Eco-epidemiology of Bovine Tuberculosis (bTB) in an African Savanna

The Conflict between Traditional Pastoralist Adaptations and Disease Transmission in the Modern Era

Sintayehu Workeneh Dejene

Propositions

1. Traditional pastoral practices to manage resource variability and uncertainty in African savannas are maladaptive in the modern era because of increased disease transmission risks.
   (this thesis)

2. Bovine Tuberculosis (bTB) infection rate is negatively correlated with mammalian species evenness.
   (this thesis)

3. The progress in science and technology during the twentieth century is not accompanied by a progress in morality.

4. The negative correlation between economic development and environmental concerns in developing countries is evidence of poor national policy.

5. The availability of medical information on the internet is a health threat.

6. Extrapolation of scientific findings without considering the social and cultural practices of society is like gathering cow dung where no cow has been.

Propositions belonging to the thesis, entitled
Eco-epidemiology of Bovine Tuberculosis (bTB) in an African Savanna: The Conflict between Traditional Pastoralist Adaptations and Disease Transmission in the Modern Era

Sintayehu Workeneh Dejene
Wageningen, 10 October 2017.

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Thesis

submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus,
Prof. Dr A.P.J. Mol,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Tuesday10 October 2017
at 1.30 p.m. in the Aula.

Sintayehu Workeneh Dejene
Eco-epidemiology of Bovine Tuberculosis (bTB) in an African Savanna: The Conflict between Traditional Pastoralist Adaptations and Disease Transmission in the Modern Era,
119 pages.

PhD thesis, Wageningen University, Wageningen, the Netherlands (2017)
With references, with summaries in English and Dutch

DOI: 10.18174/420891
ISBN: 978-94-6343-658-8

Contents

Chapter 1

General Introduction

Forty percent of sub-Saharan Africa is arid or semiarid rangeland, home to an estimated 80 million rural pastoralists (Notenbaert et al., 2009). Pastoralists livelihood depends mainly on the raising of domestic animals including cattle, camels, goats, sheep, and donkeys. Livestock plays multiple roles in the lifestyle of pastoralists, notably as livelihood sources and socio-cultural functions, and asset and security against risks (Guliye et al., 2007). The pastoralist systems evolved with practices such as livestock herd mobility, varying herd sizes and livestock exchange as adaptive mechanisms for managing resource variability and uncertainty (Alerstam et al., 2003; Manzano-Baena and Casas, 2010; Oteros-Rozas et al., 2013). Despite the acknowledged adaptive advantages of mobility, large herd sizes and livestock exchanges, these practices were recently shown to be a critical risk factor for transmission of zoonotic diseases due to higher chances of coming in contact with infected animals (Inangolet et al., 2008; Munyeme et al., 2008; Katale et al., 2013).

In the last half century, zoonotic diseases received significant attention because of the increase in Emerging of Infectious Diseases (EIDs) in humans, and wildlife and domestic species (Cleaveland et al., 2001). Zoonotic diseases are an important cause of concern for public health, veterinary services, livestock productions, and conservationists (Cleaveland et al., 2001). It has been estimated that infectious diseases are the most important threat to human health, responsible for one-quarter of worldwide mortality (WHO, 1999). For instance, over the last decades, about 20,000–30,000 cases of Lyme disease have been reported annually in USA and the average annual numbers of cases in Europe and Asia have been estimated at 65,467 and 3,450, respectively (Levy, 2013). It has been estimated that the introduced rinderpest virus rapidly reduced the ungulates in African savanna to 20% of their original abundance (Prins and van der Jeugd, 1993; Hudson et al., 2006). The outbreak of ebola hemorrhagic fever has been considered as a big threat to African ape populations (Nunn et al., 2008). Infectious zoonotic diseases also cause severe economic losses in the livestock industry (Thompson et al., 2002). For instance, the economic loss of bovine brucellosis in Argentina has been estimated at US$60 million per year (Samartino, 2002). Although the epidemiology of human diseases are relatively well-studied (Riley, 2007), the eco-epidemiology of animal diseases within the wildlife-livestock interface are largely unknown in Africa.

Disease at the wildlife-livestock-human interface

There is a growing recognition of the importance of multi-species interaction for the emergence and re-emergence of pathogens in wildlife, livestock and humans (Cleaveland et al., 2001; Daszak et al., 2001; Chomel, 2007). With two-thirds of human diseases being multi-host pathogens (Cleaveland et al., 2001; Taylor et al., 2001) and three-quarter of emerging human diseases being zoonotic (Taylor et al., 2001), there is a strong public health interest in better understanding the dynamics of multi-species pathogens (Daszak et al., 2000). For instance, the emergence of Severe Acute Respiratory Syndrome (SARS) coronavirus, Hendra virus, hantavirus pulmonary syndrome, Lyme disease and Ebola hemorrhagic fever are some of the most recent examples illustrating the risk of pathogen transmission at the wildlife-livestock-human interface (Mathews, 2009). A large proportion (77%) of domestic animal pathogens infect multiple hosts, including wildlife species (Cleaveland et al., 2001; Jones et al., 2008). The importance of wildlife as a source of avian influenza, foot and mouth disease (FMD), rift valley fever, malignant catarrhal fever, bovine tuberculosis and Nipah virus for domestic animal species is well documented (Alexander 2000; Worthington and Bigalke, 2001; Vosloo et al., 2002; Epstein et al., 2006). In other cases, the spill-over of domestic animal pathogens to wildlife caused severe outbreaks with great concerns for conservation, such as pasteurellosis and Sierra Nevada outbreak in Bighorn sheep (George et al., 2008; Clifford et al., 2009), rabies in Ethiopian wolves (Randall et al., 2006), and bovine brucellosis and tuberculosis in bison (Tessaro et al., 1990). Therefore, diseases that arise from the livestock–wildlife interface are of paramount importance and must be an area of focus for animal and human health authorities (Siembieda et al., 2011). Improving our understanding of the biological and anthropogenic processes that promote contact between hosts is critical for limiting pathogen transmission at this interface. As a consequence, a new approach to the studies on complex multi-host and multi-pathogen systems are needed to better understand the transmission of the diseases and from there develop appropriate disease control strategies.

Bovine tuberculosis

Bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, is an important zoonotic disease affecting many mammal species and human health (de Vos et al., 2001; Skuce et al., 2012). The World Health Organisation (WHO, 2012) identified bTB as one of the eight worldwide neglected zoonoses needing urgent attention, especially in developing countries. Although control programmes have eliminated or nearly eliminated this disease from domestic animals in most developed countries, bTB is still widespread in Great Britain, Ireland, New Zealand and many developing countries, especially in Africa (Renwick et al., 2007; Shitaye et al., 2007; Humblet et

al., 2009). bTB in animals has been reported from 33 of 43 African countries. Nevertheless, published information indicate that bTB is probably widespread in cattle at the continental level (Ayele et al., 2004; Boukary et al., 2011), whereas the status and risk factors associated with transmission of bTB in multi-host communities have only been documented in few countries in Africa (Renwick et al., 2007; de Garine-Wichatitsky et al., 2013).

Host species for M. bovis

Cattle are considered to be the main hosts for *M. bovis*, but other domesticated and wild mammals can also be infected. Goats, sheep (Paling et al., 1988), camels (Mamo et al., 2011), dogs (Gay et al., 2000) and cats (Wilesmith et al., 1994) are also susceptible to an infection with *M. bovis,* but no transmission from these to other animals has been reported so far. Wildlife hosts are classified as either maintenance hosts or spill-over hosts or dead-end hosts (Huang et al., 2013; Barron et al., 2015). The brushtail possum (*Trichosurus vulpecula*; Coleman et al., 1999), European badger (*Meles meles*; Delahay et al., 2001), bison (*Bison bison*; Rhyan and Spraker, 2010), African buffalo (*Syncerus caffer*; de Vos et al., 2001), Kafue lechwe (*Kobus leche*; Munyeme et al., 2008), greater kudu (*Tragelaphus strepsiceros*; Keet et al., 2001), and white-tailed deer (*Odocoileus virginianus*; Quigley et al., 1997), are already identified maintenance hosts for bTB, however, some authors now believe these species may only be spillover hosts that maintains the pathogen only when their population densities are high (Raghvendra et al., 2010). However, lions (*Panthera leo*), leopards (*P. pardus*), cheetahs (*Acinonyx jubatus*) and other carnivore species do not appear to be able to maintain infection in the absence of an infected maintenance host in the system (Renwick et al., 2007).

Transmission of bTB

The transmission of bTB between animals is mainly aerogenic, close contact between animals either via aerosol contamination or sharing of feed between infected and non-infected animals are major risk factors for transmission of bTB (Cleaveland et al., 2001; Menzies and Neill, 2000; Ameni et al., 2007). Ameni et al. (2007) indicated that the severity of bTB prevalence was significantly greater in cattle kept indoors together with different livestock species at a higher population density than in cattle kept alone on pastures, as close contact facilitates the transmission of infective aerosols between animals (Ameni et al., 2007). The disease can be transmitted by indirect contact between animals when cattle graze on grass that is contaminated by infected animal faeces, urine or wound discharges (Johnston et al., 2005).

Economic impact of bTB

Bovine tuberculosis (bTB) poses an important economic burden to society, linked with losses of productivity of infected animals (e.g. reduced milk yields, meat production and fertility), international trade of animals and animal products restrictions, control and eradication programs, and human health costs (Cosivi et al., 1998; Ayele et al., 2004). Studies found that bTB infection in cattle was associated with a 18% decrease in milk production in Bangladesh (Rahman and Samad, 2008) and 4% in USA (Hermandez and Baca, 1998). A study in Ireland also reported a significant difference in milk yield between bTB positive and negative cattle (Boland et al., 2010). In Tanzania a study found a TB reactor cattle provide less milk production than the non-reactor (DFID, 1999). The economic impact of bTB to the agriculture and health sector in Turkey has been estimated between 15-59 million US$ per year (Barwinek and Taylor, 1996). In UK and Ireland, the disease still has a major economic impact, approximately £100 million is spent annually in efforts to control the disease (Matthews et al., 2006). The WHO estimated total TB control costs in Ethiopia at US$14.2 million per year (WHO, 2004). In most developed countries, the disease has almost been eradicated after the implementation of preventive and control measures, such as testing, culling or pasteurisation of milk (Humblet et al., 2009). Nevertheless, bTB remains a problem in some countries with a well-developed veterinary control system (e.g., UK, Ireland, New Zealand, USA; Kaneene et al., 2002; Humblet et al., 2009), and in most developing countries, where surveillance and control activities are often inadequate or unavailable (Cosivi et al., 1998; Ayele et al., 2004; Renwick et al., 2007).

Risk factors for transmission of bTB

In Africa, the evidence suggests that bTB has a wide distribution with a high prevalence in both domestic (Figure 1.1a) and wild animals (Figure 1.1b; de Garine-Wichatitsky et al., 2013). The disease is endemic in sub-Saharan African cattle (de Garine-Wichatitsky et al., 2013). In multi-species grazing systems, the transmission of pathogens is influenced by a large number of interacting factors, related to the pathogen and host characteristics, the ecology and behaviour of hosts, livestock management and land-use.

Host movement have been repeatedly identified as one of the major risk factor for bTB transmission. Gilbert et al. (2005) showed that movement of animals was a critical risk factor for transmission of bTB. This specific factor has a major impact if animals are moved from an endemic zone to a bTB free one (Gilbert et al., 2005). In Tanzania, a high prevalence of bTB was reported in pastoral cattle with high numbers of cattle kept under extensive husbandry practice

(Kazwala et al., 2001), whereas in Uganda, the prevalence was higher in agro-pastoral than in pastoral production systems, probably because of the closer contact between cattle and the more humid conditions in agro-pastoral systems (Faye et al., 2005). When cattle herds move more, the probability of sharing water holes and grazing areas with other infected animals increases, and the probability of close contact between infected animals also increases. Inhalation of *M. bovis* is the principal route to bovine infection and is facilitated by direct and indirect contact between infected and healthy animals. Ingestion of *M. bovis* directly from contaminated pasture or water is a risk factor for transmission of the disease through contamination of the local environment (Kaneene et al., 2002). In pastoral areas of Africa, the grazing strategy relies on the movement of livestock to follow grazing and water resources over considerable distances following seasonal changes, which would increase the likelihood of both direct and indirect transmission of bTB.

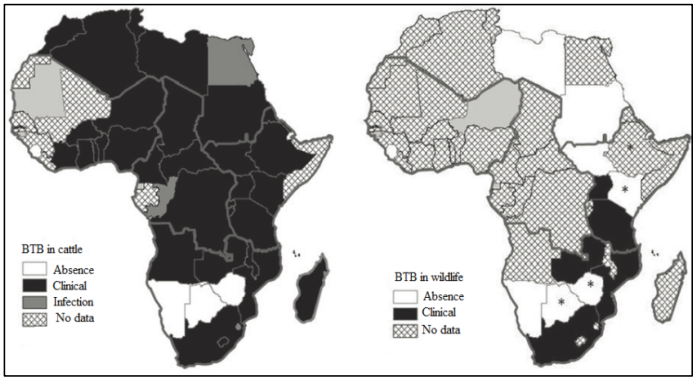

Figure 1.1. Distribution map of bTB in Africa during 1996–2011 (large grey lines indicate the African sub-regions as referred to in the text: West, Central, East and Southern Africa). (a) Cattle status at country level; (b) wildlife status at country level. Asterisk (*) indicates countries (i.e. Botswana, Ethiopia, Kenya, Zimbabwe) where suspected and confirmed wildlife bTB cases have been detected (de Garine-Wichatitsky et al., 2013).

Studies identified herd size as one of the major bTB risk factors (Griffin et al., 1996; Cleaveland et al., 2001; Munyeme et al., 2008). In East Africa, pastoralists keep multiple species, mainly cattle, sheep, goats, camels and donkeys, often in large herd sizes. Large herd sizes are common as a kind of "bank account" adaptation to uncertainty in rainfall, disease, and raid losses (Mace and Houston, 1989). The more animal there are on a farm, the greater the probability that one of them will acquire the infection (Humblet et al., 2009). Large herds generally graze on a larger area, with a higher probability to have more contiguous herds, thus increasing the risk of disease spread.

Introduction of an infected animal (exchange and purchase of animals) in a bTB free herd or area is one of the major risk factors for introducing the disease, as suggested by studies carried out in the UK, Michigan, Italy, Tanzania and Ethiopia (Marangon et al., 1998; Johnston et al., 2005; Gopal et al., 2006; Dejene et al., 2017a). Munroe et al. (1999) showed that either purchased animals from positive farms or possible contact with positive animals are large risk factors for bTB transmission (Munroe et al., 1999). Exchange of livestock within and between clans is a common practice in pastoral areas of east Africa to spread risk and build supportive relationships (Getachew, 2001; Davies and Bennet, 2007; Moritz et al., 2011). This relationship also allows pastoral nomads to share commonalities for economic relief in times of stress, and is also used for intermigration, conflict solving, hospitality and other purposes. Intermarriage within and between clans is common in pastoral areas of east Africa. The traditional marriage ceremony involves giving livestock to the family of the bride. However, these livestock exchanges may increase the risks of introduction of bTB.

Increasing evidence suggests that wildlife maintenance hosts play an important role in transmission bTB to other animals (Humblet et al., 2009; O'Brien et al., 2011; de Garine-Wichatitsky et al., 2013). Badgers (*M. meles*) in the UK and Ireland (Griffin et al., 1996; Denny and Wilesmith, 1996; Andrew et al., 2014), possum (*T. vulpecula*) in New Zealand (Julian, 1981; Jackson et al., 1995; Ryan et al., 2006; Nugent et al., 2015), white-tailed deer (*O. virginianus*) in USA and Canada (Fitzgerald and Kaneene, 2012), buffalo (*S. caffer*), kudu (*T. strepsiceros*) and lechwe (*K. leche*) in Africa (de Vos et al., 2001; Keet et al., 2001; Michel et al., 2006; Munyeme et al., 2008; de Garine-Wichatitsky et al., 2013) have been implicated in the transmission of *M. bovis* to cattle. In contrast, in British farmlands Mathews et al. (2006) found that badgers (*M. meles*) was relatively inefficient in transmitting bTB to cattle (Matthews et al., 2006). Study conducted in bTB free countries, Germany and Denmark, also showed that wildlife represents a negligible probability of infection for domestic cattle (Moser et al., 2011; Foddai et al., 2015). Mackintosh et al. (2004) indicated that the direction for transmission of bTB infection from cattle, badger and deer is not yet known. So, their role in spreading of bTB to cattle is still highly debated. In east Africa, wildlife species share resources with pastoralist livestock (Prins, 2000), and this may influence the prevalence of bTB in cattle by having direct or indirect contact (i.e., ingestion of contaminated pastures) with cattle.

Africa is recognized as a hotspot for biodiversity, but is suffering from rapid and extensive loss of that diversity (Myer et al., 2000; Gorenflo et al., 2012; Di Marco et al., 2014). Current theories on diversity-disease relationships describe host species diversity as important factor influencing

disease risk, either diluting or amplifying disease prevalence in a community. The dilution effect predicts that species diversity decreases pathogen prevalence through mechanisms such as decreased host density, reduced encounters between hosts, or reduced host survival (Keesing et al., 2006; Huang et al., 2013; Johnson et al., 2013). In contrast, the amplification effect predicts increased pathogen prevalence with greater species diversity, through increased encounters between hosts, or through the presence of secondary hosts (LoGiudice et al., 2003; Keesing et al., 2006). Despite the fact that the dilution effect occurs far more frequently than the amplification effect, our knowledge of which specific systems conform to the dilution effect and the mechanisms underlying the effects of diversity, is incomplete (Ostfeld and Keesing, 2012; Randolph and Dobson, 2012; Huang et al., 2013; Ostfeld, 2013; Johnson et al., 2013; Miller and Huppert, 2013; Huang et al., 2014; Hofmeester et al., 2016). Moreover, most studies that examine the diversity–disease relationship focus principally on species richness as a measure of biodiversity (Keesing et al., 2006). In fact, biodiversity can be measured in many different ways, as species richness, species evenness, or a combination of richness and evenness (Magurran, 1988; Tucker and Cadotte, 2013). Thus, despite many studies of the relationship between diversity and diseases, evaluating the effects of different diversity metrics on disease risk has proven to be rare (Chen and Zhou, 2015). Thus, these different metrics of diversity may have different predictive powers for predicting disease risk in the target population.

Changes in land use and associated ecosystem change have been described as one of the casual driver in the current emerging and re-emerging of infectious diseases. Land use can be the result of the introduction of invasive species, could potentially influence bTB prevalence through direct effects on the host community composition, host densities and host contact networks. For instance, invasive plant species may increase the risk of infection to tick-borne diseases by increasing the density as well as the rate of encounter rate between ticks and their vertebrate hosts (Allan et al., 2010; Williams and Ward, 2010). Similarly, land use changes as a result of plant species invasions can increase the risk of mosquito-borne diseases by providing favourable habitat for vector species (Conley et al., 2011), and providing high-quality nutritional fruits and leaves for mosquito larvae (Reiskind and Zarrabi, 2011). Thus, introduction of invasive plant species has been associated with a number of ecological complications, resulting in reductions of species diversity (Chu et al., 2003; Turner et al., 2003), and this may increase the prevalence and transmission of bTB, as non-competent mammalian hosts at higher levels of biodiversity may dilute pathogen transmission (Huang et al., 2013; Huang et al., 2014; Dejene et al., 2017b).

Most eco-epidemiological studies on bTB were conducted in developed countries, where control and/or eradication programmes have been implemented since a long time as well as with intensive farming practices. Disease transmission and risk factors associated with host and their ecological interactions may vary in space and time, and in relation to management interventions. Contacts between animals, and the rate of biodiversity loss and land use change differ substantially among developed and developing countries, and the ecological setting is completely different. Most ecological studies that analysed the role of host-pathogen systems have largely focused on a single host species (Renwick et al., 2007; de Garine-Wichatitsky et al., 2013), but more efforts are needed to improve our understanding of the mechanisms underlying the prevalence of diseases, like in bTB that infect multiple species of hosts in multi-host communities from pastoral livestock production context where interactions between different livestock species and with wild animal hosts are very common. Therefore, the aim of this PhD project is to quantify the role of ecological and societal factors that determine the prevalence of bTB in cattle under the influence of wildlife-livestock interactions.

