans. A veterinarian was the first to suggest a similar cause for the two outbreaks, but was initially ignored. The result was more dying birds and humans and a significant delay in discovering the etiology of disease (Heinrich et al., 2000).

Nonetheless, the list of disease situations where more than collaborative medicine is needed is exponential. Animal models of non-infectious diseases can help explain the biology and intrinsic risk factors for disease but not the how and why groups are exposed to external risk factors. For example, studies in mice can explain why mutations to the *agouti* gene play a physiological role in obesity and how obesity leads to other diseases such as insulin-resistant diabetes (Steppan et al., 2001; Carroll et al., 2004). The research is often translatable to people and beneficial to veterinary medicine. However, these studies do not explain how income, ethnicity, gender and social standing limit one's ability to afford good food or have access to it. These social problems are a key player in addressing these health issues (Hossain et al., 2007). Cholesterol-improving drugs and studies on animal obesity will not solve this health issue. Severe malnutrition specifically with protein malnutrition leads to diseases in youth called kwashiorkor. These individuals experience stunted growth, mental retardation, poor immune function, and often die from the illness (Stephenson et al., 2000). The reasons for starvation go beyond access to food. Numerous factors play a role and include social standing, ecosystem

health, education, poverty, women's rights, and more. Clearly, just improving animal health and providing handout nutrition is not a long-term solution for such complex problems.

The same situation also exists for infectious diseases. Lyme disease and Bluetongue Disease will be used as an illustration. Lyme disease is carried by *Ixodid* ticks with hosts that include white-tailed deer, white-footed mice and chipmunks (Steere, 2010). Veterinarians and human physicians have preached the need for tick preventive measures and the veterinary field has even developed a vaccination protecting dogs from infection. Yet, these measures are purely preventive. They do not remove the threat from the environment. Increased encroachment on wildlife habitats and landscape fragmentation are considerable contributors to this disease problem (Steere, 2010). Therefore incorporating urban planning, wildlife conservation, and other environmental expertise in the reduction of Lyme disease is critical. Bluetongue disease is spreading northward into Europe, impacting sheep and sheep farmers across the continent. A suggested cause is climate change and a greater geographic range for the *Culicoides* vector (Wilson and Mellor, 2008). Without the knowledge of climatologists, meteorologists, entomologists and atmospheric scientists the efforts to control disease would be limited. Medicine in these situations is reactionary, but not wholly preventive. Medicine alone is not the answer.

The combined efforts of human and animal medicine can lead to greater disease control, treatment and prevention in the world. This collaboration will promote health from both the animal and human side in unison, leading to overall improved health in

both. This cooperation—One Medicine—is crucial to One Health, but it cannot stand alone (World Organization for Animal Health, 2006).

Suggestions for the Future

Since each country has unique resources, people, cultures, diseases, and stressors, we cannot rely on a pre-constructed solution if we want to achieve a sustainable improvement in health. Plans must be developed on the existing infrastructure and take into account cultural components, economic factors, indigenous knowledge, and current food systems and supplies, while engaging related disciplines at all levels of the local population and government. This development is an adaptive approach—One Health—that is needed to achieve optimal human and animal health worldwide. Since microbial, environmental, and social systems intersect, the One Health movement and its systemic, inter-disciplinary engagement is the only method that can effectively improve health. This system approach moves away from reductionism toward a more holistic approach to problem solving and future prevention (Holling et al., 1998). Greater collaboration between natural and social scientists will achieve a broader understanding of the interaction of human behavior, culture and environment on health. This method for specific projects recognizes the need for individuals from many levels of society and government to be involved (Folke et al., 2005). With this approach, knowledge from all levels—local as well as international—is valued and used to construct the best plan, rather than relying on a centralized resource management solution as a global cure-all (Holling et al., 1998; Kerkhoff and Lebel, 2006).

Such an inclusive approach is site-specific and must be tailored to the specific needs and characteristics of the population, health issue, and environment of interest.

This is not a simple process, and the international community working to improve health in many parts of the world is challenged by it. In addition, the medical community currently lacks the structure and experience with this method, and this limits their ability to respond. Yet, if we fail to adapt we run the risk of developing a brittle infrastructure that may not respond well to future health emergencies or allow for better global health in both human and animal at all.

One Health's aim is to achieve optimal health in animals, humans, and the environment and while veterinary and human medicines play key roles, they cannot succeed alone. Local knowledge and needs must be incorporated along with Western knowledge, cultural understanding, and ecological, political, economic and societal issues as well. This is a complex and demanding task that will only be successful when there is true commitment and cooperation on the part of those involved.

Chapter 4: Simulation Modeling

One of the most insidious and nefarious properties of scientific models is their tendency to take over, and sometimes supplant, reality.

— *Erwin Chargaff*

Epidemiology has multiple goals and purposes one of which is to estimate the potential effect of interventions and control programs on the state of disease or to determine the appropriate level of surveillance in an area or region. One of the tools used to estimate effects or to determine appropriate levels of surveillance are simulation models.

A Brief History of Infectious Disease Modeling

Simulation modeling entered mainstream usage in the epidemiology profession as microcomputers and their associated processors became more readily available, user-friendly and powerful. Spreadsheet programs with mathematical capabilities have allowed for the development of desktop models. This has allowed models such as the Reed-Frost Model of contact rate-based transmission between susceptible, infected and recovered individuals, and the Markov Chain Model that studies the probability of transitions between states of disease to gain increasing complexity from their original forms (Carpenter, 1988a). Yet, the roots of modeling came prior to the advent of personal computing.

The original epidemiological models were used to model small pox, rinderpest and malaria and these models developed concepts still used today (Carpenter, 1984; Brauer, 2009). The reproductive ratio essentially shows that if the number of secondary infections that occur from a single infected individual is more than one then the disease will propagate, and if it is less than one then the disease will die out. The concept of the reproductive ratio was born out of the idea of threshold values for disease occurrence. Threshold values arose from early models of malaria. Another concept learned from epidemiology's initial foray into modeling is the use of the law of mass-action, which describes disease transmission and contact rates based on population density. The epidemiologic community has learned that contact rates are independent of population density and tend to be a standard incidence within a given population or a saturation point. These concepts are used in state transition models, which function on the premise that individuals move between states of health and illness. More specifically, individuals move between susceptible, infected, immune and recovered states based on certain probabilities. Via the use of mass-action application in this model, movement of individuals between compartments was found to be proportional to the population size with a time-component for the duration of "exposure" to that transition rate. Yet, when the population disease state equilibrium is not static (i.e., changes over time) the modeling of state-transition models becomes more complicated (Abbey, 1952; Brauer, 2009).

For many populations, population size, contact rates and reproductive ratios remain unknown. This paucity of data for "hidden" social populations, such as drug-users and wildlife, led to the use of network infectious disease models. These models

trace contacts or interactions between individuals in a population. Network models are limited by the fact that they are not dynamic (Danon et al., 2011). Dynamic models incorporate the concept of the change of model parameters, such as infection rates, birth rates and death rates over time in populations or population densities (May and Anderson, 1979; Howitt, 1982; Carpenter, 1988a; Thieme, 2003a). Since only one time point is often traced or evaluated in the sampling used for network epidemiology modeling, this dynamic nature is not possible. Another option has been agent-based models, which model behaviors, actions and their associated patterns in society among simulated individuals or units (Danon et al., 2011; Grimm, 1999; Grimm et al., 2005). Agent-based modeling requires a great deal of computer processing power and since agent-based models deal with behavior they have an intrinsic level of uncertainty based on the detail of behavior that can be and is needed to be modeled. Finally, epidemiologists recognize that movement patterns of people and animals often explain spread of disease on a regional or global scale and that these patterns can be modeled. This has led to the use of four movement patterns that act as proxies for human movement; they are airline networks, commuter networks, movement of dollar bills and cattle movement patterns. These models make no assumption about transmission rate, but they speak about the flow of people in a population. They are known as meta-population models (Danon et al., 2011; Carpenter and Sattenspiel, 2009).

Simulation modeling has become a key tool for epidemiology as well as a broad range of other disciplines specifically since the advancement in computer technology. Over the years it has developed from simplistic forms that could be done by hand to complicated models that require significant computing power to run. The use of models

is well established in epidemiology and models continue to evolve as we better learn to solve epidemiologic problems. The purpose of this section is to discuss basic modeling concepts and types in order to familiarize and prepare the reader to be able to critically evaluate model usage in epidemiology.

Deterministic versus Stochastic Modeling

Modeling is an effort to study a population via simulation in order to assess a hypothesis or evaluate a program. Models can be divided into deterministic or stochastic models. These definitions are based upon the ability of the model to simulate the variability that is present in a system. The earliest models were criticized for using point estimates as variables, rather than incorporating variation or considering prior states or values of variables in the model. Static parameters do not allow one to understand the effect of prior states of disease or to reflect the variation that is intrinsic to biological systems (Anderson and May, 1979; Carpenter, 1988a).

All of life and biological processes are filled with variation. Statistically, we describe this variability as variance or standard deviations within the sample and standard error around the sample summary statistic through specific distributions of the data. Parametric data are data with a known mathematically derived distribution, a point measurement and its variance. Non-parametric data does not have a known distribution, but must be fit to a distribution using computer software programs if the variation in the distribution wants to be used in a model. These programs return the distribution of best fit, the associated parameters and their variance (Vose, 2008c). In a normally distributed or Gaussian data set, 50% of the data is found on either side of the mean or average. Non-Gaussian data can be skewed and can have either a left tail, with the majority of data

points found at the high end of the measured scale, or a right tail, with a majority of data points found at the low end of the measured scale. Common distributions are the normal distribution, which applies to most biological measurements; the log normal distributions which are useful for data that are normalized when the log value is taken; the binomial distribution for proportional data or yes/no data; the negative binomial for over-dispersed incident or count data; the Poisson for incidence or count data; uniform distributions which are synonymous with the random number generator where every value in the distribution's range has an equal chance of being selected; and beta distributions for prior probabilities in Latent Class Analysis. There are numerous others as well (Vose, 2008a). Stochasticity returns measures of center, distribution shape and measures of variability as well, so that the whole range of the effect can be seen. This provides a broader picture of the problem under study and more information to decision makers as well.

With non-Gaussian distributions, data points are not necessarily distributed with 50% of the data on either side of the mean, and the median is a better measure of center. The median is the center point of the data when all of the data are listed in numerical order (Kleinbaum et al., 2008a). The shape of a variable's distribution gives one an understanding of the probability of having a certain value of the variable. This probability can be determined mathematically using differential calculus as a probability density function. Several software programs will sample from a distribution with a given set of parameters that define it based on this probability distribution so that the sampled values over the course of many iterations mirrors the distribution that was sampled. This is what occurs with Monte-Carlo sampling (Vose, 2008d, b).

The reason that distribution sampling is now commonly used is that it captures the variability of a system being examined. Using a point estimate to describe the data alone can be misleading without also knowing the variability associated with that point measurement. When this is done it is considered deterministic. Deterministic models typically use and report measures of center, but do not incorporate any of the variability around them. It limits the reality of the simulation and thus effects the decision-making power that a model can provide (Carpenter, 1988b). Stochastic models incorporate all of the variability associated with a particular parameter. This is either done through the use of the appropriate distribution or via random-number generators.

The output of deterministic models, models that use only averages for input variables yield only a single expected outcome and thus have no variability. Yet, there are reasons for the use of deterministic models. Originally, it required more knowledge and advanced programming and software capability to yield stochastic results (Carpenter, 1988b). So, many early models were deterministic. Deterministic models allowed science to realize that vaccinating 70% to 80% of the population could result in “herd immunity” to small pox, which gave credence to the concept of eradication through vaccination (Danon et al., 2011). When information is only available without the knowledge of the distribution of the inputs and outputs associated with the phenomenon of interest in the population of interest, simulation is not without value, but it is less informative.

Stochastic models provided variability to the model that more closely resembles real life, but with increased mathematical complexity. Simulation models are able to introduce variability with the advent of random-number generators and their use in these

spreadsheet models (Carpenter, 1988a). Stochastic models have further developed and now computing power allows for the Markov-Chain Monte Carlo model. This is a stochastic method where the state transition probabilities (Markov Chain component) pull from a distribution (Monte Carlo component) around a variable and the process is repeated numerous times to develop a distribution for the outcome (Carpenter, 1988a; Vose, 2008b). Newer stochastic modeling techniques include Bayesian modeling which incorporates prior probabilities into estimates of unknown variables such as diagnostic test sensitivity and specificity. Agent-based modeling on the other hand, simulates heterogeneous groups within a landscape to better represent human behavior and provide a more realistic method to model diverse environments.

Population-Based Modeling

Models are meant to mimic reality. They are built with explicit assumptions and definite limitations. Their function resembles the system being analyzed, but they remain simplified versions of reality. Typically in epidemiology models that mimic populations are described. The outcome of the model is based on assumptions about the population and its interactions.

Population biology has its own mathematical discipline. Infectious disease spread and modeling falls within this framework. Within population biology, the formulation of models is meant to transform a biological or scientific problem into a mathematical construct (Thieme, 2003c). As is true of most systems, these biological and scientific problems are not completely understood; thus these mathematical equations can just mimic reality. As the model becomes more complex or realistic, the mathematics respond in kind (Parunak et al., 1998). The analysis of the model involves mathematical

equations that represent the population and the goal is to have a result that can be explained in biological terms and not mathematical terms. A desirable model returns a conceptual insight and not a mathematical proof (Parunak et al., 1998; Thieme, 2003c). An equation-based or population-based model is able to do this because characteristics of the group or individuals are modeled via equations that represent links between individuals or groups. The observable trends based on these linked equations can be discussed as biological phenomenon; these equation-based interactions are the model output (Parunak et al., 1998).

In order for population-based models to work they focus on aggregate characteristics. Modeling at the population level or system level allows for determining appropriate equations to fit the system under study. The more refined the model becomes in terms of granularity, the greater the potential of having unknown parameters and an inability to mathematically define the grouping. This can make the model less accurate and useful (Thieme, 2003c). Further, the more detail or granularity that one wants the model to have, the greater the complexity that is required. For example, an agent-based model or individual based model was used to estimate the time to recognition of a brucellosis case in the dairy herd population in Japan. The researchers recognized that their limitations included using a constant herd size and a uniform distribution for health parameters (Yamamoto et al., 2008). A uniform distribution is a one where all values of the parameter have an equal probability of existing in a population (Vose, 2008a). The researchers recognized that they did not have more specific data, and that there were many unknowns. Further, they validated the parameters with many experts and previous publications and ran a sensitivity analysis on the less robust parameters as well. It is

important to know the effect of such weak parameters on the model so one can know the impact of having poor data (Yamamoto et al., 2008). Another example using a population-based model is one where the researchers were trying to determine the sampling method to detect Johne's disease or paratuberculosis in cattle with the greatest herd sensitivity and specificity. The researchers noted that the model would have been more accurate if data were available on the number of cows that contribute to the environmental sample of those that are infected. Further, they comment that the model does not take into account subclinical cases or the impact of super-shedders of this mycobacterium into the environment. In order to gain this level of detail, the researchers explicitly state the need for currently unknown parameters (Tavornpanich et al., 2008). There is a proportionate response with the detail in a model and the unknowns in the variables. These situations require greater validation of the parameters used and an understanding of the limitations in the model. This does not make a model useless, but it does provide boundaries to the reality that it describes. This can become cumbersome on a population level (Brauer, 2009). For these reasons, population models are well-suited for more homogeneous populations and physical processes with flows between groups that are not reliant or affected by individual decision-making (Parunak et al., 1998). As a result, one must be careful when modeling human processes that are impacted by human behavior and decision-making and not just biological processes. Population-based models may simplify this aspect of the system to a point where the relevance or reality can be questioned (Page, 1999). The modeler must recognize this drawback.

The Reed-Frost Model

One of the earliest dynamic population models to simulate disease is the Reed-Frost or Susceptible, Infected, and Recovery (SIR) model used for infectious disease modeling. It began as the Kermack-McKendrick Model for infectious disease spread in large populations using calculus to simulate mass-action movement between the states of SIR and has evolved into the Reed-Frost model, which incorporates the concept of an effective contact rate transmitting disease from infected individuals to susceptible individuals into the law of mass action as well as dictated by Markovian states (Abbey, 1952; Fukuda et al., 1984; Brauer, 2009, Thieme, 2003d, Carpenter, 1988a). The mathematical modeling theories found in the Reed-Frost construct are still the basis for many models today. The seven assumptions of the original Reed-Frost model are: 1) the population is closed; 2) the population is initially composed of completely immune and completely susceptible individuals; 3) an infected individual that enters the community will come into contact with other individuals all at the same contact rate; 4) the infected individual is subclinical during the entire incubation period; and 5) the infected individual only spreads disease for a short period of time called the infectious period; 6) individuals will recover with complete immunity; and 7) new infections result from sufficient contact with the infected individual (Fukuda et al., 1984). The probability of sufficient contact is a constant amongst all individuals in the community as well.

There are some inherent problems with these assumptions. A significant limitation to modeling is the determination of the reproductive ratio or reproductive number. This number is calculated based on a closed population, a constant rate of contact and lifelong immunity once an individual has become ill. Determining the

reproductive ratio in a realistic situation is much more difficult (Brauer, 2009). Such situations are those where the population is open, contact rates are not uniform and immunity and chance of illness vary across the population based on age, group, sex, or a number of other variables. Other drawbacks to the use of SIR models have been found based on the evaluation of the lack of fit of the model's simulated epidemic curve when compared to the actual outbreak's epidemic curve. It is necessary to consider the prevalence of subclinical or inapparent infections as well as the prevalence of immunity in a population (Fukuda et al., 1984). Finally, due to the complexity of the vector life cycles and potential hosts in a susceptible population or area, vector-borne diseases become too complicated for SIR models as well (Roche et al., 2008). Further, modeling of parasitic populations is necessary when modeling infestations, but this requires the researcher to model the parasite population as well, and not just the impact of the parasite on the host population, as is done with the majority of SIR models. The additional requirement of simulating the parasite population adds significantly to the complexity of the model (Thieme, 2003b).

Mathematically, the formula that is the basis for the Reed Frost Model is as follows:

$$C_{t+1} = S_t * (1-q^{C_t}) \quad \text{(Formula 1)}$$

where t is the time period, C_{t+1} is the number of infectious cases in a time period and C_t is the number of infectious cases in the previous time period. S_t is the number of susceptible individuals in the previous time period. The q^{C_t} is the probability of not being sick in the previous time period and is calculated as $(1-p)^{C_t}$. The p is the probability of an individual contracting the illness and is uniform throughout the population; therefore, the

probability defined by q is also uniform throughout the population. The probability (p) is calculated as:

$$p = k/n-1 \quad \text{(Formula 2)}$$

where k is the effective contacts made by an individual with other individuals in the community in the time period and n is the population size (Carpenter, 1988b).

As the model progresses through time periods the number of immune, infected and susceptible individuals change. As individuals become sick they are removed from the susceptible pool and placed into the infected pool, where they can then cause others to get sick in the next time period. As infected individuals become immune they are placed into the immune pool and are resistant to infection in the next time period. If the immunity is not long lasting, then immune individuals can become susceptible again, and are prone to be re-infected in the next time period. Even greater complexity can be introduced by modeling the vaccination of susceptible individuals, so that vaccinated susceptible individuals cannot become infected despite never having disease. The duration of action for the vaccination can also be input into the model. This vaccination would be added protection along with the acquired immunity if an animal recovered (Carpenter, 1988b). Further, a case fatality rate could be added to the infected as well if there is mortality associated with the disease.

Variations of the Reed Frost Model have been developed to incorporate birth and death rates, incubation periods, re-infections and return to susceptibility as well.

Nonetheless, such models look at populations and groups and their parameters and not the behaviors associated with infection spread (Abbey, 1952). Therefore it is necessary

to have the appropriate population data and agent data to successfully model diseases using this platform.

Agent-Based Modeling

Using individuals rather than populations as the focus point of a model to quantify an outcome first began in the 1970s in ecology research. Its usage increased exponentially in the 1980s and came into use in the social sciences in the 1990s (Grimm, 1999). Agent-based models are those where the environment and the individuals of interest are virtually simulated and their interactions are used to determine population or system level outcomes. These models simulate individual behavior and interactions rather than their aggregate characteristics. This can be very useful when behavior patterns are known and specific population parameters are not. In addition, agent-based models are easier to construct because they do not require a high degree of mathematical knowledge and proofs (Parunak et al., 1998).

Agent-based models are useful when the population is heterogeneous. More specifically, they are appropriate when: 1) behaviors of individuals are poorly represented by aggregate transition rates due to heterogeneity in the population; 2) individual behavior is complex and mathematical equations poorly represent the behavior as a result; 3) activities of individuals are a more realistic way to describe the system and assign stochasticity rather than processes; 4) the model must be understood and validated by expert judgment and therefore must be understandable to said experts; 5) stochasticity is better applied to an agent behavior than as an overall population variable; and 6) when attempting to assess and understand emergent phenomenon of a system (Bonabeau, 2002). These models require a greater amount of information and knowledge of the

individuals as well as the system (Janssen and Ostrom, 2006). Since they attempt to model the interaction and behaviors of individuals within a system and not its aggregate form they also have computer processing limitations (Grimm, 1999). In comparison to population models, the output is the aggregate characteristics that arise from individual behaviors, rather than the recognition of how aggregate characteristics describe interactions

Ecological studies often use agent-based models to simulate complex environments or communities in order to test hypotheses. Pattern oriented models are used to model the realistic complexity with its concurrent uncertainty inherent in models that are meant to resemble actual systems. This allows for the building of a model that is not too unwieldy for use. Using patterns at multiple levels of organization or hierarchy allows for the best representation of a reality and allows for a more reliable evaluation of outcomes in the model. Pattern oriented modeling states that the underlying interactions or behaviors are shared, but the characteristics and risks of those interactions differ between groups (Grimm et al., 2005).

Dealing with uncertainty in agent-based models remains a challenge. How to model uncertainty and to manage it within the model structure has yet to be fully determined. Using patterns at multiple levels of organization best mimics reality, reducing uncertainty intrinsic to a model that attempts to replicate reality. Uncertainty associated with the parameters of the model can be evaluated via sensitivity analyses. Such an analysis determines the change in the outcome based on the change in the input parameter. This concept is also used in population-based modeling (Grimm et al., 2005).

The use of a model and its design is based on the subject under study, the purpose of the study and the information available. Agent based models are an alternative to population-based studies when individual behaviors or heterogeneous group patterns are critical to an outcome or when population parameters are unknown while behaviors are well-described.

Justifications for Using an Agent-Based Model

The purpose of this thesis is to assess the disease ecology of brucellosis and the human-animal interaction in the Kakheti region of Georgia for the purposes of identifying transmission routes, education needs and intervention potentials. Kakheti is a diverse area of Georgia in its geography and ethnicities. The livestock owning component of the society works with sheep and cattle and can be divided into sheep shepherds, cattle herders, people who milk dairy cows, and cow milk dairy producers, and which further differ based on a person's gender. Sheep shepherds conduct the milking and dairy product production from milking sheep. These groups have different exposures and risks and their make-up differs between the municipalities of Kakheti (Havas et al., 2011a; Havas et al., 2011b).

The purpose of the modeling component of this thesis is to determine the impact of animal level interventions on human incidence of brucellosis annually. Infection is primarily associated with occupational exposure, although contaminated dairy product consumption can still play a role (Havas et al., 2011a; Havas et al., 2011b). For Kakheti, specific data on patterns of human and animal interactions and behavior patterns between ethnic groups is lacking, but if it was available then it should also be modeled. Using a pattern-oriented approach on the level of the individual, herd/flock, village, and

municipality of Kakheti, an agent-based model can emulate the system and return aggregate systems information based on these individual interactions among humans and livestock. Its validity is determined by comparison to the results of a natural system and by validation of the behaviors seen in rural Kakheti. Building interactions based on behaviors determined by a questionnaire survey conducted in Kakheti and the interactions defined by the assessment were further validated through and by collaboration with local Georgians during the project (Havas et al., 2011b). To model each municipality at all these levels using a population-based model would have been mathematically complex and would have required information on effective contact rates between flocks and herds and humans. A more conventional population-based model would have been more difficult to validate due to lack of population data and more difficult to explain to local policy makers as well.

Zoonotic disease occurs because of the interaction of agents, which can be simulated using agent-based models. Boneabeau states that, “At the simplest level, an agent-based model consists of a system of agents and the relationships between them.” (Bonabeau, 2002). These agents can be animals, people, vectors, fomites, infectious agents or any things or individuals that are responsible for the spread of disease. Agent based models require a great deal of information if one is to model a society and the disease within a society, but if that level of data is available, the results are more realistic. This is not to say that agent-based models control for all uncertainty, but they reduce the uncertainty associated with extrapolating disease transmission and disease events to population levels when transmission occurs at the individual level. An agent- based model was used for this project because of the complexity of the environment, the

different groups at risk at different time steps and at different magnitudes and the need for the model to be understandable by policy makers.

Chapter 5: Risk factors associated with human brucellosis in the country of Georgia: A case-control study

Karyn A. Havas^{1,*}, Marine Ramishvili², Archil Navdarashvili², Ashley E. Hill¹,

Shota Tsanava², Paata Imnadze², and Mo D. Salman¹

¹ Animal Population Health Institute
Department of Veterinary Medicine and Biomedical Sciences
Colorado State University
Fort Collins, Colorado
United States

² National Center for Disease Control and Public Health,
Department of Anthrax, Zoonotic and Anaerobic Infections
Tbilisi, Georgia.

Summary

Human brucellosis occurs when humans ingest or contact *Brucella spp.* from shedding animals or contaminated environments and food. Georgia has livestock and human brucellosis, but the epidemiology has not been fully characterized. A case-control study was conducted to identify risk factors for brucellosis in humans that present with at least six clinical signs and test positive through antibody-agglutination tests. A multivariable logistic regression was run using age as a categorical variable and another with age as a continuous variable. The significant variables were similar in the magnitude of the odds ratios. For the regression using age as the categorical variable the following risk factors for humans with brucellosis were identified: among those who work with animals as their primary employment (OR: 77.8, 95% confidence interval (95% CI: 2.8,2184.5), among those who do not work with animals as their primary employment (OR: 12.7, 95% CI: 0.7, 239.6), being unemployed or a pensioner (OR: 13.1, 95% CI: 1.1,149.9), sheep ownership (OR: 19.3, 95% CI: 4, 93.5), making dairy products (OR 12.4, 95% CI: 0.9, 172.6), living in eastern Georgia (Kakheti) (OR: 278.1, 95% CI: 5, 15,454.4), and being older than 44 years (OR: 9.3, 95% CI: 0.7, 128.9). Cattle ownership was not significant, nor were individual animal related tasks such as assisting in livestock births and milking. Education of at-risk groups about risk factors and control of disease in sheep may reduce the human disease risk. This is the first study of its kind in Georgia since the collapse of the Soviet Union.

Introduction

Brucellosis is an infectious disease of ruminant livestock, swine, dogs, rats, horses, and humans. There are six *Brucella spp.*, four of which are zoonotic. The zoonotic species in order of decreasing virulence in humans are *B. melitensis*, *B. suis*, *B. abortus* and *B. canis*. *Brucella spp.* can remain latent within the host's macrophages and cause chronic illness (Araj, 1999; Dornand et al., 2002). Each *Brucella spp.* has a preferential host but can infect others either as a dead-end or incidental host. Humans are a dead-end host and very rarely infect another human. *B. melitensis* is commonly found in sheep and goats. It can also infect cattle and dogs, and cattle can shed the organism in their milk (Radostits et al., 2007). *B. abortus* is commonly found in cattle and can infect sheep and dogs. Sheep can shed the bacteria as well (Radostits et al., 2007). *B. suis* is commonly found in swine, but can be transmitted to cattle and horses which are dead-end hosts (Radostits et al., 2007).

Human brucellosis is under-reported worldwide, but is most prevalent in Mediterranean countries, Central Asia, the Caucasus, Latin America and sub-Saharan Africa (McDermott and Arimi, 2002; Radostits et al., 2007). *B. melitensis* is the most common cause of human brucellosis in the Mediterranean and Central Asia. *B. abortus* is most common in Latin America and sub-Saharan Africa (McDermott and Arimi, 2002; Radostits et al., 2007). Reasons for under-reporting of human brucellosis include lack of access to medical care, vague clinical signs and symptoms resulting in misdiagnosis, and the need for complicated laboratory techniques for correct diagnosis (Mantur et al., 2006; Franco et al., 2007). Cases of chronic brucellosis can significantly reduce the quality of

life. A decrease in productive life years can result from sequelae of chronic infections, including endocarditis, osteomyelitis, arthritis and meningitis (Dornand et al., 2002).

Brucellosis in animals is most symptomatic in primary infections. Certain *Brucella* spp. have a propensity for the pregnant uterus and in primary infected animals this results in a late term abortion. Animals with recurrent infections do not repeatedly abort, but all infected animals have tissues, including aborted material, afterbirth and vaginal discharge, that are laden with bacteria. The bacteria are also shed in the milk for up to two months post parturition in sheep and for the duration of lactation in cattle. Infected tissues and milk are the main source of environmental contamination that spreads the disease horizontally to other members of the flock or herd, and to humans (Radostits et al., 2007).

Human infection is associated with factors and behaviors related to exposure to contaminated food products and shedding animals. Occupational exposure for shepherds, animal caretakers, veterinarians and milkers occurs when the individual is exposed to vaginal discharges, milk, aborted fetuses and infectious afterbirth tissues (Corbel, 2006; Radostits et al., 2007). Consumption of raw milk and contaminated dairy products also cause disease (Corbel, 2006; Mantur et al., 2006). Individuals who own livestock, have family members with the disease, consume raw dairy products or have a greater occupational exposure to the disease are at greater risk of contracting brucellosis (Avdikou et al., 2005; Mantur et al., 2006; Franco et al., 2007; Sofian et al., 2008; Earhart et al., 2009). In addition, preventive measures among humans can include education (Kozukeev et al., 2006).

Brucellosis in humans is directly related to disease in animals and in Georgia cattle and sheep specifically. The country of Georgia lies in a region of the world with a history of brucellosis. It is estimated that the annual human incidence is 27.6 cases per one million persons (Pappas et al., 2006). Georgian society is heavily dependent upon milk and meat from cattle and sheep. In addition, sheep exports to countries in Southwest Asia are growing; if infected these exports could spread brucellosis throughout the region.

To be effective, brucellosis control programs need to be tailored to the country in which they are applied. Control of brucellosis in animals in Georgia may be challenging because after the collapse of the Soviet Union, all active animal health programs were discontinued and have yet to be fully re-initiated. Thus, minimizing human brucellosis in this country requires prevention of spread from animals to humans and understanding which risk factors for human brucellosis are most important.

The purpose of this study is to identify potential risk factors associated with human brucellosis infection in the country of Georgia. Since this is the first study of its kind available in international peer-reviewed journals, the results can also be used as a guide to further research in human brucellosis in Georgia. More specifically, this study explores animal-related, occupational, ethnic and regional diversity factors. The goal is to use the collected data and available information about the country in order to propose practical and effective control measures to reduce the spread of the disease in the country, to highlight needs for further research and to aid local medical professionals in understanding the epidemiology of brucellosis.

Methods

Study Design

We conducted a case-control study. The online OpenEpi unmatched Case Control Sample Size calculator was used to calculate the necessary sample size for 80% power and 95% confidence for the smallest risk difference between the proportion of cases and controls exposed to the different risk factors investigated. The smallest risk difference was 20% as estimated by expert opinion at the Georgian National Center for Disease Control and Public Health and Institute of Parasitology and Tropical Medicine (Dean et al., 2009). This yielded a sample size of 83 cases and 83 controls. The sample size was rounded to 100 in each group to ensure adequate numbers should some questionnaires be incomplete.

Case and Control Definitions

Both cases and controls were from the Institute of Parasitology and Tropical Medicine (IP) in Tbilisi, Georgia from February to September 2010. Cases were incident cases of brucellosis and were defined as patients referred to the IP that upon examination presented clinically for brucellosis and were positive on both the plate and tube agglutination tests. Clinical cases were those that had at least six clinical symptoms of brucellosis. The IP is the only center in the country of Georgia that provides definitive diagnosis and treatment for human brucellosis. Therefore, it is necessary for individuals to travel from their home location to Tbilisi for care if brucellosis is suspected. Recurrent brucellosis infections were excluded. Controls were defined as incident cases of parasite infestation diagnosed at the IP from February to September 2010. Parasite infestations include malaria, amoebiasis, trichinellosis, leishmaniasis, ascariasis, enterobiasis,

fasciolosis, among others. Individuals who were previously diagnosed with brucellosis were excluded from the controls. The IP is the primary diagnostic and treatment center for parasitic infections for Georgia. Only ascariasis and enterobiasis patients can be treated at other medical facilities in Georgia, so the vast majority of patients were similar in their use of the IP for diagnosis and treatment. Brucellosis is the only non-parasitic infection treated in the hospital. Patients come from all over Georgia for treatment of these diseases. The purpose of using hospital controls was to reduce the bias associated with subjects being able to travel to Tbilisi for treatment; therefore they are more representative of the population from which cases would arise, but are not likely representative of all Georgians (Wakefield et al., 1992; Rothman et al., 2008).