Outline of the Thesis

Bovine tuberculosis (bTB) infection is generally correlated with individual cattle's age, sex, body condition, and with husbandry practices such as herd composition, herd movement, herd size, production system and proximity to wildlife - including bTB maintenance hosts. In chapter two, I test the correlation between those factors and the prevalence of bTB in Ethiopian cattle in a pastoral area where cattle and wildlife species share grazing lands and water sources, and quantified the direct and indirect relationships between risk factors using a structural equation model.

Current theories on diversity-disease relationships describe host species diversity and species identity as important factors influencing disease risk, either diluting or amplifying disease prevalence in a community. Whereas the simple term 'diversity' embodies a set of animal community characteristics, it is not clear how different measures of species diversity are correlated with disease risk. In chapter three, I therefore test the effects of species richness, Pielou's evenness and Shannon's diversity of wild herbivores on bTB risk in cattle. I also extend the analyses and included the study of an identity effect of greater kudu and the effect of host habitat use overlap between wildlife maintenance host (greater kudu) and cattle on bTB risk.

Changes in land use and associated ecosystem changes have been described as one of the casual driver in the current emerging and re-emerging of infectious diseases. In chapter four, I test the

relation between land use cover - including the invasive woody plant species *Prosopis juliflora* - and bTB prevalence in cattle and examined the potential underlying mechanisms by which ecological consequences of land use cover changes, such as an invading woody species, alters the risk of bTB infection.

In East Africa, pastoralists may receive or bestow livestock to create and strengthen social relationships. In chapter five, I use network analyses to examine the relative importance of cattle transfer action on bTB infection.

Finally, chapter 6 reviews the key finding of previous chapters and ties them together in a discussion to predict changes in pathogen transmission and provide alternative and new insights with regard to interventions and disease control measures.

Description of the study area

The study was carried out in Awash National Park and in the neighbouring Afar Region, Ethiopia. Study sites were selected based on a gradient of wildlife-livestock interactions, livestock production systems, concentrations of livestock and wildlife, and the presence of common grazing and water resources. In the southern tip of the region, most of the grazing land and watering points are shared by livestock and wild animals from the Awash National Park (Figure 1.2). It is very common to observe livestock grazing in close proximity to wild animals in the study area, but wildlife-livestock co-grazing is less frequently observed when moving away from the park to the north of the study area.

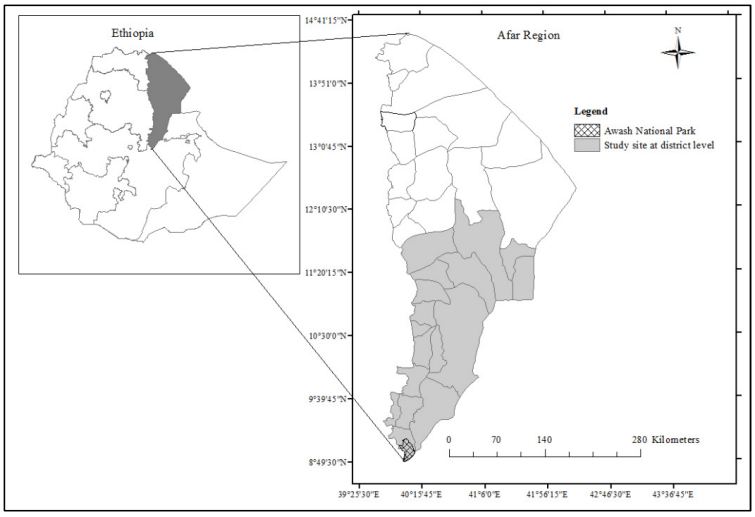

Figure 1.2. Map of the study area, the Afar Region in Ethiopia (small inset) and 17 districts (larger map). The location of Awash National Park in the South is indicated by the cross-hatched area.

Awash National Park (ANP) is located in the Ethiopian Rift valley (between 39° 20' to 40° 65'E longitude and 8° 50 to 9° 50'N latitude, 960 to 1050 m above sea level) with a semi-arid savanna vegetation grazing areas. The long rainy season is from July to September and the short rainy season is from February to April. The long dry period is from October to January and the short dry period is from May to June (EMA, 2013). Livestock grazing, crop production and settlement construction inside Awash National Park have become common practice in the area. Two thirds of the area demarcated as the Awash National Park is inhabited and utilized by local people for grazing of livestock in the presence of wildlife species (Franks et al., 2003), such greater kudu (*Tragelaphus strepsiceros*), which are known wildlife hosts of *M. bovis* (Keet et al., 2001).

The Afar region is located in the north-eastern part of Ethiopia (between 39° 34' to 42° 28'E longitude and 8° 49' to 14° 30'N latitude; Figure 1.2) and covers about 70,000 km^2 (CSA, 2008). It is characterized by an arid and semi-arid climate with low and erratic rainfall. Rainfall is bimodal throughout the region, with a mean annual rainfall of 500 mm in the semi-arid western escarpments, decreasing to 150 mm in the arid zones to the east. The minimum and maximum annual temperature of the area is 20°C and 40°C, respectively (EMA, 2013). The altitude ranges from 120 m below sea level in the Danakil depression to 1500 m above sea level. It has an estimated population of 1.5 million of which 90% are pastoralists and 10% are agro-pastoralists (CSA, 2008). Afar communities traditionally keep herds of cattle, sheep, goats and camels. There are about 1.9 million cattle in the Afar region, of which 90% are managed under a pastoral production system (ANRS, 2009). Afar pastoralists form a highly traditional society that has received less development attention than many comparable societies in Africa where traditional practices and institutions remain strong. In Afar society the clan is the most important political and social unit. A clan is formed by an extended group of families, and serves as a nucleus for administration and cooperation to conduct social activities among clan members. The clan is also the lowest social unit which can hold communal property rights over land and other natural resources.

Chapter 2

Risk factors for bovine tuberculosis (bTB) in cattle in Ethiopia

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Modified from PLoS One, 2016, 11(7): e0159083

Bovine tuberculosis (bTB) infection is generally correlated with individual cattle's age, sex, body condition, and with husbandry practices such as herd composition, cattle movement, herd size, production system and proximity to wildlife - including bTB maintenance hosts. We tested the correlation between those factors and the prevalence of bTB, which is endemic in Ethiopia's highland cattle, in the Afar Region and Awash National Park between November 2013 and April 2015. A total of 2550 cattle from 102 herds were tested for bTB presence using the comparative intradermal tuberculin test (CITT). Data on herd structure, herd movement, management and production system, livestock transfer, and contact with wildlife were collected using semi-structured interviews with cattle herders and herd owners. The individual overall prevalence of cattle bTB was 5.5%, with a herd prevalence of 46%. Generalized Linear Mixed Models with a random herd-effect were used to analyse risk factors of cattle reactors within each herd. The older the age of the cattle and the lower the body condition the higher the chance of a positive bTB test result, but sex, lactation status and reproductive status were not correlated with bTB status. At herd level, Generalized Linear Models showed that pastoral production systems with transhumant herds had a higher bTB prevalence than sedentary herds. A model averaging analysis identified herd size, contact with wildlife, and the interaction of herd size and contact with wildlife as significant risk factors for bTB prevalence in cattle. A subsequent Structural Equation Model showed that the probability of contact with wildlife was influenced by herd size, through herd movement. Larger herds moved more and grazed in larger areas, hence the probability of grazing in an area with wildlife and contact with either infected cattle or infected wildlife hosts increased, enhancing the chances for bTB infection. Therefore, future bTB control strategies in cattle in pastoral areas should consider herd size and movement as important risk factors.

Introduction

Bovine tuberculosis (bTB) caused by *Mycobacterium bovis* is a zoonotic disease, and remains a cause of concern for livestock, wildlife and human health (Cosivi et al., 1998; Humblet et al., 2009; Huang et al., 2013). Cattle serve as the main host for *M. bovis* worldwide (de Lisle et al., 2002; Tschopp et al., 2010; Gumi et al., 2011), while other domestic animals such as pigs, cats, dogs, horses and sheep are considered to be spill-over hosts. The transmission of bTB between animals is mainly aerogenic, and close contact between animals or sharing of feed between infected and non-infected animals are major risk factors for transmission of bTB (Cleaveland et al., 2001; Menzies and Neill, 2000; Ameni et al., 2007). Ingestion of *M. bovis* from contaminated pasture or water is also a risk factor for transmission of the disease (Kaneene et al., 2002).

Bovine tuberculosis (bTB) outbreaks can trigger large economic costs to society since it can affect international trade of animals and animal products, create productivity losses (e.g., reduced milk yields and meat production, reduced fertility), call for expensive animal market restriction measures, trigger large control and eradication programs, and increase human health costs (Cosivi et al., 1998; Ayele et al., 2004). Studies found that bTB infection in cattle was associated with a 18% decrease in milk production in Bangladesh (Rahman and Samad, 2008) and 4% in USA (Hermandez and Baca, 1998). A study in Ireland also reported a significant difference in milk yield between bTB positive and negative cattle (Boland et al., 2010). In developed countries, it is controlled through a test-and-slaughter policy. Nevertheless, bTB remains a problem in some countries with a well-developed veterinary control system (e.g., UK, Ireland, New Zealand, USA; Kaneene et al., 2002; Humblet et al., 2009), and in most developing countries, where surveillance and control activities are often inadequate or unavailable (Cosivi et al., 1998; Ayele et al., 2004; Renwick et al., 2007).

In Africa, the evidence suggests that bTB has a wide distribution with a high prevalence in both domestic and wild animals. The disease is endemic in sub-Saharan African cattle (de Garine-Wichatitsky et al., 2013). Strikingly, risk factors for bTB transmission are not well known in most developing countries, as most studies were conducted in developed countries where farming practices are more intensive and control and/or eradication programmes have been implemented since decades. In Africa, most comprehensive epidemiological studies have been done in Zambia (Cook et al., 1996; Munyeme et al., 2008; Munyeme et al., 2009; Munyeme et al., 2010), Tanzania (Kazwala et al., 2001; Mfinanga et al., 2004; Cleaveland et al., 2007; Bugwesa et al., 2013) and Uganda (Oloya et al., 2007). These studies have identified various risk factors for bTB

transmission at different levels. At individual animal level, the prevalence of tuberculosis-like lesions increased with age and decreased with increasing body condition (Caron et al., 2003; Ameni et al., 2007; Cleaveland et al., 2007). At herd level, herd size and movement have been identified as risk factors increasing bTB transmission. In Tanzania, a high prevalence of bTB was reported in pastoral cattle with high numbers of cattle kept under intensive husbandry practice (Kazwala et al., 2001), whereas in Uganda, the prevalence was higher in agro-pastoral than in pastoral production systems, probably because of the closer contact between cattle and the more humid conditions in agro-pastoral systems (Faye et al., 2005). Introduction of infected animals into the herd could also increase bTB transmission (Humblet et al., 2009). Considering the introduced animals, Reilly and Courtenay (2007) demonstrated that the risk of bTB spread can be reduced by introduction of animals from a non-endemic area, minimising the number of animals introduced, and introducing more calves and yearlings than adults. In East Africa, pastoralists keep multiple species, mainly cattle, sheep, goats, camels and donkeys, often in large herd sizes. The grazing strategy in the area relies on the movement of livestock to follow grazing and water resources over considerable distances following seasonal changes. Most studies focusing on risk factors associated with pathogens that infect multiple host species involved single species and neglected the effect of multiple hosts (Renwick et al., 2007; de Garine-Wichatitsky et al., 2013). It is therefore necessary to explore the relation between pastoral livestock production system with transmission of bTB in a multiple host community.

Previous studies have been carried out to investigate the roles that wildlife species play on the dynamics of bTB transmission (Corner, 2006; Renwick et al., 2007). Wildlife hosts are classified as either spill-over hosts or maintenance hosts (Humblet et al., 2009). Spill-over hosts can be infected by bTB, and do not transmit the pathogen to other animals efficiently (Corner, 2006; Renwick et al., 2007; Huang et al., 2013). Increasing evidence suggests that wildlife maintenance hosts play an important role in transmission bTB to other animals (O'Brien et al., 2011; de Garine-Wichatitsky et al., 2013), however, some authors now believe they may be a spill-over host that maintains the organism only when its population density is high (Raghvendra et al., 2010). So, their role in spreading of bTB to cattle is still highly debated. In East Africa, humans encroach into wildlife habitats with their livestock in search of grazing areas and water, particularly during the dry season. Wildlife species that share resources with pastoralist livestock (Prins, 2000; Sitters et al., 2009) may influence the prevalence of bTB in cattle by having direct or indirect contact (i.e., ingestion of contaminated pastures) with cattle. More studies are required to better understand the effects of interactions between ecological and animal management risk factors in multi-host communities.

Most studies focusing on ecological risk factors associated with pathogens that infect multiple host species tend to concentrate on industrialized countries, whereas the epidemiology of bTB in the developing world, especially in Africa, remains largely unknown (Humblet et al., 2009). Moreover, livestock production systems and contacts between livestock and wildlife also differ substantially between developed and developing countries (Keet et al., 2001). bTB has been shown to be endemic in cattle from Ethiopia (Ameni et al., 2003; Asseged et al., 2004; Teklul et al., 2004). Given the complexity of factors affecting bTB at the individual and herd level, a study is required that quantifies the effects of multiple independent variables in a single analysis, distinguishing among the direct and indirect effects. This study therefore aimed to test which risk factors were associated with bTB prevalence in Ethiopian cattle in a pastoral area where cattle and wildlife species share grazing lands and water sources, and quantified the direct and indirect relationships between risk factors using a structural equation model.

Materials and Methods

Study Area

The study was carried out in Awash National Park and in the neighbouring Afar Region, Ethiopia (Figure 1.2). Study sites were selected based on a gradient of wildlife-livestock interactions, livestock production systems, concentrations of livestock and wildlife, and the presence of common grazing and water resources. In the southern tip of the region, most of the grazing land and watering points are shared by livestock and wild animals from the Awash National Park. It is very common to observe livestock grazing in close proximity to wild animals in the study area, but wildlife-livestock co-grazing is less frequently observed when moving away from the park to the north of the study area.

Livestock production systems

In the Afar region two types of pastoralism are recognized: resident or agro-pastoralists, in which animals are grazed within village perimeters without migration in search of pasture, while transhumance is the most common grazing system in the region, which involves the seasonal movements of livestock to follow suitable grazing and water resources over considerable distances in the dry season, coming back to the villages in the rainy season (Kassa et al., 2005). In the wet season, animals are kraaled at night, and in most cases herds owned by two to five close relatives or clan members are joined. The animals are released in the morning and the herds graze separately during the day in rangelands around the homesteads. In the dry season, some herds remain sedentary, whereas transhumant herds often migrate together, sharing grazing areas and

watering sources along the way. Migratory distances vary from 3 to 55 km and the routes follow rivers or water sources.

Livestock transfer

Afar pastoralists have adapted to their harsh environment through customized social organisation. An important element of this social organisation is livestock transfer by which pastoralists may receive or bestow livestock to create and strengthen social relationships and establish trust through gifts, loans and herding contracts (Moritz, 2013). Livestock animals are transferred in many ways in Afar society (Getachew, 2001; Davies and Bennett, 2007). The most important livestock transfer mechanism for post-crisis herd recovery is called "*iribu*". A second important mechanism of livestock transfer is "*ala*" which is a system of bond-friendship entailing gifts of livestock. Beyond *iribu* and *ala*, animals are transferred on many other occasions, such as during a marriage, on other celebrations and ceremonies, or as compensation for injured parties.

Study design

A cross sectional multi-stage sampling with 'sub-region' as the highest stage followed by 'district' (n=17), and 'sub-district' (n=34) as lowest sampling stages was used to select study villages. Study animals were obtained using a three-stage random sampling procedure. The 'village' within the sub-district was regarded as the primary unit, the 'herd' as secondary unit and 'individual animal' as tertiary unit. Herds of livestock in each sub-district were stratified into three groups based on herd size (large, medium and small) after calculating the average herd size of the sub-district. Herds (one herd from each stratum) and individual animals were selected randomly. A total of 102 livestock herds from 34 sub-districts (3 in each sub-district, one large, medium and small herd) were selected. Informed consent was sought verbally from all livestock herders and herd owners.

Sample size determination

The sample size was determined by assuming that the average expected prevalence of bTB was 11% (Mamo et al., 2013). The desired sample size was calculated using the 95% confidence interval and at 5% absolute precision following the method of Thrusfield (Thrusfield, 1995). The total sample size per district was calculated, which gave us a total number of required animals of 2550 for all 17 districts, or 75 animals for each of the 34 sub-districts. A complete list of sub-districts and villages within the sub-district was obtained from each district pastoral and agro-pastoral office. Sub-district within the district and villages within sub-district were selected using

random numbers. We excluded animals younger than 1 year, since they are herded around the home-base; cows at a late stage of gestation, because they are difficult to handle, and clinically sick animals which, at late stages of the disease showed false negative responses.

Single comparative intradermal tuberculin test

Tuberculin skin testing was performed using aliquots of 0.1 ml of 2500 IU/ml bovine purified protein derivative (PPD) and 0.1 ml of 2500 IU/ml avian PPD (Prionics Lelystad B.V, Lelystad, The Netherland). Bovine and avian PPDs were injected by veterinary staff intradermally at two sites approximately 12 centimetres apart at the border of the anterior and middle thirds of one side of the neck. This was done after shaving the two sites using a razor blade. The skin thickness was measured with digital callipers prior to and 72 h after PPD injection. An animal was considered bTB positive if the reaction at the bovine site minus the reaction at the avium site ≥ 4 mm cut-off, according to the recommendations of the World Animal Health Organization (OIE, 2009). In this study, livestock owned by one owner and/or his close relatives, in which the animals shared common grazing sites, watering points, kept at night at a common site and moved together during migration, was considered as a single herd in the calculation of the herd prevalence. A herd was considered bTB positive if it had at least one tuberculin reactor animal (Ameni et al., 2007; OIE, 2009; Tschopp et al., 2009). In addition to the comparative intradermal tuberculin test, information was collected for each tested animal: sex, age, lactation and reproduction status, parity number and body condition score. Animals were categorized into three age groups: juveniles between one and three years, reproductive animals between three and ten years and animals older than ten years. Body condition of the animals was scored on a 1 (thin) to 3 (fat) scales.

Questionnaire survey

To identify risk factors associated with bTB prevalence, semi-structured interviews with the herders and herd owners were conducted, gathering information on general herd management practices, livestock movement and transfer, introduction of animals into the herd, pastoral production system, other livestock species kept, types and levels of herd contacts, water sources (during the wet and dry season) and contact with wild animals (Table 2.1). Local agricultural officers, knowledgeable on local farming practices and who had received prior training on the administration and the scope of the questions, assisted us during the interviews. All herd owners and herders of tuberculin tested cattle were interviewed by means of pre-tested questionnaires. Information on contacts between cattle herds and wildlife species was obtained from

questionnaires to herders. The wildlife-livestock interaction section of the questionnaires included questions on the observation of wildlife in the grazing land and/or watering areas of cattle herds. 'Contact with wildlife' was defined as wildlife species being visible to the herders in the grazing and/or watering areas of cattle herds.

Table 2.1. Descriptions, predicted effect and units of the predictors used in the analysis.

Description of data sets	Predicted effect	Unit
Average herd movement in a day	positive	km (7.3km)
Herd size	Positive	Number
Number of all new animal introduced into the herd	Positive	Number
Number of animals transferred	Positive	Number
Number of sheep and goats	Positive	Number
Number of camels	Positive	Number
Number of donkeys	Positive	Number
Wild animals		Class
• Contact with wild animals	Positive	
• No contact with wild animals	Negative	
Livestock production system		Class
• Pastoral production system	positive	
• Agro-pastoral production system	negative	
Other stock		Class
• Presence	positive	
• Absence	negative	
Interaction terms		
Herd size and contact with wildlife	Positive	

Herd movement and livestock transfer

The interviewer estimated the maximum movement distance of the livestock herd by tracing the herd movement in the area based on interview-derived information, bound by roads, streams, rivers or hills, village, district, sub-district, or region, wildlife habitat or park, or other physical indicators, which were located on a georeferenced map. Subsequently, the maximum daily distance was calculated for sedentary and for the transhumance herds. For each herd, the total number of animals introduced into the herd and transferred was estimated. It is likely that herds that graze close together have similar bTB prevalence, due to mixing of animals. At larger distances, herd bTB prevalence might be different, either higher or lower, due to spatial variation in bTB prevalence. To assess the impact of scale on the effect of livestock transfer on herd bTB prevalence, the effect of the number of animals transferred to strengthen social relationships was analysed separately for herds within and for herds outside the average daily herd movement radius of 7.3 km (Table 2.1). Livestock transfer included all animals received or bestowed to create and strengthen social relationships. Some of the Afar pastoralist kept multiple species (cattle, sheep, goat, donkey and camel), so we also considered the presence or absence of stocks

other than cattle as a risk factor. Geographic coordinates and altitude were registered at the central point of each village by a global positioning system (GPS, GPSMAP 64).

Ethical Statements

This study was approved by Haramaya University, Ethiopia (Reference number HUP14/559/15).

Statistical analysis

Generalized Linear Mixed Models (GLMM) were used to examine the effects of predictors on the bTB infection probability for each animal using herd as random factor, with a binary response as a dependent variable (bTB positive/negative). Different approaches were used to study the strength and the relative importance of the risk factors on bTB prevalence at herd level. Prior to developing our candidate models for the herd-level analysis, one-by-one univariate analyses were performed to identify potential risk factors, using the bTB prevalence as dependent variable in a Generalized Linear Model (GLM) (family=binomial). The number of bTB positive as well as the numbers of bTB negative cattle were specified in a two vector response variable by combining two vectors into a single object as dependent variable, comprising the bTB positive and negative cattle in a herd. Predictor variables with $p<0.25$ were recognized as potential risk factors, and were subsequently used to construct multiple regression models. For highly correlated independent variables, only the one causing the largest change in the log-likelihood function was added to the final global model to avoid multi-collinearity, which was assessed by checking the variance inflation factors (VIFs); the final VIF-results confirmed the absence of collinearity among explanatory variables (all VIFs< 5). In addition, to investigate the effect of wildlife-livestock interactions on the prevalence of bTB, we included the interaction term between herd size and contact with wildlife after including all main factors. From the global model, candidate models were selected using ΔAIC (< 5), with the best approximating candidate model having the highest w, as described in Burnham and Anderson (Burnham and Anderson, 2002). Model averaging was used to construct the final model using the Akaike weights of the different candidate models (Anderson et al., 2000). Furthermore, structural equation modelling (SEM) was conducted using the lavaan package (Preston et al., 2012; Rosseel, 2012) to study the relative direct and indirect importance of each risk factors on the bTB prevalence. All analysis were done using R v3.2.0.

Results

Animal level risk factors for bTB prevalence

The individual animal prevalence of bTB was 5.5%, whereas the herd level prevalence was 46% (47 out of 102 herds).

Risk of bTB infection increased with increasing age, as animals older than ten years had a significantly higher probability of bTB infection. There was also a strong association between having a poor body condition score and bTB infection, but sex, lactation status and reproductive status were not related with bTB status (Table 2.2).

Table 2.2. Summary of risk factors associated with bovine tuberculosis (bTB) in traditional Afar cattle in November 2013 to April 2015 (n=2550).

Risk factor	Levels	Number of cattle tested	bTB reactor animals (%)	OR (95% CI)	χ^2	p-value
Sex	Male	272	14 (5.1)	1.0	0.1	0.770
	Female	2278	127 (5.6)	1.1 (0.62-1.92)		
Age	Juveniles	423	14 (3.3)	1.0	8.2	0.017*
	Reproductive	1776	99 (5.6)	1.7 (0.98-3.05)		
	Aged	351	28 (8.0)	2.5 (1.31-4.89)		
Lactation	Lactating	1095	54 (4.9)	1.0	1.67	0.197
	Non lactating	1183	73 (6.2)	1.3 (0.88-1.82)		
Reproduction	Gravid	821	54 (6.2)	1.0	0.95	0.377
	Non gravid	1330	73 (5.2)	1.2 (0.83-1.72)		
Body condition	Thin	414	42 (10.1)	1.0	17.6	< 0.001***
	Normal	2021	95 (4.7)	0.4 (0.30-0.64)		
	Fat	115	4 (3.5)	0.3 (0.11-0.91)		

OR= Odds Ratio, CI=95% confidence intervals; * $P< 0.05$; ** $p < 0.01$; *** $p < 0.001$

Herd level risk factors for bTB prevalence

Based on the results of the one-by-one GLM analyses, seven out of 10 variables were identified as potential bTB risk factors, namely, herd size, the average herd movement in a day, number of animals introduced into the herd, number of animals transferred between herds, number of camels, pastoral production system, and contact with wildlife. These were all positively associated with bTB prevalence (Table 2.3). However, the number of sheep and goats, the number of donkeys, and the presence of other livestock were not correlated with bTB prevalence in cattle (Table 2.3).

Spearman's correlation matrix showed that herd movement and introduction of animals into the herd were strongly correlated with herd size and number of animals transferred, respectively ($r>0.7$; Table S2.1), and therefore only the latter variables were included in the multiple variable model to avoid collinearity.

Variables included in the multiple linear regression analyses were herd size, number of animals transferred, number of camels, production system and contact with wildlife (Table S2.2). All of these variables had a significant association with bTB prevalence in the GLM analyses (Table 2.3).

Table 2.3. Results of the one-by-one GLM analysis of risk factors and summary statistics for all predictors against herd bTB prevalence (n=102).

	bTB prevalence			
Variables	b (95% CI)	OR(95% CI)	χ^2	p-value
Herd size	0.08 (0.05-0.7)	1.1 (1.04-10.6)	76.8	< 0.001***
Herd movement	0.06 (0.01-0.30)	1.2 (1.17-1.30)	63.6	< 0.001***
Number of animals introduced	0.05 (0.007-0.11)	1.1 (1.05-1.10)	36.2	< 0.001***
Number of animal transferred	0.05 (0.03-0.06)	1.0 (-1.03-1.06)	24.4	< 0.001***
Number of sheep and goats	0.01 (0.01-0.02)	1.0 (0.99-1.01)	2.3	0.264
Number of donkeys	0.00 (-0.09-0.11)	1.0 (0.93-1.13)	0.2	0.646
Number of camels	0.03 (0.03-0.06)	1.0 (1.0-1.06)	27.2	< 0.001***
Production system	0.06 (-1.04-5.42)	11.4 (1.58-81.6)	14.7	0.016*
Contact with wildlife	0.02 (-0.46-1.25)	2.0 (1.29-2.81)	9.5	<0.001***
Presence of other livestock	0.00 (-0.78-1.65)	1.8 (0.57-5.79)	1.3	0.309

b=standardized regression coefficient with 95% confidence intervals, OR=Odds Ratio with 95% confidence intervals; * $P< 0.05$; ** $p < 0.01$; *** $p < 0.001$

The results of model averaging showed that, herd size and contact with wildlife were positively correlated to bTB prevalence (Table 2.4; Figure S2.1). The model also showed that the interaction of herd size and contact with wildlife had a positive effect on the prevalence of herd bTB.

Table 2.4. Summary statistics of the final model, with standardized regression coefficient (b with 95% confidence interval), Odds Ratio (OR) with 95% confidence interval, and p-value from the GLMs for the predictors correlated with herd bTB prevalence as obtained through model averaging (n=102).

	bTB prevalence		
Variables	b (95% CI)	OR (95% CI)	*p*-value
Herd size	0.94 (0.56-1.28)	1.1 (1.04-10.9)	< 0.001***
Number of animals transferred	0.00 (0.00-0.17)	1.0 (0.96-1.01)	0.171
Number of camels	-0.11 (-0.20-0.08)	1.0 (-0.97-1.02)	0.246
Contact with wildlife	0.19 (0.05-0.33)	11.8 (1.43-96.4)	0.007**
Production system	0.28 (-0.42-0.98)	2.3 (0.29-17.48)	0.442
Herd size and contact with wildlife	0.15 (0.02-0.40)	1.0 (0.93-1.00)	0.008**

b=standardized regression coefficient with 95% confidence intervals, OR= Odds Ratio, CI=95% confidence intervals; *p<0.05; ** p<0.01; *** p<0.001

The structural equation model showed that the probability of contact with wildlife, as an important risk factor for bTB infection, was mainly influenced by herd size (b=0.9, p<0.001), through herd movement (b=0.59, p<0.01; Figure 2.1).

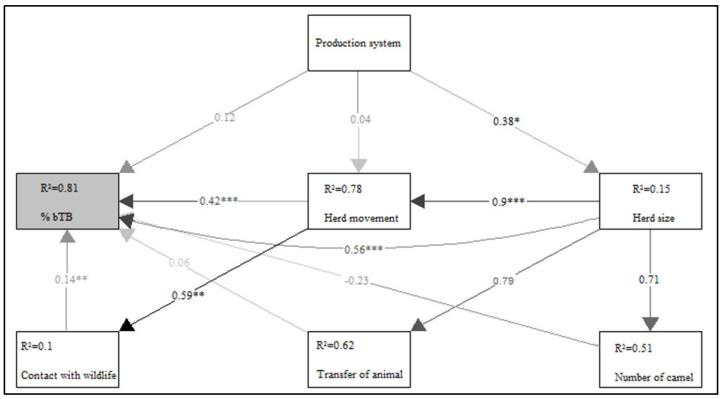

Figure 2.1. Structural equation modelling graph of the direct and indirect effects of risk factors on bTB prevalence (% bTB). The arrows indicate supported path coefficients. * $p<0.05$; ** $p<0.01$; *** $p<0.001$. Variables are herd size (size), the average herd movement in a day (movement), number of animal transferred (transfer), number of camels (camel), production system (production), and contact with wildlife (wildlife). The proportion of variance explained (R^2) appears above every response variable in the model.

The analysis of the impact of spatial scale on the effect of livestock transfer on herd bTB prevalence showed that exchange of animals among the herd from outside the average daily herd movement radius of 7.3 km (Table 2.1) reduced bTB prevalence (b=-0.11; OR=0.89; 95% CI=0.79-0.99; $p<0.05$) compared to an animal exchanged among the herd that was obtained from a transfer within the daily herd movement radius (b=0.04; OR= 0.96; 95% CI=0.91-1.03; $p>0.05$).

Discussion

The overall individual bTB prevalence was 5.5%, comparable with other results reported from Ethiopia (Ameni et al., 2003; Ameni et al., 2007; Fetene and Kebede, 2009; Gumi et al., 2011), Uganda (Bernard et al., 2005), Zambia (Munyeme et al., 2008; Munyeme et al., 2009; Munyeme et al., 2010) and Tanzania (Katale et al., 2013). Our risk factor analyses identified the age of animals and body condition scores as significant factors associated with bTB infection at individual animal level. Herd size, contact with wildlife and their interaction were identified as risk factors at herd level.

In line with previous studies (Ameni et al., 2007; Inangolet et al., 2008; Regassa et al., 2008; Gumi et al., 2011; Katale et al., 2013; Mamo et al., 2013), bTB prevalence increased with the age of the animals, probably because of the longer exposure to the agent over time of older animals. Results also showed that a poor body condition score was associated with bTB infection. Cause and

effect are not clear, however animals in poor body condition are likely more susceptible to tuberculosis infection, or tuberculosis positive animals develop a poor body condition score as a result of being infected, i.e., a clinical sign that typically follows an active infection with *M. bovis* (Kazwala et al., 2001).

Similar to what Cleaveland et al. (2007) and Ameni et al. (2003) found, herd size was positively correlated with the probability of bTB infection in the herd (Cleaveland et al., 2007; Ameni et al., 2003). Transmission of bTB, which is mainly through aerosols transmission (Renwick et al., 2007; Skuce et al., 2012) described as a density-dependent (Huang et al., 2013). Increasing herd size can lead to higher encounter rates of susceptible and infectious hosts, thereby promoting the spread of the pathogen within the herd. Our results support the hypothesis that herd size is a risk factor for the transmission of bTB.

Several wildlife species have been found bTB positive to serological rapid tests in Ethiopia (Tschopp et al., 2010; de Garine-Wichatitsky et al., 2013). Kafue lechwe (*Kobus leche*), greater kudu (*T. strepsiceros*), and African buffalo (*Syncerus caffer*) are known as wild maintenance hosts, implicated in the transmission of *M. bovis* to cattle in Africa (de Vos et al., 2001; Keet et al., 2001; de Lisle et al., 2002; Munyeme et al., 2009). In Zambia and Tanzania high prevalence rates of bTB in cattle have been recorded within and around the wildlife area, where contact between wild maintenance hosts, particularly the lechwe and buffalo, and domestic animals were high (Munyeme et al., 2009; Katale et al., 2013). Transmission of bTB between animals is due to direct contact when sharing forage or water resource, and/or indirect contact when grass is contaminated by infected faeces, or urine (Nolan and Wilesmith, 1994; Cleaveland et al., 2001; Johnston et al., 2005). We also found that contact with wildlife was a risk factor for bTB prevalence in cattle. In the north and north eastern part of Awash National Park, it is common to observe livestock grazing in close proximity to wild animals during the dry season. In Africa, species such as greater kudu are less affected by livestock presence in their habitat use (Prins, 2000). This large habitat overlap between cattle and greater kudu could play a role, and cattle could acquire bTB through grazing contaminated pastures. However, the structural equation model showed that the probability of contact with wildlife was partly confounded with herd size, through herd movement (Figure 2.1; 2.2). Pastoralists with larger herds move more during the dry season searching for water and pasture, and graze a larger area. These practices may predispose more cattle to bTB infection due to the higher chances of coming in contact with contaminated pastures or infected wild and domestic animal hosts. So, the correlation of contact with wildlife and bTB infection might not tell the whole story, as the underlying reasons for this

contact is probably the herd size and the herd movements, which are probably more important risk factors. Moreover, we do not know to what extent wildlife is directly or indirectly infected with bTB from cattle. These uncertainties need more detailed ecological and epidemiological research.

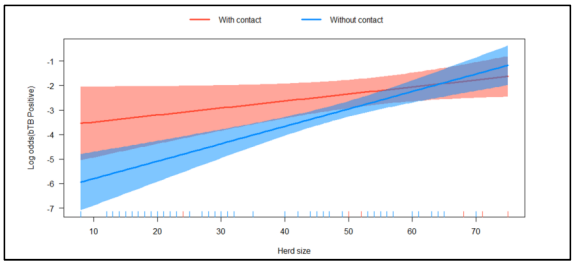

Figure 2.2. Illustration of the GLM results showing the effect of the interaction of herd size and contact with wildlife on the herd bTB prevalence (log odds scale; with 95% CLs).

The transfer of livestock, within and between clans, is a common practice in the area to spread risks, especially during periods of drought and/or conflicts. We found that transfer of animals was not positively correlated with bTB prevalence. Introduction of an infected animal into a bTB free herd or area is one of the major risk factors for introducing the disease (Marangon et al., 1998; Johnston et al., 2005; Gopal et al., 2006). We found that the number of animals exchanged outside the average daily herd movement radius (7.3 km, Table 2.1) reduced bTB prevalence. This could be due to the fact that, within a village, cattle herds share water holes and grazing areas with each other, and come in close contact with one another; this homogenizes the infection prevalence of bTB among cattle, resulting in a similar herd infection prevalence across village herds. Similarly, others found that introduction of animals from non-endemic area, or minimizing the number of animals being introduced, and introducing juveniles lowered the risk of bTB spread (Reilly and Courtenay, 2007). Therefore, we conclude that whether the transfer of livestock either positively or negatively affects bTB prevalence depends on the status of the transferred animals, the number and type (receiving or bestowing) of transferred, the age of the animal, the distances between receivers and donors, and the spatial variation of the bTB prevalence of the herds from which animals are received.

Two types of production and grazing system are practiced in the area, i.e., village resident herds with an agro-pastoral production system, and transhumant herds, following a pastoral production system. bTB prevalence in Uganda was higher in agro-pastoral production systems of Uganda (Faye et al., 2005). However, other studies indicated that a transhumant grazing system is a risk factor for infectious disease transmission (Bugwesa et al., 2013), as the pastoral production system relies on movement of livestock following grazing and water resources over considerable

distances under seasonal changes. Other studies showed that bTB occurs on both agro-pastoral and pastoral farming systems with no distinct differences in prevalence (Kazwala et al., 2001; Munyeme et al., 2010). In line with these latter studies, our study showed that cattle under a pastoral production system showed a slightly higher, but not significant, bTB prevalence than under an agro-pastoral system. This might be explained by the interactions of the two cattle husbandry systems in the area. Transhumant movements are more intensive during the dry season, when transhumant herds graze in farmlands or on cotton farms. During these moments there is a lot of interaction between agro-pastoral and the pastoral cattle on these grazing lands, possibly resulting in a similar infection prevalence.

In conclusion, this study identified that bTB prevalence increased with increasing age of cattle and with decreasing body condition at individual animal level. Herd size is an important risk factor contributing to the prevalence of bTB in cattle, because larger herds have the need to move more to look for more pastures and thus end up in the wildlife conservation area where the probability of contact with wildlife maintenance hosts harbouring bTB is higher. Thus, based on the study, it is impossible to indicate the contribution of wildlife species in the transmission of bTB to cattle, and the direction of the spread of the pathogen between wildlife and cattle. Findings from this study add useful epidemiological information regarding bTB infection at the livestock-wildlife interface in Ethiopia. In order to improve this understanding, further surveillance and research on the disease ecology, including habitat use among different wildlife species with cattle, migration ecology, and population monitoring are needed.

Acknowledgements

We are grateful to Netherlands Fellowship Programme (NUFFIC) for the financial support of the PhD scholarship through Wageningen University for the first author. We thank Haramaya University for the logistic support. We are also indebted to all field and laboratory staff who worked with us during tuberculin testing, habitat survey and laboratory work. We thank Zheng Huang and Tim Hofmeester for their help with the statistics. Finally, we are grateful to all herders and herd owners who collaborated with us during the interviews and bTB testing.

Supporting information

Table S2.1. Spearman's correlation matrix among variables (n=102)

Variable	Size	Movement	Introduction	Transfer	Camels	Production	Wildlife
Size	1						
Movement	0.89	1					
Introduction	0.77	0.79	1				
Transfer	0.69	0.66	0.84	1			
Camels	0.67	0.69	0.70	0.62	1		
Production	0.36	0.30	0.30	0.29	0.32	1	
Wildlife	0.38	0.34	0.33	0.28	0.23	0.13	1

Variables are herd size (size), the average herd movement in a day (movement), number of animals introduced into the herd (introduction), number of animal transferred (transfer), number of camels (camel), production system (production) and contact with wildlife (wildlife)

Table S2.2. Summary of the global model (top) and selected candidate models (**δ** AIC < 5 and weight >0.05) and the variables included in the model (+ indicates the inclusion of the variable in the model).