Questionnaire and Interviewing

Upon diagnosis, physicians at the IP interviewed the cases and controls using a standard form. Questions included age, sex, ethnicity, region and municipality of residence and work, income level, education, occupation, travel, assisting in livestock birthing, livestock ownership specifically sheep, cow, goat and/or swine ownership, whether other family members had brucellosis, if so, what was their age and sex and the frequency of consumption of raw milk, consumption of dairy products from raw milk, production of dairy products, milking and slaughtering. The questionnaires were reviewed for clarity and translated into Georgian by the collaborators at the National Center of Disease Control and Public Health in the country of Georgia. The questionnaire was then presented to the clinicians and they were trained on it as well. The study designed was reviewed by Colorado State University's Institutional Review Board and was approved on 4 February 2010.

Statistical Analysis

Individual factors were summarized using either proportions with 95% confidence intervals or means with their standard deviations. In addition, frequencies and characteristics of the variables of interest were compared between cases and controls for differences in proportions or means using the T-test, chi-squared test or Fisher's exact test where appropriate.

Multivariable logistic regression was used to estimate the associations between potential risk factors and the odds of brucellosis. Income was not used in the analysis because the responses varied between individual and household level information, making the individuals non-comparable. Nominal categorical variables (e.g. consumption of raw milk products) were modeled as both yes/no variables, and in their original categorical form. Variables eligible for inclusion into a multivariable model were those with a p-value ≤ 0.25 in univariable analysis. Multivariable model building was a backward selection process. Variables were retained in the model if removal significantly affected model fit (likelihood ratio ≤ 0.10). Variables with a p-value < 0.10 were discussed if considered biologically and culturally relevant. Categorical variables divided into dummy variables were evaluated using a partial likelihood ratio test and binomial categorical variables were evaluated with the Wald test. Factors that were not statistically significant during the backwards selection process were evaluated for confounding by adding them back into the model singly and factors that changed any of the model's coefficients by greater than 15% were considered confounders. In addition, biologically and culturally significant factors were evaluated as effect modifiers and were retained in the model if the p-value ≤ 0.10 . All variables were converted to categorical variables.

The Hosmer-Lemeshow Goodness of Fit test was used to evaluate the overall fit of the model. Stata and MS Excel software were used for all analyses.

Some variables were condensed due to the low numbers within some categories. The occupation category was collapsed into animal-associated employment, housewives, non-animal-associated employment, student or child, and unemployed or pensioner. The student and child group was the reference group because it best represented individuals with little or no animal contact. Age was analyzed in two different ways and required that the model be built twice with the different age variables. This was due to the fact that the case and control population resulted in very different distributions of age and although categorically age groups differ in Georgia in their societal roles (children go to school until they are 18 and therefore have less contact with animals) the groupings may be too broad and could introduce bias into the results. The first analysis looked at age as a categorical variable and the second analysis looked at age as a continuous variable. When using age as a continuous variable, the assumption of linearity was checked by plotting the Pearson residuals against age and visually assessing for a departure from linearity. In the first analysis age was categorized as zero to 17 years of age (reference group) to represent school age children; 18 to 44 years of age to represent adults; and 45 years of age and greater to represent middle aged and elderly. The provinces were categorized as western Georgia (reference), Kakheti, Kvemo Kartli, Mtskheta-Mtianeti and Shida Kartli and Tbilisi. Western Georgia was comprised of patients from Samtskhe-Javakheti, Adjara, Guria, Imereti, and Samegrelo and Zemo Svaneti (Figure 5.1). Ethnicity was categorized as Georgian and non-Georgian. Finally, two of the 100 cases were missing data on variables that were significant to the analysis and had to be dropped

from the analysis in order to be able to conduct the likelihood ratio test. The final sample size used in the study was 98 cases and 100 controls.



Figure 5.1: The administrative regions of Georgia (The Parliament of Georgia)

Results

For each case and control diagnosed at the IP from February to September 2010, a questionnaire was completed for a 100% response rate. Evaluation of the differences in frequency and percentage of the descriptive characteristics of the cases and controls used in multivariable evaluation was done and cases and controls differed in the distribution of gender, age category, occupation, province of residence and ethnicity (Table 5.1).

Finally, with age as a continuous variable, the median age among cases was 31 years and the median age among controls was 11 years.

Univariable Analysis

Univariable analysis further assessed associations between odds of brucellosis and gender, livestock ownership (specifically cattle and sheep), eating dairy products made from raw milk, making dairy products, milking, assisting livestock with birthing,

slaughtering, having family members with disease, ethnicity, occupation and province of residence (Table 5.2).

Table 5.1: Comparison of characteristics between cases (n= 98) and controls (n = 100) from the Institute of Parasitology, Tbilisi, Georgia, 2010

Factors	Cases (%)	Controls (%)	p-value*
Gender			
Male	77 (79)	52 (52)	<0.01
Female	21 (21)	48 (48)	
Age Group			
0 to 17	14 (14)	66 (66)	<0.01
18 to 44	52 (53)	27 (27)	
> 44	32 (33)	7 (7)	
Occupation			< 0.01
Student/Child	14 (14)	70 (70)	< 0.01
Animal-related	33 (34)	1 (1)	
Housewife	7 (7)	8 (8)	
Non-animal related	30 (31)	15 (15)	
Unemployed/Retired	14 (14)	6 (6)	
Province of Residence			
Western Georgia	2 (2)	31 (31)	<0.01
Kakheti	44 (45)	13 (13)	
Kvemo Kartli	30 (31)	24 (24)	
Shida Kartli/ Mtskheta Mtianeti	8 (8)	20 (20)	
Tbilisi	14 (14)	12 (12)	
Ethnicity			
Georgian	54 (55.)	77 (77)	<0.01
Armenian	3 (3)	2 (2)	
Azerbaijani	38 (39)	19 (19)	
Kist	2 (2)	0	
Russian	1 (1)	0	
Ossetian	0	2 (2)	

* A chi-squared or Fisher's exact test of association was used where appropriate.

The frequency at which the variables of interest occurred for those who performed the activity amongst cases and controls was also studied. The frequency per week that cases made dairy products was significantly higher compared to controls (Table 5.2). Cheese was the most frequently consumed raw milk dairy product and was close to being statistically significant between cases and controls. The frequency of eating raw milk dairy products was significantly higher in cases compared to controls (Table 5.2).

Multivariable Logistic Regression

Variables eligible for inclusion in a multivariable model included gender, family members with disease, livestock ownership, cattle ownership, sheep ownership, milking, assisting in livestock births, slaughtering, occupation, age groups and age, ethnicity and province of residence. Family members with disease fell out of the model because it was perfectly predictive, likely due to a low number of observations. Cattle and sheep ownership were separately placed in the model instead of livestock ownership to provide more information than livestock ownership alone. In the model that analyzed age as a categorical variable, milking and assisting in livestock birthing confounded the initial backwards-selection model and the odds ratios were calculated with and without adjusting for these variables. In the model that analyzed age as a continuous variable, age was insignificant, but age, milking and assisting in livestock birthing all confounded the initial backwards selection model and were thus kept in the final model. Interaction terms between owning sheep and making dairy products, owning sheep and assisting with livestock births, owning sheep and milking, milking and making dairy products, and making dairy products and eating raw milk dairy products were investigated. No interaction terms significantly improved either of the two model fits.

For the model that assessed age as a categorical variable, the province of residence for Kakheti, Kvemo Kartli and Tbilisi had large odds ratios when compared to Western Georgia when all other variables were controlled (Table 5.2). Cases had 19.3 (95% CI: 4, 93.5) greater odds of being sheep owners and 12.4 (95% CI: 0.9, 172.6) greater odds of being a dairy product producer (Table 5.2).

Table 5.2: Descriptive, univariable and multivariable logistic regression results for risk factors for brucellosis with p-values < 0.10 Georgia, 2010, n_{cases} = 98, n_{controls} = 100

Risk Factors	Cases, # (%)	Controls, # (%)	UV		MV ^{a,1}		MV ^{b,1}		MV ^{a,2}		MV ^{b,2}	
			OR (95% CI)	PV								
Own sheep	66 (67)	13 (13)	13.8 (7, 28)	<0.01	22.4 (5, 106)	<0.01	19.3 (4, 94)	<0.01	20.3 (5, 87)	<0.01	17.3 (4, 77)	<0.01
Eat raw dairy products	59 (60)	49 (49)	1.7 (1, 3)	0.06	0.14 (0.03, 0.6)	0.01	0.15 (0.03, 0.7)	0.01	0.13 (0.03, 0.55)	<0.01	0.16 (0.04, 0.7)	0.01
Make dairy products	49 (50)	7 (7)	13.3 (6, 32)	<0.01	23.2 (4, 151)	0.01	12.4 (1, 173)	0.06	21.4 (4, 130)	<0.01	11 (0.8, 159)	0.08
Age: continuous			1.07 (1.04, 1.09)	<0.01								
<u>Occupation</u>						<0.01		<0.01		<0.01		<0.01
Students & children			Ref.		Ref.		Ref.		Ref.		Ref.	
Animal work			165 (21, 1308)	<0.01	119.1 (5, 2970)	<0.01	77.8 (3, 2185)	0.01	243.2 (17, 3559)	<0.01	59.1 (3, 1210)	<0.01
Unemployed /pension			11.7 (4, 36)	<0.01	13.4 (1, 150)	0.04	13.1 (1, 150)	0.04	30.3 (6, 165)	<0.01	11.8 (2, 88)	0.02
Non-animal work			10 (4, 23)	<0.01	11.6 (1, 208)	<0.10	12.7 (0.7, 240)	0.09	60.2 (8, 436)	<0.01	19.8 (1.6, 242)	0.02
<u>Age groups</u>						0.06		0.08				
0 to 17 years			Ref.		Ref.		Ref.					
> 44 years			21.6 (8, 59)	<0.01	10.7 (0.8, 145)	0.08	9.3 (0.7, 129)	<0.10				
<u>Province of Residence</u>						<0.01		<0.01		<0.01		<0.01
West Georgia			Ref.		Ref.		Ref.		Ref.		Ref.	
Kakheti			52.5 (11, 249)	<0.01	385.3 (7, 21616)	<0.01	278.1 (5, 15454)	0.01	122.7 (5, 3009)	<0.01	168.5 (4, 6340)	<0.01
Kvemo Kartli			19.4 (4, 89)	<0.01	174.2 (3, 9519)	0.01	131.5 (3, 7054)	0.02	54.5 (2, 1243)	0.01	76 (2, 2667)	0.02
Shida.Kartli/ Mskheta. Mtianeti			6.2 (1, 32)	0.03	44.5 (0.7, 2913)	0.08						
Tbilisi			18.1 (4, 92)	<0.01	994.1 (13, 76803)	<0.01	755.3 (10, 56172)	<0.01	310.8 (10, 9993)	<0.01	450.4 (9, 21892)	<0.01

MV stands for multivariable and UV stands for univariable

^a The multivariable regression without adjusting for confounding

^b The multivariable regression while adjusting for assisting in birthing and milking as confounders.

¹ The analysis using age as a categorical variable

² The analysis using age as a continuous variable

PV stands for p-value; SD stands for standard deviation; and 95%CI stands for 95% confidence interval.

* The p-values for the descriptive characteristics of the variables were determined using Fisher's exact, Chi-square test or the two sample T-test.

The following variables were not found significant in the model: cow ownership, swine ownership, milking animals, assisting in animal birthing, slaughtering, drinking raw milk, foreign travel, ethnicity (Georgian versus non-Georgian), the age group of 18-44 year olds and working as a housewife.

Eating raw dairy products appears protective with cases having 0.15 times smaller odds (95% CI: 0.03, 0.7) than controls of eating raw dairy products. As compared to students and children, cases had 77.8 (95% CI: 2.8, 2184.5), 13.1 (95% CI: 1.1, 149.9) and 12.7 (95% CI: 0.7, 239.6) greater odds of working with animals, being unemployed or pensioners and being employed in non-animal occupations respectively. Finally, cases have 9.3 (95% CI: 0.7, 128.9) greater odds of disease when aged over 44 years compared to individuals aged zero to seventeen than controls (Table 5.2). The Hosmer-Lemeshow Goodness of Fit p-value was 0.28, indicating adequate model fit.

For the model that assessed age as a continuous variable, all of the same variables were significant except for age. The province of residence for Kakheti, Kvemo Kartli and Tbilisi had large odds ratios when compared to Western Georgia when all other variables were controlled (Table 5.2). Cases had 17.3 (95% CI: 3.9, 76.8) greater odds of being sheep owners and 11 (95% CI: 0.8, 159.3) greater odds of being a dairy product producer (Table 5.2). Eating dairy products appears protective with cases having 0.16 times smaller odds (95% CI: 0.04, 0.7) than controls of eating dairy products. As compared to students and children, cases had 59.1 (95% CI: 2.9, 1209.6), 11.8 (95% CI: 1.6, 87.7) and 19.8 (95% CI: 1.6, 241.9) greater odds of working with animals, being unemployed or pensioners and being employed in non-animal occupations respectively. The Hosmer-Lemeshow Goodness of Fit p-value was 0.57, indicating adequate model fit.

Discussion

For both models the most significant risk factors in this study were living in Kakheti, Kvemo Kartli and Tbilisi, any occupation other than being a student or child,

but most significantly animal-related work, sheep ownership, and making dairy products. Eating dairy products appeared protective.

Animal-related occupations included animal ownership, shepherd, milker and veterinarian and the animal-related occupations were significant. This association of occupation with brucellosis infection is similar to other studies from former Soviet states and areas adjacent to the Caucasus (Kozukeev et al., 2006; Sofian et al., 2008; Earhart et al., 2009). Men and women take general care of the cattle, but the men milk and care for sheep, shepherd all livestock and slaughter animals. In this study, 96% of the subjects that were employed in animal-related work were male. Making dairy products is a female role. Gender was represented within these animal care roles and was not significant otherwise. Also, all groups of non-student occupations were identified as significant risk factors because they likely all owned livestock. Taking part in animal care is independent of employment making employment less informative as a risk factor than animal ownership.

Those with disease had 17.3 to 19.3 greater odds of owning sheep, yet cattle ownership was not significant. Sheep are the main reservoir for *B. melitensis* infections, but they can be infected with *B. abortus*. Yet, cattle, the *B. abortus* reservoir, are more prone to *B. melitensis* infection than sheep are to *B. abortus* (Corbel, 2006; Radostits et al., 2007). It is less likely that infected sheep carry *B. abortus* and more likely that infected cattle carry *B. melitensis*. Further, the majority of human patient cultures in Georgia are *B. melitensis* and *B. melitensis* has been cultured from small ruminants (Malania et al., 2009; Onashvili et al., 2009). These findings indicate that *B. melitensis* is present in the sheep and humans in the country.

A cattle disease component is still possible. Cases had 11 to 12.4 greater odds of being dairy product producers than controls. Also, dairy products are more commonly made from cow milk (88% of cases and 100% of controls) as compared to sheep milk (18% of cases, 14% of controls). The increased disease odds associated with making dairy products combined with the high use of cow milk suggests a cow component to illness. This is strengthened by the fact that cattle are readily infected with *B. melitensis*, which also helps explain why sheep ownership was significant—they are the primary reservoir for *B. melitensis* (Corbel, 2006; Radostits et al., 2007).

The extremely high odds of brucellosis in individuals from Kakheti and Kvemo Kartli are associated with the large sheep populations in these regions. In 2009, Kakheti and Kvemo Kartli had 269,400 and 131,800 head of sheep, respectively, as compared to Samtskhe-Javakheti in Western Georgia with 87,400 head of sheep (Kvinikadze et al., 2009). Sheep milk is used to make the Gouda cheese from this region. Also, predominately Muslim ethnic groups concentrate in these regions and include the Kists and Azerbaijanis. Their fat source is butter and rendered butter, which are made from raw milk. Butter can be contaminated with *Brucella spp.* Western Georgia is comprised primarily of ethnic Georgians. Kvemo Kartli has a large Armenian and Azerbaijani presence. Kakheti has a large presence of Muslims (Population Census and Demographic Statistics Division, 2002). This ethnic distribution explains why ethnicity was not significant in the model; it was already represented by province of residence.

When age was analyzed as a categorical variable, it showed brucellosis patients to have greater odds of being older than 44 years as compared to those aged 17 and younger than parasitosis patients. Yet, age as a continuous variable was not significant in the

model, but did act as a confounder of the categorical occupational variable. Controls had a greater proportion of individuals under the age of 18 compared to cases in this study. If the majority of these controls were diagnosed with intestinal parasitism then a bias can exist in the study. This is a necessary consideration since children are more likely to exhibit pica behaviors and get intestinal parasite infestations. If children were over-represented in the controls, then in theory both the 18 to 44 year olds and > 44 year olds would have been significant, but the > 44 year olds was the only group approaching significance in the model (OR: 9.3; p-value < 0.1); this may reflect a greater propensity for disease associated with aging. Concurrent illness may make this age group susceptible to disease or disease reoccurrence. Of those involved in animal-related work, 79% are from the 18 to 44 age group and 18% are from the > 44 age group. The older age groups did not have a greater animal exposure. It would seem that the increased odds of being greater than 44 years of age amongst the cases are likely due to confounders such as health status, health care access and nutrition.

There may be respondent bias regarding dairy product consumption. All dairy products other than milk itself are made from raw milk. Milk is heated or boiled before consumption though. Eating raw milk dairy products was found to be protective compared to those who had not consumed dairy products, which is in contrast to other studies (Kozukeev et al., 2006; Sofian et al., 2008; Earhart et al., 2009). Georgians are aware of brucellosis and that un-aged cheese can carry disease. Individuals may not admit that they or their children became ill due to this well-known risk factor. Public awareness of the risk from contaminated cheese may create a bias that is more evident in the cases and results in showing that cheese consumption appears to be protective. It is

also important to consider that our controls were patients with various forms of parasite infestations and often children are over-represented in this population. If children also eat more dairy products, then this could also explain the protective seen in that category. Finally, another concern would be that only the severely ill travelled to Tbilisi for diagnosis and treatment. Based on the concept of dose-response, it could be that those working with animals directly receive a larger dose of bacteria and are therefore more ill than those that are infected from dairy products, or the lower dose present in the dairy products does not cause illness in adults. Thus, if the hospital based cases are related to the severity of disease, those who were infected through contaminated food may be under-represented. Having regional diagnostic and treatment centers rather than just one central center could allow for a more accurate reflection of this measure by increasing the ability and probability of individuals to seek care among those with less severe illness.

B. melitensis causes more severe disease in humans than *B. abortus*, so patients infected with *B. melitensis* may be more prone to seek treatment. This increased likelihood of seeking medical care could be a selection bias in the hospital-based sampling resulting in an overrepresentation of sheep ownership as compared to cattle ownership. Further, since a patient has to reach a threshold of at least six clinical symptoms before being serologically tested for disease, there is the possibility of misclassification of cases as non-cases. This would occur among patients with less severe disease and thus less clinical symptoms of disease. This may be another reason that *B. abortus* is underestimated and why the risk factors that highlight *B. melitensis*, such as living in Kakheti and sheep ownership, are overestimated.

Limitations

There are two significant limitations to this pilot study. They are the sample size and the difference in the distribution of age between cases and controls. As an initial study the size and scope of this project were limited and future studies would benefit from being multi-year studies that have sample sizes large enough to conduct analyses stratified by age or by conducting matching case-control studies on the appropriate variables made evident in this research.

The distribution of age in the study was different in cases and controls. The purpose of this study was to identify risk factors and because of this did not match cases and controls in the study design, as this would have prevented the quantification of these variables as risk factors. The impact of age could have affected a number of variables including eating dairy products. Dairy product consumption is a cultural component of Georgian life, but children may be more prone to consume raw milk or to eat more dairy products in general. The impact of age on the magnitude of the odds associated with occupations is evident by the confounding effects of age as a continuous variable on occupations as well. Nonetheless, in this study, age as a continuous variable and age as a categorical variable identify the same risk factors with similar odds ratios.

Sample size was estimated based on expert opinion of the differences of exposures of the risk factors evaluated between brucellosis cases and controls, but the sample size selected did not allow for precision in the confidence intervals. Yet, some variables, despite very wide confidence intervals, were still significant. Further, the variables that approach significance cannot be ruled out as risk factors at this sample size and require further study. Also, this study is representative of Institute of Parasitology

and Tropical Medicine patients and may not be externally valid—a common concern with hospital-based samples because selection bias could result from the ability to access medical care. Ethnic minority populations are the primary inhabitants of some villages in Georgia and the language is not Georgian. They may not pursue treatment and medical care in the current system. The same bias is likely present for the controls as well. Nonetheless, the cases and controls are comparable.

Conclusions

The centrally provided diagnostics and medical care may prevent adequate access to all citizens and this inaccessibility prevents complete identification of brucellosis cases and their associated risk factors. The high odds ratios of certain provinces reflect that the majority of Muslims and sheep are also in these regions. Working with animals is a significant risk factor, but regular animal contact in general is important to consider because occupations did not delineate who did and did not have animal contact. The other key risk factors were being older than 44 years of age, making dairy products and owning sheep. *B. melitensis* may be the causative agent since sheep are significantly associated with disease and are the natural host for this particular pathogen. However, it does not preclude human infections from *B. melitensis* and *B. abortus* from cattle. Methods to reduce disease in the human population should be focused on controlling disease at the sheep level. In addition, education of individuals who work with animals on routes of infection from shedding animals could also play a significant role in reducing disease (Kozukeev et al., 2006).

Further study in this area is warranted and needed based on these results. This research reveals that animal contact is important, but does not clearly determine if there

are different risks at different ages and how this is related to dairy product consumption. Future studies should be multi-year and/or matched for age to ensure a better precision among the risk factors. In addition, future studies may want to focus on regional differences that could exist between Kakheti, Kvemo Kartli, Western Georgia, Tbilisi and central Georgia (Mtskheta-Mtianeti and Shida Kartli).

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Chapter 6: The human-animal interface of brucellosis in the country of Georgia 2010: A rapid assessment analysis.

Karyn A Havas^{1,*}, Marine Ramishvili², Archil Navdarashvili², Paata Imnadze²,

Mo D. Salman¹

¹Animal Population Health Institute
College of Veterinary Medicine and Biomedical Sciences
Colorado State University
Campus Stop 1644
Fort Collins, CO 80523

²Department of Anthrax, Zoonotic and Anaerobic Infections,
National Center for Disease Control and Public Health of Georgia
M.Asatiani St.
Tbilisi, Georgia 0177

Summary

A rapid assessment of animal management and dairy product production and distribution was conducted in the Kakheti region of the country of Georgia during the spring of 2010. The purpose was to understand the disease ecology of brucellosis through the study of human-animal interactions and to identify high risk human and animal groups for disease, topics of education, intervention possibilities and dairy product distribution and animal movement patterns that could contribute to disease spread. A standard questionnaire was used to interview 198 villagers in the eight municipalities of Kakheti as well as key informants. The latter included physicians, veterinarians, laboratory personnel, agricultural specialists and cheese production specialists. The study revealed that animals are managed at the village level. Seasonal pastures are used with the summer pastures being throughout the highlands of Kakheti and the winter pastures, where calving and lambing occurs, are at the village or in lowland pastures found in southeastern Kakheti. Access to animals for sero-surveillance and disease control programs is best when they are on winter pastures. Male villagers take turns shepherding and herding. They also do all the sheep dairy production. Women care for and milk cattle as well as make the dairy products from cow milk, but are not involved in sheep dairy production. Georgians and Azerbaijanis are the main ethnic groups involved in animal production. The area of Kakheti with the widest cheese distribution is the Akhmeta region; the guda cheese from this area is sold all over Kakheti and in central Georgia. On average cheese is aged three days in 20% brine for white cheeses and 21 days in 20% brine for guda cheeses, neither of

which is long enough to kill *Brucella spp.* Further, the local people resist the use of heated or pasteurized milk for cheese production. Although reduction of human disease could be accomplished via changes in cheese production or via animal disease control methods, animal disease control methods are likely to face less cultural resistance.

Introduction

The country of Georgia is a former Soviet state situated between Europe and Asia on the Black Sea. Brucellosis is present in both human and animal populations in the country, but the most common *Brucella spp.* is unknown as is the distribution of animal disease. According to the limited monitoring conducted by the Georgian National Center for Disease Control, the greatest annual incidence of human brucellosis is found in eastern Georgia in the Kakheti region (Navdarashvili et al., 2005). The human disease incidence in Kakheti was anywhere from three to nine times greater than Kvemo Kartli from 2004 to 2008. Kvemo Kartli was the region in Georgia with the next highest human disease incidence. Epidemiologic methods of investigating and controlling the animal level of brucellosis have not been fully implemented in the country.

Brucella spp. are gram negative, intracellular bacteria that can cause disease in livestock, pigs, humans, dogs, horses, camels and wood rats. The four zoonotic species of *Brucella* are *B. melitensis*, *B. suis*, *B. abortus* and *B. canis*. The reservoir species for *B. melitensis* are sheep and goats (Alton, 1990); *B. abortus* cattle and *B. suis* pigs. *B. canis* has a canine reservoir but rarely causes clinical disease in humans (Radostits et al., 2007). Humans are readily infected with *Brucella spp.* through ingestion of contaminated dairy products, inhalation of aerosolized bacteria and via open skin wounds or mucous membranes from a shedding animal or infected environment (Corbel M J, 2006). Human brucellosis is characterized by nonspecific clinical signs that may lead to a chronic disease resulting in the formation of granulomas that most often result in osteo-articular

signs and endocarditis (Franco et al., 2007). In endemic areas, the disease is under-diagnosed and under-reported (Pappas et al., 2006).

Rapid assessment procedures are classified as techniques that gather qualitative data regarding agricultural issues in a time- and cost-effective manner (Harris et al., 1997). Originally agriculturally based, these are more formally called Rapid Rural Assessments (RRA) (Harris et al., 1997). RRAs are a useful intermediate between unstructured research methods and formalized surveys. An intermediate is often needed in settings where there is a paucity of background data, baselines and sampling frames. RRAs also utilize a sampling method known as ‘sampling until saturation’ and a more informal survey technique. This concept recognizes that when trying to characterize a system or program each interview in similar areas return less and less new information about the system being investigated. The informal, more open-ended survey allows for the discovery of patterns in a society (Rhoades, 1985). Saturation is said to occur at approximately 12 interviews and can return a reasonable level of understanding of the system or theme being studied in a society (Guest et al., 2006; Marshall, 1996). Saturation sampling and the following data collection techniques are utilized for RRA: focus groups, observations, individual or household interviews, key informant interviews and secondary data sources (Crawford, 1997).

The purpose of this study was to understand the brucellosis disease ecology and human-animal interface in the Kakheti region of the country of Georgia by describing the dairy production and distribution as well as animal management and pasturing practices through the use of rapid assessment techniques. The intention was to critically evaluate the social, economic and husbandry environment and to identify areas and groups with

higher risk movement patterns that could increase the spread of disease, topics of education, and methods to pursue or avoid with interventions. Findings from this study will be used to construct a zoonotic disease-spread agent-based model that will utilize the patterns of human and animal interaction and not mathematical distributions. The model's aim will be to assess the various control strategies for brucellosis.

Materials and Methods

Data Collection, Sampling Method and Sample Size

Kakheti was used as the area of research due to its extremely high incidence of human disease in comparison to other regions in Georgia. Our goal was to understand the disease ecology of brucellosis in the country's most affected region by using rapid assessment techniques. In order to understand the disease ecology three sources of data were used: observations, villager interviews and key informant interviews. An interviewer used a standard questionnaire^a to collect information about current (2010) animal management and movement, dairy production and product distribution, disease prevalence, risk factors and disease detection methods from villagers, veterinarians both public and privately employed, medical doctors, laboratory workers and dairy production experts throughout Kakheti's eight municipalities [Figure 6.1 (The Parliament of Georgia)]. Individuals also indicated on a map where they sold dairy products, bought inventory and grazed their animals by season. The questionnaire was translated into Georgian, back translated to English to evaluate the effect of translation on the researcher's intent, and pilot tested in the Tbilisi marketplace. The four interviewers (1 male, 3 female) were native Georgians who spoke fluent English as well as Georgian.

^a The questionnaire is available upon request from the corresponding author.

Two were scientists at the National Center for Disease Control and Public Health of Georgia and collaborators on this project, Archil Navdarashvili and Marika Ramishvili. The other two were local university students who were originally from western Georgia. All interviewers practiced using the questionnaires and were involved in the translation process. The first few interviews conducted by each interviewer were reviewed immediately to ensure the appropriate answers were being obtained. Interviewers worked alone unless an interview was conducted at a private home and was between a man and a woman. When this occurred, an additional interviewer of the opposite sex was also present but did not ask questions. Informed consent was obtained and the Colorado State University's Institutional Review Board approved this study on 12 February 2010.



Figure 6.1: The municipalities and town centers of the Kakheti region of Georgia

Convenience and purposive sampling were used to gather at least 12 dairy production and 12 animal management interviews per municipality. The non-probability method of sampling until saturation of the themes associated with animal management and dairy production in Kakheti was employed. Because data from the 2002 Population Census suggested that ethnicities cluster in the different municipalities and topographic

maps indicate that geography is different among municipalities, potential exists for agricultural themes to differ between municipalities (Geostat, 2002). Thus, 12 interviews among dairy product distributors and producers and 12 interviews among animal owners were conducted in each municipality in order to allow for variation within the patterns of these agricultural systems to emerge. In total 236 interviews were conducted: 98 animal management, 97 dairy production, 14 veterinary, 9 physician, 8 human laboratory, 4 veterinary laboratory, and 6 dairy production expert. Of all the villagers approached for an interview, fewer than five declined and the majority of these were in the northwest municipality of Akhmeta (Figure 6.1). In Akhmeta, some villagers were concerned that the group was doing research on account of the government; other individuals did not say why they refused to be interviewed. All of the key informants agreed to be interviewed.

Data Description and Statistical Analysis

As previously stated, the purpose of the study was to reveal trends and patterns in the regions by conducting interviews, but quantitative evaluation was also conducted to assist in recognizing any trends. Because data were not collected using probability sampling, analyses can just suggest patterns. Data were assessed for normality using the Shapiro Wilk test for Normality at $\alpha = 0.05$. For continuous data, if the data were normally distributed, then the mean and standard deviation were reported; otherwise the median and inter-quartile ranges were reported. The T-test and the Mann-Whitney test were used for statistical comparison of normally distributed and non-normally distributed data respectively. For categorical data, the frequency and 95% confidence intervals were calculated. Statistical associations were evaluated with the Chi-squared test or Fisher's exact test and proportions were compared using the Z-test. Multiple post-hoc pairwise

comparisons were assessed using a modified Bonferroni adjustment of the level of significance. Stata and MS Excel software were used. The level of significance was $p = 0.05$.

Results

Dairy Product Production and Market Distribution

Dairy product consumption was common among those interviewed and included drinking milk, boiling the milk prior to consumption and eating dairy products regularly (Table 6.1). The only ethnic difference in dairy product consumption that was noted was the use of erbo (clarified butter) and butter by Azerbaijanis more than other ethnic groups. The consumption of the rest of the dairy products had little ethnic correlation.

Numerous dairy products were made from raw milk, including white cheeses called kartuli or imeruli made from raw sheep or cow milk, although cow milk cheeses were more common. The fresh cheese was placed in brine for storage and to age. Sulguni cheese was made from cow milk and was the only cheese that was boiled during its production process and stored in brine for long term. Factories just brine to taste prior to sale to local restaurants. Guda cheese is primarily made from raw milk collected from sheep grazing on the high pastures in the summer months, and it is the most common use for sheep milk in Kakheti. Guda is generally highly fermented and stored in brine to age and keep. White cheeses are aged in brine for a median of 3 days and guda for a median of 21 days ($p = 0.02$). The median salt concentration of the brine used for all cheeses is 0.20 (IQR: 0.2, 0.2) or approximately 200 grams of salt per liter of water. Matsoni is a yoghurt-like product that is made from boiled milk; Khacho is an acidic cottage cheese;

Nadughi is boiled curd; and Arajani is made from boiled milk and is comparable to sour cream.