Model	b	Wildlife	Size	Transfer	Camel	Production	Wildlife*Size	AIC	δ AIC	weight
Global model	-7.3	+	+	+	+	+	+	178.5	4.23	0.03
Model 1	-6.5	+	+				+	174.3	0.00	0.24
Model 2	-6.7	+	+	+			+	174.8	0.55	0.18
Model 3	-7.1	+	+			+	+	175.8	1.53	0.11
Model 4	-6.1		+	+				176.3	2.01	0.09
Model 5	-6.5	+	+		+		+	176.3	2.03	0.09
Model 6	-7.3	+	+	+		+	+	176.5	2.17	0.08
Model 7	-6.7	+	+	+	+		+	176.9	2.61	0.06
Model 8	-6.9		+	+		+		177.1	2.79	0.06
Model 9	-5.8		+					177.1	2.82	0.06

Variables are herd size (size), number of animal transferred (transfer), number of camels (camel), production system (production), contact with wildlife (wildlife), and the interaction of contact with wildlife and herd size (wildlife*size)

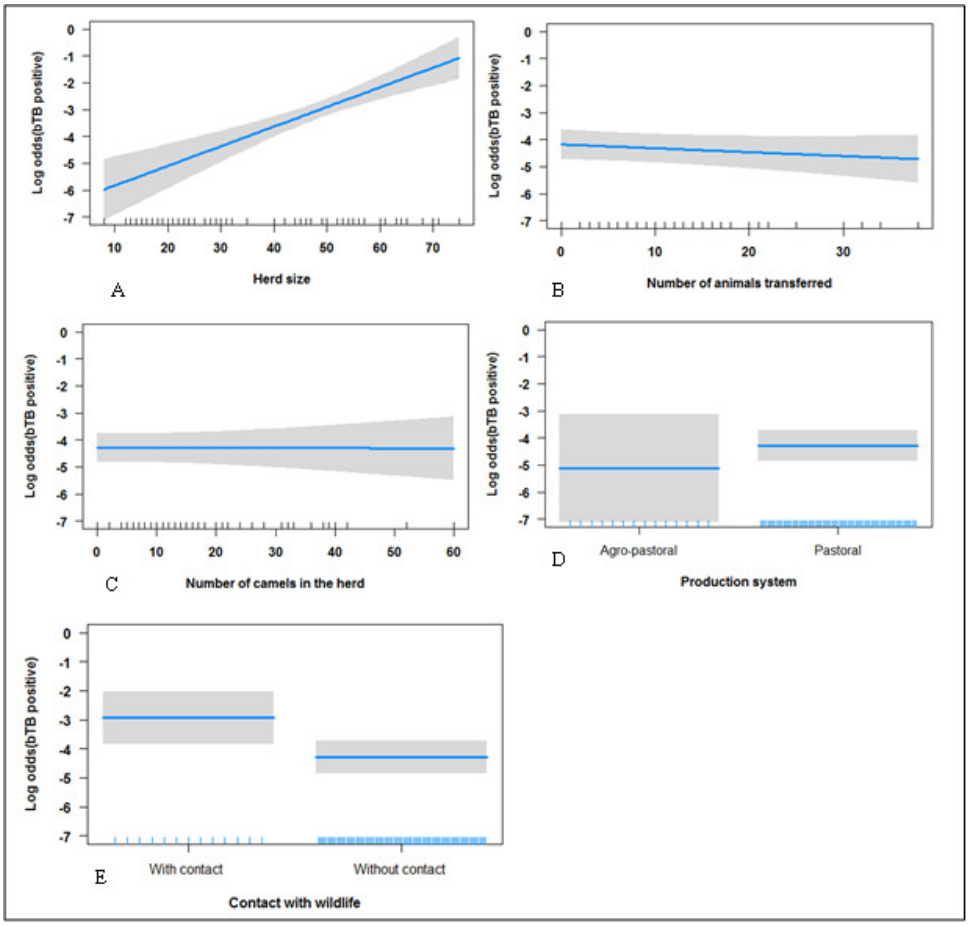

Figure S2.1. Visualization of GLM results of the effects of explanatory variables on herd bTB prevalence (log odds scale with 95% CLs) in relation to herd size (A), the number of livestock transferred (B), the number of camels (C), production system (D), and contact with wildlife (E).

Chapter 3

Effect of host diversity and species assemblage composition on bovine tuberculosis (bTB) risk in Ethiopian cattle

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Parasitology, 2017, 144: 783–792

Current theories on diversity-disease relationships describe host species diversity and species identity as important factors influencing disease risk, either diluting or amplifying disease prevalence in a community. Whereas the simple term 'diversity' embodies a set of animal community characteristics, it is not clear how different measures of species diversity are correlated with disease risk. We therefore tested the effects of species richness, Pielou's evenness and Shannon's diversity on bTB risk in cattle in the Afar Region and Awash National Park between November 2013 and April 2015. We also analysed the identity effect of a particular species and the effect of host habitat use overlap on bTB risk. We used the comparative intradermal tuberculin test to assess the number of bTB infected cattle. Our results suggested a dilution effect through species evenness. We found that the identity effect of greater kudu - a maintenance host – confounded the dilution effect of species diversity on bTB risk. bTB infection was positively correlated with habitat use overlap between greater kudu and cattle. Different diversity indices have to be considered together for assessing diversity-disease relationships, for understanding the underlying causal mechanisms. We posit that unpacking diversity metrics is also relevant for formulating disease control strategies to manage cattle in ecosystems characterized by seasonally limited resources and intense wildlife-livestock interactions.

Introduction

Bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, is an important zoonotic disease affecting many mammal species, and mainly spreads via aerosol transmission (Skuce et al., 2012). The World Health Organisation (WHO, 2012) identified bTB as one of the eight worldwide neglected zoonoses needing urgent attention, especially in developing countries. The disease is endemic in sub-Saharan African cattle (de Garine-Wichatitsky et al., 2013), and cattle are the main host for *M. bovis* (Cosivi et al., 1998). A wide range of domestic and wildlife mammals, but also humans can be infected with bTB (Munyeme et al., 2008). Although control programmes have eliminated or nearly eliminated this disease from domestic animals in most developed countries, bTB is still widespread in Great Britain, Ireland, New Zealand and many developing countries, especially in Africa (Renwick et al., 2007; Humblet et al., 2009). In fact, bTB is an important public concern, and can cause economic losses due to livestock deaths, product reduction and trade restrictions (Humblet et al., 2009).

Africa is recognized as a hotspot for biodiversity, but is suffering from rapid and extensive loss of that diversity (Myer et al., 2000; Olff et al., 2002; Gorenflo et al., 2012; Di Marco et al., 2014). The continent is also a hotspot for emerging infectious diseases as illustrated by emergence of Ebola, HIV/AIDS, MERS, among others (Morens et al., 2004). As biodiversity loss is thought to be a major explanatory factor of the increase in emergence of infectious diseases (Keesing et al., 2010; Ostfeld and Keesing, 2012; Huang et al., 2013), it is key to investigate the links between biodiversity, and biodiversity loss on the patterns of infectious diseases in Africa. Recently, several studies have shown that a reduction in biodiversity may increase the prevalence and transmission of diseases (Keesing et al., 2010; Cardinale et al., 2012; Johnson et al., 2013; Myers et al., 2013; Civitello et al., 2015). The two alternative hypotheses are the dilution and the amplification effect (Keesing et al., 2006; Huang et al., 2013; Hofmeester et al., 2016). The dilution effect predicts that species diversity decreases pathogen prevalence through mechanisms such as decreased host density, reduced encounters between hosts, or reduced host survival (Keesing et al., 2006; Huang et al., 2013; Johnson et al., 2013). In contrast, the amplification effect predicts increased pathogen prevalence with greater species diversity, through increased encounters between hosts, or through the presence of secondary hosts (LoGiudice et al., 2003; Keesing et al., 2006). A recent review of the relationships between species diversity and diseases reported dilution effects in up to 80% of the studies examined, and amplification effects in 12% of the studies (Cardinale et al., 2012; Ostfeld and Keesing, 2012). Despite the fact that the dilution effect occurs far more frequently than the amplification effect, our knowledge of which

specific systems conform to the dilution effect and the mechanisms underlying the effects of diversity, is incomplete (Ostfeld and Keesing, 2012; Randolph and Dobson, 2012; Huang et al., 2013; Ostfeld, 2013; Johnson et al., 2013; Miller and Huppert, 2013; Huang et al., 2013; Hofmeester et al., 2016). Understanding the underlying mechanisms how the risk of disease relates to the level of biodiversity is important, both for predicting disease dynamics in the context of global biodiversity decline, and to provide valuable insights into successful control measures.

Most studies that examine the diversity–disease relationship focus principally on species richness as a measure of biodiversity (Keesing et al., 2006). In fact, biodiversity can be measured in many different ways, as the number of species (species richness), the distribution of individuals over species (species evenness), or a combination of richness and evenness, as represented by diversity indices such as the Shannon index (Magurran, 1988; Tucker and Cadotte, 2013). Many studies have argued that species richness and evenness are two independent indices (Sheldon, 1969; Smith and Wilson, 1996; Gosselin, 2006; Symonds and Johnson, 2008), and suggest treating them separately (Magurran, 1988; Legendre and Legendre, 1998). Ostfeld and Keesing (2000) stated that encounter rate is proportional to the distribution of hosts. Thus, evenness which measure how evenly the individuals are distributed in the community among different species may be most appropriate measure of biodiversity to explain disease risk, because of power to detect the probability of encounter between pathogens and each host species. Thus, despite many studies of the relationship between diversity and diseases, evaluating the effects of different diversity metrics on disease risk has proven to be rare (Chen and Zhou, 2015). Thus, these different metrics of diversity may have different predictive powers for predicting disease risk in the target population. Here we tested for the effect of different diversity metrics on bTB risk in cattle.

Several recent studies suggest that the occurrence of particular species in the animal community may play an important role in disease risk, and in determining whether biodiversity amplifies or dilutes the infectious disease (Fenton and Pedersen, 2005; Keesing et al., 2010; Hamer et al., 2011; Johnson et al., 2013; Oda et al., 2014; Johnson et al., 2015). This effect of a particular species on pathogen transmission is known as the identity effect (Hantsch et al., 2013; Huang et al., 2014; Huang et al., 2016). Generally, the identity effect on pathogen transmission can be observed in two different situations (Huang et al., 2016). One is that a key species with particularly high or low reservoir competence may be present in communities when species diversity increases. The other situation is where a species can affect vector abundance (either positively or negatively) (Huang et al., 2016). To our knowledge, the generality of this pattern for

directly transmitted or aerosol-borne diseases, such as bTB, has not been established. Thus, understanding the identity effect is an important step in being able to understand the expected impacts of biodiversity loss on disease dynamics. In Africa, buffalo (*Syncerus caffer*), greater kudu (*Tragelaphus strepsiceros*) and lechwe (*Kobus leche*; Cosivi et al., 1995; de Garine-Wichatitsky et al., 2013) have been identified as maintenance hosts and implicated in the transmission of *M. bovis*. Warthog (*Phacochoerus africanus*) are also thought to be a potential reservoir for this bacteria in Africa (Tschopp, 2015). The presence of species like the greater kudu and warthog are likely to affect the type of encounters with cattle, which could then alter the relation between biodiversity and disease risk. We thus tested for the existence of an identity effect of greater kudu and warthog. We predict that bTB risk increased with the occurrence of maintenance host species.

Currently, livestock and wild herbivores graze together in many arid and semi-arid rangelands of Africa, with much resource use overlap, as livestock species are ecologically similar, with similar resource requirements as several wild herbivore species (Prins, 2000; Sitters et al., 2009). Overlapping space use can lead to interspecific interactions, and stimulate the spread and prevalence of many diseases (Riley et al., 1998), as most pathogens are able to cross-infect multiple host species. Hence, in areas where wildlife and livestock co-occur, pathogens can emerge and establish in these sympatric host populations (Gortazar et al., 2007). For example, foot and mouth disease, rabies, anthrax, brucellosis and bovine tuberculosis (bTB) have all been shown to be reciprocally transmissible between livestock and wildlife (Frolich et al., 2002; Artois, 2003; Ward et al., 2006; Cooper et al., 2010; Proffitt et al., 2011). In this context, resource use overlap between host species can play an important role in pathogen transmission by increasing contact rates and environmental exposure to the agent (Roper et al., 2003; Böhm et al., 2009). How habitat use by hosts affects direct and indirect interactions among hosts is fundamental in understanding multi-host disease transmission (Cooper et al., 2010), and is critical for designing scientifically sound disease control strategies (Hudson et al., 2002). Nevertheless, the role that spatial interactions between livestock and wildlife host play in disease transmission remains mostly unknown (Böhm et al., 2009; Martin et al., 2011; Tschopp, 2015). For instance, habitat and water resources use overlap may stimulate bTB transmission through increasing wildlife maintenance host-cattle contact, such as observed in and around Awash National Park, Ethiopia, where large numbers of livestock share their habitat with wildlife particularly during the dry season when resources are scarce. We therefore also tested whether habitat use overlap between wildlife maintenance host (greater kudu and warthog) and cattle increased bTB incidence. Beside the role of host community composition and resource overlap, a positive effect of host (e.g.,

cattle) densities (Humblet et al., 2009) has also been associated with bTB transmission risk. We also tested whether cattle densities were positively correlated with bTB incidence in cattle.

Materials and Methods

Study area

We carried out a cross-sectional study in Awash National Park and in the neighbouring Afar Region, Ethiopia (Figure 1.2). Awash National Park is situated in the Ethiopian Rift valley and had an elevation of 960 to 1050 m above sea level. It is covered in semi-arid savanna. The Afar region is found in the north-eastern part of Ethiopia with an area of about 70,000 km^2 (CSA, 2008). It is characterized by an arid and semi-arid climate with low and erratic rainfall, with a mean annual rainfall of 500 mm in the semi-arid western escarpments, decreasing to 150 mm in the arid zones to the east. Study sites were included due consideration of variation in wildlife-livestock interactions, concentrations of livestock and wildlife, and the presence of common grazing and water resources.

Study Design

A cross sectional multi-stage sampling was used to select study villages with 'sub-region' as the highest level followed by 'district' (n=17; Figure 1.2), and 'sub-district' (n=34) at the lowest level. Study animals were obtained using a three-stage random sampling procedure. The village within the sub-district was regarded as the primary unit, the herd as secondary unit and individual animal as tertiary unit, following the method of chapter 2. The desired sample size, which gave us a total of 2550 animals, was calculated following the method of Dejene et al. (2016). Tuberculin skin testing was performed using Purified Protein Derivative (PPD, supplied by Prionics Lelystad B.V, Lelystad, The Netherlands) to identify bTB positive animals following the method of chapter 2.

Dung counts

Plots for dung counts were established using stratified random sampling. First, sub-districts were stratified according to vegetation type. 204 plots (6 in each of the 34 sub-districts) of 100 x 100 m were laid out randomly in these vegetation types and were GPS geo-referenced. In each plot, we surveyed 50 transects of 100 m length and 2 m wide, and counted dung piles. Each pile of dung was attributed to a locally available wildlife species based on the size, shape and form of the pellets by using Stuart and Stuart (2000), and with the help of experienced local trackers. The relative abundances of wild herbivores were estimated based on the frequency of faecal droppings found in the plot transects following Vicente et al. (2004). We divided each 100 m

transect into 10 sectors of 10 m length. We defined sign frequency as the average number of 10 m sectors with the presence of wild herbivores droppings. Based on these frequencies, we calculated for each of the species the frequency-based indirect index (FBII):

$$\mathrm{FBII} = \frac{1}{n}\sum_{i=1}^{n} s_i$$

Where s_i is the number of sign-positive sectors in the i^{th} 100 m transect (i.e., S_i varies between 0 and 10), and n is the number of 100 m transects considered (i.e., n=50 for each plot; Vicente et al., 2004).

Ethical Statements

This study was approved by Haramaya University, Ethiopia (Reference number HUP14/559/15).

Statistical analysis

For each sub-district Pianka's Niche Overlap, mammalian species richness (S), mammalian species diversity (H') and mammalian species evenness (J') were calculated. Habitat use overlap between cattle and greater kudu was calculated according to Pianka's Niche Overlap (Pianka, 1973). This index varies from 0, no overlap, to 1, complete overlap.

$$O_{jk} = \frac{\sum p_{ij} p_{ik}}{\sqrt{\sum p_{ij}^2 \sum p_{ik}^2}}$$

where *Ojk* is the overlapping index between species *j* and *k*, and p_{ij} and p_{ik} being the proportions of use of habitat i by the species j and k.

Shannon's diversity index (H') was used to estimate mammalian species diversity as;

$$H' = -\sum_{i=1}^{S} p_i \ln p_i$$

where p_i is the proportion of species i, and S is the number of species (Hill, 1973).

Pielou's index was used to estimate mammalian species evenness (Hill, 1973), which is most widely used in ecology (Zhang et al., 2012).

$$J' = \frac{H'}{\ln(S)}$$

where H' represents the Shannon diversity index, and S is the total number of species observed. Biodiversity metrics were calculated using package vegan of R v3.2.0 (Oksanen, 2016).

Generalized Linear Mixed Models (GLMM, family=Poisson) using package lme4 were used to examine the effects of predictors (Table SI 3.1) on the sub-district bTB incidence. Prior to developing our candidate models, we performed one-by-one univariate analyses to identify potential spatial risk factors, using the number of bTB infected animals as dependent variable. Predictor variables with $p<0.25$ recognized as potential spatial risk factors (Huang et al., 2013), and subsequently used to construct multiple regression models. For highly correlated independent variables, only the one causing the largest change in the Log-Likelihood added to the final global model to avoid multi-collinearity, which was assessed by using variance inflation factors. The final variance inflation factor values were all <5 and confirmed the absence of collinearity among variables. From the global model, candidate models constructed using delta ΔAIC (<5), with the best approximating candidate model having the lowest delta AIC, as described in Burnham and Anderson (2002). Model averaging was used to construct the final model based on the lowest Akaike weights of the different candidate models (Anderson et al., 2000). In this analysis, we treated district as a random effect to account for repeated sampling. We carried out all analyses in R v3.2.2 (R Core Team, 2015).

Results

Pielou's species evenness (J') and Shannon's species diversity (H') varied between 0.46-0.90, and 0.72-2.05, respectively. Habitat use overlap between cattle and kudu varied from 0, no overlap to 0.95, high overlap. The highest Pianka's Niche Overlap index between warthog and cattle was 0.84. Relative abundances of kudu and warthog ranged from 0-0.93 and 0-0.79, respectively (Table SI 3.2).

Univariate analyses

Based on the results of the univariate analyses, we identified seven out of eight variables as potential risk factors, namely, mammalian species richness, Pielou's species evenness (J'), Shannon's species diversity (H'), habitat use overlap between cattle and greater kudu, habitat use overlap between cattle and warthog, relative density of greater kudu, and relative density of warthog (Table 3.1). Surprisingly, density of cattle dung was not associated with the number of bTB infected cattle in the sub-district (Table 3.1).

Communities that contained greater kudu had a significantly higher bTB incidence than communities without greater kudu (Table 3.1; Figure 3.1; b=0.9, 95% CI=0.5-1.2; OR=2.4, 95% CI=1.6-3.5; $p<0.001$).

Table 3.1. Results of the one-by-one GLMM analysis of all variables and summary statistics (regression coefficient b with 95% confidence intervals, Odds Ratio (OR) with 95% confidence intervals, χ^2 and p-value) for all predictors against sub-district (n=34) number of bTB positive animals from the likelihood ratio test (logLik = log likelihood) and AIC value.

	Number of bTB positive animals			
Variables	b (95%CI)	OR(95%CI)	χ^2	p-value
Habitat use overlap with kudu	1.2(0.6-1.7)	3.3(1.9-5.5)	19.6	< 0.001***
Habitat use overlap with warthog	1.4(0.7-1.9)	3.9(2.1-6.9)	20.9	< 0.001***
Relative density of kudu	1.3(0.6-1.8)	3.5(1.9-6.0)	18.9	< 0.001***
Relative density of warthog	1.4(0.8-2.0)	4.1(2.1-7.5)	19.6	< 0.001***
Species diversity (H')	0.7(0.2-1.1)	2.0(1.3-3.1)	14.3	0.002**
Species evenness (J')	-2.3(-4- 0.6)	0.9(0.2-1.5)	7.63	0.006**
Species richness	0.1(0.01-0.2)	1.1(1.0-1.2)	9.74	0.001**
Density of cattle dung	0.2(0.1-0.3)	1.0(0.9-1.1)	0.15	0.693
Presence of Kudu	0.9 (0.5-1.2)	2.4 (1.6-3.5)	17.21	< 0.001***

Kudu=greater kudu; * P< 0.05; ** p < 0.01; *** p < 0.001

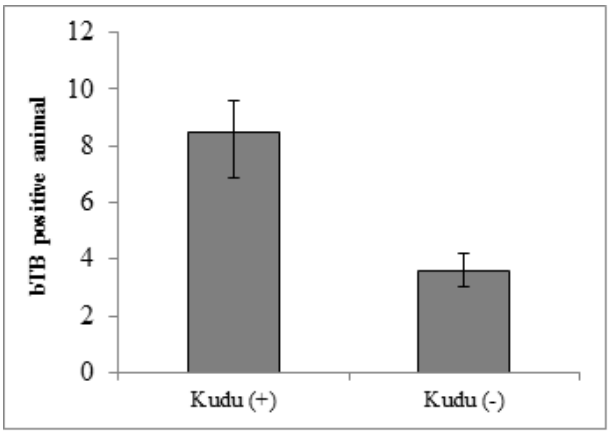

Figure 3.1. Effects of the presence of a particular host species, greater kudu, on the number bTB positive animals at sub-district level (n=34). Data shown are means with the 95% confidence intervals

The Spearman's correlation matrix showed that species richness was strongly correlated with Shannon's species diversity index. Habitat use overlap between cattle and warthog, relative density of greater kudu, and relative density of warthog were strongly correlated with habitat use overlap between cattle and greater kudu (r>0.7; Table SI 3.3). Therefore, we only included the latter two variables and species evenness in the multiple variable model to avoid collinearity.