Table 6.1: Dairy product consumption summary for 155 villagers from all municipalities in the Kakheti region of the country of Georgia

Dairy Product Intake	Consumes		Boil Milk		Consume daily	
	%	95% CI	%	95% CI	%	95% CI
Milk Consumption	78	71, 84	96	91, 99	55	47, 63
Dairy Product Consumption	99	95, 100	NA	NA	93	87, 96

The following abbreviation is in this table: 95% CI stands for 95% confidence interval.

Dairy product vendors formed three groups with distinct differences: 59% made the dairy products they sold (producers), 17% of producers also bought products to sell; and 41% bought the products that they sold (distributors). Producers got 84% of the milk from their livestock and only a small proportion used milk from their own and other villagers' livestock. In addition, 86% of producers used only cow milk, 2% used only sheep milk, and 7% used both sheep and cow milk. Both producers and distributors sold products made from cow milk, but distributors sold more sheep milk products than producers. Sheep milk was primarily used to make guda cheese; thus an association existed between guda production and sheep milk use ($p < 0.01$). Distributors more commonly sold guda and sulguni, while producers sold matsoni, arajani, khacho and butter (Table 6.2). Commercial dairy production factories in Kakheti were small to medium scale and sold products to retail sources in large towns and cities. Three cheese factories were visited in Kakheti. Pasteurization was not used in any of the factories and milk was stored in bulk tanks at temperatures between 2.5 and 4 C after delivery from local farmers who transported the milk in unrefrigerated containers.

Table 6.2: A comparison and statistical summary of vendors who make and who buy dairy products to sell from all municipalities in the Kakheti region of the country of Georgia

Dairy Product Production	Make Products to Sell		Buys Products to Sell		<i>Z-statistic</i> <i>P-value*</i>
	%	95% CI	%	95% CI	
	59	49, 69	41	31, 51	
	%	95% CI	%	95% CI	<i>Z-statistic</i> <i>P-value*</i>
Sells Dairy Products Year Round	59	49, 69	69	55, 81	> 0.05
Sell Milk Year Round	29	13, 49	22	3, 60	> 0.05
White Cheese	37	24, 51	31	19, 45	> 0.05
Imeruli	49	36, 63	47	34, 61	> 0.05
Nadughi	12	5, 24	6	1, 15	> 0.05
Arajani	7	2, 17	0	NA	0.05
Cow milk based products	98	91, 100	94	83, 99	> 0.05
Milk	53	39, 66	22	11, 35	< 0.01
Guda	16	8, 28	49	35, 63	< 0.01
Sulguni	4	0.4, 12	20	10, 33	< 0.01
Matsoni	46	32, 59	25.5	15, 39	0.03
Khacho	39	26, 52	3.6	0.4, 13	< 0.01
Sheep milk based products	9	3, 19	53	38, 68	< 0.01
Butter	21	11, 34	0	NA	< 0.01

The designation 95% CI is an abbreviation for 95% confidence interval. The sample size for villagers who bought dairy products they sold was 55 for all but milk where 51 individuals responded. There were 57 villagers that made the dairy products they sold. Ho: the proportion of those who bought the dairy product would be the same as those who made them.

*In order to be significant the pair wise comparisons used a modified Bonferroni correction for the level of significance of 0.05. The modified Bonferroni correction requires that the p-value be less than 0.037 in order to be significant.

Local village residents make most dairy products, but the main source of guda is the town of Akhmeta and its surrounding villages. Distributors commonly bought products from markets in the town of Akhmeta and its surrounding villages, and in Lagodekhi, Kvareli, Telavi and Sagarego to sell locally. Gurjaani vendors had the most geographically extensive sourcing for their dairy product inventory. Also, the roadside

markets in Sagarego are on a major roadway in Kakheti, allowing for a wide distribution of product (Figure 6.1).

Animal Ownership and Use

Among livestock owning households, 96% own cattle and 28% own sheep (Table 3). According to veterinarians and villagers, the Azerbaijani and Georgian ethnic groups most commonly own sheep and cattle, and Azerbaijanis are the most likely to own sheep. Approximately 48% of sheep- and 98% of cow-owning households milk their animals (Table 6.3). These values were similar to the population estimations made by veterinarians for sheep milked, 49%, but their estimates for cattle-milking were much less (69 %) compared to the 98% determined from the survey. Most un-milked cows were heifers, and they will be milked after their first calving. Veterinarian key informants further corroborated these animal uses and stated that Azerbaijanis milked more of their sheep than did other groups. Sheep were used for milk, meat and as a live export commodity, and were slaughtered at a greater rate than cattle. Slaughtering is a male-dominant chore, whether sheep or cattle are being slaughtered. Typically the male head of the household, sons, neighbors or local butchers did the slaughtering. Butchers typically slaughtered cattle but not sheep. Cattle are usually slaughtered at the end of their useful milking life.

Table 6.3: A summary of animal ownership, use, and seasonal pasture from all municipalities in the Kakheti region of the country of Georgia

Animal Ownership	Sheep			Cattle		
	<i>%</i>	<i>95% CI</i>	<i>N</i>	<i>%</i>	<i>95% CI</i>	<i>N</i>
% HH owning livestock	28	19, 37	98	96	90, 99	98
% Livestock-owning HH that milk	48	28, 69	25	98	93, 100	94
% Animal pop used for milk	37	35, 38	3473	55	52, 59	676
% Use seasonal pastures, owning-HH	41	22, 61	27	17	10, 26	98
% Use seasonal pastures, Pop	38	37, 40	3473	37	33, 41	676
	<i>Median</i>	<i>N</i>	<i>IQR</i>	<i>Median</i>	<i>N</i>	<i>IQR</i>
# per owning HH	50	27	7.5, 155	3	94	2, 9
# Animals in village grazing flocks and herds	300	6	250, 1000	50	67	40, 100
# Animals in grazing herds on seasonal pastures	1250	6	1000, 1500	75	13	40, 100
# Months milk	4	19	3, 4	8	106	6, 9

The following abbreviations are in this table: HH stands for household; 95% CI stands for 95% confidence interval; N stands for the number of respondents that the question applied to and responded and IQR stands for inter-quartile range.

Animal Grazing

Local grazing pastures surround the settlements. Sheep and cattle typically grazed separately, but grazing lands were shared by all species. For villagers that owned sheep and cattle, a majority of the species had physical contact. Contact did not typically occur on the pasture, but most of the household's livestock species used the same pasture at different times. And some animals had contact at night. Mingling of livestock from different households is very common while grazing. Village level grazing herds were

more common than household level herds (Table 6.3). Summer and winter pastures were often used as well, and twice the number of sheep flocks used seasonal pastures than did cattle herds. The median flock size in seasonal pasture flocks is 1250 sheep, compared to 300 sheep in village pasture flocks and approximately 50 sheep owned by the typical household. As for cattle, 82 cows grazed together on seasonal pastures in comparison to 50 cows on the village pastures with the typical household owning three cattle. Intermingling of household flocks and herds was very common in the village. Some mingling of animals from different villages and ethnic groups also existed. The most common ethnic group that individuals, who defined themselves as ethnically Georgian, grazed their flocks and herds with were the Azerbaijanis (Table 6.3 & 6.4).

Shepherding and Animal Husbandry

Four shepherds or herders were used on village pastures and five shepherds or herders were used on the seasonal pastures per day for each herd or flock. Shepherds and herders were adult men, either villagers that take turns, animal owners, or hired help and the type of shepherd or herder that a household used on seasonal pasture was not statistically different from the type a household used on village pastures (Table 6.4). Hired shepherds and herders were Azerbaijani and Georgian.

If calving and lambing does not occur at the household, then shepherds and herders are responsible for its oversight. The majority of lambing occurs in January and February, with lambing season from November through May. The majority of calving occurs from January to May. Cattle could birth year round, though birthing normally occurred on winter pastures. Therefore, it would be the shepherds and herders on the local pastures that would be responsible

Table 6.4: A summary of shepherding and mingling of livestock from all municipalities in the Kakheti region of the country of Georgia

	Village Pasture			Seasonal Pasture		
	%	95% CI	N	%	95% CI	N
Overall animal mingling	80	71, 88	95	80	59, 93	25
Animals intermingle from same village	78	68, 86	95	84	60, 97	19
Animals intermingle between villages	3	0.7, 9	95	11	1, 33	19
<u>Shepherds</u>						
<i>Hired</i>	39	28, 50	88	43	23, 66	23
<i>Villagers by turn</i>	35	25, 46	88	30	13, 53	23
<i>Male owner</i>	24	15, 34	88	22	8, 44	23
<u>Species Mingling</u>						
<i>Come into contact</i>	63	53, 73	93	62	41, 80	26
<i>Same pasture at different times</i>	98	87, 100	41	93	68, 100	15
<i>Contact at night</i>	36	23, 51	50	38	14, 68	13

The following abbreviation is in this table: 95% CI stands for 95% confidence interval. N stands for the number of respondents that the question applied to and responded. Percentages may not sum to 100, because only the most common categories were reported among the trends identified.

Both men and women milk by hand. A significant association between the use of seasonal pastures and the gender of the milker was found. Men more often milk when they use seasonal pastures and women more often milk when animals are grazed on village pastures (Table 6.5). The gender association of milking with specific pastures corroborates the use of sheep on seasonal pastures more often, as men are the only ones who milk sheep. Cattle and sheep were milked a median of eight and four months, respectively (Table 6.3). The peak of the cow-milking season is June, July and August, and the sheep-milking season is May, June, July, and August. Women made the cow

milk products, but shepherds (men) made the highland cheese from sheep milk. All family members assist with animal care otherwise.

Table 6.5: A summary of gender roles in milking from all municipalities in the Kakheti region of Georgia

	Males who milk n = 17		Females who milk n = 36	
	% (#)	95% CI	% (#)	95% CI
Use Seasonal Pasture	33 (7)	15, 57	19 (4)	5, 42
Don't Use Seasonal Pasture	14 (10)	7, 24	43 (31)	31, 55
P-value	0.04		0.05	

The abbreviation 95% CI stands for 95% confidence interval and the p-value are results from the statistical test of association between males and females who milk and those who do not and the use of seasonal pastures.

Discussion

This RRA was conducted in the Kakheti region of Georgia. Kakheti had the highest incidence of brucellosis in comparison to the other regions of Georgia according to the National Center for Disease Control and Public Health (Navdarashvili et al., 2005). Little is published about the agricultural and the dairy industry in this region, yet have these industries are the main sources for human brucellosis infection. An RRA allowed for a time and cost-effective assessment and provided a better understanding of these vital components of the brucellosis human-animal interface. RRA was a useful method to gain critical information that can assist in simulation model building, intervention determination and surveillance program implementation when working with a constrained time and budget allowance.

Regular human-animal contact is likely the primary source of human brucellosis infection, while dairy product consumption is secondary. However, people in larger towns and villages have less daily animal contact and may have a greater risk of disease from dairy products. A majority of the population in the smaller villages own livestock,

and towns with fewer livestock are reliant on the smaller villages to supply dairy products. Other than what is made to sell, the production in these smaller villages is for subsistence. Akhmeta, which contains the majority of high-mountain pastures, has a widespread guda cheese distribution. Approximately 45.5% of sheep and cattle owners from Akhmeta use seasonal pastures in this municipality. Animals from Kakheti and other regions in Georgia also use these pastures, increasing the potential for brucellosis disease spread within Akhmeta and from here to the rest of Kakheti and Georgia via animals and guda cheese. *B. melitensis* can last for 45 days in 27% brine in sheep cheese, thus the guda consumer does experience some risk (International Commission on Microbiological Specifications for Foods, 1996; Corbel M J, 2006).

The spread of disease among livestock and to humans is likely seasonal and based on calving and lambing cycles as well as milking periods. Sheep shed *B. melitensis* on average for two months, but can excrete the bacteria for up to 180 days post-parturition (Alton, 1990). Lambs generally nurse until May and then sheep are milked from May to August. Because of the duration of bacterial shedding, sheep cheese produced in the May to June time period is more likely to be infected compared to that made later in the season. Cattle can shed continuously through their lactation, so cheese made from cow milk is always a risk. Sheep milk cheese is safest to consume in the winter months, both because it is past the period when sheep are likely to shed bacteria and because the cheese has aged since the end of the sheep-milking season in late summer.

White cheeses and butter are consumed freshly made and are the products with the highest risk for *Brucella* spp. transmission. The cheeses are made of unpasteurized milk, brined for a median of three days, consumed quickly and bought on a regular basis.

Brining can effectively kill bacteria if there is a long enough exposure, but this abbreviated period of brining seen in Kakheti does not provide sufficient time to kill the bacteria. Butter is made from raw milk and is not salted or aged, making it a viable source of infection. In addition, *Brucella* spp. can survive in unsalted butter for up to 13 months (International Commission on Microbiological Specifications for Foods, 1996). Azerbaijanis consume more butter and erbo than others and are thus at increased risk of brucellosis. The lowest risk dairy products are those that are boiled or acidic: sulguni, matsoni, arajani and nadughi. The arajani and matsoni are similar to yogurt products, in which *Brucella* spp. survive for approximately four days (Falenski et al., 2010). Boiling milk will kill *Brucella* spp. However, villagers resist using boiled milk for making cheese because of quality concerns. The same attitudes about boiled milk extend to factory cheeses as well. Industrial production, pasteurization, and associated food standards will be difficult to enforce, especially while individuals have milk-producing animals and while a strong informal economy exists. Therefore dairy products remain a brucellosis health threat.

Brucellosis transmission associated with meat consumption is exceedingly rare, especially from properly cooked meat (International Commission on Microbiological Specifications for Foods, 1996; Corbel M J, 2006). Individuals who slaughter animals are at an increased risk of brucellosis infection, and old Soviet-era teachings have created a general fear of consuming meat from animals infected with brucellosis. Villagers suspect animals that abort of having brucellosis and they sell or slaughter them and dispose of the carcass, but they do not suspect other animals in the herd. These practices spread the infection and affect food security. Both the issues of the ability to safely consume meat

from an infected sheep or cow, the likelihood of other infected animals in the flock and herd, and the spreading of brucellosis through the sale of infected animals should be addressed in an education campaign.

The geo-agricultural framework in Kakheti is around the village. Households are clustered in settlements and the surrounding land is used as pasture, vineyards or cropland and a majority of the public land is used for grazing. Due to shared land resources villagers mix their herds on the pastures and share the work as well. As a result, it is likely that shepherds and other village members can be infected with brucellosis. The exceptions are those who graze their animals separately, but if the grazing land is not private those animals are exposed as well. Thus the village herd or flock is the more meaningful unit of study.

Seasonal pasturing maintains livestock infections and contributes to the spread of infection by preventing closed village herds. Parturition occurs on winter pastures and animal movement occurs shortly thereafter. The contamination of these pastures with parturient discharge occurs during the winter when the mean temperature is approximately 4 C (39 F) (Weather Underground, 2010). *Brucella* spp. can survive for up to 66 days on wet soils at less than 10 C (50 F) (Corbel M J, 2006). During this time period where the weather is cool but temperatures are above freezing, the contaminated environment is a source of infection to the grazing animals. More animals are mixed during movement to either summer or home pastures, increasing the exposure from animals that are shedding due to a late lambing or abortion in April.

Men are most likely to contract the disease from sheep because they are the individuals that handle, milk, and make dairy products from these animals. Both women

and men handle the cattle so all family members are at risk from cattle. Georgian and Azerbaijani ethnic groups are at highest risk of infection because they more commonly own livestock and work as hired shepherds.

Canine vectors in the rural environment are shepherd and feral dogs. These canines are exposed to animals on pasture and the shepherds regularly feed afterbirth to the dogs. Dogs likely eat the afterbirth that is “thrown away” as well, both on pastures and in the village. In rural Kakheti, there is no formal sanitation system and garbage is disposed of in the most convenient method. If dumpsites are used animals can access dumpsites. Therefore, canines can be infected with the Rev 1 strain of *B. melitensis* and with *B. abortus*, so the biological threat that this poses needs further study (Baek et al., 2003; Hinic et al., 2010).

Georgian laboratories have primarily cultured *B. melitensis* and an occasional *B. abortus* from human blood samples. Both *B. melitensis* and *B. abortus* have been cultures from animal samples, sheep and cattle respectively. Cattle can be incidental hosts of *B. melitensis* and can contribute to its zoonotic disease spread. Sheep can be infected with *B. abortus*, but they are not as readily infected with *B. abortus* as cattle are with *B. melitensis* (Corbel, 2006; Radostits et al., 2007).

Limitations

The sampling method and sample size may impact representativeness and power in this study. The quantitative summary used here is not meant to necessarily accurately estimate the frequency of animal management practices in this region, but to identify common practices and common dairy production and animal management methods. To further clarify some of these trends and to identify any variation, the input of social

scientists as well as representative sampling of villages and towns with a comprehensive survey would need to be done.

Conclusions

A Brucellosis Control Plan is feasible despite the animal movement and environmental contamination that occurs. Animals are likely the greatest source of human disease and a control program focused on the herds and flocks will be more successful rather than interventions focused on food products, especially since there is strong public resistance to pasteurized milk products. Animals are available in the winter while on lowland seasonal pastures or near the village. This is an ideal time to conduct serologic surveys, vaccination or test and slaughter. Many of the grazing lands are public lands maintained by the Georgian government. Implementation of testing and vaccination requirements can help reduce the spread of disease on these pastures. The local private veterinarians that know the local community are unemployed and can act as a workforce to undertake these activities in partnership with the government veterinary services. In addition, Georgia is developing a live sheep export market. These animals must be certified brucellosis free prior to being exported. The flocks in which brucellosis has been successfully controlled could be a source of replacement animals in a test and slaughter program. This would require meeting the World Organization for Animal Health's Terrestrial Code testing standards.

Conflict of interest statement:

Dr. M.D. Salman is the Editor in Chief of this journal. He receives honorarium for this role. The decision of acceptance or rejection of this manuscript, however, is not handled by Dr. Salman.

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Chapter 7: A brucellosis disease control strategy for the country of Georgia: an agent-based model

Karyn A. Havas¹, Randall B. Boone², Ashley E. Hill³, and Mo D. Salman¹

¹ Animal Population Health Institute, College of Veterinary Medicine and Biomedical Sciences
Campus Stop 1644
Colorado State University
Fort Collins, Colorado 80523-1644

² Natural Resources Ecology Laboratory
Campus Stop 1499
Colorado State University
Fort Collins, Colorado 80523-1499

³ California Animal Health and Food Safety Laboratory System
University of California
PO Box 1770
Davis, CA, 95617

Summary

Brucellosis is the most common bacterial zoonotic disease and is present in livestock and humans in the country of Georgia. By far the most common bacterial species cultured in both groups is *B. melitensis* (Malania et al., 2009; Onashvili et al., 2009). Georgia currently lacks sufficient data to build a reliable population-based simulation model of human-animal interactions in the country. Therefore using previous studies that investigated animal management and dairy production practices in Georgia as well as human risk factors, an agent-based simulation model was constructed to evaluate the effect of animal-level infection control program on human incidence and sheep flock and cattle prevalence of brucellosis in the Kakheti region of Georgia. This model simulates the patterns of interaction of individual agents, specifically human animal workers, sheep flocks and cattle herds to return population-based data that is a summation of all of these interactions (Bonabeau, 2002; Grimm et al., 2005). This is a model with primarily deterministic population-level variables that randomly designates characteristics to agents as is appropriate based on the agent's intrinsic societal patterns of interaction. The model was able to estimate the current livestock brucellosis prevalence by municipality, which was used as the baseline brucellosis prevalence in cattle herds and sheep flocks. The infection control program was run until the herd and flock prevalence fell below 2% a

level that is acceptable in an important reduction in the spread of the disease. Among the animal-workers, shepherds had the greatest disease reduction as a result of the infection control program. Cattle had the greatest influence on the incidence of human disease. Control strategies should include sheep and cattle, confirmation of the species of brucellosis present in the cattle population, and should be conducted at the municipality level. This approach can be considered as a model to other countries and regions when assessment of control strategies are needed but data are scattered.

Introduction

The country of Georgia is a former Soviet state with a highly agrarian society. The region of Kakheti with its eight municipalities has the highest incidence of brucellosis in the country (Navdarashvili et al., 2005) (Figure 7.1). This region is home to the majority of the national sheep flock and also has a large proportion of the country's cattle herd (Kvinikadze et al., 2010). Brucellosis is present in both animals and humans. Data show the presence of both *B. melitensis* and *B. abortus* infections in the human population, with *B. melitensis* being cultured much more commonly than *B. abortus* from human cases (Malania et al., 2009; Havas et al., 2011b). A case control study showed the highest human risk was associated with animal-related work, dairy production and sheep ownership (Havas et al., 2011a). Population level disease parameters and contact rates were not available for the various populations at the municipality level, but patterns of human-animal interaction have been studied, thus allowing for simulation of the activities at the individual level despite the inability to simulate disease through the processes of the population.



Figure 7.1: The municipalities and town centers of the Kakheti region of Georgia

Countrywide infection control campaigns programs are economically challenging, so it is important for policy makers to understand the cost and benefits when planning such campaigns. Simulation modeling can be an effective way to estimate the potential outcomes, costs of control program or effective surveillance program parameters. These models are commonly equation-based and estimated at the population level (Gonzalez-Guzman and Naulin, 1994; Roth et al., 2003; Zinnstag et al., 2005). Simulation modeling in veterinary medicine and public health is often done by applying inputs and their parameters at the population level to assess various outputs. Another option is to conduct agent-based modeling, where the behaviors and interactions of individuals and smaller units within a population aggregate to yield population-level outputs. Agent-based models are useful for simulating complex and heterogeneous populations where population-level parameters are unknown or inadequate (Parunak et al., 1998; Page, 1999; Grimm et al., 2005; Janssen and Ostrom, 2006).

Brucellosis is the most common zoonotic bacterial disease worldwide (World Health Organization, 2006). It is difficult to differentiate it from other similar infections

and difficult to successfully treat because it can recrudescence due to the fact that it is an intracellular pathogen that can cause recurring bacteremia and requires sustained antimicrobial therapy. Acute human disease is characterized by recurring fever, malaise and lethargy. Chronic disease most commonly results in endocarditis and osteoarticular complications that have long-term health impacts (Corbel, 2006; Pappas et al., 2006; Franco et al., 2007). The source of human infection is direct or indirect contact with livestock, specifically sheep and goats, swine and cattle—the host species for the zoonotic species *Brucella melitensis*, *B. suis*, and *B. abortus*, respectively (Moreno et al., 2002; Corbel, 2006). Transmission is via ingestion of the bacteria in contaminated dairy products (International Commission of Microbiological Specifications for Foods, 1996) or by occupational exposures that result in ingestion, inhalation or through broken skin by direct contact with an infected animal or the contaminated environment (Corbel, 2006; Blasco and Molina-Flores, 2011). Infected animals contaminate the environment with bacteria through their milk, afterbirth, post-parturition vaginal discharges and aborted tissues, which are the primary route of spread of infection amongst livestock (Corbel, 2006; Olsen and Tatum, 2010). Human-to-human transmission has occurred only on rare occasions and is primarily associated with sexual transmission (Corbel, 2006; Blasco and Molina-Flores, 2011).

Control of human brucellosis is only through control of the infection in livestock. Control programs rely on test and slaughter in conjunction with vaccination strategy (Corbel, 2006; Blasco and Molina-Flores, 2011). The Rev 1 vaccine is used against *B. melitensis* in small ruminants and interferes with serologic testing (Blasco, 1997; Olsen and Tatum, 2010), and the S19 strain and RB51 are used in cattle. The S19 vaccine

interferes with serologic tests while the RB51 does not, but both confer similar immunity (Shurig et al., 2002; Blasco and Molina-Flores, 2011). All vaccines are capable of causing abortions in pregnant livestock and protect approximately 65% of the livestock that are vaccinated (Alton, 1990; Zinnstag et al., 2005). *B. suis* does not have an effective vaccine (Radostits et al., 2007).

The purpose of this study was to assess specific control strategies by applying an agent-based model to reflect the interaction of different types of animal workers with their livestock and the associated risk of brucellosis. This type of study is important since there is a paucity of national-, regional-, or municipality-level data on animal prevalence or effective contact rates. This model was used to estimate the reduction in human disease that could be achieved via a Rev 1 vaccination campaign among sheep flocks, and a test and slaughter and RB51 vaccination campaign within cattle herds at the municipality level. The Georgia Ministry of Agriculture Veterinary Services can use this study to assist in the planning of a control program in the Kakheti region of Georgia. Further use of this approach can be used in other countries and regions with similar situations with lack of reliable field data.

Materials and Methods

Sources of data

Data from previous studies and the 2004 Agricultural Census and Georgian statistical yearbooks from 2009 and 2010 were used to create a conception of society via an agent-based model (National Statistics Office of Georgia, 2005; Kvinikadze et al., 2009; National Statistics Office of Georgia, 2010; Havas et al., 2011a; Havas et al., 2011b). The annual human brucellosis incidence risks for the municipalities in Kakheti

used to calculate the baseline risk were provided by the Georgian National Center for Disease Control and Public Health, Zoonotic and Anthrax Branch (Navdarashvili et al., 2005) (Table 7.1 & 7.2).

Table 7.1: Deterministic input data for populations and demographics for livestock ownership in the Kakheti region of Georgia by municipality

Kakheti Municipality	Annual Incidence per 100,000*	Ag. Pop. that own sheep, %	Ag. Pop. that own cattle, %	Total Human Population	% pop. involved in agriculture	# Flocks	# Herds
Akhmeta	63	8	53	42,000	94	976	6,837
Dedoplistkaro	98	10	40	30,500	95	907	3,809
Gurjaani	31	3	20	69,900	95	606	4,332
Kvareli	16	4	36	37,100	95	438	4,187
Lagodekhi	45	3	45	51,800	94	444	7,296
Sagarego	7	7	40	59,400	98	1,236	7,468
Signagi	10	10	23	43,300	83	1,343	3,134
Telavi	3	3	18	70,500	90	575	3,942

Ag. Pop. is abbreviated for the agricultural population. The incidence of human brucellosis reports the maximum incidence reported in the municipality between 2004 and 2008.

References: (National Statistics Office of Georgia, 2005; Kvinikadze et al., 2009; National Statistics Office of Georgia, 2010; Navdarashvili et al., 2005).

* per 100,000 persons from 2004 to 2008

Table 7.2: Equations and input variables used to build the agents and their risk of disease within the disease control model for brucellosis for Kakheti, Georgia

Equations

Town population: (deterministic data)*

$$\text{pop}_{\text{ag,town}} = \text{pop}_{\text{town}} \times \%_{\text{ag,m}}$$

Eq. 1

$$\text{pop}_{\text{non-ag,town}} = \text{pop}_{\text{town}} - \text{pop}_{\text{ag}}$$

Eq. 2

$$\text{pop}_{\text{non-ag,male,town}} = \text{pop}_{\text{non-ag,town}} \times \%_{\text{male}}$$

Eq. 3

$$\text{pop}_{\text{non-ag,female,town}} = \text{pop}_{\text{town}} - \text{pop}_{\text{non-ag,male,town}}$$

Eq. 4

Town agricultural population: HH is an abbreviation of household (deterministic data)*

$$\text{pop}_{\text{ag,male,town}} = \text{pop}_{\text{ag,town}} \times \%_{\text{male}}$$

Eq. 5

$$\text{pop}_{\text{ag,fem,town}} = \text{pop}_{\text{town}} - \text{pop}_{\text{ag,male,town}}$$

Eq. 6

$$\#HH_{\text{ag,town}} = \text{pop}_{\text{ag,town}} / \#Person_{\text{HH}} \text{ specific for each municipality}$$

Eq. 7

$$\#HHag_{sh/town} = \#HHag_{town} \times \%ag_{sh} = \# \text{ sheep shepherds/town} \rightarrow \text{male only}$$

Eq. 8

$$\#HHag_{catt/town} = \#HHag_{town} \times \%ag_{catt} = \# \text{ cattle shepherds/town} \rightarrow \text{male only}$$

Eq. 9

$$\#HHsh_{milk/town} = \#HHag_{town} \times \%ag_{sh} \times \%sh_{milk} = \# \text{ sheep milkers/town} \rightarrow \text{male only}$$

Eq. 10

$$\#HHcatt_{milk/town} = \#HHag_{town} \times \%ag_{catt} \times \%catt_{milk} = \# \text{ cow milkers/town}$$

Eq. 11

Village populations: (deterministic data)*

$$pop_{vill} = pop_m - pop_{town} = \text{total } pop_{vill} / \#vill_m$$

Eq. 12

$$pop_{ag, vill} = pop_{vill} \times \%ag_m$$

Eq. 13

$$pop_{non-ag, vill} = pop_{vill} - pop_{ag}$$

Eq. 14

$$pop_{non-ag, male, vill} = pop_{non-ag, vill} \times \%_{male}$$

Eq. 15

$$pop_{non-ag, fem, vill} = pop_{vill} - pop_{non-ag, male, vill}$$

Eq. 16

Village agricultural population: HH is an abbreviation of household (deterministic data)*

$$pop_{ag, male, vill} = pop_{ag, vill} \times \%_{male}$$

Eq. 17

$$pop_{ag, fem, vill} = pop_{vill} - pop_{ag, male, vill}$$

Eq. 18

$$\#HHag_{vill} = pop_{ag, vill} / \#Person_{HH} \rightarrow \text{specific for villages in a municipality}$$

Eq. 19

$$\#HHag_{sh/vill} = \#HHag_{vill} \times \%ag_{sh} = \# \text{ sheep shepherds/village} \rightarrow \text{male only}$$

Eq. 20

$$\#HHag_{catt/vill} = \#HHag_{vill} \times \%ag_{catt} = \# \text{ cattle shepherds/village} \rightarrow \text{male only}$$

Eq. 21

$$\#HHsh_{milk/vill} = \#HHag_{vill} \times \%ag_{sh} \times \%sh_{milk} = \# \text{ sheep milkers/village} \rightarrow \text{male only}$$

Eq. 22

$$\#HHcatt_{milk/vill} = \#HHag_{vill} \times \%ag_{catt} \times \%catt_{milk} = \# \text{ cow milkers/village}$$

Eq. 23

Risk equations:

$$Risk_{dairy-producers} = risk_{baseline} \times OR_{dairy-producers}$$

Eq. 24

$$Risk_{cow-milkers} = risk_{baseline} \times OR_{animal-owners}$$

Eq. 25

$$Risk_{sheep-milkers} = risk_{baseline} \times OR_{sheep-owners} * OR_{animal-owners}$$

Eq. 26

$$Risk_{sheep-shepherds} = (risk_{baseline} \times OR_{sheep-owners} * OR_{animal-owners}) / \# \text{ days shepherd per year}$$

Eq. 27

$$Risk_{cow-shepherds} = (risk_{baseline} \times OR_{animal-owners}) / \# \text{ days shepherd per year}$$

Eq. 28

$$\text{Risk}_{\text{baseline}} = B$$

Solved for B by municipality:

$$\text{Annual human incidence risk}^{**} = (\% \text{pop}_{\text{nonag}} \times B) + (\% \text{pop}_{\text{ag-nolivestock}} \times B) + (\% \text{pop}_{\text{ag}} \times \% \text{ag}_{\text{sh}} \times \% \text{pop}_{\text{male}} \times B \times \text{OR}_{\text{sheep}} \times \text{OR}_{\text{animalwork}}) + (\% \text{pop}_{\text{ag}} \times \% \text{ag}_{\text{cow}} \times \% \text{pop}_{\text{male}} \times B \times \text{OR}_{\text{animalwork}}) + (\% \text{pop}_{\text{ag}} \times \% \text{ag}_{\text{cow}} \times \% \text{pop}_{\text{female}} \times B \times \text{OR}_{\text{makedairyproducts}})$$

If an individual randomly comes into contact with an infected herd or flock, then the individual risk was assessed by using a random number generator that sampled from Uniform (0,100). If the randomly selected number was \leq to the assigned risk for that individual that individual developed brucellosis.

Deterministic baseline human brucellosis annual incidence risks by municipality (risk_{baseline} by municipality)

Akhmeta	= 0.8 per 100,000 persons
Dedoplistkaro	= 1 per 100,000 persons
Gurjaani	= 1 per 100,000 persons
Kvareli	= 0.4 per 100,000 persons
Lagodekhi	= 1 per 100,000 persons
Sagarego	= 1 per 100,000 persons
Signagi	= 0.1 per 100,000 persons
Telavi	= 1 per 100,000 persons

Deterministic odds ratios of risk factors used in the model^a

OR _{dairy-producers}	= 12.4
OR _{animal-owners}	= 77.8
OR _{sheep-owners}	= 19.3

Deterministic values for interventions used in the disease control program

RB51 and Rev 1 vaccine field efficiency = 65%[‡]
 Percentage of population immune based on population vaccination coverage:
 (% vaccine efficiency x % pop. coverage x 100)
 50% population coverage: 32.5%
 60% population coverage: 39%
 80% population coverage: 52%
 100% population coverage: 65%
 Rose Bengal Test Sensitivity Range[†]: 21% to 98.3%
 Rose Bengal Test Specificity Range[†]: 68.8% to 100%

Population level deterministic data was applied to agents using a random-number generator that sampled from Uniform (0, 100). If the randomly selected number was \leq to the population-level variable, the agent was classified as having that characteristic.

References for data: * (National Statistics Office of Georgia, 2005; Kvinikadze et al., 2009; National Statistics Office of Georgia, 2010); ** (Navdarashvili et al., 2005); ‡ (Alton, 1990; Zinnstag et al., 2005); and † (Nielson, 2002)

** Data was obtained from the National Statistics Center of Georgia (GeoStat) without any associated variation

^a These odds ratios were obtained from a pilot case-control study with a limited sample size (Havas et al. 2011a). The risk factors were significant but there was little precision and the wide confidence intervals precluded their use in the model.

Overview of model organization

In summary the model was built to simulate human cases in the following manner. A village within each municipality contains village level and household level sheep flocks and cattle herds, all of which are the units of interest. Both levels of animal groupings interact with human workers within the village. The interaction of people with the livestock through shepherding, herding, milking and dairy product production activities accounted for contact of individuals with livestock. Household sheep flocks and cattle herds were randomly defined as infected at the start of each simulated year. When individual humans came into contact with the infected livestock based on patterns of human-animal interaction in Kakheti, the human either developed infection or did not based on their risk of infection (Table 7.2). Disease spread between flocks and herds was not simulated. The model also simulates an infection control program that utilizes Rev 1 sheep flock vaccination for the duration of the control program and cattle Rose Bengal Test and culling of positive cattle herds (first 2 yrs) as well as herd RB51 vaccination (yrs 3 to 5). Figure 7.2 depicts the visual representation of the model. This model is constructed of primarily population-based deterministic variables that used random selection to apply the variable characteristics to the appropriate agents as defined by the intrinsic societal patterns of interaction. This is done throughout the simulation by comparing the value of the variable of interest to a randomly selected number. The random number is selected by using a random-number generator, which samples from a uniform distribution with a range from 0 to 100. If the randomly selected number was less than or equal to the deterministic variable, then characteristic (infected, vaccine immune, milking flock or herd, Rose Bengal test positive, etc) was true for that agent.

The available data were not adequate to provide more specific distributions. Even data on human disease incidence is used only from a four-year period due to political upheavals in the country that prevent assuming a uniform data collection method from before and after 2004. The variation seen in the model develops more from the patterns of interaction based on the individual's type of work and whether they interact with sheep flocks and cattle herds at the village or household level collected from a rapid rural assessment (Havas et al, 2011b) rather than from distributions.

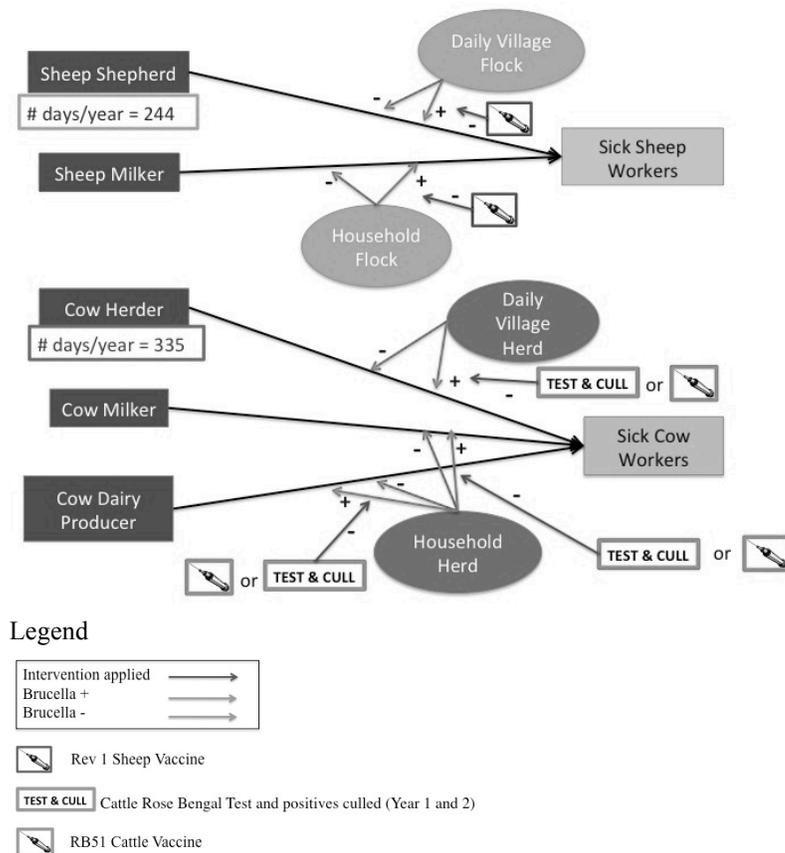


Figure 7.2: The conceptual map for the agent-based model of brucellosis in the Kakheti region of Georgia

The model virtually recreated Kakheti's eight municipalities. The number of villages for each municipality were known and created in the model along with their human and animal populations (personal communication Giorgi Kvinikadze, GeoStat

data on the number of villages and towns per municipality) (Kvinikadze et al., 2009, 2010). Table 7.2 depicts the data used as deterministic inputs to the model. For each municipality the percentage of households involved in sheep and cattle farming and the household size were collected from the 2004 Agricultural Census. The units of interest were the humans who worked with animals, the sheep flock and cattle herd. The number of animals per flock and cattle herd in each municipality was estimated by standardizing animal population numbers from 2004 to 2010 by assuming that the same number of households owned animals and those animals were evenly divided among the households. This assumption was applied at the municipality level, so the differences in herd sizes between municipalities still included in the model. The human risk was assessed based on the animal work done by the human and whether or not the sheep flock or cattle herd was infected and not how many animals within a flock or herd were infected. Based on results from a questionnaire survey conducted by the senior author, it was determined that four shepherds or herders were used per 300 sheep and per 75 cattle on grazing lands (Havas et al., 2011b). Open source NetLogo software was the platform used to create the model (Wilensky, 1999).

Several human animal-work roles in each community were simulated. The number of shepherds and herders in a village as well as the number needed each day were calculated based on sheep flock or cattle herd number and size and grazing flock or herd size at the village level (Havas et al., 2011b). Shepherds and herders interacted with village level animal groups. It was assumed that every household that had a sheep flock or cattle herd also had a shepherd or herder. Households in the village shared grazing responsibilities and households rotated shepherding and herding duties. All of the

shepherds were male (Havas et al., 2011b). Sheep milkers milked and made the dairy products and both activities were characterized in their disease risk. Only 48% of households milk their sheep flocks, and each household was assigned a male sheep milker and each sheep milker was randomly assigned a household milking flock. Of the cattle-owning households, 98% of them were milked (Havas et al., 2011b). Each household that owned milking cows had a milker and a dairy product producer. Making dairy products from cow milk is a female role. In the model, women are just categorized as cow milk dairy product producers and cow milkers were men, and each one of these individuals was randomly assigned a household herd. The reason was that the respondents of the case-control study that provided the odds ratio for the risk factors only had men who milked the cows and women who made the dairy products despite other studies indicated that both genders typically milked cows (Havas et al., 2011b). Therefore, to ensure that the odds ratio used reflected the appropriate groups, cow milking was defined as a male role in the model.

At the start of every model run, all humans were assumed to be uninfected. There were no data available regarding the prevalence of chronic disease or the incidence of recurring infections. The incidence data used came from patients diagnosed and treated (presumably successfully) at the Institute of Parasitology in Tbilisi, Georgia. For milking and dairy product production an individual from a cattle and sheep owning household was randomly assigned to a milking sheep flock or milking cattle herd. If the animal group was infected, then the risk of infection to the milker or dairy producer was assessed on the first day of the year (Eq. 24-26, Table 7.2). The shepherds' and herders' risk were distributed throughout the time period of risk based on how many days they worked and

whether they randomly came into contact with an infected sheep flock and cattle herd that was part of the village level flock and herd with which they worked. If they came in contact with an infected animal group, then their risk of disease was assessed. The time period of risk is from the beginning of the calving or lambing season through milking season given the knowledge about the spread of the disease to the human population. For sheep flocks this is from January through August (244 days), and for cattle herds this is from January through November (335 days) (Havas et al., 2011b) (Eq. 27 and Eq. 28, Table 7.2). For agricultural families who did not own livestock and for non-agricultural populations, a calculated baseline incidence was applied to them and their risk of disease was from ingestion of contaminated dairy products (Table 7.2).

Current data show a higher odds of being infected if an individual owns sheep (odds ratio = 19 (Havas et al., 2011a) and positive human and small ruminant cultures have been primarily *B. melitensis* (Malania et al., 2009; Onashvili et al., 2009). Therefore, this model assumes that the primary cause of human infection is *B. melitensis*, including incidental cattle *B. melitensis*. Interventions included Rev 1 vaccination for sheep flocks and a Rose Bengal Test and cull of positive cattle herds for the first two years and an RB51 vaccination every additional year. RB51 vaccination replaced herd test and cull after two years because the model assumes that at that point in the program cattle herd *B. abortus* infections will be a greater public health risk than incidental *B. melitensis* infections. The lesser risk of *B. melitensis* in cattle is through the control of disease in the sheep populations, the natural host of *B. melitensis*, which spread disease to the cattle and the reduction in the *B. melitensis* infected herds through culling of herds that were test positive during the first two years.

The range of the sensitivities and specificities for the Rose Bengal Test (RBT) were obtained from Nielson (2002) and modeled as a uniform distribution (Table 7.2). Ranges from the literature had to be used due to the fact that the laboratories in Georgia that conduct RBT testing have not established their testing sensitivity and specificity ranges, and when asked do not differentiate between the two terms. The cattle herds that tested positive were culled.

Determination of risk and baseline incidence rates

The highest incidence rate per 100,000 in each municipality from 2004 to 2008 was used to calculate the baseline human disease incidence (Table 7.2). There has been no change in surveillance and monitoring for human or animal brucellosis during that time period and the disease is often under-reported. Therefore, the highest incidence is likely a better representation of the true disease risk in the municipality. The risk of disease within different groups of animal workers was determined by using odds ratios calculated from an incident case control study of brucellosis in Georgia in 2010. Since brucellosis is a rare disease by epidemiological definition (<5%), the odds ratios are similar to the relative risks had a cohort study been done (Dohoo et al., 2003) (Table 7.2).

Estimation of sheep flock and cattle herd prevalence per municipality

The model ran an animal level prevalence of 5% for both flocks and herds, assuming that initially animal groups had the same prevalence. The human incidence output of the model was then compared to the actual incidence data. The goal was to have the model's human incidence be close to but greater than the median of the 2004 to 2008 data. For Lagodekhi, the highest incidence was used because of a large variation in the year-to-year reported incidence rate. The model was not able to simulate human

incidence close to the actual reported median of the incidence from 2004 to 2008. This is likely due to under-reporting of disease from this area. Fifteen iterations of the model at 0.5% increments if above 2% or at 0.25% increments if below 2% of the animal prevalence were run until the human incidence closest to the median was returned.

Since sheep flock and cattle herd prevalence were input variables which baseline was estimated using the model, it was important to note the effect that could be had on the human disease incidence output. Therefore, a variation analysis was run to assess for trends in the human disease incidence output when varying the input cattle herd and sheep flock prevalence by increments of 5% from zero to 50% while holding the other prevalence constant at the level in the model for that year. Five iterations per level were run and such a small sample size precludes the use of statistics on the output data. Thus, the trends are assessed visually. This is not considered a sensitivity analysis due to the limited number of iterations that could be feasibly run by the agent-based model on the available computer processors.

Control strategies as scenarios

Thirty iterations per scenario were run for each of the eight municipalities for each scenario. The mean human brucellosis incidence, sheep flock prevalence and cattle herd prevalence were calculated from the 30 iterations run for each scenario along with the standard error of the mean. This model output was summarized at the municipality level, but the pattern of daily human-herd or human-flock interactions within all the villages and towns of the municipality created each municipality's output. A baseline was run with no animal level interventions and the outputs from this baseline scenario were used to assess the efficacy of the infection control program. After the baseline

outputs were established the cattle herd and sheep flock level interventions were included until the municipality-level cattle herd and sheep flock prevalence fell below two percent, at which point a more intensive eradication program should be initiated in the municipality (Alton, 1990; Hegazy et al., 2009).

The first two years of the infection control program simultaneously used sheep vaccination and cattle herd RBT testing. The Rev 1 vaccination was set at 65% effectiveness (Alton, 1990; Zinnstag et al., 2005) and scenarios with a 50%, 60%, 80% and 100% flock vaccination coverage were run (Table 7.2). Each level of population coverage also tested 50% of the cattle herds using the RBT. Test positive herds were culled.

The most advantageous vaccination coverage was the scenario with the greatest reduction in sheep worker cases (and cattle worker cases after the second year of the intervention) based on non-overlapping standard errors of the mean number of workers, and if no scenario met this definition, then the vaccination coverage with the greatest mean sheep flock (and cattle herd after the second year) prevalence reduction with non-overlapping standard errors was chosen. Since mass vaccination of an entire population is not always economically feasible (Banai, 2002), a program could instead choose annual benchmarks that provide the greatest benefit to the human population and still result in a reduced animal group prevalence. Since complex agent-based models are limited by computer processing speeds only 30 iterations could be run. Due to this small number of iterations, the non-overlapping standard errors were the appropriate method for identifying the effective control strategies; more stringent statistical tests and the use of 90% or 95% confidence intervals would not have been able to identify an

advantageous scenario. This same scenario was duplicated for year two of the program. For the first two years of the program, 0%, 75% and 100% cattle herd-testing coverages were run for 30 iterations with the most ideal vaccination population coverage for that year. In the third year cattle herd test and cull was replaced with an RB51 vaccination with 65% vaccine effectiveness (Zinnstag et al., 2005) and the same population coverage as the sheep flocks. The dual vaccine intervention was repeated until the flock and herd prevalence fell below 2%.

The mean municipality-level human brucellosis incidence, sheep flock prevalence and cattle herd prevalence were compared within a municipality for each year of the infection control program using an one-way ANOVA or Kruskal-Wallis based on the normality of the data with a 5% level of significance. Then the within municipality-level human brucellosis incidence, flock prevalence and herd prevalence were compared by year using pair-wise comparisons that used a Bonferroni adjustment test with an overall level of significance of 0.05 (Kleinbaum et al., 2008b). The normality of the output data was assessed using the Shapiro-Wilk Normality for each year of the intervention.

Results

Animal level prevalence

Prior to running simulations to evaluate the animal level interventions, the model was used to estimate the animal level prevalence for each municipality (Table 7.3). Akhmeta had the highest prevalence followed by Telavi, Signagi, Lagodekhi, Dedoplistkaro, Sagarego, Gurjaani, and Kvareli.

Table 7.3: Human brucellosis incidence and the associated estimated animal level prevalence used as the input for the baseline animal prevalence for each municipality in Kakheti, Georgia

Kakheti Municipality	Reported Median Human Incidence*	Estimated Herd/Flock Prevalence	Simulated Human Incidence*
Akhmeta	41	22%	41
Dedoplistkaro	23	4%	24
Gurjaani	14	0.75%	17
Kvareli	8	1.75%	12
Lagodekhi**	46	5%	48
Sagarego	44.4	2%	51
Signagi	5	5%	6
Telavi	26	12%	27

The herd or flock prevalence was estimated by choosing the prevalence that best approximated the median human incidence from data collected from 2004 to 2008.

* per 100,000 persons (Navdarashvili et al, 2005)

**Lagodekhi's incidence was determined using the maximum end of the range of the data from 2004 to 2008, due to large variation between yearly reported numbers.

Rev 1 flock vaccination and cattle rose bengal test and cull

Three municipalities began with a sheep flock and cattle herd prevalence below 2%—Kvareli, Gurjaani and Sagarego and were excluded from the infection control program. In the first year, a 50% flock vaccination coverage was the best for all but Dedoplistkaro and Akhmeta, which used 60% and 80% respectively. No other municipality had both their animal groups' prevalence fall below 2%.

In the second year Akhmeta used 60% flock vaccination coverage and the remaining municipalities used 80% coverage. Dedoplistkaro, Signagi and Lagodekhi all had sheep flock prevalence that fell below 2%, but cattle herd prevalence remained above 2%. Even if cattle testing and positive animal culls covered 100% of the populations during the second year, the prevalence still would not have been less than 2%.

Rev 1 flock vaccination and RB51 herd vaccination

The third year used the cattle RB51 vaccination in lieu of test and cull procedures. Dedoplistkaro, Signagi and Lagodekhi required herd vaccination coverage of 50%, 60%

and 80%, respectively, and Akhmeta and Telavi required 80% flock and herd coverage. At the end of this year, Signagi, Dedoplistkaro and Lagodekhi had sheep flock and cattle herd prevalence below 2%. Overall, the estimated human incidence rates, flock and herd prevalences for all three municipalities were significant and the greatest impact was seen among the shepherds and herders (Table 7.4 & 7.5).

The fourth year of the program used vaccination for both animal groups again. Akhmeta's most beneficial vaccination coverage was 60%, which dropped the sheep flock prevalence to below 2%. Telavi used 80% coverage of animal groups, which dropped the sheep flock and cattle herd prevalence to 1% and 1.6% respectively and again the greatest impact on the incidence was seen among shepherds and herders (Table 7.4 & 7.5).

For the final year, Akhmeta used 80% vaccination coverage of cattle herds and the herd prevalence fell below 2%. Overall, flock and herd prevalence fell from 22% to 1.7% and 1.9% and the benefits of the control program were seen among shepherds and herders, just as all the other municipalities (Table 7.4 & 7.5).

Table 7.4: Mean annual estimates of output for human disease incidence risk, flock prevalence and herd prevalence for the baseline and final years in the brucellosis disease control program for municipalities in the Kakheti region of Georgia

Kakheti Municipality		Akhmeta	Dedoplistkaro	Lagodekhi*	Signagi	Telavi
Baseline						
Baseline Human Incidence^a		41 ¹⁻⁵ (2)	24 ²⁻³ (2)	48 ²⁻³ (2)	6 ¹⁻³ (1)	27 ¹⁻⁴ (1)
Baseline Prevalence, %	<i>Herd</i>	22 ¹⁻⁵ (0.1)	4 ¹⁻³ (0.1)	5 ¹⁻³ (0.04)	5 ¹⁻³ (0.1)	12 ¹⁻⁴ (0.1)
	<i>Flock</i>	22 ¹⁻⁵ (0.2)	4 ¹⁻³ (0.1)	5 ¹⁻³ (0.2)	5 ¹⁻³ (0.1)	12 ¹⁻⁴ (0.2)
Year 3						
Vaccination Coverage, %	<i>Herd</i>	80	50	80	60	80
	<i>Flock</i>	80	TS	TS	TS	80
Post Intervention Human Incidence		19 ⁴⁻⁵ (1)	11 (1)	29 (1)	2 (0.4)	18 (1)
Post Intervention Prevalence, %	<i>Herd</i>	6 ⁴⁻⁵ (0.1)	1 (0.04)	1 (0.02)	<2 (0.03)	3 ⁴ (0.1)
	<i>Flock</i>	3 ⁴⁻⁵ (0.1)	1 (0.1)	<2 (0.1)	<2 (0.1)	2 ⁴ (0.1)
Year 4						
Herd and Flock Vaccination Coverage, %	<i>Herd</i>	60	--	--	--	80
	<i>Flock</i>	60	--	--	--	80
Post Intervention Human Incidence		13 (1.03)	--	--	--	15 (0.7)
Post-Intervention Prevalence, %	<i>Herd</i>	4 ⁵ (0.04)	--	--	--	<2 (0.04)
	<i>Flock</i>	2 (0.1)	--	--	--	1 (0.1)
Year 5						
Vaccination Coverage, %	<i>Herd</i>	80	--	--	--	--
	<i>Flock</i>	TS	--	--	--	--
Post Intervention Human Incidence		11 (1)	--	--	--	--
Post-Intervention Prevalence, %	<i>Herd</i>	<2 (0.03)	--	--	--	--
	<i>Flock</i>	<2 (0.08)	--	--	--	--

The mean human disease incidence and herd and flock brucellosis prevalence of the 30 iterations of the associated scenario is reported with the standard error in parenthesis. Sagarego, Gurjaani and Kvareli had flock and herd brucellosis prevalence below 2% and were not included in the disease control program. The superscript number is the significant pair-wise comparisons. Comparisons are within the same municipality comparing the variable of interest.

^a per 100,000

* Used the maximum of the range

^c 50% of cattle are tested and the positives are culled

TS: Sheep flocks and cattle herds are included into a test and slaughter program for populations with prevalence below 2%.

Table 7.5: The percent reduction of the model outputs from baseline through the completion of the disease control program by municipality in the Kakheti region of Georgia

Municipality	Human Incidence	Flock Prevalence	Herd Prevalence	# Shepherds	# Herders	# Milkers	# Dairy Producers	Duration Control Program
Akhmeta	73%	91%	92%	77%	71%	--	--	5 yrs
Dedoplistkaro	57%	70%	65%	62%	59%	--	--	3 yrs
Lagodekhi	39%	68%	74%	--	69%	No difference	No difference	3 yrs
Signagi	76%	67%	68%	75%	84%	--	--	3 yrs
Telavi	43%	92%	87%	91%	82%	No difference	--	4 yrs

The percent differences from baseline were reported in this table for animal workers who had a mean baseline output > 1 sick person per year. Means with overlapping standard errors between the output at baseline and at the end of the disease control program were classified as having “no difference” in the number of cases reported. The municipalities of Gurjaani, Kvareli and Sagarego were excluded from the disease control program since their baseline flock and herd prevalence was $\leq 2\%$.

As for the omnibus multiple group comparisons that used ANOVA or Kruskal-Wallis test as well as pairwise comparisons for the output within the municipalities across the years of the control program, overall all municipalities had statistically significant differences in the output of the mean human disease incidence, flock prevalence and cattle herd prevalence between the years of the control program. Pair wise comparisons of the output among the years of the infection control program revealed that for each year in each municipality there was a statistically significant reduction in flock and cattle herd prevalence. Yet, human disease incidence did not change significantly between the years within a municipality. This lack of difference in the human incidence of disease between years of the control program was seen in all municipalities involved in the control program (Table 7.6).

Table 7.6: The comparisons of the statistical difference between the mean municipality-level human disease incidence outputs between the years of the control program by municipality

	Akhmeta	Dedoplistkaro	Lagodekhi	Signagi	Telavi
	<i>Year of Program</i>				
Years in Program	5	3	3	3	4
<i>Reference Year</i>					
Baseline	All	All	Years 2 - 3	All	All
Year One	Years 3 - 5	Years 2-3	Years 2-3	None	Years 3 - 4
Year Two	Years 3 - 5	None	Year 3	None	Year 4
Year Three	Years 4 - 5	End	End	End	None
Year Four	None				End
Year Five	End				

The years that are significantly different and that came after the reference year are listed in the table. Any unlisted years were not significant. The overall omnibus test was significant for all municipalities and a Bonferroni adjustment was used in the multiple comparisons within the municipality. Animal group prevalence was not included because the level of disease difference was significant for every year of the program that the interventions were run.

Varying livestock prevalence

The variation analysis visually assessed how the change in the estimated baseline sheep flock and cattle herd prevalence would impact human disease incidence. In Akhmeta, Telavi, Lagodekhi, Gurjaani and Kvareli, the variation of flock prevalence appeared to have minimal impact on human incidence, while changes in the cattle herd prevalence resulted in a wide variation in the incidence of human disease. In Sagarego and Dedoplistkaro, the impact of varying flock and herd prevalence appeared to have the same effect on human incidence. Signagi’s human disease incidence showed a greater sensitivity to changes in sheep flock prevalence than cattle herd prevalence. In all municipalities except Signagi and Gurjaani, a herd prevalence of zero resulted in a much lower human incidence than a flock prevalence of zero. Finally, in Dedoplistkaro, Telavi, Gurjaani and Kvareli, livestock flock or herd prevalence of > 20% did not appear to change human incidence as much as flock or herd prevalence < 20% (Figure 7.3).

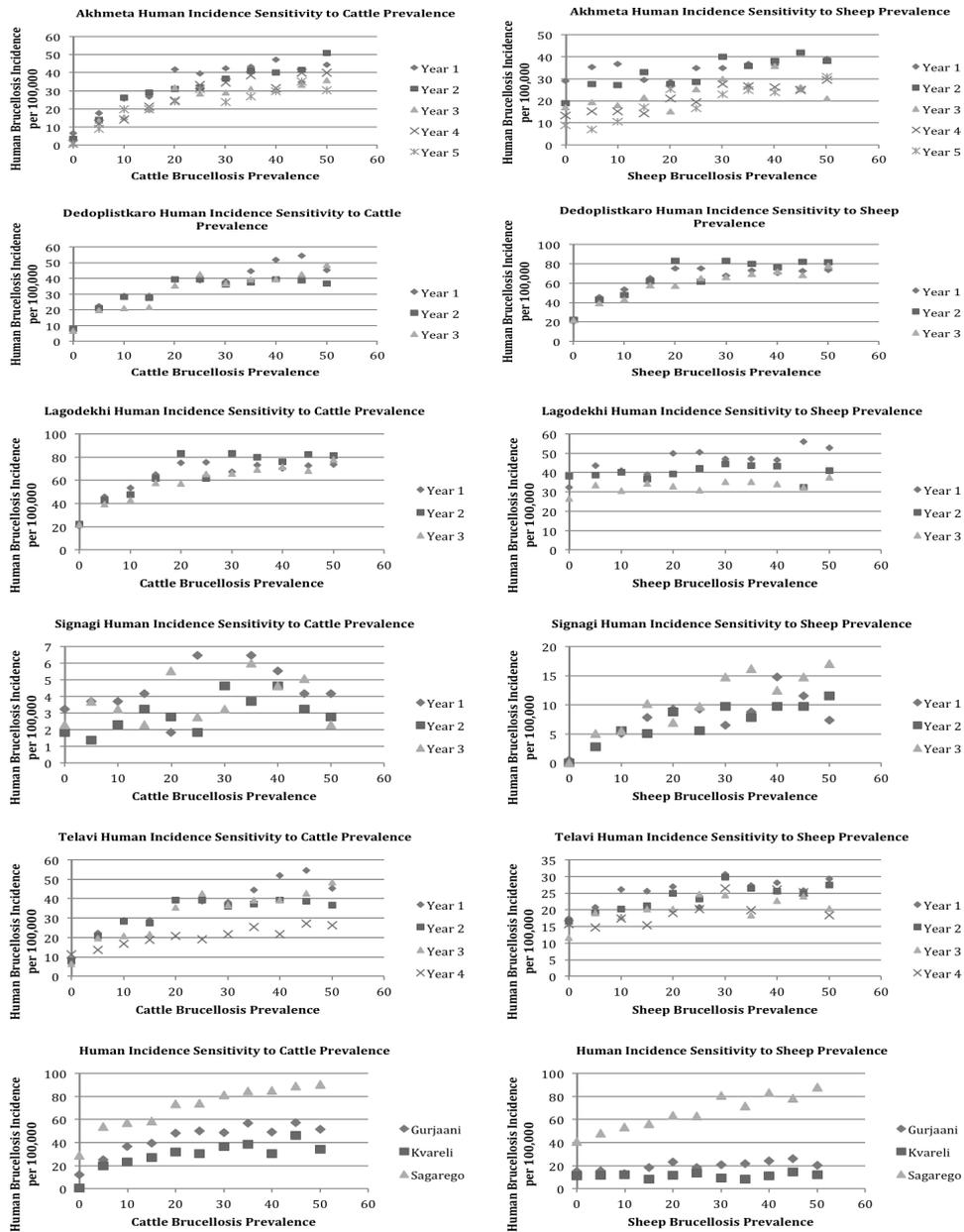


Figure 7.3: Estimated effect from the variation analysis of herd and flock prevalence of *B. abortus* and *B. melitensis* on human brucellosis incidence within Georgia

Discussion

The agent-based model

Brucellosis is endemic among livestock in the Kakheti region of Georgia and it regularly spills into the human population. Transitioning nations face several dilemmas

while identifying optimal control strategies for livestock diseases, especially those of zoonotic significance. Without population parameters such as sheep flock and cattle herd incidence or prevalence, values for cattle herd and flock mixing within the village and between villages and information on effective contact rates, modeling at population levels using equation-based techniques is difficult. This is compounded by the fact that there are differences in flock and herd structures between municipalities in highly agrarian societies, which are not seen in a highly industrialized farming society. The decentralized nature of farming in these regions adds variability to the modeling of the farming. Yet, with the knowledge of human-animal interactions on individual, village and municipality levels, it is possible to use agent-based modeling techniques. Such a model derived from social patterns associated with animal and dairy product production at the various levels of the community can assist in the development of infection control strategies without using overly cumbersome or overly simplified equation-based models. Further validation of the model is possible via the stakeholders because it models individual's patterns of activity rather than processes, making it easier to understand.

Recommendations for a brucellosis control program

The data demonstrate that variation exists among the municipalities. Therefore, the seroprevalence of flocks and cattle herds should be conducted at the municipality level. Gurjaani, Kvareli and Sagarego all had a baseline prevalence $\leq 2\%$. Past eradication efforts have shown that at this level of brucellosis, vaccination alone is ineffective, and that vaccination of replacement livestock and test and slaughter of adults is more successful (Alton, 1990; Hegazy et al., 2009). Therefore, these efforts could be undertaken immediately in municipalities with low prevalence.

Further, in municipalities of moderate prevalence (2% to < 10% infection rates), a three-year campaign with 50% cattle culling was effective. Increasing cattle testing may allow the prevalence to fall below 2% within two years. Yet, in high prevalence ($\geq 10\%$) municipalities, the duration of the intervention cannot be less than three years, even with increased cattle testing. Also, year-to-year livestock interventions did not necessarily result in a significant decrease in the human disease incidence. This was seen primarily when the population involved in agriculture was $\leq 90\%$. Benchmarks beyond using human incidence rates as a proxy for livestock prevalence will be needed to measure progress in an infection control program.

Vaccination was the most efficacious control strategy in this model as evidenced by flocks reaching a prevalence of 2% more quickly than cattle herds. The model's test and cull program did not have laboratory-determined sensitivities and specificities to use, so the complete range was chosen from the literature. In addition, serology is inherently inaccurate with false positives being culled as well as true positives, and this rate can be quite high using a screening test such as the Rose Bengal Test that maximizes sensitivity reducing false negatives, but not specificity (Nielson, 2002; World Animal Health Organization, 2008b, a). Care must be used if implementing this strategy at high prevalence levels so that testing is done properly to maximize sensitivity and specificity. This can help protect needed resources. If cultures reveal that the cattle herd infections are a mixture of *B. melitensis* and *B. abortus*, then vaccination of cattle with RB51 or potentially Rev 1 from the beginning of the control program should be considered (Alton, 1990; Banai, 2002; Shurig et al., 2002).

Shepherds and herders within the municipalities benefited most from the infection control program. Disease incidence within the shepherds and herders decreased more than the incidence within milkers and cow milk dairy producers. The owners of the sheep flocks and cattle herds milk their own animals and make dairy products from their milk. In reality as it was observed by the senior author, there is some sharing of milk in a village, but people primarily produce products from their livestock's milk supply. The same is not true with shepherding and herding. Even if an individual shepherds or herds only his animals, the environment is often shared and the shepherding and herding duties are commonly shared at the village level using public grazing lands. Thus a shepherd or herder is exposed to many flocks and herds, both by direct contact as well as indirect contact by sharing the same environment.