Multiple variable analyses

Variables included in the multiple variable analysis were Pielou's species evenness, Shannon's species diversity and habitat use overlap between cattle and greater kudu.

The results of model averaging showed always a negative relationship between Pielou's species evenness and the number of bTB positive cattle, but we did not find a significant relationship between Shannon's species diversity and the number of bTB positive cattle, although the effect of species diversity was always positive in the models. In addition, our analysis also identified habitat use overlap between cattle and greater kudu as a significant risk factor for the number bTB positive cattle in the sub-districts (Table 3.2; Figure 3.1).

Table 3.2. Summary statistics of the final model, obtained through model averaging, with regression coefficient (b ± SE), Odds Ratio (OR, 95% confidence interval) and p-value from the likelihood ratio test for the effect of species evenness (J'), species diversity (H') and cattle-greater kudu habitat use overlap on the number of bTB infected Ethiopian cattle in the sub-districts (n=34).

	Number of bTB positive animal		
Variables	b (95%CI)	OR (95%CI)	p-value
Pielou's species evenness	-2.01(-3.9-0.1)	0.2(0.02-0.82)	0.036*
Shannon's species diversity	0.46(0.3-1.2)	1.6(0.77-3.30)	0.221
Habitat use overlap with kudu	1.14(0.3-1.8)	2.8(1.35-5.94)	0.008**

Kudu=greater kudu; * $P< 0.05$; ** $p < 0.01$; *** $p < 0.001$

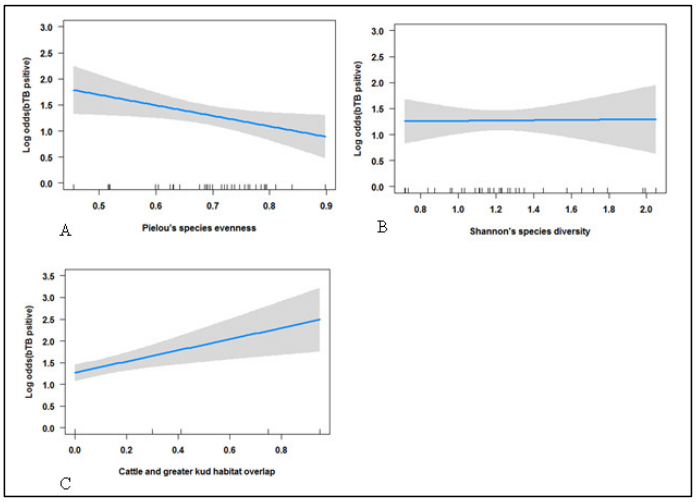

Figure 3.2. GLMM results of the effects of explanatory variables on the number of bTB positive cattle at sub-district level (log odds scale) in relation to Pielou's species evenness (A), Shannon's species diversity (B) and resource overlap between greater kudu and cattle (C).

Discussion

Our study showed that bTB infection rate was negatively associated with mammalian species evenness (J'), in line with our predictions derived from the dilution effect hypothesis. However, contrary to our expectation we did not find a significant relationship between mammalian species

diversity (H') and the number of bTB infected cattle. There was also a positive effect of habitat use overlap between cattle and greater kudu on bTB incidence in cattle. As proposed by Ostfeld and Keesing (2000), if the encounter rate is proportional to the distribution of the host species, species evenness would seem most appropriate for disease risk, because evenness, not richness, would capture the probability of encounter between pathogens and each host species (Ostfeld and Keesing, 2000; Chen and Zhou, 2015). Our study detected a dilution effect of Pielou's species evenness on the risk of bTB incidence, an influential aerosol-borne disease. This dilution effect is possibly explained by encounter reduction, in that the addition of alternative hosts may decrease the risk of pathogen transmission by reducing encounter rates between susceptible and infected hosts (Keesing et al., 2006; Chen and Zhou, 2015). In pastoral areas of East Africa, the distribution and abundance of large grazers is negatively associated with the presence of cattle (Voeten and Prins, 1999; de Leeuw et al., 2001; Bonnington et al., 2007). For instance, de Leeuw et al. (2001) observed a significant reduction of species like Oryx, gerenuk, and gazelle in the presence of cattle in Kenya (de Leeuw et al., 2001), and Odadi et al. (2007) found that the preference of foraging habitat for cattle was lower in the presence of wild grazers (Odadi et al., 2007). Many mammal species that can be infected by bTB are spill-over or dead-end hosts and do not transmit the pathogen efficiently (Corner, 2006; Renwick et al., 2007). The presence of these non-competent or spill-over mammalian species might act as barriers to cattle herd movement and distribution, and reduce encounter rates among cattle herds by changing the grazing behaviour and habitat preference (e.g., avoidance of sites contaminated by faeces or different preferences for feeding patches). Such an "encounter reduction" (Keesing et al., 2006) might lead to decreased probabilities of bTB infection risk, although the exact mechanism behind these correlations needs more attention. Similarly other studies demonstrated that the presence of non-competent species can have profound effects on the behaviour of deer mice, that is shift microhabitat use to avoid encounters with other species and reduce encounter rates among deer mice, thereby reducing the prevalence of Sin Nombre virus in deer mice (Clay et al., 2009; Dizney and Ruedas, 2009).

We did not detect significant effects of host species diversity (H') on the bTB infection level. The lack of a significant association between host species diversity and disease risk might occur because the index we chose, the Shannon index, stresses the number of species and presence of rare species (McGarigal and Marks, 1994; Haines-Young and Chopping, 1996; Riitters et al., 2000; Magurran et al., 2004). Thus, this metric might fail to weigh in the specific importance of particular species that are not rare, which might be addressed better by focusing on the effects of host identity (Hamer et al., 2011). Moreover, studies also criticizing the dilution effect argued that

pathogen transmission might increase in high-diversity communities (Randolph and Dobson 2012; Wood and Lafferty, 2013; Huang et al., 2016) due to the increased chance of including a particular species that has a positive effect on pathogen transmission (Hantsch et al., 2013; Huang et al., 2014). For instance, a recent study on bTB suggested that the presence of buffalo increased disease risk due to its high bTB competence (Huang et al., 2016). Power and Mitchell (2004) also demonstrated how the identity effect of particular host species influence the diversity–disease relationship, and found that more diverse systems had higher rates of infection (i.e., amplification effect), because these species rich (high richness) assemblages contained highly competent reservoir hosts (Power and Mitchell, 2004). Bouchard et al. (2013) found that the occurrence of white-tailed deer (*Odocoileus virginianus*), an important host for adult ticks, increase the abundance tick and thus increased the risk of tick-borne diseases (Bouchard et al., 2013). Similarly, we found that the presence of greater kudu and habitat use overlap between cattle and greater kudu was positively associated with the number of bTB infection. In Africa, species habitat use such as of greater kudu is not strongly affected by cattle presence (Prins, 2000), because kudus are almost exclusively browsers and the kudu-cattle dietary niche overlap is relatively small (Fritz et al., 1996). High habitat use overlap between cattle and kudu could increase encounter rates between them and create a positive identity effect of kudu on transmission of bTB, as a known wildlife bTB reservoir host. On the contrary, the presence of opossums created a negative identity effect on tick abundance (Keesing et al., 2009). Thus, high species diversity (high Shannon's species diversity and species richness) may amplify or dilute pathogen prevalence depending on the occurrence of a particular species. If the occurrence of the particular species had a negative identity effect, it may enhance the strength of the negative diversity-disease relationship; when the identity effect is positive, it may weaken the negative diversity-disease relationship and lead to a dilution effect (Huang et al., 2016). Another example is the influence of warthogs, which are predominantly grazers and compete with cattle for high-quality food in African savannas (Treydte et al., 2006). The species is also recognized as a hosts for ticks, which are vectors of various diseases including African Swine Fever in eastern Africa (Osofsky et al., 2005). Thus livestock keepers tend to avoid the areas that are used by warthog for fear of diseases **(**Maleko et al., 2012). This could decrease the encounter rate between cattle and warthog, and lead to a non-significant identity effect on bTB transmission. This might be the reason for a non-significant negative diversity–disease relationship. We recognize that our conclusions are based on correlative studies and that further studies with experimental manipulation including host behaviour change and contact rates among hosts are required to thoroughly test this hypothesis. However, our results are a necessary first step towards

understanding the role of community structure on bTB risk and identifying the underlying mechanisms.

In addition to direct transmission, which requires close contact between host species, indirect transmission via environmental contamination is also possibility for bTB transmission. In the north and north eastern part of Awash National Park, particularly in the northern part of the Park at the hot spring and kudu valley areas, it is common to observe livestock grazing in close proximity with kudu during the dry season. *M. bovis* has been detected in environmental samples in East Africa (Roug et al., 2014), and experimental studies have confirmed that the bacteria can survive for multiple days outside hosts (Fine et al., 2011). Kelly and Collins (1978) suggested that the major factors influencing survival of the bacteria in soil is soil temperature and moisture, as high temperature causes desiccation, and negatively influence survival of the bacteria. Environmental persistence of *M. bovis* has been proposed to play a role in the transmission of bTB in the UK (Courtenay et al., 2006). Wetlands or humid areas are also potential risk factors, and areas around pounds are generally moister, with greater amounts of shade, which are favourable conditions for *M. bovis* survival (Jackson et al., 1995). In Africa, flooding or soil humidity have also been suggested as propagating factors for *M. bovis* in the environment, as demonstrated in Tanzania (Cleaveland et al., 2007) and Zambia (Munyeme et al., 2009) by creating favourable conditions for *M. bovis* survival. The humid marsh-shrub wetland habitat near the hot-spring and kudu valley of Awash National Park and the surrounding water holes may act as potentially high risk areas for *M. bovis* infection, as these areas are generally moist, with greater amounts of shade. So, the correlation of habitat use overlap between greater kudu and cattle with bTB infection in the GLMM analyses might not tell the whole story, as the underlying reasons for this correlation is that it is possible that environmental transmission occurs among African wildlife and livestock. These uncertainty and complex eco-epidemiological scenarios and possible confounding factors require further investigation of the transmission network.

Our results highlight aspects of mammalian species evenness and spatial differences in species assemblage that are likely to affect the risk of disease. Our results support the idea that a greater mammalian species evenness acts as a buffer against disease outbreaks. Our findings also demonstrate that the presence of a particular reservoir hosts can affect the diversity-disease relationship. Hence it is a prerequisite to understand the identity effect, and predict future outbreaks and minimize the risk of disease transmission. Ecologists, epidemiologists and policy makers need to understand the complex interactions among potential host species to identify risk factors for disease transmission and identify efficient management actions. In order to improve

this understanding, further ecological and epidemiological research on disease transmission and contact networks is required.

Acknowledgements

We are grateful to NUFFIC for the financial support of the PhD scholarship through Wageningen University for the first author. We thank Haramaya University for the logistical support. We thank Richard S. Ostfeld for his valuable comments on the manuscript. We are also indebted to all field and laboratory staff who worked with us during tuberculin testing, habitat survey and laboratory work. Finally, we are grateful to all herders and herd owners who collaborated with us during the interviews and bTB testing.

Supporting information

Table SI 3.1. Descriptions, predicted effects, variable and units of the predictors used in the analysis.

Predictor	Predicted effect	Variable	Units
Habitat overlap with kudu	positive	Pianka's index	-
Habitat overlap with warthog	Positive	Pianka's index	-
Relative density of kudu	Positive	FBII	-
Relative density of warthog	Positive	FBII	-
Mammalian species richness	Negative	Species richness	N
Mammalian species evenness	Negative	Pielou's index	-
Mammalian species diversity	Negative	Shannon's index	-
Density of cattle dung	Positive	Density of cattle	N/km^2

Table SI 3.2. Summary statistics per sub-district in the Afar Region, Ethiopia, of cattle habitat use overlap with greater kudu and warthog (Pianka's Niche Overlap), relative abundance of greater kudu and warthog (FBII), and species richness, evenness (Pielou's species evenness, J') and diversity (Shannon's diversity index, H').

Sub-district	Cattle habitat overlap		Relative abundance		Species			Cattle dung density /km²
	Kudu	Warthog	Kudu	Warthog	Richness	Evenness	Diversity	
Abosena A	0	0	0	0	5	0.79	1.28	6.0 X 10^3
Adalalita	0	0	0	0	5	0.70	1.13	5.7 X 10^3
Adalil	0	0	0	0	5	0.69	1.12	4.8 X 10^3
Ade Ar Ena	0	0	0	0	5	0.78	1.26	6.4 X 10^3
Adibaro	0	0	0	0	5	0.69	1.11	6.5 X 10^3
Askomana	0	0	0	0	3	0.90	1.03	4.7 X 10^3
Beri	0	0	0	0	5	0.71	1.15	6.8 X 10^3
Bilu	0	0	0	0	5	0.72	1.16	4.8 X 10^3
Bira Eforo	0	0	0	0	5	0.64	1.03	4.7 X 10^3
Bolhamo	0	0	0	0	5	0.68	1.09	4.8 X 10^3
Buri	0.3	0.47	0.29	0.18	18	0.69	1.98	9.3 X 10^3
Daleti	0	0	0	0	5	0.74	1.19	4.2 X 10^3
Deaka	0	0	0	0	5	0.76	1.23	6.8 X 10^3
Debel Na H	0	0	0	0	5	0.84	1.35	8.2 X 10^3
Diduba	0.95	0.84	0.93	0.79	17	0.72	2.05	5.3 X 10^3
Dikikana B	0	0	0	0	4	0.52	0.72	3.6 X 10^3
Gahertu	0	0	0	0	5	0.76	1.23	5.4 X 10^3
Geberoch	0	0	0	0	4	0.79	1.11	6.7 X 10^3
Halbina Wale	0	0	0	0	5	0.63	1.02	7.2 X 10^3
Haresa	0	0	0	0	5	0.81	1.30	6.7 X 10^3
Hintmegetana	0	0	0	0	5	0.72	1.17	6.9 X 10^3
Hotemerona	0	0	0	0	5	0.81	1.3	4.0 X 10^3
Kahrtuna T	0	0	0	0	4	0.63	0.87	6.9 X 10^3
Kailuna A K	0	0.21	0	0.31	12	0.75	1.79	4.6 X 10^3
Rumaytu	0	0	0	0	4	0.60	0.84	4.3 X 10^3
Sabure	0.75	0.65	0.67	0.52	12	0.69	1.66	7.2 X 10^3
Sebulina W	0	0	0	0	4	0.52	0.72	5.6 X 10^3
Seganto	0	0	0	0	5	0.76	1.22	4.9 X 10^3
Sehana M	0	0	0	0	4	0.73	1.02	3.2 X 10^3
Sergat	0	0	0	0	4	0.79	0.96	3.7 X 10^3
Serkamo	0.41	0.47	0.29	0.61	16	0.52	1.99	6.0 X 10^3
Wantuna F	0	0	0	0	4	0.78	1.08	5.5 X 10^3
Weydelelina	0	0	0	0	5	0.46	0.73	5.5 X 10^3
Wiena A	0	0	0	0	5	0.6	0.97	5.2 X 10^3

Table SI 3.3. Spearman's correlation (r_s) matrix among independent variables

	Species evenness (J')	Species diversity (H')	Habitat overlap with warthog	Relative density of kudu	Relative density of warthog	Species richness	Habitat overlap with kudu
Species evenness (J')	1.0						
Species diversity (H')	0.4	1.0					
Habitat overlap with warthog	-0.2	0.6	1.0				
Relative density of kudu	-0.3	0.5	0.9	1.0			
Relative density of warthog	-0.2	0.6	0.9	0.8	1.0		
Species richness	-0.1	0.8	0.6	0.6	0.4	1.0	
Habitat overlap with kudu	-0.3	0.5	0.9	0.9	0.8	0.7	1.0

Chapter 4

Beyond invasion: land use influencing bovine tuberculosis (bTB) infection

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Changes in land use and associated ecosystem change have been described as one of the causal drivers in current emerging and re-emerging of infectious diseases, but there is a notable scarcity of scientific knowledge to show whether, and how, land use changes play this role. Land use change can be the result of the introduction of the non-native *Prosopis juliflora*, a most powerful invasive woody species, threatening biodiversity and mammalian host community structure. We hypothesized that higher proportion of *P. juliflora* cover may increase bTB risk by changing the composition of the host community. We tested the relation between land use - including the invasive woody plant species *P. juliflora* - and bovine tuberculosis (bTB) prevalence in cattle in the Afar Region, Ethiopia between November 2013 and April 2015. We examined the potential underlying mechanisms by which ecological consequences of land use/cover, such as an invading woody species, alters the risk of bTB transmission. A total of 2550 cattle from 102 herds were investigated for the presence of bTB using the comparative intradermal tuberculin test (CITT). Landsat images from 2014 were used to quantify the proportion of different land cover by applying a k-means unsupervised classification, and analyzing this within a buffer of 16km from the location of each cattle herd. A generalized linear model was used to quantify the relationship between bTB prevalence and the proportion of land use classes. Then, multiple regression tree analyses were used to identify the most important land use predictor accounting for the variation in bTB prevalence. A model averaging analysis identified the proportion of *P. juliflora* cover, as a significant risk factor for increasing bTB prevalence in cattle ($b=12.2$, 95%CI=8.9-15.5, $p<0.001$). Multiple regression tree analysis also identified the proportion of Prosopis as the most important land use predictor. This could explain the variation in bTB prevalence by altering the mammalian species evenness and movement of livestock herds, which can change contact rates and hence disease transmission risk between hosts. Given the projected spread of Prosopis, land use/cover changes and associated changes in host community composition could affect the risk of infectious diseases, which is important for decision makers when formulating disease control strategies.

Introduction

Emerging infectious diseases (EID) have increased significantly over time, and most EIDs over the past 70 years were zoonoses (Jones et al., 2008). In recent years, evidence suggest that land use practices drive emerging, re-emerging and further spreading of zoonotic diseases (Patz et al., 2000; OIE, 2004; Nicole et al., 2014). For instance, high percentages of forest edges nearby herbaceous cover increased exposure to tick vectors (Hernandez et al., 2007). Vittor et al. (2006) also found that the conversion of rain forests for agriculture has led to a re-emergence and increased incidence of malaria. Cleared land creates areas where water can become stagnant, providing breeding sites for mosquito species capable of parasite transmission (Vittor et al., 2006). A low proportion of riparian forest has been identified as an important predictor of whirling disease, due to increase stream sedimentation, thus, increasing habitat for oligochaetes (McGinnis and Kerans, 2013). Increased forest fragmentation was also associated with an increased risk of Lyme disease by creating suitable habitats for hosts (Jackson et al., 2006). Therefore, understanding the underlying mechanisms how the risk of disease relates to the land use is important, both for predicting disease dynamics, and to provide valuable insights into successful control measures. Despite growing awareness that disease emergence may be related, at least in part, to land use (Woolhouse and Gowtage-Sequeria, 2005), these studies usually focus on vector-borne diseases, while there is a notable scarcity of scientific knowledge to show whether, and how, ecological change plays this role on aerosol-borne animal diseases. Here, we examined the relation between land use/cover and bovine tuberculosis (bTB) prevalence in cattle, and explored the underlying mechanisms accounting for these observations.

Bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, is a zoonotic disease of human, livestock and wildlife (O'Reilly and Daborn, 1995; Humblet et al., 2009), and cattle serve as the main host for the bacteria (de Garine-Wichatitsky et al., 2013). It is an important case of concern with potential public health, conservation and economic importance, since it can affect international trade of animals and animal products (Ayele et al., 2004). Inhalation of *M. bovis* is the principal route to bTB transmission and is facilitated by direct or indirect contact between infected and healthy animals. The direct or indirect interactions between hosts has several points at which alteration of the landscape could influence disease dynamics. Land use/cover could potentially influence bTB prevalence through direct effects on the host community composition, host densities and host contact networks. For instance, low proportion of forest, grassland, and other natural habitat due to anthropogenic influence has been associated with a number of ecological

complications, resulting in reductions of biodiversity (Chu et al., 2003; Turner et al., 2003). Recent studies have shown that a reduction in biodiversity may increase the prevalence and transmission of bTB, as non-competent mammalian hosts at higher levels of biodiversity may dilute pathogen transmission, a mechanism termed the dilution effect (Huang et al., 2013; Huang et al., 2014; Dejene et al., 2017b). Thus, it is important to distinguish between these processes, to understand how changes in land use indirectly affect pathogen transmission, and to be able to formulate control actions that minimize future disease outbreaks.