The variation of cattle herd and sheep flock prevalence independently indicated some critical information as well. In most municipalities, cattle herds appear to be the main source of human disease. The current evidence points to *B. melitensis* as the major cause of disease (Malania et al., 2009; Onashvili et al., 2009). Cattle are likely a major contributor because of their greater role in dairy production—98% of cattle herds versus 48% of sheep flocks are used for dairy (Havas et al., 2011b). Thus, there are more individuals exposed to cattle. Cattle are typically the host species for *B. abortus*, yet they can be infected with and shed *B. melitensis* and control of the infection in flocks is key to disease control overall (Banai, 2002; Moreno et al., 2002). In light of this, at the very least, the focus should be on flock vaccination, and required vaccination should take place in order for flocks to share common grazing lands with cattle and to reduce incidental infection in cattle (Banai, 2002). Another option that requires more study

would be to vaccinate cattle with the Rev 1 vaccine (Alton, 1990; Banai, 2002; Shurig et al., 2002).

Assumptions

The majority of the agricultural data were from the last agricultural census in 2004. It is assumed that the same number of households per municipality currently own sheep and cattle as did in 2004, and that the decline in the livestock population is due to a decline in sheep flock and cattle herd size. Cattle herds and flocks were assumed to have equal prevalence at the start of the simulation because these species share the same environment, susceptibility, and grazing patterns and therefore have similar levels of infection. Every household that owns a flock has an assigned shepherd and if the flock is used for dairy, then a milker. The flock milker milks the animals and makes the dairy products. Every household that has a herd also has a herder, and for dairy herds, a cow milker and cow milk dairy producer. In reality, more than one member of the household may do these chores or a shepherd or herder may be hired. The model assumes that the household size of 3.2 persons per household based on data from 2004 has remained the same while using a population size from 2010.

At the start of every simulation, the human population is assumed to be susceptible to disease, and the model does not account for the recurring infections, chronic disease or immunity that may exist in the population. Data on incidence were taken from the only brucellosis diagnostic and treatment center in Georgia, so we are assuming all patients are treated. Although there is likely some immunity, the duration of the immunity is unknown and not found in the literature. Further recurrence of chronic disease is debilitating and it is assumed that animal work would then have to be passed to

other individuals capable of performing the tasks. Even if the debilitation is not severe and people can still work, the brucellosis incidence is very low and affects $\leq 0.1\%$ of the population; therefore the impact from this assumption is likely minimal. As for the animal population simulated by this model, individual animals were not modeled and the computer processing ability to model individual animals was not available for a model with this many interacting agents. Therefore, the model simulated flocks and herds as either entirely infected, susceptible, or vaccine immune.

The percentage of milking flocks and herds was assumed to be uniform across Kakheti and, due to a lack of data, did not integrate potential municipality differences. The model assumed equal exposure and equal workload of all shepherds and herders within the village. The model does not account for differences that occur if the shepherds or herders were hired versus if households shepherd or herders do the work themselves. Differences that may occur due to the use of seasonal pasture are not included because the work is often shared on these pastures as well. The model also assumed that culled household cattle herds are replaced prior to the start of every year based on the small herd sizes. Household herd sizes range from one to three animals, so this is a more practical assumption than would be true in an area with large herd sizes. Further, local individuals often slaughter or sell animals they believe are infected with brucellosis (Havas et al., 2011b), so there may be less social resistance to the animal culling. The model also assumed that the baseline risk is evenly distributed among the agricultural population that does not own livestock, the non-agriculturally employed population, and between villages. Further, it assumed that infection is randomly spread among all villages and does not cluster within villages. Since brucellosis has gone uncontrolled since the fall of

the Soviet Union and mass animal movement occurs when seasonal pastures are used, the livestock infection very well may not cluster at the village level.

Limitations

This model does not account for the spread of infection among livestock, nor does it model an eradication control program that would have to be implemented as the seroprevalence falls to levels below 2%. The potential effect of this limitation is dependent upon the stability of the prevalence within the population. If the reproductive ratio is approximately equal to one, then the disease prevalence is stable in the population, but if it is greater than one, the prevalence of disease is increasing and the model may overestimate the disease control programs effect. The odds ratios used to estimate the risk of infection based on risk factor for disease in the human population were determined using a multivariable logistic regression from a pilot study of risk factors. The pilot study examined incident cases of brucellosis reported to a central national treatment facility in 2010 and may not be representative of all of Georgia. However, it is the only study that estimates the effect of these risk factors in this country. The resulting confidence intervals suggest the significance of the risk factors since they do not overlap with one, but they are large. This precludes the use of variation in the odds ratios in the model. A uniform distribution was used in numerous locations in the model due to the fact that limited data was available, thus inhibiting the determination of the true distribution. Yet, a uniform distribution was used even with RBT specificity and sensitivity, which could have used a triangular distribution. There are limited distribution options available for use in NetLogo. Finally, agent-based models require significant

computer processing power that imposes a severe limitation on the number of iterations run based on the model's complexity and the available computing processor speed.

Conclusions

Agent-based modeling is a useful tool for planning and assessing infection control programs in countries with little national or population level data. This study reveals that despite this lack of population-level data, an understanding of the social patterns that surround animals and animal products can allow for the modeling of disease or infection within a system. The computer processor limitations do preclude the ability to run the same number of iterations as seen with population-based models, but do not prevent the return of useful information. The use of this agent-based model for the Kakheti region of Georgia has revealed that in a five-year time span, brucellosis can be significantly controlled in that region and that the greatest benefit falls to the shepherds and herders. The model also revealed that the impact of a program will differ by municipality, and that any control program should be tailored to each municipality to ensure the best use of resources.

Conflict of interest statement:

Dr. M.D. Salman is the Editor in Chief of this journal. He receives honorarium for this role. The decision of acceptance or rejection of this manuscript, however, is not handled by Dr. Salman.

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Conclusions of a Systematic Review of Brucellosis in the country of Georgia

We have the duty of formulating, of summarizing, and of communicating our conclusions, in intelligible form, in recognition of the right of other free minds to utilize them in making their own decisions.

- Ronald Fisher

Brucellosis in the Kakheti region of Georgia is a multi-faceted problem that arises from ethnic group differences, animal management practices that spread disease, dairy production that places food items at risk, misinformation about the threat of disease from different food sources and the ongoing need for adequate disease monitoring.

Village level grazing and seasonal pasture use aggravate disease spread among livestock. Thus flocks and herds, for the purposes of disease control and surveillance, are not as important epidemiologically as individual units so much as collective units at the village or pasture level. Co-mingling of animals among villagers and villages is common throughout the area. Spread of disease among animals is likely greatest on the winter pastures, as this is where calving and lambing occurs, although transmission on seasonal pastures cannot be ruled out. Sheep ownership carries a higher associated risk of contracting disease according to the case-control study, but the agent based model reveals that cattle also contribute significantly to disease. Although cattle and sheep are often separated while grazing they nonetheless share the same pastures. In addition, cattle are readily infected with *B. melitensis* and can subsequently shed the bacteria. In light of all of this information all livestock need to be included in the control program. Yet,

laboratory identification of the *Brucella* spp. in the livestock population will be critical for disease control planning, specifically in regards to vaccination use versus test and slaughter in the cattle population.

Use of shared pastures should be restricted to livestock that have been vaccinated in order to stem the spread of disease among the livestock. This will require permanent identification of vaccinated livestock and an initial determination of each municipality's flock and herd seroprevalence prior to vaccinating the livestock as well. Livestock are most available for serologic testing and vaccination in the fall, prior to heavy snows and after all animals have returned to lowland or village pastures from summer or highland pastures. At this time the replacement lambs and even perhaps the calves are also old enough to be vaccinated. The calving season is more variable than the lambing season, so greater care must be taken with the cattle population in ensuring calves are greater than four months of age. There are numerous private sector veterinarians who are currently unemployed throughout Kakheti. These professionals could be hired by the government to provide additional manpower for veterinary services in support of the disease surveillance and control program and they would also provide assurance to local inhabitants with whom they are familiar. This latter benefit could theoretically increase the compliance within the program.

As for disease control to human populations, the reduction of disease in the livestock population will also protect the individuals that work with the livestock. According to the results of the agent-based model, the greatest protective benefits will be among the shepherds and herders. Reduction of disease associated with contaminated dairy product consumption is a secondary concern to direct contact with livestock in

Kakheti. A large majority of individuals own animals and are likely infected via direct contact. Nonetheless, the risk from dairy products made from raw milk and that are improperly aged can still contain viable *Brucella* organisms capable of causing illness.

There is significant resistance to the use of pasteurization, especially for milk that is to be used for cheese and other dairy products. It is a quality concern among the population of Kakheti. Local inhabitants are well aware of the risk of drinking raw milk and very rarely drink raw milk without straining and heating or boiling it. Many individuals also understand that cheese products can be contaminated with *Brucella* spp. if not aged for a long enough period in brine, but the risk is likely under-estimated, because many people readily consume fresh cheese. Due to the high resistance to using pasteurized milk for cheese-making and popularity of fresh cheese, disease control in dairy products may require determining flock or herd disease freedom and certification. Yet, due to the fact that the food industry is highly informal, any programs that would have an impact on a large portion of the population would be difficult to implement. Therefore, control at the animal level would be the most advantageous.

Finally, individuals whose animals are brucellosis positive either sell or kill their infected livestock, or the government kills the livestock. The meat is not incorporated into the market for fear of brucellosis transmission. Overall, the risk of contracting brucellosis from meat products, excluding pork infected with *B. suis*, is very low, and essentially non-existent if properly cooked and handled. An education campaign encouraging the use of meat from infected animals and slaughter of these animals by individuals with proper training and personal protection could help sustain the food security of the region as well.

This multi-faceted approach to understanding disease in Georgia, a country transitioning to democracy and development, was critical. This three-pronged approach used analytical (case control study and agent based simulation model) and descriptive epidemiology (rapid assessment) that allowed us to recognize the role of sheep and cattle in human disease and disease propagation; the ethnic groups that need to be communicated with and listened to when implementing control programs; the shortcomings in dairy production processes; the routes of spread among animals based on management strategies; necessary areas of education to increase food security and allay unnecessary fears; and methods and seasons that are appropriate for implementing disease control programs. As a result of the work presented in this dissertation the understanding of brucellosis in Kakheti goes beyond medical and basic epidemiological understanding, but incorporates understanding of food security issues, the ethnic groups at risk, economic components and agricultural practices.

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Appendix 1: NetLogo Code for the Agent-Based Model

```
extensions [gis
            array]
globals [pasture-layer
          district-layer
          male-code
          female-code
          sick
          gender
          ORanimalwork ; OR approaches RR when disease is rare
          ORSheep
          ORmdp
          DedoRisk ; baseline risk of disease (incidence rate)
          SigRisk
          SagRisk
          TelRisk
          GurjRisk
          AkhRisk
          LagoRisk
          KvarRisk
          mn           ; month
          ]
```

```
patches-own [pasture district ];
```

```
.;*****Define Breeds*****
```

```
breed [ cattle cow ]
breed [ ewe aewe ]
breed [people ]
breed [ villagea villageas ]
breed [ villageb villagebs ]
breed [ villagec villagecs ]
breed [ villaged villageds ]
breed [ villagee villagees ]
breed [ villagef villagefs ]
breed [ villageg villagegs ]
breed [ villageh villagehs ]
breed [ towna townas ]
breed [ townb townbs ]
```

breed [townc towncs]
 breed [townd townds]
 breed [towne townes]
 breed [townf townfs]
 breed [towng towngs]
 breed [townh townhs]
 breed [towni townis]
 breed [agppl agppls]
 breed [nonagppl nonagppls]
 towna-own [role
 whotown]
 townb-own [role
 whotown]
 townc-own [role
 whotown]
 townd-own [role
 whotown]
 towne-own [role
 whotown]
 townf-own [role
 whotown]
 towng-own [role
 whotown]
 townh-own [role
 whotown]
 towni-own [role
 whotown]
 villagea-own [role ; array for role
 whovill]
 villageb-own [role ; array for role
 whovill]
 villagec-own [role ; array for role
 whovill]
 villaged-own [role ; array for role
 whovill]
 villagee-own [role ; array for role
 whovill]
 villagef-own [role ; array for role
 whovill]
 villageg-own [role
 whovill]
 villageh-own [role ; array for role
 ;gender ; array for gender
 ;sick ; array for sick
 whovill]
 nonagppl-own [

```

        whoag
        sex
        infected_person? ]
agppl-own [ whoag
        sex
        no_livestock
        shpshep
        cowshep
        shpmilk
        cowmilk
        makedp
        spshp
        spcow
        infected_person?
        activeshepewe
        activeshepcow ]
ewe-own [ whoshp
        infected?
        milkshp
        vaccinated? ]
cattle-own [ whocatt
        infected?
        milkcow
        vaccinated?
        dead ]

;*****SETUP*****
to setup
clear-all
ask agppl [set infected_person? false ]
clear-drawing
clear-all-plots
clear-output

setup-gis

populate_townvill
populate
populate_livestock

end
;*****END SETUP*****

```

```

;*****SETUP GIS*****
to setup-gis
;set data from temporary variables into patches
set district-layer gis:load-dataset "/Users/karynhavas/ASCII/kakh_dist.asc"
;next command makes the world the same size as gis layers
gis:set-world-envelope gis:envelope-of district-layer
;command to move temp variable data into patches plane
gis:apply-raster pasture-layer pasture
gis:apply-raster district-layer district

;to color each municipality individually
ask patches [
  if (district = 26 ) [ set pcolor grey - 2 ] ;akhmeta
  if (district = 27 ) [ set pcolor orange + 1 ] ;dedoplistkaro
  if (district = 28 ) [ set pcolor brown - 2 ] ;gurjaani
  if (district = 29 ) [ set pcolor yellow ] ;kvareli
  if (district = 30 ) [ set pcolor 58 + 1 ] ;lagodekhi
  if (district = 31 ) [ set pcolor green ] ;sagarego
  if (district = 32 ) [ set pcolor blue + 2 ] ;signagi
  if (district = 33 ) [ set pcolor pink - 1 ] ;telavi
]
end

;*****Creation of villages and towns*****
to populate_townvill
let i 0
;*****
;*****NON_AG_DEDOPLISTKARO*****
if District_Population = "Dedoplistkaro" or District_Population = "All" [
  create-towna 1 [ ;; dedoplistkaro town
    set i 0
    ask towna [
      while [ district != 27 ] [ setxy random-xcor random-ycor]
      set color red
      set whotown 0
      set shape "circle"
      set size 1
    ]
  ]
]
create-villagea 15 [
  set i 1
  ask villagea [
    while [district != 27 ] [ setxy random-xcor random-ycor ]
    set color blue
    set whovill i
    set shape "circle"
  ]
]

```

```

    set size 1
    set i ( i + 1 )
  ]
]
]
;*****
;***** POPULATE NON_AG SIGNAGI*****
if District_Population = "Signagi" or District_Population = "All" [
;*****SIGNAGI*****
create-townb 1 [                               ;; signagi
  ask townb [
    while [ district != 32 ] [ setxy random-xcor random-ycor]
    set color red
    set whotown 16
    set shape "circle"
    set size 1
  ]
]
create-townc 1 [                               ;; Tsnori
  ask townc [
    while [ district != 32 ] [ setxy random-xcor random-ycor]
    set color red
    set whotown 17
    set shape "circle"
    set size 1
  ]
]
;*****VILLAGES OF SIGNAGI*****
create-villageb 19 [
  set i 18
  ask villageb [
    while [district != 32 ] [ setxy random-xcor random-ycor ]
    set color blue - 10
    set whovill i
    set shape "circle"
    set size 1
    set i ( i + 1 )
  ]
]
]
;*****
;*****NONAG POP SAGAREGO*****
if District_Population = "Sagarego" or District_Population = "All" [
  create-townd 1 [                               ;; sagarego town
    set i 37
    ask townd [

```

```

while [ district != 31 ] [ setxy random-xcor random-ycor]
set color red
set whotown 37
set shape "circle"
set size 1
]
]
create-villagec 43 [
set i 38 ;38 to 80 are v_id for Sagarego
ask villagec [
while [district != 31 ] [ setxy random-xcor random-ycor ]
set color blue
set whovill i
set shape "circle"
set size 1
set i ( i + 1 )
]
]
]
,*****
,*****POP_NONAG_GURJAANI*****
if District_Population = "Gurjaani" or District_Population = "All" [
create-towne 1 [ ;; Gurjaani town
set i 0
ask towne [
while [ district != 28 ] [ setxy random-xcor random-ycor]
set color red
set whotown 81 ;v_id 81
set shape "circle"
set size 1
]
]
create-villaged 29 [
set i 82
ask villaged [
while [district != 28 ] [ setxy random-xcor random-ycor ]
set color blue
set whovill i
set shape "circle"
set size 1
set i ( i + 1 )
]
]
]
]

```

```

.*****
;
.*****Non_Ag_Akhmeta*****
if District_Population = "Akhmeta" or District_Population = "All" [
  create-townf 1 [                               ;; Akhmeta town v_id
    set i 0
    ask townf [
      while [ district != 26 ] [ setxy random-xcor random-ycor]
      set color red
      set whotown 111                               ;v_id = 111
      set shape "circle"
      set size 1
    ]
  ]
create-villagee 60 [
  set i 112                                       ;v_id 112 to 171
  ask villagee [
    while [district != 26 ] [ setxy random-xcor random-ycor ]
    set color blue
    set whovill i
    set shape "circle"
    set size 1
    set i ( i + 1 )
  ]
]
]
.*****
;
.*****Non_Ag_Telavi*****
if District_Population = "Telavi" or District_Population = "All" [
  create-towng 1 [                               ;; Telavi town v_id
    set i 0
    ask towng [
      while [ district != 33 ] [ setxy random-xcor random-ycor]
      set color red
      set whotown 172                               ;v_id = 172
      set shape "circle"
      set size 1
    ]
  ]
create-villagef 29 [
  set i 173                                       ;v_id 173 to 201
  ask villagef [
    while [district != 33 ] [ setxy random-xcor random-ycor ]
    set color blue
    set whovill i
    set shape "circle"
    set size 1
  ]
]

```

```

    set i ( i + 1 )
  ]
]
]
;*****
;*****Non_Ag_Kvareli*****
if District_Population = "Kvareli" or District_Population = "All" [
  create-townh 1 [                               ;; Kvareli town v_id
    set i 0
    ask townh [
      while [ district != 29 ] [ setxy random-xcor random-ycor]
      set color red
      set whotown 202                               ;v_id = 202
      set shape "circle"
      set size 1
    ]
  ]
  create-villageg 21 [                             ;v_id 203 to 223
    set i 203
    ask villageg [
      while [district != 29 ] [ setxy random-xcor random-ycor ]
      set color blue
      set whovill i
      set shape "circle"
      set size 1
      set i ( i + 1 )
    ]
  ]
]
;*****
;*****Non_Ag_Lagodekhi*****
if District_Population = "Lagodekhi" or District_Population = "All" [
  create-towni 1 [                               ;; Lagodekhi town v_id
    set i 0
    ask towni [
      while [ district != 30 ] [ setxy random-xcor random-ycor]
      set color red
      set whotown 224                               ;v_id = 224 to 287
      set shape "circle"
      set size 1
    ]
  ]
  create-villageh 63 [                             ;v_id 225 to 287
    set i 225
    ask villageh [
      while [district != 30 ] [ setxy random-xcor random-ycor ]

```

```

    set color blue
    set whovill i
    set shape "circle"
    set size 1
    set i ( i + 1 )
  ]
]
]
end

;*****pop agriculture people*****
to populate
;*****
;*****AG_DEDO_POP*****
if District_Population = "Dedoplistkaro" or District_Population = "All" [
;*****TOWN*****
let v_id 0 ; Dedop v_id 0 to 15
while [ v_id = 0 ] [
  create-nonagppl 335 [
    set infected_person? false
    ifelse random-float 1.0 <= 0.474 [ set sex male-code ] [set sex female-code ]
    if random-float 1.0 <= 0.00001 [ set infected_person? true ]
    set whoag v_id
    move-to one-of towna with [ whotown = v_id ]
  ]
  create-agppl 6965 [ ; 95.4% pop engaged in ag 2004 Data- based on % of
HH engaged in ag 7300 (Geostat 2010) * 0.954
    set infected_person? false
    set no_livestock true
    ;assign gender at town level
    ifelse random-float 1.0 <= 0.474 [ set sex male-code ] [set sex female-code ]
;2002Census Pop Sex and Municipality for rural dedo
    set whoag v_id
    move-to one-of towna with [ whotown = v_id ]
  ]
  ;assign roles at town level
  let HHpop ( 6965 / Household_Size )
  ask n-of ( HHpop * 0.1 ) agppl with [sex = male-code and whoag = v_id ] ;sheep
shepherd in town 6965/3.2 x 0.1 = 218
    [ set shpshep true
      set no_livestock false
    ]
  ask n-of ( HHpop * 0.42 ) agppl with [ sex = male-code and whoag = v_id ] ; cow
shepherd 6965/3.2 x 0.42 = 914
    [ set cowshep true
      set no_livestock false
    ]
  ]
]
]
]
end

```

```

]
ask n-of ( HHpop * 0.1 * 0.48 ) agppl with [ sex = male-code and shpshep = true and
whoag = v_id ] ; sheep milker: males milk 6965/3.2 x 0.1 x 0.48 = 105
[ set shpmilk true
set no_livestock false
]
ask n-of ( HHpop * 0.42 * 0.98 ) agppl with [ whoag = v_id and sex = male-code ]
;cow milker: male and female 6965/3.2 x 0.42 x 0.98 = 896
[ set cowmilk true
set no_livestock false
]
ask n-of ( HHpop * 0.42 * 0.98 ) agppl with [ sex = female-code and whoag = v_id ]
;dairy producer are female and cow only - sheep mdp encompassed by milkers
[ set makedp true
set no_livestock false
]
set v_id 1
]
;*****VILLAGES*****
set v_id 1
while [ v_id > 0 and v_id <= 15 ] [
create-nonagppl 71 [
set infected_person? false
ifelse random-float 1.0 <= 0.474 [ set sex male-code ] [set sex female-code ]
if random-float 1.0 <= 0.00001 [ set infected_person? true ]
set whoag v_id
move-to one-of villagea with [ whovill = v_id ]
]
create-agppl 1476 [ ; 95.4% pop engaged in ag in Dedo- based on % of HH
engaged in ag 1547 * 0.954
set infected_person? false
set no_livestock true
;assign gender at village level
ifelse random-float 1.0 <= 0.474 [ set sex male-code ] [set sex female-code ]
;2009Yearbook overall Georgia
set whoag v_id
move-to one-of villagea with [ whovill = v_id ]
]
;assign roles at village level
let HHpop ( 1476 / Household_Size )
ask n-of ( HHpop * 0.1 ) agppl with [ sex = male-code and whoag = v_id ] ;sheep
shepherd in town 1476/3.2 x 0.1 = 46
[ set shpshep true
set no_livestock false
]
]

```

```

ask n-of ( HHpop * 0.42 ) agppl with [ sex = male-code and whoag = v_id ] ; cow
shepherd 1476/3.2 x 0.42 = 194
[ set cowshep true
  set no_livestock false
]
ask n-of ( HHpop * 0.1 * 0.48 ) agppl with [ sex = male-code and shpshep = true and
whoag = v_id ] ; sheep milker: males milk 1476/3.2 x 0.1 x 0.48 = 22
[ set shpmilk true
  set no_livestock false
]
ask n-of ( HHpop * 0.42 * 0.98 ) agppl with [ whoag = v_id and sex = male-code ]
;cow milker: male and female 1476/3.2 x 0.42 x 0.98 = 190
[ set cowmilk true
  set no_livestock false
]
ask n-of ( HHpop * 0.42 * 0.98 ) agppl with [ sex = female-code and whoag = v_id ]
;dairy producer are female and cow only - sheep mdp encompassed by shpmilkers
[ set makedp true
  set no_livestock false
]
set v_id (v_id + 1 )
]
]
;*****
;*****SIGNAGI AG POP*****
if District_Population = "Signagi" or District_Population = "All" [
let v_id 16 ; Signagi is v_id 16
if v_id = 16 [ ; SIGNAGI
create-nonagppl 395 [
set infected_person? false
ifelse random-float 1.0 <= 0.474 [ set sex male-code ] [set sex female-code ]
if random-float 1.0 <= 0.000001 [ set infected_person? true ]
set whoag v_id
move-to one-of townb with [ whotown = v_id ]
]
create-agppl 1905 [ ; 82.8% pop engaged in ag 2004 Data- based on % of
HH engaged in ag 2300 (Geostat 2010) * 0.828
set infected_person? false
set no_livestock true
;assign gender at town level
ifelse random-float 1.0 <= 0.474 [ set sex male-code ] [set sex female-code ]
;2002Census Pop Sex and Municipality for rural dedo
set whoag v_id
move-to one-of townb with [ whotown = v_id ]
]
;assign roles at town level

```

```

let HHpop ( 1905 / Household_Size )
  ask n-of ( HHpop * 0.12 ) agppl with [ sex = male-code and whoag = v_id ] ;sheep
shepherd in town 1905/3.2 x 0.12 = 71
  [ set shpshep true
    set no_livestock false
  ]
  ask n-of ( HHpop * 0.28 ) agppl with [ sex = male-code and whoag = v_id ] ; cow
shepherd 1905/3.2 x 0.28 = 167
  [ set cowshep true
    set no_livestock false
  ]
  ask n-of ( HHpop * 0.12 * 0.48 ) agppl with [ sex = male-code and shpshep = true
and whoag = v_id ] ; sheep milker: males milk 1905/3.2 x 0.12 x 0.48 = 34
  [ set shpmilk true
    set no_livestock false
  ]
  ask n-of ( HHpop * 0.28 * 0.98 ) agppl with [ whoag = v_id and sex = male-code ]
;cow milker: male and female 1905/3.2 x 0.28 x 0.98 = 164
  [ set cowmilk true
    set no_livestock false
  ]
  ask n-of ( HHpop * 0.28 * 0.98 ) agppl with [ sex = female-code and whoag = v_id ]
;dairy producer are female and cow only - sheep mdp encompassed by shpmilkers
  [ set makedp true
    set no_livestock false
  ]
]
set v_id 17 ; TSNORI is v_id 17
if v_id = 17 [ ; TSNORI
  create-nonagppl 1049 [
    set infected_person? false
    ifelse random-float 1.0 <= 0.474 [ set sex male-code ] [set sex female-code ]
    if random-float 1.0 <= 0.000001 [ set infected_person? true ]
    set whoag v_id
    move-to one-of townc with [ whotown = v_id ]
  ]
  create-agppl 5051 [ ; 82.8% pop engaged in ag 2004 Data- based on % of
HH engaged in ag 6100 (Geostat 2010) * 0.828
    set infected_person? false
    set no_livestock true
    ;assign gender at town level
    ifelse random-float 1.0 <= 0.474 [ set sex male-code ] [set sex female-code ]
;2002Census Pop Sex and Municipality for rural dedo
    set whoag v_id
    move-to one-of townc with [ whotown = v_id ]
  ]
]

```

```

;assign roles at town level
let HHpop ( 5051 / Household_Size )
ask n-of ( HHpop * 0.12 ) agppl with [ sex = male-code and whoag = v_id ] ;sheep
shepherd in town 5051/3.2 x 0.12 = 189
[ set shpshep true
  set no_livestock false
]
ask n-of ( HHpop * 0.28 ) agppl with [ sex = male-code and whoag = v_id ] ; cow
shepherd 5051/3.2 x 0.28 = 442
[ set cowshep true
  set no_livestock false
]
ask n-of ( HHpop * 0.12 * 0.48 ) agppl with [ sex = male-code and shpshep = true
and whoag = v_id ] ; sheep milker: males milk 5051/3.2 x 0.12 x 0.48 = 91
[ set shpmilk true
  set no_livestock false
]
ask n-of ( HHpop * 0.28 * 0.98 ) agppl with [ whoag = v_id and sex = male-code ]
;cow milker: male and female 5052/3.2 x 0.28 x 0.98 = 433
[ set cowmilk true
  set no_livestock false
]
ask n-of ( HHpop * 0.28 * 0.98 ) agppl with [ sex = female-code and whoag = v_id ]
;dairy producer are female and cow only - sheep mdp encompassed by shpmilkers
[ set makedp true
  set no_livestock false
]
]
;*****VILLAGES OF SIGNAGI*****
set v_id 18 ; 19 villages v_id 18 to 36
while [ v_id <= 36 ] [
  create-nonagppl 315 [
    set infected_person? false
    ifelse random-float 1.0 <= 0.474 [ set sex male-code ] [set sex female-code ]
    if random-float 1.0 <= 0.000001 [ set infected_person? true ]
    set whoag v_id
    move-to one-of villageb with [ whovill = v_id ]
  ]
  create-agppl 1521 [ ; 82.8% pop engaged in ag in signagi- based on % of
HH engaged in ag 1837 * 0.828
  set infected_person? false
  set no_livestock true
  ;assign gender at village level
  ifelse random-float 1.0 <= 0.474 [ set sex male-code ] [set sex female-code ]
;2009Yearbook overall Georgia
  set whoag v_id

```

```

    move-to one-of villageb with [ whovill = v_id ]
  ]
;assign roles at village level
let HHpop ( 1521 / Household_Size )
ask n-of ( HHpop * 0.12 ) agppl with [ sex = male-code and whoag = v_id ] ;sheep
shepherd in town 1521/3.2 x 0.12 = 57
  [ set shpshep true
    set no_livestock false
  ]
ask n-of ( HHpop * 0.28 ) agppl with [ sex = male-code and whoag = v_id ] ; cow
shepherd 1521/3.2 x 0.28 = 133
  [ set cowshep true
    set no_livestock false
  ]
ask n-of ( HHpop * 0.12 * 0.48 ) agppl with [ sex = male-code and shpshep = true
and whoag = v_id ] ; sheep milker: males milk 1521/3.2 x 0.12 x 0.48 = 27
  [ set shpmilk true
    set no_livestock false
  ]
ask n-of ( HHpop * 0.28 * 0.98 ) agppl with [ whoag = v_id and sex = male-code ]
;cow milker: male and female 1521/3.2 x 0.28 x 0.98 = 130
  [ set cowmilk true
    set no_livestock false
  ]
ask n-of ( HHpop * 0.28 * 0.98 ) agppl with [ sex = female-code and whoag = v_id ]
;dairy producer are female and cow only - sheep mdp encompassed by shpmilkers
  [ set makedp true
    set no_livestock false
  ]
set v_id (v_id + 1 )
]
]
;*****
;*****AGPOP_SAGAREGO*****
if District_Population = "Sagarego" or District_Population = "All" [
;*****TOWN*****
let v_id 37 ; Sagarego v_id 37 to 80
if v_id = 37 [
create-nonagppl 195 [
set infected_person? false
ifelse random-float 1.0 <= 0.474 [ set sex male-code ] [set sex female-code ]
if random-float 1.0 <= 0.00001 [ set infected_person? true ]
set whoag v_id
move-to one-of townd with [ whotown = v_id ]
]
]
]

```

```

create-agppl 11305 [          ; 98.3% pop engaged in ag 2004 Data- based on % of
HH engaged in ag 11500 (Geostat 2010) * 0.983
  set infected_person? false
  set no_livestock true
;assign gender at town level
  ifelse random-float 1.0 <= 0.474 [ set sex male-code ] [set sex female-code ]
;2002Census Pop Sex and Municipality for rural sag
  set whoag v_id
  move-to one-of townd with [ whotown = v_id ]
]
;assign roles at town level
let HHpop ( 11305 / Household_Size )
  ask n-of ( HHpop * 0.07 ) agppl with [ sex = male-code and whoag = v_id ] ;sheep
shepherd in town 11305/3.2 x 0.07 = 247
  [ set shpshep true
    set no_livestock false
  ]
  ask n-of ( HHpop * 0.41 ) agppl with [ sex = male-code and whoag = v_id ] ; cow
shepherd 11305/3.2 x .41 = 1449
  [ set cowshep true
    set no_livestock false
  ]
  ask n-of ( HHpop * 0.07 * 0.48 ) agppl with [ sex = male-code and shpshep = true
and whoag = v_id ] ; sheep milker: males milk 11305/3.2 x 0.07 x 0.48 = 119
  [ set shpmilk true
    set no_livestock false
  ]
  ask n-of ( HHpop * 0.41 * 0.98 ) agppl with [ whoag = v_id ]
;cow milker: male and female 11305/3.2 x 0.41 x 0.98 = 1420
  [ set cowmilk true
    set no_livestock false
  ]
  ask n-of ( HHpop * 0.41 * 0.98 ) agppl with [ sex = female-code and whoag = v_id ]
;dairy producer are female and cow only - sheep mdp encompassed by shpmilkers
  [ set makedp true
    set no_livestock false
  ]
]
;*****VILLAGES*****
set v_id 38
while [ v_id <= 80 ] [
  create-nonagppl 19 [
    set infected_person? false
    ifelse random-float 1.0 <= 0.474 [ set sex male-code ] [set sex female-code ]
    if random-float 1.0 <= 0.00001 [ set infected_person? true ]
    set whoag v_id

```

```

    move-to one-of villagetc with [ whovill = v_id ]
  ]
  create-agppl 1095 [          ; 98.3% pop engaged in ag in sag- based on % of HH
engaged in ag 1114 * 0.983 = 1122
  set infected_person? false
  set no_livestock true
  ;assign gender at village level
  ifelse random-float 1.0 <= 0.474 [ set sex male-code ] [set sex female-code ]
;2009Yearbook overall Georgia
  set whoag v_id
  move-to one-of villagetc with [ whovill = v_id ]
  ]
;assign roles at village level
let HHpop ( 1095 / Household_Size )
ask n-of ( HHpop * 0.07 ) agppl with [ sex = male-code and whoag = v_id ] ;sheep
shepherd in town 1095/3.2 x 0.07 = 24
  [ set shpshep true
  set no_livestock false
  ]
  ask n-of ( HHpop * 0.41 ) agppl with [ sex = male-code and whoag = v_id ] ; cow
shepherd 1095/3.2 x .41 = 140
  [ set cowshep true
  set no_livestock false
  ]
  ask n-of ( HHpop * 0.07 * 0.48 ) agppl with [ sex = male-code and shpshep = true
and whoag = v_id ] ; sheep milker: males milk 1095/3.2 x 0.07 x 0.48 = 11
  [ set shpmilk true
  set no_livestock false
  ]
  ask n-of ( HHpop * 0.41 * 0.98 ) agppl with [ whoag = v_id ]
;cow milker: male and female 1095/3.2 x 0.41 x 0.98 = 137
  [ set cowmilk true
  set no_livestock false
  ]
  ask n-of ( HHpop * 0.41 * 0.98 ) agppl with [ sex = female-code and whoag = v_id ]
;dairy producer are female and cow only - sheep mdp encompassed by shpmilkers
  [ set makedp true
  set no_livestock false
  ]
  set v_id (v_id + 1 )
]
]
.*****
,
.*****GURJAANI_AGPOP*****
if District_Population = "Gurjaani" or District_Population = "All" [
,*****TOWN*****

```

```

let v_id 81 ; Gurj v_id 81 to 110
if v_id = 81 [
  create-nonagppl 494 [
    set infected_person? false
    ifelse random-float 1.0 <= 0.474 [ set sex male-code ] [set sex female-code ]
    if random-float 1.0 <= 0.00001 [ set infected_person? true ]
    set whoag v_id
    move-to one-of towne with [ whotown = v_id ]
  ]
  create-agppl 9006 [ ; 94.8% pop engaged in ag 2004 Data- based on % of
HH engaged in ag 9500 (Geostat 2010) * 0.948 = 9006
  set infected_person? false
  set no_livestock true
  ;assign gender at town level
  ifelse random-float 1.0 <= 0.474 [ set sex male-code ] [set sex female-code ]
;2002Census Pop Sex and Municipality for rural dedo
  set whoag v_id
  move-to one-of towne with [ whotown = v_id ]
  ]
  ;assign roles at town level
  let HHpop ( 9006 / Household_Size )
  ask n-of ( HHpop * 0.03 ) agppl with [ sex = male-code and whoag = v_id ]
;sheep shepherd in town 9006/3.2 x 0.03 = 84
  [ set shpshep true
  set no_livestock false
  ]
  ask n-of ( HHpop * 0.21 ) agppl with [ sex = male-code and whoag = v_id ] ; cow
shepherd 9006/3.2 x 0.21 = 591
  [ set cowshep true
  set no_livestock false
  ]
  ask n-of ( HHpop * 0.03 * 0.48 ) agppl with [ sex = male-code and shpshep = true
and whoag = v_id ] ; sheep milker: males milk 9006/3.2 x 0.03 x 0.48 = 40
  [ set shpmilk true
  set no_livestock false
  ]
  ask n-of ( HHpop * 0.21 * 0.98 ) agppl with [ whoag = v_id ]
;cow milker: male and female 9006/3.2 x 0.21 x 0.98 = 579
  [ set cowmilk true
  set no_livestock false
  ]
  ask n-of ( HHpop * 0.21 * 0.98 ) agppl with [ sex = female-code and whoag = v_id ]
;dairy producer are female and cow only - sheep mdp encompassed by shpmilkers
  [ set makedp true
  set no_livestock false
  ]
]

```

```

]
;*****VILLAGES*****
set v_id 82
while [ v_id <= 110 ] [
  create-nonagppl 395 [
    set infected_person? false
    ifelse random-float 1.0 <= 0.474 [ set sex male-code ] [set sex female-code ]
    if random-float 1.0 <= 0.00001 [ set infected_person? true ]
    set whoag v_id
    move-to one-of villaged with [ whovill = v_id ]
  ]
  create-agppl 1975 [ ; 94.8% pop engaged in ag in Gurj- based on % of HH
engaged in ag 2083 * 0.948 = 1975
    set infected_person? false
    set no_livestock true
    ;assign gender at village level
    ifelse random-float 1.0 <= 0.474 [ set sex male-code ] [set sex female-code ]
;2009Yearbook overall Georgia
    set whoag v_id
    move-to one-of villaged with [ whovill = v_id ]
  ]
  ;assign roles at village level
  let HHpop ( 1975 / Household_Size )
  ask n-of ( HHpop * 0.03 ) agppl with [ sex = male-code and whoag = v_id ] ;sheep
shepherd in town 1975/3.2 x 0.03 = 19
  [ set shpshep true
    set no_livestock false
  ]
  ask n-of ( HHpop * 0.21 ) agppl with [ sex = male-code and whoag = v_id ] ; cow
shepherd 1975/3.2 x .21 = 130
  [ set cowshep true
    set no_livestock false
  ]
  ask n-of ( HHpop * 0.03 * 0.48 ) agppl with [ sex = male-code and shpshep = true
and whoag = v_id ] ; sheep milker: males milk 1975/3.2 x 0.03 x 0.48 = 9
  [ set shpmilk true
    set no_livestock false
  ]
  ask n-of ( HHpop * 0.21 * 0.98 ) agppl with [ whoag = v_id ]
;cow milker: male and female 1975/3.2 x 0.21 x 0.98 = 127
  [ set cowmilk true
    set no_livestock false
  ]
  ask n-of ( HHpop * 0.21 * 0.98 ) agppl with [ sex = female-code and whoag = v_id ]
;dairy producer are female and cow only - sheep mdp encompassed by shpmilkers
  [ set makedp true

```

```

        set no_livestock false
    ]
    set v_id (v_id + 1 )
]
]
.*****
,
.*****AG_POP_AKHMETA*****
if District_Population = "Akhmeta" or District_Population = "All" [
,
.*****TOWN*****
let v_id 111 ; akhm v_id 111 to 171
if v_id = 111 [
    create-nonagppl 529 [
        set infected_person? false
        ifelse random-float 1.0 <= 0.474 [ set sex male-code ] [set sex female-code ]
        if random-float 1.0 <= 0.000008 [ set infected_person? true ]
        set whoag v_id
        move-to one-of townf with [ whotown = v_id ]
    ]
    create-agppl 7871 [ ; 93.7% pop engaged in ag 2004 Data- based on % of
HH engaged in ag 8400 (Geostat 2010) * 0.937 = 7871
        set infected_person? false
        set no_livestock true
        ;assign gender at town level
        ifelse random-float 1.0 <= 0.474 [ set sex male-code ] [set sex female-code ]
;2002Census Pop Sex and Municipality for rural dedo
        set whoag v_id
        move-to one-of townf with [ whotown = v_id ]
    ]
    ;assign roles at town level
    let HHpop ( 7871 / Household_Size )
    ask n-of ( HHpop * 0.08 ) agppl with [ sex = male-code and whoag = v_id ] ;sheep
shepherd in town 7871//3.2 x 0.08 = 197
        [ set shpshep true
        set no_livestock false
        ]
        ask n-of ( HHpop * 0.56 ) agppl with [ sex = male-code and whoag = v_id ] ; cow
shepherd 7871/3.2 x .56 = 1377
        [ set cowshep true
        set no_livestock false
        ]
        ask n-of ( HHpop * 0.08 * 0.48 ) agppl with [ sex = male-code and shpshep = true and
whoag = v_id ] ; sheep milker: males milk 7871/3.2 x 0.08 x 0.48 = 95
        [ set shpmilk true
        set no_livestock false
        ]
]
]

```

```

    ask n-of ( HHpop * 0.56 * 0.98 ) agppl with [ whoag = v_id ]
;cow milker: male and female 7871/3.2 x 0.56 x 0.98 = 1350
    [ set cowmilk true
      set no_livestock false
    ]
    ask n-of ( HHpop * 0.56 * 0.98 ) agppl with [ sex = female-code and whoag = v_id ]
;dairy producer are female and cow only - sheep mdp encompassed by shpmilkers
    [ set makedp true
      set no_livestock false
    ]
  ]
;*****VILLAGES*****
set v_id 112
while [ v_id <= 171 ] [
  create-nonagppl 35 [
    set infected_person? false
    ifelse random-float 1.0 <= 0.474 [ set sex male-code ] [set sex female-code ]
    if random-float 1.0 <= 0.000008 [ set infected_person? true ]
    set whoag v_id
    move-to one-of villagee with [ whovill = v_id ]
  ]
  create-agppl 525 [          ; 93.7% pop engaged in ag in akhmeta based on % of
HH engaged in ag 560 * 0.937
    set infected_person? false
    set no_livestock true
    ;assign gender at village level
    ifelse random-float 1.0 <= 0.474 [ set sex male-code ] [set sex female-code ]
;2009Yearbook overall Georgia
    set whoag v_id
    move-to one-of villagee with [ whovill = v_id ]
  ]
  ;assign roles at village level
  let HHpop ( 525 / Household_Size )
  ask n-of ( HHpop * 0.08 ) agppl with [ sex = male-code and whoag = v_id ] ;sheep
shepherd in town 525/3.2 x 0.08 = 13
    [ set shpshep true
      set no_livestock false
    ]
    ask n-of ( HHpop * 0.56 ) agppl with [ sex = male-code and whoag = v_id ] ; cow
shepherd 525/3.2 x .56 = 92
    [ set cowshep true
      set no_livestock false
    ]
    ask n-of ( HHpop * 0.08 * 0.48 ) agppl with [ sex = male-code and shpshep = true
and whoag = v_id ] ; sheep milker: males milk 525/3.2 x 0.08 x 0.48 = 6
    [ set shpmilk true

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```

        set no_livestock false
    ]
    ask n-of ( HHpop * 0.56 * 0.98 ) agppl with [ whoag = v_id ]
;cow milker: male and female 525/3.2 x 0.56 x 0.98 = 90
    [ set cowmilk true
      set no_livestock false
    ]
    ask n-of ( HHpop * 0.56 * 0.98 ) agppl with [ sex = female-code and whoag = v_id ]
;dairy producer are female and cow only - sheep mdp encompassed by shpmilkers
    [ set makedp true
      set no_livestock false
    ]
    set v_id (v_id + 1 )
]
]
;*****
;*****AG_POP_TELAVI*****
if District_Population = "Telavi" or District_Population = "All" [
;*****TOWN*****
let v_id 172 ; telavi v_id 172 to 201
if v_id = 172 [
create-nonagppl 2030 [
set infected_person? false
ifelse random-float 1.0 <= 0.474 [ set sex male-code ] [set sex female-code ]
if random-float 1.0 <= 0.00001 [ set infected_person? true ]
set whoag v_id
move-to one-of towng with [ whotown = v_id ]
]
create-agppl 18070 [ ; 89.9% pop engaged in ag 2004 Data- based on % of
HH engaged in ag 20100 (Geostat 2010) * 0.899 = 18070
set infected_person? false
set no_livestock true
;assign gender at town level
ifelse random-float 1.0 <= 0.474 [ set sex male-code ] [set sex female-code ]
;2002Census Pop Sex and Municipality for rural dedo
set whoag v_id
move-to one-of towng with [ whotown = v_id ]
]
;assign roles at town level
let HHpop ( 18070 / Household_Size )
ask n-of ( HHpop * 0.03 ) agppl with [ sex = male-code and whoag = v_id ] ;sheep
shepherd in town 18070/3.2 x 0.03 = 169
[ set shpshep true
set no_livestock false
]
]
]

```

```

ask n-of ( HHpop * 0.2 ) agppl with [ sex = male-code and whoag = v_id ] ; cow
shepherd 18070/3.2 x .2 = 1129
[ set cowshep true
  set no_livestock false
]
ask n-of ( HHpop * 0.03 * 0.48 ) agppl with [ sex = male-code and shpshep = true
and whoag = v_id ] ; sheep milker: males milk 18070/3.2 x 0.03 x 0.48 = 81
[ set shpmilk true
  set no_livestock false
]
ask n-of ( HHpop * 0.2 * 0.98 ) agppl with [ whoag = v_id ]
;cow milker: male and female 18070/3.2 x 0.2 x 0.98 = 1106
[ set cowmilk true
  set no_livestock false
]
ask n-of ( HHpop * 0.2 * 0.98 ) agppl with [ sex = female-code and whoag = v_id ]
;dairy producer are female and cow only - sheep mdp encompassed by shpmilkers
[ set makedp true
  set no_livestock false
]
]
;*****VILLAGES*****
set v_id 173
while [ v_id <= 201 ] [
  create-nonagppl 175 [
    set infected_person? false
    ifelse random-float 1.0 <= 0.474 [ set sex male-code ] [set sex female-code ]
    if random-float 1.0 <= 0.00001 [ set infected_person? true ]
    set whoag v_id
    move-to one-of villagef with [ whovill = v_id ]
  ]
  create-agppl 1563 [ ; 89.9% pop engaged in ag in telavi based on % of HH
engaged in ag 1738 * .899 = 1563
  set infected_person? false
  set no_livestock true
  ;assign gender at village level
  ifelse random-float 1.0 <= 0.474 [ set sex male-code ] [set sex female-code ]
;2009Yearbook overall Georgia
  set whoag v_id
  move-to one-of villagef with [ whovill = v_id ]
]
;assign roles at village level
let HHpop ( 1563 / Household_Size )
ask n-of ( HHpop * 0.03 ) agppl with [ sex = male-code and whoag = v_id ] ;sheep
shepherd in town 1563/3.2 x 0.03 = 15
[ set shpshep true

```

```

        set no_livestock false
    ]
    ask n-of ( HHpop * 0.2 ) agppl with [ sex = male-code and whoag = v_id ] ; cow
shepherd 1563/3.2 x .2 = 98
    [ set cowshep true
      set no_livestock false
    ]
    ask n-of ( HHpop * 0.03 * 0.48 ) agppl with [ sex = male-code and shpshep = true
and whoag = v_id ] ; sheep milker: males milk 1563/3.2 x 0.03 x 0.48 = 7
    [ set shpmilk true
      set no_livestock false
    ]
    ask n-of ( HHpop * 0.2 * 0.98 ) agppl with [ whoag = v_id ]
;cow milker: male and female 1563/3.2 x 0.2 x 0.98 = 96
    [ set cowmilk true
      set no_livestock false
    ]
    ask n-of ( HHpop * 0.2 * 0.98 ) agppl with [ sex = female-code and whoag = v_id ]
;dairy producer are female and cow only - sheep mdp encompassed by shpmilkers
    [ set makedp true
      set no_livestock false
    ]
    set v_id (v_id + 1 )
]
]
.*****
,
.*****AG_POP_KVARELI*****
if District_Population = "Kvareli" or District_Population = "All" [
,
.*****TOWN*****
let v_id 202 ; kvareli v_id 202 to 223
if v_id = 202 [
create-nonagppl 396 [
set infected_person? false
ifelse random-float 1.0 <= 0.474 [ set sex male-code ] [set sex female-code ]
if random-float 1.0 <= 0.000004 [ set infected_person? true ]
set whoag v_id
move-to one-of townh with [ whotown = v_id ]
]
create-agppl 8204 [ ; 95.4% pop engaged in ag 2004 Data- based on % of
HH engaged in ag 8600 (Geostat 2010) * 0.954 = 8204
set infected_person? false
set no_livestock true
;assign gender at town level
ifelse random-float 1.0 <= 0.474 [ set sex male-code ] [set sex female-code ]
;2002Census Pop Sex and Municipality for rural dedo
set whoag v_id

```

```

    move-to one-of townh with [ whotown = v_id ]
  ]
;assign roles at town level
let HHpop ( 8204 / Household_Size )
ask n-of ( HHpop * 0.04 ) agppl with [ sex = male-code and whoag = v_id ] ;sheep
shepherd in town  $8204/3.2 \times 0.04 = 103$ 
  [ set shpshep true
    set no_livestock false
  ]
ask n-of ( HHpop * 0.38 ) agppl with [ sex = male-code and whoag = v_id ] ; cow
shepherd  $8204/3.2 \times .38 = 974$ 
  [ set cowshep true
    set no_livestock false
  ]
ask n-of ( HHpop * 0.04 * 0.48 ) agppl with [ sex = male-code and shpshep = true
and whoag = v_id ] ; sheep milker: males milk  $8204/3.2 \times 0.04 \times 0.48 = 49$ 
  [ set shpmilk true
    set no_livestock false
  ]
ask n-of ( HHpop * 0.38 * 0.98 ) agppl with [ whoag = v_id ]
;cow milker: male and female  $8204/3.2 \times 0.38 \times 0.98 = 955$ 
  [ set cowmilk true
    set no_livestock false
  ]
ask n-of ( HHpop * 0.38 * 0.98 ) agppl with [ sex = female-code and whoag = v_id ]
;dairy producer are female and cow only - sheep mdp encompassed by shpmilkers
  [ set makedp true
    set no_livestock false
  ]
]
;*****VILLAGES*****
set v_id 203
while [ v_id <= 223 ] [
  create-nonagppl 21 [
    set infected_person? false
    ifelse random-float 1.0 <= 0.474 [ set sex male-code ] [set sex female-code ]
    if random-float 1.0 <= 0.000004 [ set infected_person? true ]
    set whoag v_id
    move-to one-of villageg with [ whovill = v_id ]
  ]
  create-agppl 1295 [ ; 95.4% pop engaged in ag in kvaerli- based on % of
HH engaged in ag  $1357 * .954 = 1295$ 
    set infected_person? false
    set no_livestock true
    ;assign gender at village level

```

```

    ifelse random-float 1.0 <= 0.474 [ set sex male-code ] [set sex female-code ]
;2009Yearbook overall Georgia
    set whoag v_id
    move-to one-of villageg with [ whovill = v_id ]
]
;assign roles at village level
let HHpop ( 1295 / Household_Size )
ask n-of ( HHpop * 0.04 ) agppl with [ sex = male-code and whoag = v_id ] ;sheep
shepherd in town 1295/3.2 x 0.04 = 16
[ set shpshep true
  set no_livestock false
]
ask n-of ( HHpop * 0.38 ) agppl with [ sex = male-code and whoag = v_id ] ; cow
shepherd 1295/3.2 x .38 = 154
[ set cowshep true
  set no_livestock false
]
ask n-of ( HHpop * 0.04 * 0.48 ) agppl with [ sex = male-code and shpshep = true
and whoag = v_id ] ; sheep milker: males milk 1295/3.2 x 0.04 x 0.48 = 8
[ set shpmilk true
  set no_livestock false
]
ask n-of ( HHpop * 0.38 * 0.98 ) agppl with [ whoag = v_id ]
;cow milker: male and female 1295/3.2 x 0.38 x 0.98 = 151
[ set cowmilk true
  set no_livestock false
]
ask n-of ( HHpop * 0.38 * 0.98 ) agppl with [ sex = female-code and whoag = v_id ]
;dairy producer are female and cow only - sheep mdp encompassed by shpmilkers
[ set makedp true
  set no_livestock false
]
set v_id (v_id + 1 )
]
]
.*****
,
.*****AG_POP_LAGODEKHI*****
if District_Population = "Lagodekhi" or District_Population = "All" [
,*****TOWN*****
let v_id 224 ; lagodekhi v_id 224 to 287
if v_id = 224 [
create-nonagppl 435 [
set infected_person? false
ifelse random-float 1.0 <= 0.474 [ set sex male-code ] [set sex female-code ]
if random-float 1.0 <= 0.00001 [ set infected_person? true ]
set whoag v_id

```

```

    move-to one-of towni with [ whotown = v_id ]
  ]
  create-agppl 7065 [          ; 94.2% pop engaged in ag 2004 Data- based on % of
HH engaged in ag 7500 (Geostat 2010) * 0.942 = 7065
  set infected_person? false
  set no_livestock true
;assign gender at town level
  ifelse random-float 1.0 <= 0.474 [ set sex male-code ] [set sex female-code ]
;2002Census Pop Sex and Municipality for rural dedo
  set whoag v_id
  move-to one-of towni with [ whotown = v_id ]
  ]
;assign roles at town level
  let HHpop ( 7065 / Household_Size )
  ask n-of ( HHpop * 0.03 ) agppl with [ sex = male-code and whoag = v_id ]
;sheep shepherd in town 7065/3.2 x 0.03 = 66
  [ set shpshep true
  set no_livestock false
  ]
  ask n-of ( HHpop * 0.48 ) agppl with [ sex = male-code and whoag = v_id ] ; cow
shepherd 7065/3.2 x .48 = 1060
  [ set cowshep true
  set no_livestock false
  ]
  ask n-of ( HHpop * 0.03 * 0.48 ) agppl with [ sex = male-code and shpshep = true
and whoag = v_id ] ; sheep milker: males milk 7065/3.2 x 0.03 x 0.48 = 32
  [ set shpmilk true
  set no_livestock false
  ]
  ask n-of ( HHpop * 0.48 * 0.98 ) agppl with [ whoag = v_id ]
;cow milker: male and female 7065/3.2 x 0.48 x 0.98 = 1039
  [ set cowmilk true
  set no_livestock false
  ]
  ask n-of ( HHpop * 0.38 * 0.98 ) agppl with [ sex = female-code and whoag = v_id ]
;dairy producer are female and cow only - sheep mdp encompassed by shpmilkers
  [ set makedp true
  set no_livestock false
  ]
  ]
;*****VILLAGES*****
set v_id 225
while [ v_id <= 287 ] [
  create-nonagppl 41 [
  set infected_person? false
  ifelse random-float 1.0 <= 0.474 [ set sex male-code ] [set sex female-code ]

```

```

if random-float 1.0 <= 0.00001 [ set infected_person? true ]
set whoag v_id
move-to one-of villageh with [ whovill = v_id ]
]
create-agppl 662 [ ; 94.2% pop engaged in ag in kvaerli- based on % of
HH engaged in ag 703 * .942 = 662
set infected_person? false
set no_livestock true
;assign gender at village level
ifelse random-float 1.0 <= 0.474 [ set sex male-code ] [set sex female-code ]
;2009Yearbook overall Georgia
set whoag v_id
move-to one-of villageh with [ whovill = v_id ]
]
;assign roles at village level
let HHpop ( 662 / Household_Size )
ask n-of ( HHpop * 0.03 ) agppl with [ sex = male-code and whoag = v_id ]
;sheep shepherd in town 662/3.2 x 0.03 = 6
[ set shpshep true
set no_livestock false
]
ask n-of ( HHpop * 0.48 ) agppl with [ sex = male-code and whoag = v_id ] ; cow
shepherd 662/3.2 x .48 = 99
[ set cowshep true
set no_livestock false
]
ask n-of ( HHpop * 0.03 * 0.48 ) agppl with [ sex = male-code and shpshep = true
and whoag = v_id ] ; sheep milker: males milk 662/3.2 x 0.03 x 0.48 = 3
[ set shpmilk true
set no_livestock false
]
ask n-of ( HHpop * 0.48 * 0.98 ) agppl with [ whoag = v_id ]
;cow milker: male and female 662/3.2 x 0.48 x 0.98 = 97
[ set cowmilk true
set no_livestock false
]
ask n-of ( HHpop * 0.48 * 0.98 ) agppl with [ sex = female-code and whoag = v_id ]
;dairy producer are female and cow only - sheep mdp encompassed by shpmilkers
[ set makedp true
set no_livestock false
]
set v_id (v_id + 1 )
]
]
end

```

```

;*****populate livestock*****
to populate_livestock
;*****DEDO_LIVESTOCK*****
****
if District_Population = "Dedoplistkaro" or District_Population = "All" and Livestock =
" Sheep" [
  let v_id 0
  if v_id = 0 [
    let HHpop ( 6965 / Household_Size )
    create-ewe ( HHpop * 0.1 ) [ ; 6965/3.2 (HHsizeJTdata x.1 = 218 represents 38 sheep
per unit
    set color grey
    set whoshp v_id
    set infected? false
    move-to one-of towna with [ whotown = v_id ]
  ]
  ask n-of ( HHpop * 0.1 * 0.48 ) ewe with [ whoshp = v_id ] [ set milkshp true ]
]
set v_id 1
let HHpop ( 1476 / Household_Size )
while [ v_id <= 15 ] [
  create-ewe ( HHpop * 0.1 ) [ ; 1476 / 3.2 (HH size Jtdata) x .1 (pop in ag with
shpAg2004) = 46 represents 38 sheep per unit
  set color grey
  set whoshp v_id
  set infected? false
  move-to one-of villagea with [ whovill = v_id ]
]
  ask n-of ( HHpop * 0.1 * 0.48 ) ewe with [ whoshp = v_id ] [ set milkshp true ]
  set v_id (v_id + 1 )
]
]
if District_Population = "Dedoplistkaro" or District_Population = "All" and Livestock =
" Cattle" [
  let v_id 0
  let HHpop ( 6965 / Household_Size )
  if v_id = 0 [
    create-cattle ( HHpop * 0.42 ) [ ; 6965/3.2 (HHsizeJTdata x 0.42 = 914 represents 3
cows per unit
    set color brown
    set whocatt v_id
    set infected? false
    move-to one-of towna with [ whotown = v_id ]
  ]
  ask n-of ( HHpop * 0.42 * 0.98 ) cattle with [ whocatt = v_id ] [ set milkcw true ]
]
]

```

```

set v_id 1
set HHpop ( 1476 / Household_Size )
while [ v_id <= 15 ] [
  create-cattle ( HHpop * 0.42 ) [ ;1476 / 3.2 (HH size Jtdata) x .42 (pop in ag with
shpAg2004) = 194 and represents 3 cows per unit
  set color brown
  set whocatt v_id
  set infected? false
  move-to one-of villagea with [ whovill = v_id ]
]
ask n-of ( HHpop * 0.42 * 0.98 ) cattle with [ whocatt = v_id ] [ set milkcow true ]
set v_id (v_id + 1 )
]
]
if District_Population = "Dedoplistkaro" or District_Population = "All" and Livestock =
"All" [
  let v_id 0
  let HHpop ( 6965 / Household_Size )
  if v_id = 0 [
    create-ewe ( HHpop * 0.1 ) [ ; 6965/3.2 (HHsizeJTdata x.1 = 218 represents 38 sheep
per unit
    set color grey
    set whoshp v_id
    set infected? false
    move-to one-of towna with [ whotown = v_id ]
  ]
  ask n-of ( HHpop * 0.1 * 0.48 ) ewe with [ whoshp = v_id ] [ set milkshp true ]
  create-cattle ( HHpop * 0.42 ) [ ; 6965/3.2 (HHsizeJTdata x 0.42 = 914 represents 3
cows per unit
  set color brown
  set whocatt v_id
  set infected? false
  move-to one-of towna with [ whotown = v_id ]
  ]
  ask n-of ( HHpop * 0.42 * 0.98 ) cattle with [ whocatt = v_id ] [ set milkcow true ]
  ]
  set v_id 1
  set HHpop ( 1476 / Household_Size )
  while [ v_id <= 15 ] [
    create-ewe ( HHpop * 0.1 ) [ ;1476 / 3.2 (HH size Jtdata) x 0.1 (pop in ag with
shpAg2004) = 46 represents 38 sheep per unit
    set color grey
    set whoshp v_id
    set infected? false
    move-to one-of villagea with [ whovill = v_id ]
  ]
]
]

```

```

    ask n-of ( HHpop * 0.1 * 0.48 ) ewe with [ whoshp = v_id ] [ set milkshp true ]
    create-cattle ( HHpop * 0.42 ) [ ;1476 / 3.2 (HH size Jtdata) x 0.42 (pop in ag with
shpAg2004) = 194 and represents 3 cows per unit
    set color brown
    set whocatt v_id
    set infected? false
    move-to one-of villagea with [ whovill = v_id ]
  ]
  ask n-of ( HHpop * 0.42 * 0.98 ) cattle with [ whocatt = v_id ] [ set milkcow true ]
  set v_id (v_id + 1 )
]
]
.*****SIGNAGI LIVESTOCK*****
if District_Population = "Signagi" or District_Population = "All" and Livestock =
" Sheep" [
  let v_id 16
  let HHpop ( 1905 / Household_Size )
  if v_id = 16 [ ; SIGNAGI
    create-ewe ( HHpop * 0.12 ) [ ; 1905/3.2 (HHsizeJTdata x 0.12 = 71 represents 17
sheep per unit
    set color grey
    set whoshp v_id
    set infected? false
    move-to one-of townb with [ whotown = v_id ]
  ]
  ask n-of ( HHpop * 0.12 * 0.48 ) ewe with [ whoshp = v_id ] [ set milkshp true ]
]
  set v_id 17
  set HHpop ( 5051 / Household_Size )
  if v_id = 17 [ ; TSNORI
    create-ewe ( HHpop * 0.12 ) [ ; 5051/3.2 (HHsizeJTdata x.12 = 189 represents 17
sheep per unit
    set color grey
    set whoshp v_id
    set infected? false
    move-to one-of townc with [ whotown = v_id ]
  ]
  ask n-of ( HHpop * 0.12 * 0.48 ) ewe with [ whoshp = v_id ] [ set milkshp true ]
]
  set HHpop ( 1521 / Household_Size )
  set v_id 18
  while [ v_id <= 36 ] [
    create-ewe ( HHpop * 0.12 ) [ ;1521 / 3.2 (HH size Jtdata) x 0.12 (pop in ag with
shpAg2004) = 57 represents 17 sheep per unit
    set color grey
    set whoshp v_id

```

```

    set infected? false
    move-to one-of villageb with [ whovill = v_id ]
  ]
  ask n-of ( HHpop * 0.12 * 0.48 ) ewe with [ whoshp = v_id ] [ set milkshp true ]
  set v_id (v_id + 1 )
]
]
if District_Population = "Signagi" or District_Population = "All" and Livestock =
"Cattle" [
  let v_id 16
  let HHpop ( 1905 / Household_Size )
  if v_id = 16 [          ;SIGNAGI CATTLE
    create-cattle ( HHpop * 0.28 ) [ ; 1905/3.2 (HHsizeJTdata x 0.28 = 167 represents 2
cows per unit
    set color brown
    set whocatt v_id
    set infected? false
    move-to one-of townb with [ whotown = v_id ]
  ]
  ask n-of ( HHpop * 0.28 * 0.98 ) cattle with [ whocatt = v_id ] [ set milkcw true ]
]
  set HHpop ( 5051 / Household_Size )
  set v_id 17
  if v_id = 17 [          ;TSNORI CATTLE
    create-cattle ( HHpop * 0.28 ) [ ; 5051/3.2 (HHsizeJTdata x 0.28 = 442 represents 2
cows per unit
    set color brown
    set whocatt v_id
    set infected? false
    move-to one-of townc with [ whotown = v_id ]
  ]
  ask n-of ( HHpop * 0.28 * 0.98 ) cattle with [ whocatt = v_id ] [ set milkcw true ]
]
  set v_id 18
  set HHpop ( 1521 / Household_Size )
  while [ v_id <= 36 ] [
    create-cattle ( HHpop * 0.28 ) [ ;1521 / 3.2 (HH size Jtdata) x 0.28 (pop in ag with
shpAg2004) = 133 and represents 2 cows per unit
    set color brown
    set whocatt v_id
    set infected? false
    move-to one-of villageb with [ whovill = v_id ]
  ]
  ask n-of ( HHpop * 0.28 * 0.98 ) cattle with [ whocatt = v_id ] [ set milkcw true ]
  set v_id (v_id + 1 )
]
]