One of the other important pathways by which land use changes alter ecosystem is by the introduction of non-native species into novel landscapes (Hobbs and Humphries, 1995; Crooks, 2002). Incursion of non-native species is one of the most serious threats to natural ecosystems worldwide (Sharma et al., 2005; Butchart et al., 2010; Simberloff et al., 2013). Recent evidence showed that land use changes due to invasion by invasive plant species affect the risk of exposure to diseases. For example, invasive plant species may increase the risk of infection to tick-borne diseases by increasing the density as well as the rate of encounter rate between ticks and their vertebrate hosts (Allan et al., 2010; Williams and Ward, 2010). Similarly, land use changes as a result of plant species invasions increase the risk of mosquito-borne diseases by providing favourable habitat for vector species (Conley et al., 2011), and providing a high-quality nutritional fruits and leaves for mosquito larvae (Reiskind and Zarrabi, 2011). *Prosopis juliflora* (further referred to as Prosopis) is among the most extremely invasive plants species globally, infesting over four million hectares of lands in arid and semi-arid part of Africa (Pasiecznik et al., 2001; Gallahar and Merlin, 2010; Wakie et al., 2014). It was introduced in Ethiopia in the 1970s (Zollner, 1986; Coppock et al., 2005) as a control mechanism to combat desertification (Haregeweyn et al., 2013; Mehari, 2015). Around Awash in the Afar Region, about 30,000 ha of grassland, rangelands, water points and open acacia woodland were estimated to be occupied by Prosopis (Mehari, 2015). By 2020, approximately 31% of the area is projected to be covered by Prosopis (Haregeweyn et al., 2013). Currently, the species is a most powerful invasive species, threatening biodiversity and mammalian host community structure because of its weedy and invasive nature (Mehari, 2015). As biodiversity loss is a predictor for the increase in bTB outbreaks (Huang et al., 2013; Huang et al., 2014; Dejene et al., 2017b), we expected that invasion of Prosopis might increase the bTB prevalence through loss of non-competent or spillover mammalian hosts. The invasion by Prosopis reduces availability of palatable herbaceous species (Mehari, 2015) which could increase the movement of cattle herds in search for pasture and increase the probability of contact with infected hosts. Moreover, areas that are currently invaded by Prosopis were important sources of forage for livestock in the dry season, leading to shrinkage

of the range- and grasslands, and altering local host densities, and contact among cattle herds. For instance, host movement and herd size have been repeatedly identified as one of the major risk factor for bTB infection in African cattle populations (Oloya, et al., 2007; Boukary et al., 2011; Dejene et al., 2016). We, therefore, hypothesize that increasing Prosopis invasion can increase the risk of bTB infection by increasing contact among cattle herds. However, no study, to our knowledge, examined the effect of Prosopis invasion on the risk of disease transmission. Here, we explore the impacts of Prosopis invasion on bTB prevalence in cattle, and how these invasions may influence the key parameters of this host–pathogen interaction, leading to changes in bTB prevalence.

Materials and Methods

Study area

The field study was conducted in the Afar Region, Ethiopia, from November 2013 to April 2015, located in the Horn of Africa between 39°34' to 42°28'E longitude and 8°49' to 14°30' N latitude (Figure 1.2). The region has an area of 70,000 km^2, divided into five sub-regions administratively, each of them comprising of several districts. The area has a bimodal rainfall pattern, with a long rainy season from July-September and a short rainy season from February-April. The mean annual rainfall ranges from 500 mm in the western escarpment to 150 mm in the lowlands. The minimum and maximum annual temperature is 20°C and 40°C, respectively. The altitude ranges from 120 m below sea level to 1500 m above sea level (EMA, 2013).

Study design and sample size determination

A cross sectional multi-stage sampling was used to select study 'sub-region' as the highest level followed by 'district' (n=17; Figure 1.2), and 'sub-district' (n=34) at the lowest level. Study animals were obtained using a three-stage random sampling procedure following. The 'villages' within the sub-district were regarded as the primary unit, the 'herd' as secondary unit and 'individual animals' as tertiary unit, following the method of chapter 2. A total of 102 livestock herds from 34 sub-districts (3 in each sub-district, one large, medium and small herd) were selected to identify bTB positive animals following the method described in chapter 2. The total sample size per district was calculated using a 95% confidence interval at a 5% absolute precision following the method of Thrusfield (1995), which gave a total number of required animals of 2550. Herds (one herd from each stratum) and individual animals within herds were selected randomly.

Tuberculin testing of cattle

To identify bTB positive animals, tuberculin skin testing was performed using Purified Protein Derivative (PPD, supplied by Prionics Lelystad B.V, Lelystad, The Netherlands). PPD was administered in two shaved sites, 12 cm apart from each other in the middle neck region, after having recorded skin thickness with a calliper following the method of chapter 2. Skin thickness was measured again at both injection sites after 72 h. The reaction at each site was derived by measuring the difference of the skin thickness before and 72 h after the injection. An animal was considered bTB positive if the reaction at the bovine site minus the reaction at the avium site was ≥ 4 mm, according to the recommendations of the World Animal Health Organization (OIE, 2009).

Dung counts

Dung count was used to calculate Shannon's diversity and Pielou's species evenness index following the method of Dejene et al. (2017b). Plots for dung counts were established using stratified random sampling. First, sub-districts were stratified according to vegetation type. 204 plots (6 in each of the 34 sub-districts) of 100 x 100 m were laid out randomly in these vegetation types and were GPS geo-referenced. In each plot, we surveyed 50 transects of 100 m length and 2 m wide, and counted dung piles. Each pile of dung was attributed to a locally available wildlife species based on the size, shape and form of the pellets by using Stuart and Stuart (2000), and with the help of experienced local trackers. The relative abundances of wild herbivores were estimated based on the frequency of faecal droppings found in the plot transects following Vicente et al. (2004) and Dejene et al. (2017b).

Questionnaire survey

Semi-structured interviews with the herders and herd owners were conducted, gathering information on general herd management practices, livestock movement and herd size. Local agricultural officers, knowledgeable on local farming practices and who had received prior training on the administration and the scope of the questions, assisted us during the interviews. The interviewer estimated the maximum movement distance of the livestock herd by tracing the herd movement in the area based on interview-derived information, bound by roads, streams, rivers or hills, village, district, sub-district, or region, wildlife habitat or park, or other physical indicators, which were located on a georeferenced map. Subsequently, the maximum daily distance was calculated for sedentary and for the transhumance herds.

Land cover classification

A land cover map was derived from six Landsat 8 images (December 2014) with no cloud cover in the area of interest, obtained via the Centre Science Processing Architecture ordering interface (espa.cr.usgs.gov). The description of the land cover classes was based on the standard classes defined by the US Geological Survey (Mohan et al. 2011). Accordingly, eight classes were identified, namely, farmland, acacia woodland, bushland, grassland, area covered by Prosopis, wetland, water bodies, and bare land (Table 4.1).

Table 4.1. The description of the land use land cover (LULC) classes used classification in the Afar Region, Ethiopia.

LULC classes	General description
Farmland	Areas of land ploughed or prepared for crop growing (i.e., both areas identifiably under crop agriculture and land under preparation).
Bushland	Areas with shrubs, bush and small trees in which multiple stems and branches are produced from the base of the main stem.
Grassland	Areas covered primarily with different grass and with herbaceous plant species, which are used as a natural grazing land, with scattered shrubs, bushes and trees.
Prosopis	Areas covered with *P. juliflora*, an invasive plant species with dark evergreen leaves and closed canopy cover throughout the year.
Acacia woodland	Areas dominated by Acacia species found along the major perennial rivers, mainly the Mile and Awash River. The vegetation is usually evergreen (due to continuous water supply from the rivers) and serves as a dry season feed for livestock.
Wetland	Areas covered with swamps and wetlands
Water bodies	Areas with water bodies
Bare land	Areas with essentially no vegetative cover

The images were classified by means of a k-means unsupervised classification, using Erdas Imagine. Initially, 36 clusters were requested, for which the maximum number of iterations was set to 20, and the convergence threshold to 0.950. Subsequently, each of the 36 clusters was given one of the eight class labels, based on Google Earth imagery, resulting in a map with eight classes.

Farmland and Prosopis were often grouped together, and therefore a principal component analysis (PCA) was performed using Landsat 8 bands 3 and 4 to separate these two classes. After the PCA, another unsupervised classification was applied to the first component. The appropriate classes were then labelled as Prosopis. Farmland was difficult to classify, as different crops are in different developmental stages and have different spectral information. Therefore, the large commercial irrigation farms present in the area were traced manually via Google Earth, and added to the classification.

A reference set of 61 points was constructed, using Google Earth images. These reference points served as 'ground truth data' to define the accuracy of the classified map, and calculate the kappa coefficient of agreement. The proportions of the land cover classes within a buffer of 16 km around each of the 102 study herds, which is the daily average distance of herd movement (Dejene et al., 2016), was determined using ArcGIS.

Statistical analysis

Shannon's diversity index (H') and Pielou's index were callculated following the method of Dejene et al. (2017b). Relationships between bTB prevalence and proportion of land use / cover were quantified in two separate analyses. First, a Generalized Linear Model was used to examine the effects of the proportion of land use on bTB prevalence as a binary response. From the global model, candidate models were constructed using $\Delta AIC < 5$, with the best approximating model having the lowest ΔAIC (Burnham and Anderson, 2002). Model averaging was used to construct the final model based on the lowest Akaike weights of the different candidate models (Anderson et al., 2000). So, we analysed differences in host community composition as function of land use / cover type to examine the possible underlying mechanisms through which land use / cover type altered bTB prevalence. Second, regression tree analyses were used to identify the most important land use / cover predictors accounting for the variation in bTB prevalence. Regression tree analysis was used because of its ability to deal with non-normal data, numerical and categorical data, as well as its ability to automatically consider the interactions among explanatory variables (De'ath and Fabricus, 2000). We carried out all analyses in R v3.2.2 (R Core Team, 2015).

Results

The proportion of grassland was by far the dominant land cover followed by the proportion of bushland (Figure 4.1). Proportion of farmland, Prosopis and acacia woodland were often minor components of the landscape, but the major proportion of the main dry season grazing land, which is found in the Awash River basin, was covered by Prosopis, followed by acacia woodland (Figure 4.1). The overall map accuracy of classification was 70.2%, and the kappa coefficient was 0.63.

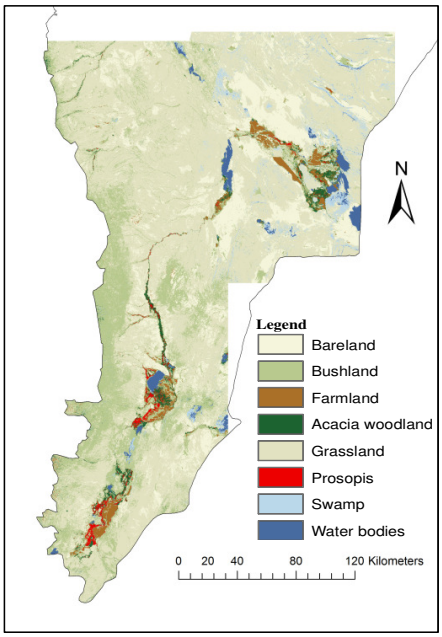

Figure 4.1. Classification of land cover types in the Afar Region, Ethiopia, on the basis of 2014 Landsat images.

Land use and bTB prevalence

There was a significant positive relationship between the proportion of Prosopis cover and bTB prevalence, but bTB prevalence tended to be relatively lower in areas with a higher proportion of bushland (Table 4.2).

Table 4.2. Summary statistics of the final model, obtained through model averaging, with regression coefficient (b, 95% CI), Odds Ratio (OR, 95% CI) and p-value from the likelihood ratio test for the effect of proportion of land use on bTB prevalence in Ethiopian cattle.

Land use type	b (95% CI)	OR(95% CI)	p-value
Prosopis	12.2 (8.9-15.5)	20.4(7.9-52.7)	<0.001*
Bushland	-0.06 (-1.2-0.6)	0.7(0.3-1.8)	0.279
Grassland	-0.01(-1.1-0.9)	0.9(0.5-2.5)	0.958
Acacia woodland	0.01(-2.8-6.8)	1.2(0.6-6.7)	0.935
Farmland	0.48 (-5.2-6.6)	7.2(6.3-8.2)	0.447

OR= Odds Ratio, CI=95% confidence intervals; * $P< 0.05$; ** $p < 0.01$; *** $p < 0.001$

The multivariate regression tree (MRT) analysis indicated that the proportion of Prosopis and bushland in the landscape were correlated with bTB prevalence (Figure 4.2), with a higher bTB prevalence in cattle in areas with a higher Prosopis cover. The first split of the multivariate regression tree was determined by the proportion of Prosopis, grouping 67 herds (average bTB

prevalence was 1.7%) that had less than or equal to 1.3% Prosopis cover. The second split was determined by proportion of bushland, grouping 12 herds (average bTB prevalence: 2.1%) that were located in areas with a bushland cover <26.6% (Figure 4.2).

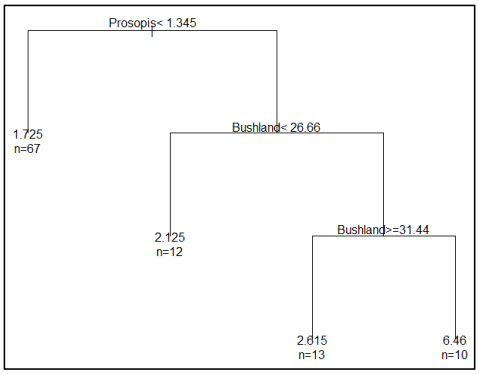

Figure 4.2: Pruned regression tree for predicting the prevalence of bTB prevalence with explanatory land use / cover variables. Each partition is labelled with the splitting rule and its value. Splitting statements are true to the left and false to the right. Terminal nodes are labelled with the predicted (mean) bTB prevalence value of the observations in the terminal group and the number of observations (n).

The regression tree analyses confirmed that the variable ranking first, i.e. the proportion of Prosopis cover, was also the variable that had a significant association with the bTB prevalence in the GLM. The regression tree analysis also included bushland as the second important predictor variable after Prosopis, similar to the GLM.

Land use and host community

Regression tree models also identified Prosopis cover as the most important land use/cover predictor accounting for the variation in species evenness (Figure 4.3A) and livestock herd movement (Figure 4.4A). We found that the mean species evenness (Pielou's species evenness, J'=0.76) was the lowest recorded in the area with the highest Prosopis cover (proportion of Prosopis >7%), indicating that Prosopis cover was correlated with a low species evenness in the landscape. When a Prosopis cover <7%, species evenness was positively correlated with acacia woodland cover (Figure 3A).

The best explanatory variable correlated with Shannon's species diversity index was grassland cover. When grass cover was ≥55% the predicted mean species diversity was relatively high at 1.8 (Figure 4.3B).

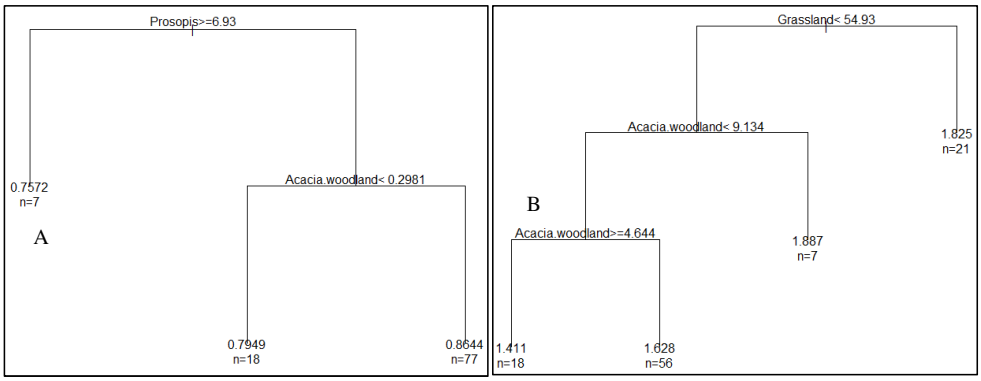

Figure 4.3. Pruned regression tree using land use / cover type as a predictor for predicting A) species evenness, J' and B) Shannon's diversity, H'. Each partition is labelled with the splitting rule and its value. Splitting statements are true to the left and false to the right. Terminal nodes are labelled with the predicted (mean) value of the observations in the terminal group and the number of observations (n).

The movement of cattle herd was also correlated to Prosopis cover, with the greatest herd movement in areas with a higher Prosopis cover (Figure 4.4A). Herds in areas with a Prosopis cover <1.3% had the lowest mean herd movement (5.8 km/day). The regression tree showed that the proportion of bushland cover was the primary predictor differentiating herd size, eventually splitting into five groups of herd size, with smaller herds in areas with relatively more bushland (i.e. ≥47%; Figure 4.4B).

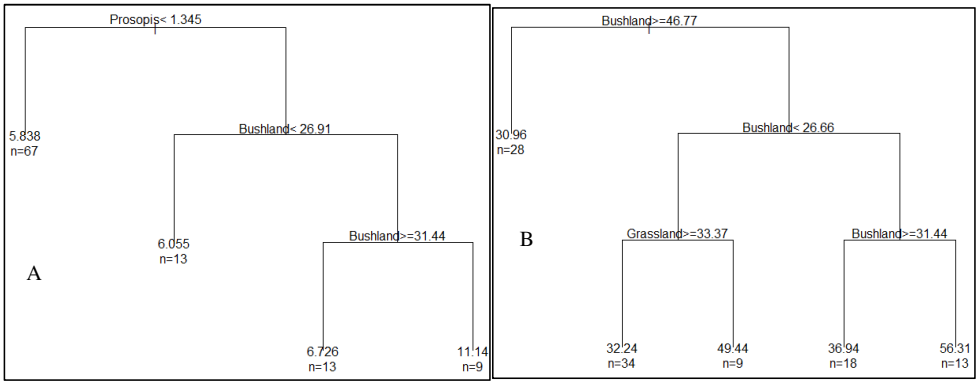

Figure 4.4. Pruned regression tree using land use / cover type as a predictor for predicting A) herd movement and B) herd size. Each partition is labelled with the splitting rule and its value. Splitting statements are true to the left and false to the right. Terminal nodes are labelled with the predicted (mean) value of the observations in the terminal group and the number of observations (n).

Discussion

Measures of land use may be useful predictors of bTB risk at various spatial scales. Our study showed that bTB prevalence was positively associated with the invasion of Prosopis. Similarly, a regression tree model identified the proportion of Prosopis as important land use predictor for the prevalence of bTB. The invasive Prosopis, although only a minor proportion of the total area of the landscape, has invaded the majority of the dry season pastureland of the Awash River Basin and causes significant environmental damage and habitat destruction (Haregeweyn et al., 2013), and thereby indirectly regulates host community composition. The loss in host species evenness and the increase in cattle movement as a consequence of the loss of palatable grasses in Prosopis areas could be potential mechanisms accounting for the observed higher bTB prevalence in these areas.

Invasive species have the ability to change ecosystem processes (Ehrenfeld, 2010), decrease the abundance, structure and diversity of native species through predation, competition, hybridization as well as indirect effects (Blackburn et al., 2004; Gaertner, et al., 2009). Similarly, invasion of Prosopis has been shown to suppress the growth of grasses and other herbaceous species and reduce species diversity (Getachew et al., 2012; El-Keblawy and Al-Rawai, 2007; Haregeweyn et al., 2013). The low diversity of herbaceous plant species observed under the dense Prosopis canopy is associated with a lower light intensity and shorter light period received by the understorey native plant species as well through competition for soil moisture (Getachew et al., 2012). The influence of Prosopis on the palatable herbaceous species in highly invaded areas may contribute to the reduction in mammal species evenness that we observed, as abundance and distribution of mammal species is partly determined by the availability and quality of palatable plant species (Treydte et al., 2013; Young et al., 2013). Haregeweyn et al. (2013) also reported negative impacts of Prosopis invasion on biodiversity in the Afar Region, as the invasion of Prosopis reduced the densities of wild animal species, such as *Oryx gazelle*, *Equus grevyi*, *Madoqua saltiana*, and *Kobus ellipsiprymnus* in response to a reduction of palatable plant species (Haregeweyn et al., 2013). As biodiversity loss is an explanatory factor of the increase in the bTB risk (Huang et al., 2013; Huang et al., 2014; Dejene et al., 2017b), bTB prevalence might increase through losses of non-competent or spillover mammalian host species. In pastoral areas of East Africa, the distribution and abundance of large grazers is negatively associated with the presence of cattle (Voeten and Prins, 1999; de Leeuw et al., 2001; Bonnington et al., 2007). Odadi et al. (2007) found that the preference of foraging habitat for cattle was lower in the presence of wild grazers (Odadi et al., 2007). Loss of wild grazers due to Prosopis invasion might increase encounter rates

between susceptible and infected cattle herds. Such an "encounter increase" (Keesing et al., 2006) might increase bTB infection risk.