```

```

]
if District_Population = "Signagi" or District_Population = "All" and Livestock = "All" [
  let v_id 16
  let HHpop ( 1905 / Household_Size )
  if v_id = 16 [          ; SIGNAGI
    create-ewe ( HHpop * 0.12 ) [ ; 1905/3.2 (HHsizeJTdata x 0.12 = 71 represents 17
sheep per unit
    set color grey
    set whoshp v_id
    set infected? false
    move-to one-of townb with [ whotown = v_id ]
  ]
  ask n-of ( HHpop * 0.12 * 0.48 ) ewe with [ whoshp = v_id ] [ set milkshp true ]
  create-cattle ( HHpop * 0.28 ) [ ; 1905/3.2 (HHsizeJTdata x 0.28 = 167 represents 2
cows per unit
    set color brown
    set whocatt v_id
    set infected? false
    move-to one-of townb with [ whotown = v_id ]
  ]
  ask n-of ( HHpop * 0.28 * 0.98 ) cattle with [ whocatt = v_id ] [ set milkcow true ]
]
set v_id 17
set HHpop ( 5051 / Household_Size )
if v_id = 17 [          ; TSNORI
  create-ewe ( HHpop * 0.12 ) [ ; 5051/3.2 (HHsizeJTdata x 0.12 = 189 represents 17
sheep per unit
    set color grey
    set whoshp v_id
    set infected? false
    move-to one-of townc with [ whotown = v_id ]
  ]
  ask n-of ( HHpop * 0.12 * 0.48 ) ewe with [ whoshp = v_id ] [ set milkshp true ]
  create-cattle ( HHpop * 0.28 ) [ ; 5051/3.2 (HHsizeJTdata x 0.28 = 442 represents 2
cows per unit
    set color brown
    set whocatt v_id
    set infected? false
    move-to one-of townc with [ whotown = v_id ]
  ]
  ask n-of ( HHpop * 0.28 * 0.98 ) cattle with [ whocatt = v_id ] [ set milkcow true ]
]
set HHpop ( 1521 / Household_Size )
set v_id 18
while [ v_id <= 36 ] [

```

```

    create-ewe ( HHpop * 0.12 ) [ ;1521 / 3.2 (HH size Jtdata) x 0.12 (pop in ag with
shpAg2004) = 57 represents 17 sheep per unit
    set color grey
    set whoshp v_id
    set infected? false
    move-to one-of villageb with [ whovill = v_id ]
  ]
  ask n-of ( HHpop * 0.12 * 0.48 ) ewe with [ whoshp = v_id ] [ set milkshp true ]
  create-cattle ( HHpop * 0.28 ) [ ;1521 / 3.2 (HH size Jtdata) x 0.28 (pop in ag with
shpAg2004) = 133 and represents 2 cows per unit
    set color brown
    set whocatt v_id
    set infected? false
    move-to one-of villageb with [ whovill = v_id ]
  ]
  ask n-of ( HHpop * 0.28 * 0.98 ) cattle with [ whocatt = v_id ] [ set milkcw true ]
  set v_id (v_id + 1 )
]
]
,*****SAGAREGO_LIVESTOCK*****
*
if District_Population = "Sagarego" or District_Population = "All" and Livestock =
" Sheep" [
  let v_id 37
  let HHpop ( 11305 / Household_Size )
  if v_id = 37 [ ; town of Sagarego
    create-ewe ( HHpop * 0.07 ) [ ; 11305/3.2 (HHsizeJTdata x 0.07 = 247 represents
22 sheep per unit
    set color grey
    set whoshp v_id
    set infected? false
    move-to one-of townd with [ whotown = v_id ]
  ]
  ask n-of ( HHpop * 0.07 * 0.48 ) ewe with [ whoshp = v_id ] [ set milkshp true ]
]
  set v_id 38
  set HHpop ( 1095 / Household_Size )
  while [ v_id <= 80 ] [
    create-ewe ( HHpop * 0.07 ) [ ; 1095 / 3.2 (HH size Jtdata) x 0.07 (pop in ag with
shpAg2004) = 24 and represents 22 sheep per unit
    set color grey
    set whoshp v_id
    set infected? false
    move-to one-of villagec with [ whovill = v_id ]
  ]
  ask n-of ( HHpop * 0.07 * 0.48 ) ewe with [ whoshp = v_id ] [ set milkshp true ]

```

```

    set v_id (v_id + 1 )
  ]
]
if District_Population = "Sagarego" or District_Population = "All" and Livestock =
"Cattle" [
  let v_id 37
  let HHpop ( 11305 / Household_Size )
  if v_id = 37 [          ; town of Sagarego
    create-cattle ( HHpop * 0.41 ) [ ; 11305/3.2 (HHsizeJTdata x 0.41 = 1449 represents 2
cows per unit
    set color brown
    set whocatt v_id
    set infected? false
    move-to one-of townd with [ whotown = v_id ]
  ]
  ask n-of ( HHpop * 0.41 * 0.98 ) cattle with [ whocatt = v_id ] [ set milkcw true ]
]
set v_id 38
set HHpop ( 1095 / Household_Size )          ;villages in Sagarego
while [ v_id <= 80 ] [
  create-cattle ( HHpop * 0.41 ) [ ;1095 / 3.2 (HH size Jtdata) x 0.41 (pop in ag with
shpAg2004) = 140 represents 2 cows per unit
  set color brown
  set whocatt v_id
  set infected? false
  move-to one-of villagec with [ whovill = v_id ]
]
  ask n-of ( HHpop * 0.41 * 0.98 ) cattle with [ whocatt = v_id ] [ set milkcw true ]
  set v_id (v_id + 1 )
]
]
if District_Population = "Sagarego" or District_Population = "All" and Livestock = "All"
[
  let v_id 37
  let HHpop ( 11305 / Household_Size )
  if v_id = 37 [          ; town of Sagarego
    create-ewe ( HHpop * 0.07 ) [          ; 11305/3.2 (HHsizeJTdata x 0.07 = 247 represents
22 sheep per unit
    set color grey
    set whoshp v_id
    set infected? false
    move-to one-of townd with [ whotown = v_id ]
  ]
  ask n-of ( HHpop * 0.07 * 0.48 ) ewe with [ whoshp = v_id ] [ set milkshp true ]
  create-cattle ( HHpop * 0.41 ) [ ; 11305/3.2 (HHsizeJTdata x 0.41 = 1449 represents 2
cows per unit

```

```

set color brown
set whocatt v_id
set infected? false
move-to one-of townd with [ whotown = v_id ]
]
ask n-of ( HHpop * 0.41 * 0.98 ) cattle with [ whocatt = v_id ] [ set milkcow true ]
]
set v_id 38
set HHpop ( 1095 / Household_Size )
while [ v_id <= 80 ] [
  create-ewe ( HHpop * 0.07 ) [ ; 1095 / 3.2 (HH size Jtdata) x 0.07 (pop in ag with
shpAg2004) = 24 and represents 22 sheep per unit
  set color grey
  set whoshp v_id
  set infected? false
  move-to one-of villagec with [ whovill = v_id ]
]
ask n-of ( HHpop * 0.07 * 0.48 ) ewe with [ whoshp = v_id ] [ set milkshp true ]
create-cattle ( HHpop * 0.41 ) [ ; 1095 / 3.2 (HH size Jtdata) x 0.41 (pop in ag with
shpAg2004) = 140 represents 2 cows per unit
set color brown
set whocatt v_id
set infected? false
move-to one-of villagec with [ whovill = v_id ]
]
ask n-of ( HHpop * 0.41 * 0.98 ) cattle with [ whocatt = v_id ] [ set milkcow true ]
set v_id (v_id + 1 )
]
]
.*****GURJAANI_LIVESTOCK*****
if District_Population = "Gurjaani" or District_Population = "All" and Livestock =
" Sheep" [
let v_id 81
let HHpop ( 9006 / Household_Size )
if v_id = 81 [ ; Town of Gurjaani
create-ewe ( HHpop * 0.03 ) [ ; 9006/3.2 (HHsizeJTdata x 0.03 = 84
represents 29 sheep per unit
set color grey
set whoshp v_id
set infected? false
move-to one-of towne with [ whotown = v_id ]
]
ask n-of ( HHpop * 0.03 * 0.48 ) ewe with [ whoshp = v_id ] [ set milkshp true ]
]
set v_id 82
set HHpop ( 1975 / Household_Size )

```

```

while [ v_id <= 110 ] [
  create-ewe ( HHpop * 0.03 ) [          ;1975 / 3.2 (HH size Jtdata) x 0.03 (pop in ag
with shpAg2004) = 19 represents 29 sheep per unit
  set color grey
  set whoshp v_id
  set infected? false
  move-to one-of villaged with [ whovill = v_id ]
]
ask n-of ( HHpop * 0.03 * 0.48 ) ewe with [ whoshp = v_id ] [ set milkshp true ]
set v_id (v_id + 1 )
]
]
if District_Population = "Gurjaani" or District_Population = "All" and Livestock =
"Cattle" [
  let v_id 81 ;town of Gurjaani livestock
  let HHpop ( 9006 / Household_Size )
  if v_id = 81 [
    create-cattle ( HHpop * 0.21 ) [          ; 9006/3.2 (HHsizeJTdata x 0.21 = 591
represents 1 cows per unit
    set color brown
    set whocatt v_id
    set infected? false
    move-to one-of towne with [ whotown = v_id ]
  ]
  ask n-of ( HHpop * 0.21 * 0.98 ) cattle with [ whocatt = v_id ] [ set milkcw true ]
]
set v_id 82
set HHpop ( 1975 / Household_Size )
while [ v_id <= 110 ] [
  create-cattle ( HHpop * 0.21 ) [          ;1975 / 3.2 (HH size Jtdata) x 0.21 (pop in ag with
shpAg2004) = 130 and represents 1 cows per unit
  set color brown
  set whocatt v_id
  set infected? false
  move-to one-of villaged with [ whovill = v_id ]
]
ask n-of ( HHpop * 0.21 * 0.98 ) cattle with [ whocatt = v_id ] [ set milkcw true ]
set v_id (v_id + 1 )
]
]
if District_Population = "Gurjaani" or District_Population = "All" and Livestock = "All"
[
  let v_id 81
  let HHpop ( 9006 / Household_Size )
  if v_id = 81 [          ; Town of Gurjaani

```

```

    create-ewe ( HHpop * 0.03 ) [          ; 9006/3.2 (HHsizeJTdata x 0.03 = 84
represents 29 sheep per unit
    set color grey
    set whoshp v_id
    set infected? false
    move-to one-of towne with [ whotown = v_id ]
]
ask n-of ( HHpop * 0.03 * 0.48 ) ewe with [ whoshp = v_id ] [ set milkshp true ]
create-cattle ( HHpop * 0.21 ) [          ; 9006/3.2 (HHsizeJTdata x 0.21 = 591
represents 1 cows per unit
    set color brown
    set whocatt v_id
    set infected? false
    move-to one-of towne with [ whotown = v_id ]
]
ask n-of ( HHpop * 0.21 * 0.98 ) cattle with [ whocatt = v_id ] [ set milkcow true ]
]
set v_id 82
set HHpop ( 1975 / Household_Size )
while [ v_id <= 110 ] [
    create-ewe ( HHpop * 0.03 ) [          ;1975 / 3.2 (HH size Jtdata) x 0.03 (pop in ag
with shpAg2004) = 19 represents 29 sheep per unit
    set color grey
    set whoshp v_id
    set infected? false
    move-to one-of villaged with [ whovill = v_id ]
]
ask n-of ( HHpop * 0.03 * 0.48 ) ewe with [ whoshp = v_id ] [ set milkshp true ]
create-cattle ( HHpop * 0.21 ) [          ;1975 / 3.2 (HH size Jtdata) x 0.21 (pop in ag with
shpAg2004) = 130 and represents 1 cows per unit
    set color brown
    set whocatt v_id
    set infected? false
    move-to one-of villaged with [ whovill = v_id ]
]
ask n-of ( HHpop * 0.21 * 0.98 ) cattle with [ whocatt = v_id ] [ set milkcow true ]
set v_id (v_id + 1 )
]
]
,*****AKHMETA_LIVESTOCK*****
*****
if District_Population = "Akhmeta" or District_Population = "All" and Livestock =
" Sheep" [
    let v_id 111
    let HHpop ( 7871 / Household_Size )
    if v_id = 111 [

```

```

    create-ewe ( HHpop * 0.08 ) [ ; 7871/3.2 (HHsizeJTdata x 0.08 = 197 represents 72
sheep per unit
    set color grey
    set whoshp v_id
    set infected? false
    move-to one-of townf with [ whotown = v_id ]
]
ask n-of ( HHpop * 0.03 * 0.48 ) ewe with [ whoshp = v_id ] [ set milkshp true ]
]
set v_id 112
set HHpop ( 525 / Household_Size )
while [ v_id <= 171 ] [
    create-ewe ( HHpop * 0.08 ) [ ;525 / 3.2 (HH size Jtdata) x 0.08 (pop in ag with
shpAg2004) = 13 represents 72 sheep per unit
    set color grey
    set whoshp v_id
    set infected? false
    move-to one-of villagee with [ whovill = v_id ]
]
ask n-of ( HHpop * 0.03 * 0.48 ) ewe with [ whoshp = v_id ] [ set milkshp true ]
set v_id (v_id + 1 )
]
]
if District_Population = "Akhmeta" or District_Population = "All" and Livestock =
"Cattle" [
    let v_id 111
    let HHpop ( 7871 / Household_Size )
    if v_id = 111 [
        create-cattle ( HHpop * 0.56 ) [ ; 7871/3.2 (HHsizeJTdata x 0.56 = 1377 represents 3
cows per unit
        set color brown
        set whocatt v_id
        set infected? false
        move-to one-of townf with [ whotown = v_id ]
        ]
ask n-of ( HHpop * 0.56 * 0.98 ) cattle with [ whocatt = v_id ] [ set milkcw true ]
]
set v_id 112
set HHpop ( 525 / Household_Size )
while [ v_id <= 171 ] [
    create-cattle ( HHpop * 0.56 ) [ ;525 / 3.2 (HH size Jtdata) x 0.56 (pop in ag with
shpAg2004) = 92 and represents 3 cows per unit
    set color brown
    set whocatt v_id
    set infected? false
    move-to one-of villagee with [ whovill = v_id ]
]
]
]

```

```

]
  ask n-of ( HHpop * 0.56 * 0.98 ) cattle with [ whocatt = v_id ] [ set milkcow true ]
  set v_id (v_id + 1 )
]
]
if District_Population = "Akhmeta" or District_Population = "All" and Livestock = "All"
[
  let v_id 111
  let HHpop ( 7871 / Household_Size )
  if v_id = 111 [
    create-ewe ( HHpop * 0.08 ) [ ; 7871/3.2 (HHsizeJTdata x 0.08 = 197 represents 72
sheep per unit
      set color grey
      set whoshp v_id
      set infected? false
      move-to one-of townf with [ whotown = v_id ]
    ]
    ask n-of ( HHpop * 0.08 * 0.48 ) ewe with [ whoshp = v_id ] [ set milkshp true ]
    create-cattle ( HHpop * 0.56 ) [ ; 7871/3.2 (HHsizeJTdata x 0.56 = 1377 represents 3
cows per unit
      set color brown
      set whocatt v_id
      set infected? false
      move-to one-of townf with [ whotown = v_id ]
    ]
    ask n-of ( HHpop * 0.56 * 0.98 ) cattle with [ whocatt = v_id ] [ set milkcow true ]
  ]
  set v_id 112
  set HHpop ( 525 / Household_Size )
  while [ v_id <= 171 ] [
    create-ewe ( HHpop * 0.08 ) [ ;525 / 3.2 (HH size Jtdata) x 0.08 (pop in ag with
shpAg2004) = 13 represents 72 sheep per unit
      set color grey
      set whoshp v_id
      set infected? false
      move-to one-of villagee with [ whovill = v_id ]
    ]
    ask n-of ( HHpop * 0.08 * 0.48 ) ewe with [ whoshp = v_id ] [ set milkshp true ]
    create-cattle ( HHpop * 0.56 ) [ ;525 / 3.2 (HH size Jtdata) x 0.56 (pop in ag with
shpAg2004) = 92 and represents 3 cows per unit
      set color brown
      set whocatt v_id
      set infected? false
      move-to one-of villagee with [ whovill = v_id ]
    ]
    ask n-of ( HHpop * 0.56 * 0.98 ) cattle with [ whocatt = v_id ] [ set milkcow true ]
  ]
]

```

```

    set v_id (v_id + 1 )
  ]
]
,*****TELAVI_LIVESTOCK*****
****
if District_Population = "Telavi" or District_Population = "All" and Livestock = "Sheep"
[
  let v_id 172
  let HHpop ( 18070 / Household_Size )
  if v_id = 172 [
    create-ewe ( HHpop * 0.03 ) [ ; 18070/3.2 (HHsizeJTdata x 0.03 = 169 represents 28
sheep per unit
    set color grey
    set whoshp v_id
    set infected? false
    move-to one-of towng with [ whotown = v_id ]
  ]
  ask n-of ( HHpop * 0.03 * 0.48 ) ewe with [ whoshp = v_id ] [ set milkshp true ]
]
set v_id 173
set HHpop ( 1563 / Household_Size )
while [ v_id <= 201 ] [
  create-ewe ( HHpop * 0.03 ) [ ; 1563/ 3.2 (HH size Jtdata) x 0.03 (pop in ag with
shpAg2004) = 15 represents 28 sheep per unit
  set color grey
  set whoshp v_id
  set infected? false
  move-to one-of villagef with [ whovill = v_id ]
]
  ask n-of ( HHpop * 0.03 * 0.48 ) ewe with [ whoshp = v_id ] [ set milkshp true ]
  set v_id (v_id + 1 )
]
]
if District_Population = "Telavi" or District_Population = "All" and Livestock = "Cattle"
[
  let v_id 172
  let HHpop ( 18070 / Household_Size )
  if v_id = 172 [
    create-cattle ( HHpop * 0.2 ) [ ; 18070/3.2 (HHsizeJTdata x 0.2 = 1129 represents 2
cows per unit
    set color brown
    set whocatt v_id
    set infected? false
    move-to one-of towng with [ whotown = v_id ]
  ]
  ask n-of ( HHpop * 0.2 * 0.98 ) cattle with [ whocatt = v_id ] [ set milkcw true ]
]
]

```

```

]
set v_id 173
set HHpop ( 1563 / Household_Size )
while [ v_id <= 201 ] [
  create-cattle ( HHpop * 0.2 ) [ ;1563 / 3.2 (HH size Jtdata) x 0.2 (pop in ag with
shpAg2004) = 98 and represents 2 cows per unit
  set color brown
  set whocatt v_id
  set infected? false
  move-to one-of villagef with [ whovill = v_id ]
]
ask n-of ( HHpop * 0.2 * 0.98 ) cattle with [ whocatt = v_id ] [ set milkcow true ]
set v_id (v_id + 1 )
]
]
if District_Population = "Telavi" or District_Population = "All" and Livestock = "All" [
  let v_id 172
  let HHpop ( 18070 / Household_Size )
  if v_id = 172 [
    create-ewe ( HHpop * 0.03 ) [ ; 18070/3.2 (HHsizeJTdata x 0.03 = 169 represents 28
sheep per unit
    set color grey
    set whoshp v_id
    set infected? false
    move-to one-of towng with [ whotown = v_id ]
  ]
  ask n-of ( HHpop * 0.03 * 0.48 ) ewe with [ whoshp = v_id ] [ set milkshp true ]
  create-cattle ( HHpop * 0.2 ) [ ; 18070/3.2 (HHsizeJTdata x 0.2 = 1129 represents 2
cows per unit
  set whocatt v_id
  set infected? false
  set color brown
  move-to one-of towng with [ whotown = v_id ]
]
ask n-of ( HHpop * 0.2 * 0.98 ) cattle with [ whocatt = v_id ] [ set milkcow true ]
]
set v_id 173
set HHpop ( 1563 / Household_Size )
while [ v_id <= 201 ] [
  create-ewe ( HHpop * 0.03 ) [ ; 1563/ 4.2 (HH size Jtdata) x 0.03 (pop in ag with
shpAg2004) = 15 represents 28 sheep per unit
  set color grey
  set whoshp v_id
  set infected? false
  move-to one-of villagef with [ whovill = v_id ]
]
]

```

```

    ask n-of ( HHpop * 0.03 * 0.48 ) ewe with [ whoshp = v_id ] [ set milkshp true ]
    create-cattle ( HHpop * 0.2 ) [ ;1563 / 3.2 (HH size Jtdata) x 0.2 (pop in ag with
shpAg2004) = 98 and represents 2 cows per unit
    set color brown
    set whocatt v_id
    set infected? false
    move-to one-of villagef with [ whovill = v_id ]
  ]
  ask n-of ( HHpop * 0.2 * 0.98 ) cattle with [ whocatt = v_id ] [ set milkcw true ]
  set v_id (v_id + 1 )
]
]
.*****KVARELI_LIVESTOCK*****
*****
if District_Population = "Kvareli" or District_Population = "All" and Livestock =
" Sheep" [
  let v_id 202
  let HHpop ( 8204 / Household_Size )
  if v_id = 202 [
    create-ewe ( HHpop * 0.04 ) [ ; 8204/3.2 (HHsizeJTdata x 0.04 = 103 represents 54
sheep per unit
    set color grey
    set whoshp v_id
    set infected? false
    move-to one-of townh with [ whotown = v_id ]
  ]
  ask n-of ( HHpop * 0.04 * 0.48 ) ewe with [ whoshp = v_id ] [ set milkshp true ]
]
  set v_id 203
  set HHpop ( 1295 / Household_Size )
  while [ v_id <= 223 ] [
    create-ewe ( HHpop * 0.04 ) [ ; 1295/ 3.2 (HH size Jtdata) x 0.04 (pop in ag with
shpAg2004) = 16 represents 54 sheep per unit
    set color grey
    set whoshp v_id
    set infected? false
    move-to one-of villageg with [ whovill = v_id ]
  ]
  ask n-of ( HHpop * 0.04 * 0.48 ) ewe with [ whoshp = v_id ] [ set milkshp true ]
  set v_id (v_id + 1 )
]
]
if District_Population = "Kvareli" or District_Population = "All" and Livestock =
" Cattle" [
  let v_id 202
  let HHpop ( 8204 / Household_Size )

```

```

if v_id = 202 [
  create-cattle ( HHpop * 0.38 ) [ ; 8204/3.2 (HHsizeJTdata x 0.38 = 974 represents 1
cows per unit
  set color brown
  set whocatt v_id
  set infected? false
  move-to one-of townh with [ whotown = v_id ]
]
  ask n-of ( HHpop * 0.38 * 0.98 ) cattle with [ whocatt = v_id ] [ set milkcw true ]
]
set v_id 203
set HHpop ( 1295 / Household_Size )
while [ v_id <= 223 ] [
  create-cattle ( HHpop * 0.38 ) [ ;1295/ 3.2 (HH size Jtdata) x 0.38 (pop in ag with
shpAg2004) = 154 and represents 1 cows per unit
  set color brown
  set whocatt v_id
  set infected? false
  move-to one-of villageg with [ whovill = v_id ]
]
  ask n-of ( HHpop * 0.38 * 0.98 ) cattle with [ whocatt = v_id ] [ set milkcw true ]
  set v_id (v_id + 1 )
]
]
if District_Population = "Kvareli" or District_Population = "All" and Livestock = "All" [
  let v_id 202
  let HHpop ( 8204 / Household_Size )
  if v_id = 202 [
    create-ewe ( HHpop * 0.04 ) [ ; 8204/3.2 (HHsizeJTdata x 0.04 = 103 represents 54
sheep per unit
    set color grey
    set whoshp v_id
    set infected? false
    move-to one-of townh with [ whotown = v_id ]
  ]
    ask n-of ( HHpop * 0.04 * 0.48 ) ewe with [ whoshp = v_id ] [ set milkshp true ]
    create-cattle ( HHpop * 0.38 ) [ ; 8204/3.2 (HHsizeJTdata x 0.38 = 974 represents 1
cows per unit
    set color brown
    set whocatt v_id
    set infected? false
    move-to one-of townh with [ whotown = v_id ]
  ]
    ask n-of ( HHpop * 0.38 * 0.98 ) cattle with [ whocatt = v_id ] [ set milkcw true ]
  ]
  set v_id 203

```

```

set HHpop ( 1295 / Household_Size )
while [ v_id <= 223 ] [
  create-ewe ( HHpop * 0.04 ) [ ; 1295/ 3.2 (HH size Jtdata) x 0.04 (pop in ag with
shpAg2004) = 16 represents 54 sheep per unit
  set color grey
  set whoshp v_id
  set infected? false
  move-to one-of villageg with [ whovill = v_id ]
]
ask n-of 8 ewe with [ whoshp = v_id ] [ set milkshp true ]
create-cattle ( HHpop * 0.38 ) [ ; 1295/ 3.2 (HH size Jtdata) x 0.38 (pop in ag with
shpAg2004) = 154 and represents 1 cows per unit
  set color brown
  set whocatt v_id
  set infected? false
  move-to one-of villageg with [ whovill = v_id ]
]
ask n-of ( HHpop * 0.38 * 0.98 ) cattle with [ whocatt = v_id ] [ set milkcow true ]
set v_id (v_id + 1 )
]
]
,*****LAGODEKHI_LIVESTOCK*****
*****
if District_Population = "Lagodekhi" or District_Population = "All" and Livestock =
" Sheep" [
  let v_id 224
  let HHpop ( 7065 / Household_Size )
  if v_id = 224 [
    create-ewe ( HHpop * 0.03 ) [ ; 7065/3.2 (HHsizeJTdata x 0.03 = 66 represents 31
sheep per unit
    set color grey
    set whoshp v_id
    set infected? false
    move-to one-of towni with [ whotown = v_id ]
  ]
  ask n-of ( HHpop * 0.03 * 0.48 ) ewe with [ whoshp = v_id ] [ set milkshp true ]
]
set v_id 225
set HHpop ( 662 / Household_Size )
while [ v_id <= 287 ] [
  create-ewe ( HHpop * 0.03 ) [ ; 662/ 3.2 (HH size Jtdata) x 0.03 (pop in ag with
shpAg2004) = 6 represents 31 sheep per unit
  set color grey
  set whoshp v_id
  set infected? false
  move-to one-of villageh with [ whovill = v_id ]
]
]

```

```

]
ask n-of ( HHpop * 0.03 * 0.48 ) ewe with [ whoshp = v_id ] [ set milkshp true ]
set v_id (v_id + 1 )
]
]
if District_Population = "Lagodekhi" or District_Population = "All" and Livestock =
"Cattle" [
let v_id 224
let HHpop ( 7065 / Household_Size )
if v_id = 224 [
create-cattle ( HHpop * 0.48 ) [ ; 7065/3.2 (HHsizeJTdata x 0.48 = 1060 represents 2
cows per unit
set color brown
set whocatt v_id
set infected? false
move-to one-of towni with [ whotown = v_id ]
]
ask n-of ( HHpop * 0.48 * 0.98 ) cattle with [ whocatt = v_id ] [ set milkcw true ]
]
set v_id 225
set HHpop ( 662 / Household_Size )
while [ v_id <= 287 ] [
create-cattle ( HHpop * 0.48 ) [ ;662/ 3.2 (HH size Jtdata) x 0.48 (pop in ag with
shpAg2004) = 99 and represents 2 cows per unit
set color brown
set whocatt v_id
set infected? false
move-to one-of villageh with [ whovill = v_id ]
]
ask n-of ( HHpop * 0.48 * 0.98 ) cattle with [ whocatt = v_id ] [ set milkcw true ]
set v_id (v_id + 1 )
]
]
if District_Population = "Lagodekhi" or District_Population = "All" and Livestock =
"All" [
let v_id 224
let HHpop ( 7065 / Household_Size )
if v_id = 224 [
create-ewe ( HHpop * 0.03 ) [ ; 7065/3.2 (HHsizeJTdata x 0.03 = 66 represents 31
sheep per unit
set color grey
set whoshp v_id
set infected? false
move-to one-of towni with [ whotown = v_id ]
]
ask n-of ( HHpop * 0.03 * 0.48 ) ewe with [ whoshp = v_id ] [ set milkshp true ]

```

```

    create-cattle ( HHpop * 0.48 ) [ ; 7065/3.2 (HHsizeJTdata x 0.48 = 1060 represents 2
cows per unit
    set color brown
    set whocatt v_id
    set infected? false
    move-to one-of towni with [ whotown = v_id ]
  ]
  ask n-of ( HHpop * 0.48 * 0.98 ) cattle with [ whocatt = v_id ] [ set milkcow true ]
]
set v_id 225
set HHpop ( 662 / Household_Size )
while [ v_id <= 287 ] [
  create-ewe ( HHpop * 0.03 ) [ ; 662/ 3.2 (HH size Jtdata) x 0.03 (pop in ag with
shpAg2004) = 6 represents 31 sheep per unit
  set color grey
  set whoshp v_id
  set infected? false
  move-to one-of villageh with [ whovill = v_id ]
]
  ask n-of ( HHpop * 0.03 * 0.48 ) ewe with [ whoshp = v_id ] [ set milkshp true ]
  create-cattle ( HHpop * 0.48 ) [ ; 662/ 3.2 (HH size Jtdata) x 0.48 (pop in ag with
shpAg2004) = 99 and represents 2 cows per unit
  set color brown
  set whocatt v_id
  set infected? false
  move-to one-of villageh with [ whovill = v_id ]
]
  ask n-of ( HHpop * 0.48 * 0.98 ) cattle with [ whocatt = v_id ] [ set milkcow true ]
  set v_id ( v_id + 1 )
]
]
ask ewe [
  set heading 0
  rt random 179
  let dist random-float 5.0
  fd dist
]
ask cattle [
  set heading 180
  rt random 180
  let dist random-float 5.0
  fd dist
]
if Sheep_vaccinated [
  ask ewe with [ infected? = false ] [
    if ( random-float 100 ) <= ( ( Vax_Pop_Coverage * Vaccine_Efficacy) / 100 ) [

```

```

    set vaccinated? true
    set color green
  ]
]
]
ask ewe [
  if ( random-float 100 ) <= Ewe_infected [
    if vaccinated? != true [
      set infected? true
      set color blue
    ]
  ]
]
ask cattle [
  if ( random-float 100 ) <= Cattle_infected [
    set infected? true
    set color black
  ]
]
;*****FOR YEARS ONE AND TWO OF THE PROGRAM*****
if Cattle_culled [
  ask cattle with [ infected? = true ] [
    if random-float 100 <= Cattle_Test_Coverage [
      let TruePos random-float 98.3 ; Nielson 2002 Se (21 to 98.3 ) (FN: 1.7 to 79 )
      if random-float 100 <= TruePos
        [ set shape "x"
          set dead true
          set color red ]
    ]
  ]
  ask cattle with [ infected? = false ] [
    if random-float 100 <= Cattle_Test_Coverage [
      let FalsePos random-float 31.2 ; Nielson 2002 Sp (68.8 to 100 ) (FP: 0 to
31.2)
      if random-float 100 <= FalsePos
        [ set shape "x"
          set dead true
          set color red ]
    ]
  ]
]
end
;*****ABOVE FOR YEARS ONE AND TWO OF THE PROGRAM*****

;*****FOR YEARS THREE THROUGH FIVE OF THE PROGRAM*****
if Cattle_vaccinate [

```

```

ask cattle with [ infected? = false ] [
  if random-float 100 <= ( (Cattle_Vax_Pop_Coverage * Vaccine_Efficacy) / 100 ) [
    set vaccinated? true
    set color green
  ]
]
]
ask cattle [
  if ( random-float 100 ) <= Cattle_infected [
    if vaccinated? != true [
      set infected? true
      set color black
    ]
  ]
]
end
,*****ABOVE FOR YEARS THREE THROUGH FIVE OF THE PROGRAM*****

,*****TO GO*****
to go
set ORSheep 19.3
set ORAnimalwork 77.8
set ORmdp 12.4
set DedoRisk 0.00001
set SigRisk 0.000001
set SagRisk 0.00001
set GurjRisk 0.00001
set AkhRisk 0.000008
set TelRisk 0.00001
set KvarRisk 0.000004
set LagoRisk 0.00001
get_date
agppl_nolivestock
actsheepmilk
actsheepshep
actcowmilk
actcowmdp
actcowshep
tick
if ticks > 365 [
  result
  stop
]
end

```

```

to agppl_nolivestock
  if ticks = 0 [
    if District_Population = "Dedoplistkaro" or District_Population = "Sagarego" or
    District_Population = "Gurjaani" or District_Population = "Telavi" or District_Population
    = "Lagodekhi" or District_Population = District_Population = "All" [
      let v_id 0
      while [ v_id <= 15 or ( v_id > 36 and v_id <= 110 ) or ( v_id > 171 and v_id <= 201 )
      or ( v_id > 223 and v_id <= 287 ) ] [
        ask agppl with [ no_livestock = true and whoag = v_id ] [
          if random-float 1.0 <= 0.00001 [
            set infected_person? true
          ]
        ]
        set v_id v_id + 1
      ]
    ]
    if District_Population = "Signagi" or District_Population = "All" [
      let v_id 16
      while [ v_id <= 36 ] [
        ask agppl with [ no_livestock = true and whoag = v_id ] [
          if random-float 1.0 <= SigRisk [
            set infected_person? true
          ]
        ]
        set v_id v_id + 1
      ]
    ]
    if District_Population = "Akhmeta" or District_Population = "All" [
      let v_id 111
      while [ v_id <= 171 ] [
        ask agppl with [ no_livestock = true and whoag = v_id ] [
          if random-float 1.0 <= AkhRisk [
            set infected_person? true
          ]
        ]
        set v_id v_id + 1
      ]
    ]
    if District_Population = "Kvareli" or District_Population = "All" [
      let v_id 202
      while [ v_id <= 223 ] [
        ask agppl with [ no_livestock = true and whoag = v_id ] [
          if random-float 1.0 <= KvarRisk [
            set infected_person? true
          ]
        ]
      ]
    ]
  ]

```

```

        set v_id v_id + 1
    ]
]
]
end

to actsheepmilk
  if Livestock = "Sheep" or Livestock = "All" [
    if ticks = 1 [
      link_sheepmilk
      infect_sheepmilk
      move_home
    ]
  ]
end

to actsheepshep
  if Livestock = "Sheep" or Livestock = "All" [
    if ticks <= 244 [
      move_shepherdsew
      infect_shepherdsew
      move_home
    ]
  ]
end

to actcowmilk
  if Livestock = "Cattle" or Livestock = "All" [
    if ticks = 1 [
      link_cowmilk
      infect_cowmilk
      move_home
    ]
  ]
end

to actcowmdp
  if Livestock = "Cattle" or Livestock = "All" [
    if ticks = 1 [
      link_cowmdp
      infect_cowmdp
      move_home
    ]
  ]
end

```

```

to actcowshep
  if Livestock = "Cattle" or Livestock = "All" [
    if ticks <= 335 [
      move_shepherdschow
      infect_shepherdschow
      move_home
    ]
  ]
end

```

```

to link_sheepmilk
  let v_id 0
  while [ v_id <= 287 ] [
    ask agppl with [ shpmilk = true and whoag = v_id ] [
      move-to one-of ewe with [ milkshp = true and whoshp = v_id ]
      create-link-with one-of ewe-here with [ milkshp = true and whoshp = v_id ]
    ]
    set v_id v_id + 1
  ]
end

```

```

to infect_sheepmilk
  if District_Population = "Dedoplistkaro" or District_Population = "Sagarego" or
  District_Population = "Gurjaani" or District_Population = "Telavi" or District_Population
  = "Lagodekhi" or District_Population = District_Population = "All" [
    ask ewe with [ milkshp = true and infected? = true and whoshp <= 15 or ( whoshp > 36
    and whoshp <= 110 ) or ( whoshp > 171 and whoshp <= 201 ) or ( whoshp > 223 and
    whoshp <= 287 ) ] [
      ask link-neighbors [
        if random-float 1.00000 <= ( 0.00001 * ORSheep * ORAnimalwork ) [
          set infected_person? true
          set color pink
          set shape "face sad"
          set size 5
        ]
      ]
    ]
  ]
  if District_Population = "Signagi" or District_Population = "All" [
    ask ewe with [ milkshp = true and infected? = true and whoshp > 15 and whoshp <= 36
  ] [
    ask link-neighbors [
      if random-float 1.00000 <= ( SigRisk * ORSheep * ORAnimalwork ) [
        set infected_person? true
      ]
    ]
  ]
end

```

```

    set color pink
    set shape "face sad"
    set size 5
  ]
]
]
]
if District_Population = "Akhmeta" or District_Population = "All" [
  ask ewe with [ milkshp = true and infected? = true and whoshp > 110 and whoshp <=
171 ] [
    ask link-neighbors [
      if random-float 1.00000 <= ( AkhRisk * ORSheep * ORanimalwork ) [
        set infected_person? true
        set color pink
        set shape "face sad"
        set size 5
      ]
    ]
  ]
]
if District_Population = "Kvareli" or District_Population = "All" [
  ask ewe with [ milkshp = true and infected? = true and whoshp > 201 and whoshp <=
223 ] [
    ask link-neighbors [
      if random-float 1.00000 <= ( KvarRisk * ORSheep * ORanimalwork ) [
        set infected_person? true
        set color pink
        set shape "face sad"
        set size 5
      ]
    ]
  ]
]
end

```

```

to move_shepherdsewe
  let shep# ( ( ( (count ewe with [ whoshp = 0 ] ) * 38 ) / 300 ) * 4 )
  if District_Population = "Dedoplistkaro" or District_Population = "All" [
    ask n-of shep# agppl with [ shpshep = true and whoag = 0 ] [
      set activeshepewe true
    ]
  ]
  let i 1
  set shep# ( ( ( (count ewe with [ whoshp = 1 ] ) * 38 ) / 300 ) * 4 )
  while [ i <= 15 ] [
    ask n-of shep# agppl with [ shpshep = true and whoag = i ] [
      set activeshepewe true
    ]
  ]
end

```

```

]
  set i i + 1
]
]
set shep# ( ( ( (count ewe with [ whoshp = 16 ] ) * 17 ) / 300 ) * 4 )
if District_Population = "Signagi" or District_Population = "All" [
  ask n-of shep# agppl with [ shpshep = true and whoag = 16 ] [
    set activeshepewe true
  ]
  set shep# ( ( ( (count ewe with [ whoshp = 17 ] ) * 17 ) / 300 ) * 4 )
  ask n-of shep# agppl with [ shpshep = true and whoag = 17 ] [
    set activeshepewe true
  ]
  set shep# ( ( ( (count ewe with [ whoshp = 18 ] ) * 17 ) / 300 ) * 4 )
  let i 18
  while [ i <= 36 ] [
    ask n-of shep# agppl with [ shpshep = true and whoag = i ] [
      set activeshepewe true
    ]
    set i i + 1
  ]
]
set shep# ( ( ( (count ewe with [ whoshp = 37 ] ) * 22 ) / 300 ) * 4 )
if District_Population = "Sagarego" or District_Population = "All" [
  ask n-of shep# agppl with [ shpshep = true and whoag = 37 ] [
    set activeshepewe true
  ]
  let i 38
  set shep# ( ( ( (count ewe with [ whoshp = 38 ] ) * 22 ) / 300 ) * 4 )
  while [ i <= 80 ] [
    ask n-of shep# agppl with [ shpshep = true and whoag = i ] [
      set activeshepewe true
    ]
    set i i + 1
  ]
]
set shep# ( ( ( (count ewe with [ whoshp = 81 ] ) * 29 ) / 300 ) * 4 )
if District_Population = "Gurjaani" or District_Population = "All" [
  ask n-of shep# agppl with [ shpshep = true and whoag = 81 ] [
    set activeshepewe true
  ]
  let i 82
  set shep# ( ( ( (count ewe with [ whoshp = 82 ] ) * 29 ) / 300 ) * 4 )
  while [ i <= 110 ] [
    ask n-of shep# agppl with [ shpshep = true and whoag = i ] [
      set activeshepewe true
    ]
  ]
]

```

```

]
  set i i + 1
]
]
set shep# ( ( ( (count ewe with [ whoshp = 111 ]) * 72 ) / 300 ) * 4 )
if District_Population = "Akhmeta" or District_Population = "All" [
  ask n-of shep# agppl with [ shpshep = true and whoag = 111 ] [
    set activeshepewe true
  ]
]
let i 112
set shep# ( ( ( (count ewe with [ whoshp = 112 ]) * 72 ) / 300 ) * 4 )
while [ i <= 171 ] [
  ask n-of shep# agppl with [ shpshep = true and whoag = i ] [
    set activeshepewe true
  ]
  set i i + 1
]
]
]
set shep# ( ( ( (count ewe with [ whoshp = 172 ]) * 28 ) / 300 ) * 4 )
if District_Population = "Telavi" or District_Population = "All" [
  ask n-of shep# agppl with [ shpshep = true and whoag = 172 ] [
    set activeshepewe true
  ]
]
let i 173
set shep# ( ( ( (count ewe with [ whoshp = 173 ]) * 28 ) / 300 ) * 4 )
while [ i <= 201 ] [
  ask n-of shep# agppl with [ shpshep = true and whoag = i ] [
    set activeshepewe true
  ]
  set i i + 1
]
]
]
set shep# ( ( ( (count ewe with [ whoshp = 202 ]) * 54 ) / 300 ) * 4 )
if District_Population = "Kvareli" or District_Population = "All" [
  ask n-of shep# agppl with [ shpshep = true and whoag = 202 ] [
    set activeshepewe true
  ]
]
let i 203
set shep# ( ( ( (count ewe with [ whoshp = 203 ]) * 54 ) / 300 ) * 4 )
while [ i <= 223 ] [
  ask n-of 12 agppl with [ shpshep = true and whoag = i ] [
    set activeshepewe true
  ]
  set i i + 1
]
]
]

```

```

set shep# ( ( ( (count ewe with [ whoshp = 224 ] ) * 31 ) / 300 ) * 4 )
if District_Population = "Lagodekhi" or District_Population = "All" [
  ask n-of 28 agppl with [ shpshep = true and whoag = 224 ] [
    set activeshepewe true
  ]
]
let i 225
set shep# ( ( ( (count ewe with [ whoshp = 225 ] ) * 31 ) / 300 ) * 4 )
while [ i <= 287 ] [
  ask n-of 4 agppl with [ shpshep = true and whoag = i ] [
    set activeshepewe true
  ]
  set i i + 1
]
]
ask agppl with [ shpshep = true and activeshepewe = true ] [
  set heading 0
  rt random 180
  fd random-float 5.0
]
end

to infect_shepherdsewe
if District_Population = "Dedoplistkaro" or District_Population = "All" [
  ask agppl with [ activeshepewe = true and infected_person? = false and whoag >= 0
and whoag <= 15] [
  if any? ewe-here with [ infected? = true ] [
    if random-float 1.00000 <= (( DedoRisk * ORSheep * ORAnimalwork ) / 126) [
      set infected_person? true
      set color red
      set shape "face sad"
      set size 5
    ]
  ]
]
]
]
if District_Population = "Signagi" or District_Population = "All" [
  ask agppl with [ activeshepewe = true and infected_person? = false and whoag > 15 and
whoag <= 36 ] [
  if any? ewe-here with [ infected? = true ] [
    if random-float 1.00000 <= (( SigRisk * ORSheep * ORAnimalwork ) / 53) [
      set infected_person? true
      set color red
      set shape "face sad"
      set size 5
    ]
  ]
]
]
]

```

```

]
]
if District_Population = "Sagarego" or District_Population = "All" [
  ask agppl with [ activeshepewe = true and infected_person? = false and whoag > 36 and
whoag <= 80 ] [
  if any? ewe-here with [ infected? = true ] [
    if random-float 1.00000 <= (( SagRisk * ORSheep * ORAnimalwork ) / 76) [
      set infected_person? true
      set color red
      set shape "face sad"
      set size 5
    ]
  ]
]
]
]
if District_Population = "Gurjaani" or District_Population = "All" [
  ask agppl with [ activeshepewe = true and infected_person? = false and whoag > 80 and
whoag <= 110 ] [
  if any? ewe-here with [ infected? = true ] [
    if random-float 1.00000 <= (( GurjRisk * ORSheep * ORAnimalwork ) / 98) [
      set infected_person? true
      set color red
      set shape "face sad"
      set size 5
    ]
  ]
]
]
]
if District_Population = "Akhmeta" or District_Population = "All" [
  ask agppl with [ activeshepewe = true and infected_person? = false and whoag > 110
and whoag <= 171 ] [
  if any? ewe-here with [ infected? = true ] [
    if random-float 1.00000 <= (( AkhRisk * ORSheep * ORAnimalwork ) / 229) [
      set infected_person? true
      set color red
      set shape "face sad"
      set size 5
    ]
  ]
]
]
]
]
if District_Population = "Telavi" or District_Population = "All" [
  ask agppl with [ activeshepewe = true and infected_person? = false and whoag = 172 ] [
  if any? ewe-here with [ infected? = true ] [
    if random-float 1.00000 <= (( TelRisk * ORSheep * ORAnimalwork ) / 92) [
      set infected_person? true

```

```

    set color red
    set shape "face sad"
    set size 5
  ]
]
]
ask agppl with [ activeshepewe = true and infected_person? = false and whoag > 172
and whoag <= 201 ] [
  if any? ewe-here with [ infected? = true ] [
    if random-float 1.00000 <= (( TelRisk * ORSheep * ORAnimalwork ) / 130) [
      set infected_person? true
      set color red
      set shape "face sad"
      set size 5
    ]
  ]
]
]
]
if District_Population = "Kvareli" or District_Population = "All" [
  ask agppl with [ activeshepewe = true and infected_person? = false and whoag > 201
and whoag <= 223 ] [
    if any? ewe-here with [ infected? = true ] [
      if random-float 1.00000 <= (( KvarRisk * ORSheep * ORAnimalwork ) / 182) [
        set infected_person? true
        set color red
        set shape "face sad"
        set size 5
      ]
    ]
  ]
]
]
]
if District_Population = "Lagodekhi" or District_Population = "All" [
  ask agppl with [ activeshepewe = true and infected_person? = false and whoag = 224 ] [
    if any? ewe-here with [ infected? = true ] [
      if random-float 1.00000 <= (( LagoRisk * ORSheep * ORAnimalwork ) / 104) [
        set infected_person? true
        set color red
        set shape "face sad"
        set size 5
      ]
    ]
  ]
]
]
]
ask agppl with [ activeshepewe = true and infected_person? = false and whoag > 223
and whoag <= 287 ] [
  if any? ewe-here with [ infected? = true ] [
    if random-float 1.00000 <= (( LagoRisk * ORSheep * ORAnimalwork ) / 163) [

```

```

    set infected_person? true
    set color red
    set shape "face sad"
    set size 5
  ]
]
]
]
end

```

```

to link_cowmilk
  let v_id 0
  while [ v_id <= 287 ] [
    ask agppl with [ cowmilk = true and whoag = v_id ] [
      move-to one-of cattle with [ milkcow = true and whocatt = v_id ]
      create-link-with one-of cattle-here with [ milkcow = true and whocatt = v_id ]
    ]
    set v_id v_id + 1
  ]
end

```

```

to infect_cowmilk
  if District_Population = "Dedoplistkaro" or District_Population = "Sagarego" or
  District_Population = "Gurjaani" or District_Population = "Telavi" or District_Population
  = "Lagodekhi" or District_Population = District_Population = "All" [
    ask cattle with [ milkcow = true and dead != true and infected? = true and whocatt <=
    15 or ( whocatt > 36 and whocatt <= 110 ) or ( whocatt > 171 and whocatt <= 201 ) or (
    whocatt > 223 and whocatt <= 287 ) ] [
      ask link-neighbors [
        if infected_person? = false [
          if random-float 1.00000 <= ( 0.00001 * ORanimalwork ) [
            set infected_person? true
            set color green
            set shape "face sad"
            set size 5
          ]
        ]
      ]
    ]
  ]
  if District_Population = "Signagi" or District_Population = "All" [
    ask cattle with [ milkcow = true and dead != true and infected? = true and whocatt > 15
    and whocatt <= 36 ] [
      ask link-neighbors [
        if infected_person? = false [

```

```

    if random-float 1.00000 <= ( SigRisk * ORanimalwork ) [
      set infected_person? true
      set color green
      set shape "face sad"
      set size 5
    ]
  ]
]
]
]
]
if District_Population = "Akhmeta" or District_Population = "All" [
  ask cattle with [ milkcow = true and dead != true and infected? = true and whocatt >
110 and whocatt <= 171 ] [
  ask link-neighbors [
    if infected_person? = false [
      if random-float 1.00000 <= ( AkhRisk * ORanimalwork ) [
        set infected_person? true
        set color green
        set shape "face sad"
        set size 5
      ]
    ]
  ]
]
]
]
if District_Population = "Kvareli" or District_Population = "All" [
  ask cattle with [ milkcow = true and dead != true and infected? = true and whocatt >
201 and whocatt <= 223 ] [
  ask link-neighbors [
    if infected_person? = false [
      if random-float 1.00000 <= ( KvarRisk * ORanimalwork ) [
        set infected_person? true
        set color green
        set shape "face sad"
        set size 5
      ]
    ]
  ]
]
]
]
end

to link_cowmdp
  let v_id 0
  while [ v_id <= 287 ] [
    ask agppl with [ makedp = true and whoag = v_id ] [

```

```

    move-to one-of cattle with [ milkcow = true and whocatt = v_id ]
    create-link-with one-of cattle-here with [ milkcow = true and whocatt = v_id ]
  ]
  set v_id v_id + 1
]
end

to infect_cowmdp
  if District_Population = "Dedoplistkaro" or District_Population = "Sagarego" or
  District_Population = "Gurjaani" or District_Population = "Telavi" or District_Population
  = "Lagodekhi" or District_Population = District_Population = "All" [
    ask cattle with [ milkcow = true and infected? = true and dead != true and whocatt <=
    15 or ( whocatt > 36 and whocatt <= 110 ) or ( whocatt > 171 and whocatt <= 201 ) or (
    whocatt > 223 and whocatt <= 287 ) ][
      ask link-neighbors [
        if infected_person? = false [
          if random-float 1.00000 <= ( 0.00001 * ORmdp ) [
            set infected_person? true
            set color blue
            set shape "face sad"
            set size 5
          ]
        ]
      ]
    ]
  ]
  if District_Population = "Signagi" or District_Population = "All" [
    ask cattle with [ milkcow = true and dead != true and infected? = true and whocatt > 15
    and whocatt <= 36 ] [
      ask link-neighbors [
        if infected_person? = false [
          if random-float 1.00000 <= ( SigRisk * ORmdp ) [
            set infected_person? true
            set color blue
            set shape "face sad"
            set size 5
          ]
        ]
      ]
    ]
  ]
  if District_Population = "Akhmeta" or District_Population = "All" [
    ask cattle with [ milkcow = true and dead != true and infected? = true and whocatt >
    110 and whocatt <= 171 ] [
      ask link-neighbors [
        if infected_person? = false [

```

```

    if random-float 1.00000 <= ( AkhRisk * ORmdp ) [
      set infected_person? true
      set color blue
      set shape "face sad"
      set size 5
    ]
  ]
]
]
]
]
if District_Population = "Kvareli" or District_Population = "All" [
  ask cattle with [ milkcow = true and dead != true and infected? = true and whocatt >
201 and whocatt <= 223 ] [
  ask link-neighbors [
    if infected_person? = false [
      if random-float 1.00000 <= ( KvarRisk * ORmdp ) [
        set infected_person? true
        set color blue
        set shape "face sad"
        set size 5
      ]
    ]
  ]
]
]
]
end

```

```

to move_shepherds_cow
let shep# ( ( (count cattle with [ whocatt = 0 ] ) * 3 ) / 50 ) * 4 )
if District_Population = "Dedoplistkaro" or District_Population = "All" [
  ask n-of shep# agppl with [ cowshep = true and whoag = 0 ] [
    set activeshepcow true
  ]
let i 1
set shep# ( ( (count cattle with [ whocatt = 1 ] ) * 3 ) / 50 ) * 4 )
while [ i <= 15 ] [
  ask n-of shep# agppl with [ cowshep = true and whoag = i ] [
    set activeshepcow true
  ]
  set i i + 1
]
]
set shep# ( ( (count cattle with [ whocatt = 16 ] ) * 2 ) / 50 ) * 4 )
if District_Population = "Signagi" or District_Population = "All" [
  ask n-of shep# agppl with [ cowshep = true and whoag = 16 ] [
    set activeshepcow true
  ]
]

```

```

]
set shep# ( ( ( (count cattle with [ whocatt = 17 ] ) * 2 ) / 50 ) * 4 )
ask n-of shep# agppl with [ cowshep = true and whoag = 17 ] [
  set activeshepcow true
]
]
set shep# ( ( ( (count cattle with [ whocatt = 18 ] ) * 2 ) / 50 ) * 4 )
let i 18
while [ i <= 36 ] [
  ask n-of shep# agppl with [ cowshep = true and whoag = i ] [
    set activeshepcow true
  ]
  set i i + 1
]
]
]
set shep# ( ( ( (count cattle with [ whocatt = 37 ] ) * 2 ) / 50 ) * 4 )
if District_Population = "Sagarego" or District_Population = "All" [
  ask n-of shep# agppl with [ cowshep = true and whoag = 37 ] [
    set activeshepcow true
  ]
]
let i 38
set shep# ( ( ( (count cattle with [ whocatt = 38 ] ) * 2 ) / 50 ) * 4 )
while [ i <= 80 ] [
  ask n-of shep# agppl with [ cowshep = true and whoag = i ] [
    set activeshepcow true
  ]
  set i i + 1
]
]
]
set shep# ( ( ( (count cattle with [ whocatt = 81 ] ) * 1 ) / 50 ) * 4 )
if District_Population = "Gurjaani" or District_Population = "All" [
  ask n-of shep# agppl with [ cowshep = true and whoag = 81 ] [
    set activeshepcow true
  ]
]
let i 82
set shep# ( ( ( (count cattle with [ whocatt = 82 ] ) * 1 ) / 50 ) * 4 )
while [ i <= 110 ] [
  ask n-of shep# agppl with [ cowshep = true and whoag = i ] [
    set activeshepcow true
  ]
  set i i + 1
]
]
]
set shep# ( ( ( (count cattle with [ whocatt = 111 ] ) * 3 ) / 50 ) * 4 )
if District_Population = "Akhmeta" or District_Population = "All" [
  ask n-of shep# agppl with [ cowshep = true and whoag = 111 ] [
    set activeshepcow true
  ]
]
]

```

```

]
let i 112
set shep# ( ( (count cattle with [ whocatt = 112 ] ) * 3 ) / 50 ) * 4 )
while [ i <= 171 ] [
  ask n-of shep# agppl with [ cowshep = true and whoag = i ] [
    set activeshepcow true
  ]
  set i i + 1
]
]
set shep# ( ( (count cattle with [ whocatt = 172 ] ) * 2 ) / 50 ) * 4 )
if District_Population = "Telavi" or District_Population = "All" [
  ask n-of shep# agppl with [ cowshep = true and whoag = 172 ] [
    set activeshepcow true
  ]
]
let i 173
set shep# ( ( (count cattle with [ whocatt = 173 ] ) * 2 ) / 50 ) * 4 )
while [ i <= 201 ] [
  ask n-of shep# agppl with [ cowshep = true and whoag = i ] [
    set activeshepcow true
  ]
  set i i + 1
]
]
set shep# ( ( (count cattle with [ whocatt = 202 ] ) * 1 ) / 50 ) * 4 )
if District_Population = "Kvareli" or District_Population = "All" [
  ask n-of shep# agppl with [ cowshep = true and whoag = 202 ] [
    set activeshepcow true
  ]
]
let i 203
set shep# ( ( (count cattle with [ whocatt = 203 ] ) * 1 ) / 50 ) * 4 )
while [ i <= 223 ] [
  ask n-of shep# agppl with [ cowshep = true and whoag = i ] [
    set activeshepcow true
  ]
  set i i + 1
]
]
set shep# ( ( (count cattle with [ whocatt = 224 ] ) * 2 ) / 50 ) * 4 )
if District_Population = "Lagodekhi" or District_Population = "All" [
  ask n-of shep# agppl with [ cowshep = true and whoag = 224 ] [
    set activeshepcow true
  ]
]
let i 225
set shep# ( ( (count cattle with [ whocatt = 225 ] ) * 2 ) / 50 ) * 4 )
while [ i <= 287 ] [

```

```

ask n-of shep# agppl with [ cowshep = true and whoag = i ] [
  set activeshepcow true
]
set i i + 1
]
]
ask agppl with [ activeshepcow = true ] [
  set heading 180
  rt random 180
  fd random-float 5.0
]
end

to infect_shepherds_cow
if District_Population = "Dedoplistkaro" or District_Population = "All" [
  ask agppl with [ activeshepcow = true and infected_person? = false ] [
    if any? cattle-here with [ infected? = true and dead != true ] [
      if random-float 1.00000 <= (( DedoRisk * ORanimalwork ) / 81 ) [
        set infected_person? true
        set color orange
        set shape "face sad"
        set size 5
      ]
    ]
  ]
]
if District_Population = "Signagi" or District_Population = "All" [
  ask agppl with [ activeshepcow = true and infected_person? = false and whoag > 15
and whoag <= 36 ] [
    if any? cattle-here with [ infected? = true and dead != true ] [
      if random-float 1.00000 <= (( SigRisk * ORanimalwork ) / 54 ) [
        set infected_person? true
        set color orange
        set shape "face sad"
        set size 5
      ]
    ]
  ]
]
if District_Population = "Sagarego" or District_Population = "All" [
  ask agppl with [ activeshepcow = true and infected_person? = false and whoag > 36
and whoag <= 80 ] [
    if any? cattle-here with [ infected? = true and dead != true ] [
      if random-float 1.00000 <= (( SagRisk * ORanimalwork ) / 54 ) [
        set infected_person? true
        set color orange
      ]
    ]
  ]
]

```

```

        set shape "face sad"
        set size 5
    ]
]
]
]
if District_Population = "Gurjaani" or District_Population = "All" [
    ask agppl with [ activeshepcow = true and infected_person? = false and whoag > 80
and whoag <= 110 ] [
    if any? cattle-here with [ infected? = true and dead != true ] [
        if random-float 1.00000 <= (( GurjRisk * ORanimalwork ) / 27 ) [
            set infected_person? true
            set color orange
            set shape "face sad"
            set size 5
        ]
    ]
]
]
if District_Population = "Akhmeta" or District_Population = "All" [
    ask agppl with [ activeshepcow = true and infected_person? = false and whoag > 110
and whoag <= 171 ] [
    if any? cattle-here with [ infected? = true and dead != true ] [
        if random-float 1.00000 <= (( AkhRisk * ORanimalwork ) / 80 ) [
            set infected_person? true
            set color orange
            set shape "face sad"
            set size 5
        ]
    ]
]
]
if District_Population = "Telavi" or District_Population = "All" [
    ask agppl with [ activeshepcow = true and infected_person? = false and whoag > 172
and whoag <= 201 ] [
    if any? cattle-here with [ infected? = true and dead != true ] [
        if random-float 1.00000 <= (( TelRisk * ORanimalwork ) / 55 ) [
            set infected_person? true
            set color orange
            set shape "face sad"
            set size 5
        ]
    ]
]
]
]
if District_Population = "Kvareli" or District_Population = "All" [

```

```

ask agppl with [ activeshepcow = true and infected_person? = false and whoag > 201
and whoag <= 223 ] [
  if any? cattle-here with [ infected? = true and dead != true ] [
    if random-float 1.00000 <= (( KvarRisk * ORanimalwork ) / 27 ) [
      set infected_person? true
      set color orange
      set shape "face sad"
      set size 5
    ]
  ]
]
]
]
]
if District_Population = "Lagodekhi" or District_Population = "All" [
  ask agppl with [ activeshepcow = true and infected_person? = false and whoag > 223
and whoag <= 287 ] [
    if any? cattle-here with [ infected? = true and dead != true ] [
      if random-float 1.00000 <= (( LagoRisk * ORanimalwork ) / 54 ) [
        set infected_person? true
        set color orange
        set shape "face sad"
        set size 5
      ]
    ]
  ]
]
]
end

```

```

to move_home
if District_Population = "Dedoplistkaro" or District_Population = "All" [
  let v_id 0
  ask agppl with [ whoag = v_id ] [
    move-to one-of towna with [ whotown = v_id ]
  ]
  set v_id 1
  while [ v_id > 0 and v_id <= 15 ] [
    ask agppl with [ whoag = v_id ] [
      move-to one-of villagea with [ whovill = v_id ]
    ]
    set v_id v_id + 1
  ]
]
]
if District_Population = "Signagi" or District_Population = "All" [
  let v_id 16
  ask agppl with [ whoag = v_id ] [
    move-to one-of townb with [ whotown = v_id ]
  ]
]
]

```

```

set v_id 17
ask agppl with [ whoag = v_id ] [
  move-to one-of townc with [ whotown = v_id ]
]
set v_id 18
while [ v_id > 17 and v_id <= 36 ] [
  ask agppl with [ whoag = v_id ] [
    move-to one-of villageb with [ whovill = v_id ]
  ]
  set v_id v_id + 1
]
]
if District_Population = "Sagarego" or District_Population = "All" [
  let v_id 37
  ask agppl with [ whoag = v_id ] [
    move-to one-of townd with [ whotown = v_id ]
  ]
  set v_id 38
  while [ v_id > 36 and v_id <= 80 ] [
    ask agppl with [ whoag = v_id ] [
      move-to one-of villagec with [ whovill = v_id ]
    ]
    set v_id v_id + 1
  ]
]
]
if District_Population = "Gurjaani" or District_Population = "All" [
  let v_id 81
  ask agppl with [ whoag = v_id ] [
    move-to one-of towne with [ whotown = v_id ]
  ]
  set v_id 82
  while [ v_id > 81 and v_id <= 110 ] [
    ask agppl with [ whoag = v_id ] [
      move-to one-of villaged with [ whovill = v_id ]
    ]
    set v_id v_id + 1
  ]
]
]
if District_Population = "Akhmeta" or District_Population = "All" [
  let v_id 111
  ask agppl with [ whoag = v_id ] [
    move-to one-of townf with [ whotown = v_id ]
  ]
  set v_id 112
  while [ v_id > 110 and v_id <= 171 ] [
    ask agppl with [ whoag = v_id ] [

```

```

    move-to one-of villagee with [ whovill = v_id ]
  ]
  set v_id v_id + 1
]
]
if District_Population = "Telavi" or District_Population = "All" [
  let v_id 172
  ask agppl with [ whoag = v_id ] [
    move-to one-of towng with [ whotown = v_id ]
  ]
  set v_id 173
  while [ v_id > 171 and v_id <= 201 ] [
    ask agppl with [ whoag = v_id ] [
      move-to one-of villagef with [ whovill = v_id ]
    ]
    set v_id v_id + 1
  ]
]
]
if District_Population = "Kvareli" or District_Population = "All" [
  let v_id 202
  ask agppl with [ whoag = v_id ] [
    move-to one-of townh with [ whotown = v_id ]
  ]
  set v_id 203
  while [ v_id > 201 and v_id <= 223 ] [
    ask agppl with [ whoag = v_id ] [
      move-to one-of villageg with [ whovill = v_id ]
    ]
    set v_id v_id + 1
  ]
]
]
if District_Population = "Lagodekhi" or District_Population = "All" [
  let v_id 224
  ask agppl with [ whoag = v_id ] [
    move-to one-of towni with [ whotown = v_id ]
  ]
  set v_id 225
  while [ v_id > 224 and v_id <= 287 ] [
    ask agppl with [ whoag = v_id ] [
      move-to one-of villageh with [ whovill = v_id ]
    ]
    set v_id v_id + 1
  ]
]
]
end

```

```

to get_date
  if ticks <= 31 [ set mn "January" ]
  if ticks > 31 and ticks <= 60 [ set mn "February" ]
  if ticks > 60 and ticks <= 91 [ set mn "March" ]
  if ticks > 91 and ticks <= 121 [ set mn "April" ]
  if ticks > 121 and ticks <= 152 [ set mn "May" ]
  if ticks > 152 and ticks <= 182 [ set mn "June" ]
  if ticks > 182 and ticks <= 213 [ set mn "July" ]
  if ticks > 213 and ticks <= 244 [ set mn "August" ]
  if ticks > 244 and ticks <= 274 [ set mn "September" ]
  if ticks > 274 and ticks <= 305 [ set mn "October" ]
  if ticks > 305 and ticks <= 335 [ set mn "November" ]
  if ticks > 335 and ticks <= 365 [ set mn "December" ]
end

;*****OUTPUT*****
to result
  output-print "about to enter non-animal associated illnesses"
  no_livestocksick
  nonagsick
end

to no_livestocksick
  let cnt count agppl with [ infected_person? = true and no_livestock = true ]
  output-type "Number of sick agppl without livestock: "
  output-print cnt
end

to nonagsick
  let cnt count nonagppl with [ infected_person? = true ]
  output-type "The number of nonag sick people is "
  output-print cnt
end

```