Another pathway by which Prosopis may influence the prevalence of bTB is through livestock herd movement. Invasion of Prosopis species have been observed to suppress grass growth and reduce availability of herbaceous plant species (Brown and Archer, 1989). The low herbaceous plant species richness observed under high densities of Prosopis could be attributed to the dense Prosopis canopy, negatively affecting the intensity and duration of light received by understorey plant species. Areas that are currently invaded by Prosopis were important sources of dry season forage area for cattle in the Afar people. The invasion by Prosopis reduces availability of palatable herbaceous species (Mehari, 2015). Several useful palatable plant species such as *Chrysopogon plumulosus, Cymbopogon schoenanthus*, *C. pospischilii*, *Andropogon canaliculatus*, *Eragrostis cylindriflore* and *Terapogon cenchriformis* are now on the verge of local extinction in areas due to Prosopis invasion (Haregeweyn et al., 2013; Mehari, 2015), which could increase the movement of cattle herd in search for pasture. Our study also showed that high proportion of Prosopis cover increased the movement of livestock herd. The herd moved more and grazed in larger areas, hence the probability of grazing and contact with either infected domestic or infected wildlife hosts increased, amplifying the chances for bTB infection (Dejene et al., 2016). Furthermore, Prosopis forms dense thorny thickets, which creates landscape barriers for host movement and decreases the permeability of the landscape. This could increase the encounter rates between susceptible and infected hosts, and thereby increase bTB transmission rates.

The dense canopies of Prosopis creates a soil that better retains humidity than surrounding non-Prosopis areas. Empirical studies conducted in Sudan showed that wind speed inside Prosopis plantation was reduced by 14%, while potential evaporation was reduced by 22% (El Fadl, 1997). *M. bovis* has been detected in soil samples in East Africa (Roug et al., 2014), and experimental studies have confirmed that the bacteria can survive for multiple days outside hosts (Fine et al., 2011). Kelly and Collins (1978) suggested that the major factors influencing survival of the bacteria in soil is soil temperature and moisture, as high temperature causes desiccation, and negatively influence survival of the bacteria. Environmental persistence of *M. bovis* has been proposed to play a role in the transmission of bTB in the UK (Courtenay et al., 2006). Humid areas are also potential risk factors, and soils with higher levels of moisture and shade offer favourable conditions for *M. bovis* survival as demonstrated in Tanzania (Cleaveland et al., 2007) and Zambia (Munyeme et al., 2010). The moist and shaded conditions created by Prosopis could

also serve as propagating factors for *M. bovis*, by creating favourable conditions for the survival of the bacteria.

Our findings contribute to a growing body of literature that illustrates how land use / cover change can alter the prevalence of diseases through altering the host species community composition, and density and mobility of hosts. The loss of biological diversity and the homogenization of host communities have the potential to increase the prevalence of and risk of exposure to zoonotic diseases. Removal of the invader could mitigate disease risk, coupled with the benefits of invasive plant removal to wildlife communities, suggesting a potential win-win scenario for biodiversity conservation and disease control. However, our results are a necessary first step towards understanding the role of invasive species on bTB risk and identifying the underlying mechanisms. More experiment studies including surveys of host communities in invaded and uninvaded areas by Prosopis, coupled with a Prosopis removal experiment are needed to distinguish between these pathways.

Acknowledgements

We are grateful to NUFFIC for the financial support of the PhD scholarship through Wageningen University for the first author. We thank Haramaya University for the logistical support. We are also indebted to all field and laboratory staff who worked with us during tuberculin testing, habitat survey and laboratory work. Finally, we are grateful to all herders and herd owners who collaborated with us during the interviews and bTB testing.

Chapter 5

Disease transmission in animal transfer networks

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Preventive Veterinary Medicine, 2017, 137: 36–42

Infectious diseases transmission is strongly determined by who contacts whom. Bovine tuberculosis (bTB) caused by *Mycobacterium bovis* is a worldwide burden for animal populations One of the major transmission mechanism between herd is the transfer of infectious animal. In East Africa, pastoralists may receive or bestow livestock to create and strengthen social relationships. Here, we used a network approach to examine the relative importance of such cattle transfer in the transmission of bTB. First, a total of 2550 cattle from 102 herds were tested using the comparative intradermal tuberculin test to assess the presence of bTB infected cattle in the herd. A herd was considered bTB positive if it had at least one tuberculin reactor animal. Next, we calculated the centrality of each herd in the cattle transfer network using four established measures of social network centralization: degree, betweenness, closeness and fragmentation. The relationships between the network centrality measures and bTB infection were examined using generalized linear mixed models (GLMM). We found that a herd's in-degree in the social network was positively correlated with the risk of being infected with bTB (b=4.2, 95%CI=2.1-5.7; p<0.001). A herd that was close to many others (i.e., had a higher closeness index) had a larger chance of acquiring bTB infection (b=2.1, 95%CI=1.4-2.8; p<0.001). Betweenness centrality was also positively associated with the presence of bTB infection. There was a negative relationship between the fragmentation index and bTB infection (b=-2.7, 95%CI=-4.9-1.3; p<0.001). The study clearly demonstrated that the extent to which a herd is connected within a network has significant implications for its probability of being infected. Further, the results are in accordance with our expectation that connectivity and the probability that a herd will transmit the disease to other herds in the network are related.

Introduction

In recent years, contact patterns among hosts have received increasing attention to better understand the dynamics of diseases (Ribbens et al., 2009; Volkova et al., 2010; Lindstrom et al., 2011). These investigations are vital for improved predictions about pathogen dynamics, disease transmission, and associated risks (Kao et al., 2007) as well as to design effective disease control strategies. Recent studies demonstrated that infected animals often have more connections in a contact network (Corner et al., 2003; Godfrey et al., 2010), and that animals are at greater risk of becoming infected if they are associated to infected individuals in different ways (Drewe et al., 2009; Porphyre et al., 2011; MacIntosh et al., 2012). In humans, significant progress has been made to predict how diseases spread using network analyses (Gupta et al., 1989; Rothenberg et al., 1998; Eames and Keeling, 2002; Pourbohloul et al., 2005; Meyers et al., 2005).

Despite this, the role of contact networks in pathogen transmission dynamics has never been quantified in pastoral production systems. Most disease transmission models focused on theoretical assumptions of the influence of host ecology on disease transmission dynamics (Keeling, 2005; Lloyd-Smith et al., 2005). Yet, contact networks can play a pivotal role in the transmission of infectious diseases among domestic animals in pastoral areas, where social organisation is highly dependent on livestock transfer between herds to strengthen social relationships. Hence, understanding the role of livestock transfer networks in disease transmission is crucial to predict which herds might be more susceptible to pathogen infection as well as to develop effective disease control strategies in pastoral area of Africa.

Bovine tuberculosis (bTB), caused by *M. bovis*, is an important zoonotic disease affecting many mammal species, and mainly spreads via aerosol transmission (Skuce et al., 2012). The disease is endemic in sub-Saharan African cattle (de Garine-Wichatitsky et al., 2013), and cattle are the main host for *M. bovis* (Cosivi et al., 1998). In fact, bTB is an important public concern, and can cause economic losses due to livestock deaths, production reduction and trade restrictions (Munyeme et al., 2008; Humblet et al., 2009). Recent studies showed that the introduction of a diseased animal into a bTB free herd through purchase is one of the risk factors for bTB transmission (Johnston et al., 2005; Gopal et al., 2006). Exchange of livestock within and between clans is a common practice in pastoral areas of East Africa to build supportive relationships and spread risks (Getachew, 2001; Davies, 2006; Davies and Bennet, 2007). Although transfer of livestock may increase the risk of disease transmission, the impact of cattle exchange on transmission risks of bTB in a pastoral production system has not been studied thus far. Given the large number of cattle involved in traditional economies in Africa, there is a need to integrate information on herd

contact structures and the risk of *M. bovis* transmission for effective bTB control strategies. We investigated the relationships between cattle transfer networks and the risk of bTB infection in north-eastern Ethiopia using network analysis.

Network analysis has become increasingly popular to examine relationships between host contact patterns and disease transmission dynamics (Lindstrom et al., 2012; Makagon et al., 2012; VanderWaal et al., 2013b; Nunn et al., 2015). The centrality metrics are most commonly used to evaluate the relative importance of a node in the network, and measures the interaction intensity of a given node in comparison with other nodes in the network (Newman, 2003; Gómez et al., 2013). In disease transmission networks, herd centrality is correlated with the risk of pathogen infection (Drewe et al., 2009; Alvarez et al., 2011; Gomez et al., 2013; VanderWaal et al., 2013b). Thus, quantifying the centrality of nodes in a network is an appropriate measure to identify pathogen sources as well as pathogen sinks (Canright and Engoe-Monsen, 2006; Craft et al., 2009; Bordes and Morand, 2011). The present study quantifies the relationships between different centrality metrics in an animal transfer network and the risk of bTB infection. This information could also be valuable to formulate evidence-based intervention strategies to minimize disease transmission among cattle herds. In this study, in-degree, density, betweenness, closeness and fragmentation index of network centrality measures were calculated to investigate the centrality of nodes in the network. We expect that the risk of being infected with bTB increases with a higher in-degree, closeness and betweenness, and decreases with an increasing fragmentation index of a node in the network.

Materials and Methods

Study area

The study was carried out in the Afar Region, Ethiopia (8° 49' to 14° 30' N latitude and 39° 34' to 42° 28'E longitude), an area of about 70,000 km^2 (Figure 1.2). The altitude ranges from 120 m below sea level in the Danakil depression to 1500 m above sea level. The area is characterized by an arid and semi-arid climate with low and erratic rainfall. Rainfall is bimodal, with a mean annual rainfall of 500 mm in the semi-arid western escarpments, decreasing to 150 mm in the arid zones to the east. Temperature ranges from 20°C to 40°C. Afar communities traditionally keep herds of cattle, sheep, goats and camels. There are about 1.9 million cattle in the Afar region, of which 90% are managed under a pastoral production system (CSA, 2008).

Livestock production systems

Two types of pastoralism are recognised in the area, namely agro-pastoralists, in which cattle are grazed within village perimeters without migration, and transhumance, the most common grazing system, which involves the seasonal movements of livestock to following suitable grazing and water resources over considerable distances (Kassa et al., 2005). In the wet season, animals are kraaled at night, and in most cases herds owned by two to five close relatives or clan members are joined. The animals are released in the morning and the herds graze separately during the day in rangelands around the homesteads. In the dry season, some herds remain sedentary, whereas transhumant herds often migrate together, sharing grazing areas.

Types of livestock transfer

Afar pastoralists have adapted to their harsh environment through their social organisation. An important element of this social organisation is livestock transfer by which pastoralists may receive or bestow livestock to create and strengthen social relationships, spread risks and establish trust through gifts, loans and herding contracts (Getachew, 2001; Davies, 2006; Davies and Bennet, 2007). The most important livestock transfer mechanism is called "*iribu*", through which any kind of species of either sex is given as a permanent gift. It is commonly practiced between close relatives, but can also be sought from non-relatives that live entirely separately of the clan. Another important mechanism of livestock transfer is "*ala*", which is a system of bond-friendship entailing gifts of livestock. *Ala* enables the recipient to increase a particular species during good times and is not related to recovery after crisis. Either clan, lineage affiliation, or geographical location influence *ala*, and gifts are generally reciprocated over time (Getachew, 2001; Davies, 2006; Davies and Bennet, 2007).

Study design

A cross sectional multi-stage sampling with sub-region as the highest level followed by district (n=17), and sub-district (n=34) as lowest sampling levels was used to select study villages. Study animals were obtained using a three-stage random sampling procedure. The village within the sub-district was regarded as the primary unit, the herd as secondary unit and individual animal as tertiary unit. Herds of livestock in each sub-district were stratified into three groups based on herd size (large, medium and small), after calculating the average herd size of the sub-district. Herds (one herd from each stratum) and individual animals were selected randomly. A total of 102 livestock herds from 34 sub-districts (3 in each sub-district, one large, medium and small herd) were selected. In this study, cattle owned by one owner and/or his close relatives, shared

common grazing area, watering points, kept at night at a common site and moved together during migration, was considered as a single herd.

Sample size determination

The sample size was determined by assuming that the average expected prevalence of bTB was 11% (Mamo et al., 2013). The desired sample size was calculated using the 95% confidence interval and at 5% absolute precision following the method of Thrusfield (1995). The desired samples size of 2550 animals was calculated for all 17 districts, or 75 animals for each of the 34 sub-districts.

Single comparative intradermal tuberculin test

Tuberculin skin testing was performed using purified protein derivative (PPD) (Prionics Lelystad B.V, Lelystad, The Netherlands) to identify bTB positive animals following the method of chapter 2. A herd was considered bTB positive if it had at least one tuberculin reactor animal (Ameni et al., 2007).

Livestock transfer between herds

Cattle transfer network data were collected from 102 herds located in the Afar Region, Ethiopia using semi-structured interviews with herd owners. From the field data two types of cattle exchange were distinguished: 1) off-herd or bestowing cattle to others, 2) import or receiving cattle from others. Transfer of livestock into the herd can be regarded as risky contacts due to possible transmission of bTB, in contrast to bestowing livestock to others. In this study, only transfer of cattle into the herd to create and strengthen social relationships were drawn and selected for the analyses, which, constitutes the majority of the livestock transfers in the area and that other types of cattle exchange were hardly important for disease transmission. The mean number of cattle received into the herd per year was determined to quantify the contacts between herds over a 10-year period, from December 2004 to November 2013.

Network components

The livestock exchange was studied using graph theory, for which 'nodes' and 'edges' are defined. 'Nodes' were defined as study herds, and 'edges' to be livestock transfer by which pastoralists receive cattle, which could potentially result in bTB transmission from one herd to another.

Social network measures

'Density' quantifies the amount of edges through which a node interacts with all others nodes within the network compared to the maximum number of edges possible. The measure varies from 0 to 1, where 0 means that there is no links and 1 means that all theoretically possible links are present (Wasserman and Faust, 1994; Martínez-Lopez et al., 2009). We calculated the importance, i.e., centrality, of each herd in the transmission network using seven established measures of social network centralization: degree, clustering coefficient, density, betweenness, closeness, eigenvector and fragmentation index (Borgatti, 2003; Table 5.1). Many of these metrics are highly correlated with each other, so only degree, betweenness, closeness and fragmentation were used in this study, which are four of the most common and easily interpretable metrics (Makagon et al., 2012; VanderWaal et al., 2013b; Nunn et al., 2015). These measures are explained further below.

Table 5.1. Predicted effects of the four network centrality indices.

Index	Expected effect
In-degree	Positive
Closeness	Positive
Betweenness	Positive
Fragmentation	Negative

Degree measures the importance of a node according to the number of direct contacts a node has with other nodes within a network (Wasserman and Faust, 1994; Nunn et al., 2015). The in-degree is defined as the number of edges coming in to the node by receiving animals from other nodes (Makagon et al., 2012; VanderWaal et al., 2013b). In this study, we consider receiving of an animal into the herd as a risk factor for introducing bTB, thus in-degree was used to analyse the bTB transmission networks. A herd with a high in-degree makes more contact with other herds by receiving animals in the network and runs a larger chance to become infected with a disease.

Closeness describes the relative distance from a particular node to all other nodes in the network (Makagon et al., 2012; Gómez et al., 2013; VanderWaal et al., 2013b). It provides an index how close a given node is from the rest of the nodes in the network. Unlike degree, closeness takes into account both direct and indirect links that a node has in the network (Makagon et al., 2012). Thus, from a disease transmission point of view, herds with a higher closeness value are more susceptible to become infected (Borgatti, 2005; VanderWaal et al., 2013a; VanderWaal et al., 2013b; Nunn et al., 2015) and facilitate an epidemic through a network (Borgatti, 2005; Fournie et al., 2013).

Betweenness is the number of shortest paths from one node to another pass through a given node in the network, calculated from the number of direct and indirect connections (Borgatti, 2003; Hanneman and Riddle, 2005; VanderWaal et al., 2013b). Thus, in the context of pathogen transmission, herds with a high betweenness index in the network act as bridges for the spread of diseases between two nodes that are indirectly linked (Borgatti, 1995; Borgatti, 2005; Fournie et al., 2013; VanderWaal et al., 2013b) and potentially spread the pathogen in the network (Salathe and Jones, 2010). It has been suggested that a node with a high betweenness value plays a pivotal role in the regulation of the information flow (i.e., disease transmission) between two indirectly linked nodes within the network (Borgatti, 2005; Makagon et al., 2012; Fournie et al., 2013; Gómez et al., 2013). Fragmentation centrality is an inverse measure of the amount of connectedness or number of different connections in a network (Nöremark et al., 2009; Makagon et al., 2012). A node with greater fragmentation index means that it is more isolated from other nodes in the network (Borgatti, 2003; Nöremark et al., 2009). The network was constructed using weighted data (that is, we considered the number of animals transferred rather than simply recording the presence or absence of animal exchange). For infections such as bTB, which can be transmitted through direct and indirect contact, both the frequency of encounters and the weight of the interactions among hosts are important (Read et al., 2008).

Statistical analysis

UCInet (Analytic Technologies; http://www.analytictech.com) was used to generate social network metrics and visualize the network pattern that exist between herds. Network graphs were created using NetDraw (Analytic Technologies). To examine whether herd network characteristics (i.e., in-degree, betweenness, closeness, and fragmentation index) are a significant predictor of bTB infection status, the data were analysed using generalized linear mixed models. Sub-district was included as a random effect in order to control for potentially correlated data due to repeated sampling. Earlier to developing our candidate models, one-by-one univariate analyses were used to recognize a potential risk factors. Predictor variables with $p<0.25$ were recognized as potential risk factors, and were then used to construct multiple regression models. For highly correlated independent variables (SI-Table 1), only the one causing the largest change in the Log-Likelihood (LL) was added to the final global model to avoid multi-collinearity. From the global model, candidate models were selected using ΔAIC (< 5) and Akaike weights ($w>0.05$), with the best approximating candidate model having the highest w, as described in Burnham and Anderson (Burnham and Anderson, 2002). The final model was constructed using

model averaging (Anderson et al., 2000). Regression analyses were performed using R v3.2.2 (R Core Team, 2015).

Ethical Statements

This study was approved by Haramaya University, Ethiopia (Reference number HUP14/559/15).

Results

Bovine tuberculosis (bTB) was detected in 47 herds out of 102 (46% herd level prevalence). The network contact density was 0.028, so each node was, on average, connected to 2.8% of all other nodes. The network graph showed that herds that had more numerous connections via transfers of animals had a larger likelihood of being infected with bTB (Figure 5.1).

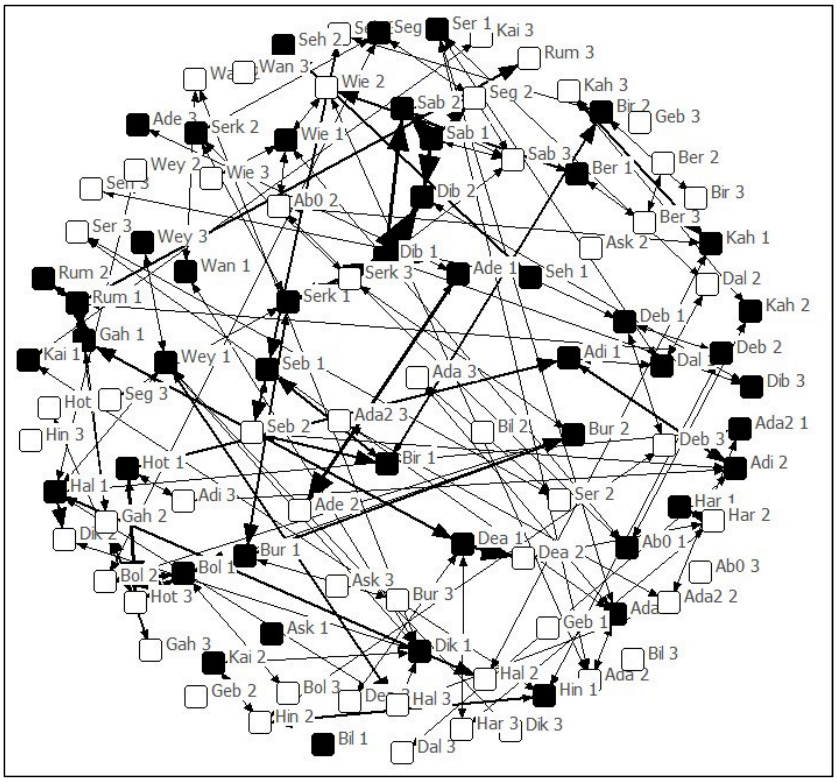

Figure 5.1. Herd network and herd bTB presence in a network of cattle herds linked through animal transfer. Each node (square) represents a herd. Arrowhead size is proportional to the node's strength, i.e., the number of cattle transferred, thus the sum of arrowheads around each node gives an indication of its in-degree centrality (i.e., the relative number of animal received by that herd). Black nodes indicate herds that tested bTB positive.

The analysis of model averaging showed that three network centrality measures (in-degree, closeness and betweenness) were positively correlated with the risk of being infected with bTB (Table 5.2; Figure 5.2).

Table 5.2. Effects of social network position on herd bTB infection (n=102) and summary statistics (standardized regression coefficient b with 95% confidence intervals, χ^2 and p-value) as obtained through GLMMs.

Centrality Measures	b (95% CI)	χ^2	p-value
In-degree	4.2 (2.14-5.67)	3.8	< 0.001***
Closeness	2.1 (1.4-2.77)	5.8	< 0.001***
Betweenness	0.18 (0.01-0.3)	3.7	0.002**
Fragmentation	-2.7(-4.9-1.3)	4.5	< 0.001***

CI=95% confidence intervals; * P< 0.05; ** p < 0.01; *** p < 0.001

A herd's in-degree in the network was positively correlated with the risk of being infected with bTB (Table 5.2; Figure 5.2A). Herds that had a higher in-degree were more likely to be part of the bTB transmission chain, characterized by a higher likelihood of being bTB infected.

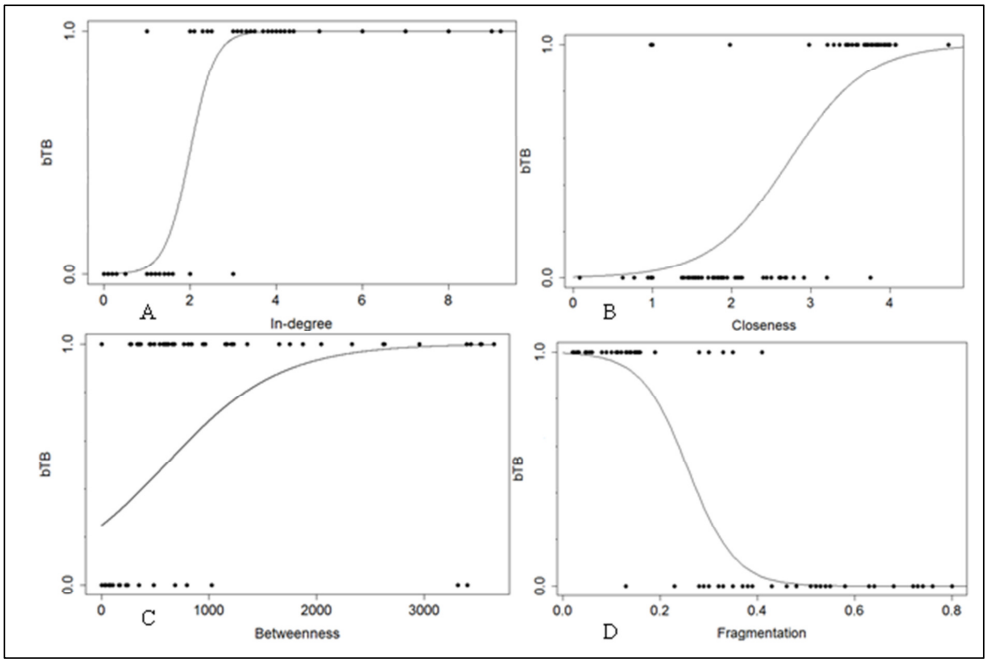

Figure 5.2. Fitted logistic regressions with the presence or absence of bTB in cattle herds as a function of in-degree (B), closeness (B), betweenness (C) and fragmentation index (D).

Increasing closeness was strongly positively correlated with the presence of bTB in the herd within the network (Table 5.2; Figure 5.2B). Social betweenness was also positively correlated

with the risk of bTB infection, likely because herds that were well connected in the animal transfer network were positioned ideally to connect the disease transmission network (Table 5.2; Figure 5.2C). As expected, fragmentation index was negatively associated with the risk of being infected with bTB (Table 5.2; Figure 5.2D). The result of the study showed that the risk of being bTB infected was more likely to occur between herds that were strongly linked in the social network (Figure 5.1; Figure 5.2; Table 5.2).

Discussion

One of the major intrinsic expenses of socialization is the increased risk of being infected with socially transmitted disease (Corner et al., 2003; Godfrey et al., 2010; Drewe et al., 2009; VanderWaal et al., 2013a). The risk of pathogen transmission is expected to increase with the exposure of a susceptible animal to infected animals. This encounter can arise from direct or indirect interactions, under influence of, e.g., animal movements. Our results demonstrated that the livestock transfer network patterns played a significant role in the risk of pathogen transmission. Our network analysis showed that a greater level of network contacts was strongly correlated with the occurrence of bTB in a herd. Moreover, our analysis showed that the position of each herd within the network can serve as a predictor for the risk of becoming infected.

In recent studies, direct network connectivity was identified as a risk factor for disease infection, and individuals with a greater in-degree were more likely to be infected with a pathogen in a social network (Godfrey et al., 2009; Drewe et al., 2011; Porphyre et al., 2011; Fournie et al., 2013; Gómez et al., 2013; VanderWaal et al., 2013b; Nunn et al., 2015). We found that herds with a greater in-degree (direct social connections) were positively correlated with the risk of being infected with bTB, indicating that greater network in-degree herds tended to have more connections in the disease transmission network, increasing the probability of becoming infected. From a bTB transmission point of view, introduction of an infected animal (exchange and purchase of animals) is one of the major risk factors for introducing a disease, as suggested by studies carried out in the UK, Michigan, Italy and Tanzania (Johnston et al., 2005; Gopal et al., 2006; Cleaveland et al., 2007; Humblet et al., 2009). Those studies showed that purchasing animals from positive farms and contacts with positive animals are large risk factors for the transmission of a disease (Munroe et al., 1999). In-degree (introduction in to the herd or receiving animals from others during livestock exchange) led to a higher risk of bTB infection.

Herds that fall close to the centre of the network (i.e., with higher closeness centrality scores), can strongly influence other herds in the network (Nöremark et al., 2011; Makagon et al., 2012;

Gómez et al., 2013). Nodes (that is, herds) with the highest closeness centrality are not only more susceptible to becoming infected with a disease but also facilitate diseases transmission through networks (Borgatti, 2005; Christley et al., 2005), potentially triggering disease epidemics. Similarly, we found that a high closeness centrality score was positively associated with the risk of becoming infected with bTB, as these herds were tightly connected to the rest of the herds in the network.

The betweenness index represents the extent to which a node (thus, a herd in our study) facilitates transmission of a disease among members of a network (Borgatti, 1995; VanderWaal et al., 2013b). Nodes with high betweenness played a pivotal role in the FMD transmission in England (Ortiz-Pelaez et al., 2006). In this study we found that a herd's bTB infection status was positively associated with its network betweenness; we think this can be explained because herds that were well-connected in the social network might have a higher probability of becoming infected with a disease. If one disconnects a herd which possesses a high betweenness centrality from the livestock exchange network, the speed of transmission between networked nodes will decrease, and the transmission of bTB within the network as a whole will be reduced. Furthermore, herds with a high betweenness score connect distinct herds (Makagon et al., 2012; VanderWaal et al., 2013b), thus some herds tend to serve as bridges in the bTB transmission network by occupying positions of high flow (high transmission betweenness). When herds with a high betweenness become infected, the pathogen may spread to new regions of the network (Salathe and Jones, 2010; Gómez et al., 2013). Thus, betweenness is an effective measure to identify high risk herds in the disease transmission network, which supposedly are ideally connected for control interventions, as, e.g., vaccination of these herds might limit disease spread over the network.

Theoretical models showed that high fragmentation generally reduces the risk of a disease to spread through a network (Nöremark et al., 2011; Makagon et al., 2012). We found that the probability of bTB infection was inhibited in more fragmented networks, likely because a high fragmentation index was related to decreased number of contacts with other nodes in the network. In the context of bTB transmission, a herd which has a low fragmentation index makes more contacts with another herds in the network, and there is greater chance that it becomes infected with bTB. If a network becomes more fragmented the opportunity of infected animals to encounter susceptible individuals decreases, limiting a wider dissemination of an infection.

In conclusion, the identification of connected and disconnected components within a network is important in terms of disease transmission. Infection of a node within a larger connected

network section would have a larger effect on the spread of diseases throughout the network than if the infected node is part of a disconnected network section. Our findings demonstrated that network centrality measures or a herd's social network position is predictive for its position in the disease transmission network. This has implications for identifying herds that function as super-spreaders or disease transmission bottlenecks in the network. It is also useful to detect and predict the risks of disease transmission which is important knowledge for formulating zoonotic disease control measures. Importantly, a simplification of the herd contact structure without considering all other possible connections (e.g., contact at water points, commercial contacts) can undermine our understanding of the transmission events at herd level, and result in a misuse of resources in seeking to prevent disease spread. More sophisticated methods are therefore required to assess the role of different network relationships in pathogen spread, such as the network analysis presented here.

Acknowledgements

We are grateful to NUFFIC for the financial support of the PhD scholarship through Wageningen University for the first author. We thank Haramaya University for the logistic support. We are also indebted to all field staff who worked with us during tuberculin testing and questionnaires survey. Finally, we are grateful to all herders and herd owners who collaborated with us during the interviews and bTB testing.

Chapter 6

Synthesis

Uncovering the ecological and social drivers of bovine tuberculosis (bTB) risk

Introduction

With increasing emergence and re-emergence of infectious diseases, there has been a growing interest for a better understanding of the dynamics of multi-host pathogens at the wildlife-livestock-human interface (Daszak et al., 2000; Jones et al., 2008; Perry et al., 2013; Wiethoelter et al., 2015). Multi-host pathogens cause two-thirds of human diseases and three-quarter of emerging human diseases are zoonotic (Cleaveland et al., 2001; Taylor et al., 2001; Engering et al., 2013). Similarly, three-quarter of livestock diseases are shared with other hosts (Cleaveland et al., 2001). So livestock diseases such as bovine tuberculosis (bTB) are shared between multiple species and represent a potential burden, including direct losses to the livestock production sector through increased mortality and reduced productivity, and indirect losses associated with cost of control, loss of trade, decreased market values, and food insecurity (Perry and Grace, 2009). Thus, improving our understanding of the biological and anthropogenic factors that promote contact between hosts is critical for limiting bTB transmission in multi-host communities.

As many answers as I have attempted to find, explain, and present in chapter 2, 3, 4 and 5 of this thesis, there are still a number of questions to follow. The complexity of this study site, the social practices and its themes are at once fascinating and challenging. I gained a better understanding of eco-epidemiology of bTB in cattle at different levels: at the animal, herd and area or landscape level on the basis of a large cross-sectional study. In this thesis, first I investigated the role of herd movements, production system, herd size and contact with wildlife on the prevalence of bTB in cattle (Chapter 2). Then, I tested the dilution and identity effect (Chapter 3) and the effect of land use, invasive plant species and underlying mechanisms (Chapter 4) on bTB prevalence. In Chapter 5 the effect of livestock transfer on disease risk was studied using a network analysis. In part I of this synthesis, I discuss and relate these results to previous studies in order to gain a better understanding of the mechanisms and generality of the ecological and social risk factors associated with bTB prevalence at animal, herd and area or landscape level in Ethiopian cattle.

Ecosystem changes, including changes in landscape characteristics, invasive species spread, biodiversity loss and changes in communities of hosts and vectors, have all been described as

major factors potentially involved in transmission of zoonotic diseases (Midgley and Thuiller, 2005; Hong and Lee, 2006; Chapter 3; 4). Such ecological changes are thought to threaten animal and public health (Daszak et al., 2001; Patz, 2001; Patz et al., 2004). Despite growing awareness that disease emergence may be related to ecological changes, there is a notable scarcity of scientific knowledge to show whether, and how, ecological changes play this role. In part II of the synthesis, I discuss the evidence on the effect of land use as a result of the introduction of the non-native *Prosopis juliflora*, a most powerful invasive woody species on disease risk to provide new insights for interventions and disease control measures.

Common social or cultural practices of traditional African pastoralists, e.g., large herd size, long distance herd mobility and exchange of livestock (among others) are strategies used to efficiently utilize available resources and adapt to uncertainties in rainfall, disease incidence, and raid losses (Getachew, 2001; Davies and Bennet, 2007; Moritz et al., 2011). Recent studies identified herd size, herd mobility and exchange of livestock as major risk factors for transmission of bTB (Munyeme et al., 2008; Humblet et al., 2009; de Garine-Wichatitsky et al., 2013; Katale et al., 2013; Mamo et al., 2013; Atehmengo and Nnagbo, 2014; Chapters 2 and 5). In part III of this thesis' synthesis, I discuss the dilemma between common social practices and scientifically supported actions to reduce disease risks, which is vital in formulating disease control strategies across African pastoral communities. In all three parts of this synthesis, I draw several conclusions and suggest a focus for management of infectious diseases and future studies.

Part I. Bovine tuberculosis risk factors

Individual animal level

At individual animal level, I found that the older the age of the cattle and the lower the body condition, the higher the chance of a positive bTB test result in the pastoral area of Afar (Chapter 2). Similarly, the prevalence of bTB increased with age and decreased with increasing body condition in other areas too (Caron et al., 2003; Ameni et al., 2007; Cleaveland et al., 2007; Inangolet et al., 2008; Regassa et al., 2008; Gumi et al., 2011; Katale et al., 2013; Mamo et al., 2013). This suggests that bTB prevalence increased with age, as the duration of exposure to the agent increases with age. Tuberculosis-positive animals are more likely develop a poor body condition score as a result of being infected, i.e., a clinical sign that typically follows an active infection with *Mycobacterium bovis*, explaining as such the relation between poor body condition and bTB prevalence.

Herd level

The result of this study corroborates other past studies that herd size, herd movement, pastoral production type, contact with wildlife and introduction of infected animal into the herd have been identified as risk factors increasing bTB transmission at the herd level (Kazwala et al., 2001; Faye et al., 2005; Cleaveland et al., 2007; Ameni et al., 2003; Tschoop et al., 2009; Mamo et al., 2013). Further to what is already known from the past studies, this study indicates that the probability of contact with wildlife was confounded with herd size, through herd movement. Pastoralists with larger herds moved more and grazed in larger areas, hence the probability of grazing in an area with bTB wildlife maintenance host and contact with either infected cattle or infected wildlife hosts increased, in turn enhancing the chances for bTB infection. However, I did not study the prevalence of bTB in wildlife and sampling of wildlife often presents difficulties for practical and ethical reasons. As a result, data on prevalence of infection in wildlife are often scarce and estimates based on convenience sampling (such as road-kills or hunter-harvest) are subject to biases (Conner et al., 2000; Courchamp et al., 2000; Nusser et al., 2008). The current knowledge about inter-species disease transmission at wildlife/livestock interface is still scarce and my results presented here also do not offer proof of bTB transmission between cattle and wildlife. These uncertainties need more detailed ecological and epidemiological research, such as molecular studies, to quantify bTB transmission routes between cattle and wildlife. However, this type of observational data and correlative study is a necessary first step towards understanding the role of host community on bTB risk and identifying the underlying mechanisms in multi-host systems. From this study, I suggests that herd size and herd movements are important risk factors in pastoral areas of Africa.

Exchange of livestock within and between clans is a common practice in pastoral areas of east Africa (Getachew, 2001; Davies and Bennet, 2007; Moritz et al., 2011) to spread risks and build supportive relationships. Although transfer of livestock may increase the risk of disease transmission, the impact of cattle exchange on transmission risk of bTB in a pastoral production system has not been studied thus far. For the first time, my network analysis showed that herds near the centre of a livestock transfer network by receiving cattle from other herd identified as an important risk for introduction of bTB, than those at the periphery (Chapter 5). The traditional adaptation strategies by receiving cattle to create and strengthen social relationships become maladapted to risks associated with bTB transmission and increased the risk of being infected with the disease. This suggests that identification of connected and disconnected components within the livestock transfer network is useful to detect and predict the risks of disease

transmission which is important for formulating disease control measures. In fact, the identification of central players in such a livestock exchange network can act as an early warning signal, and improve efficiency of control measures, in line with the results presented by Christakis and Fowler (2010).

Area or landscape level

Many pathogens are maintained by multiple host species in natural systems (Woolhouse et al., 2001), where the addition or loss of a host species in an area can influence disease risk, either diluting or amplifying disease prevalence (Dobson, 2004). The dilution effect predicts that species diversity decreases pathogen prevalence through mechanisms, such as decreased host density, reduced encounters between hosts, or reduced host survival (Keesing et al., 2006; Huang et al., 2013; Johnson et al., 2013). In contrast, the amplification effect predicts increased pathogen prevalence with greater species diversity, through increased encounters between hosts, or through the presence of secondary hosts (LoGiudice et al., 2003; Keesing et al., 2006). In fact, biodiversity can be measured in many different ways, i.e., as species richness, species evenness, or a combination of richness and evenness (Magurran, 1988; Tucker and Cadotte, 2013), and these different metrics of biodiversity may have different predictive powers for predicting disease risk. I found that bTB infection rate was negatively associated with mammalian species evenness (J'), which is in support of the dilution effect hypothesis (Chapter 3), but the underlying causal mechanism is hard to deduct from these results. The presence of these non-competent or spill-over mammalian species might act as barriers to cattle herd movement, and reduce encounter rates among cattle. The presence other host species in the community that are less competent reservoirs for bTB i.e., transmitting the pathogen ineffectively might decrease the contact between bTB wildlife reservoir hosts and cattle. Such an "encounter reduction" (Keesing et al., 2006) might lead to decreased probabilities of bTB infection risk. Species evenness can be an appropriate measure of biodiversity to explain disease risk, because the encounter rate is proportional to the distribution of the host species, thus, evenness would capture the probability of encounter between pathogens and each host species. Chen and Zhou (2015) also found that species evenness outperforms species richness as a single variable in explaining the relation between biodiversity and disease risk. This suggests that measurements of biodiversity itself play an important role to evaluate the generality of the biodiversity-disease relationship.

Land use changes as a result of plant species invasions and associated changes in the composition of the host community have been described as one of the causal drivers in current emergence and re-emergence of infectious diseases. For instance, in semi-arid African Savana, bTB prevalence

was positively associated with the invasion of Prosopis (Chapter 4). Allan et al. (2010) and Williams and Ward (2010) found that invasive plant species increased the risk of infection to tick-borne diseases by increasing the density as well as the encounter rate between ticks and their vertebrate hosts. Similarly, land use and land use changes as a result of plant species invasions increased the risk of mosquito-borne diseases by providing favourable habitat for vector species (Conley et al., 2011), and by providing high-quality nutritional fruits and leaves for mosquito larvae (Reiskind and Zarrabi, 2011). In this study I found that the loss in host species evenness and the increase in cattle movement as a consequence of the loss of palatable grasses in Prosopis areas could be potential mechanisms accounting for the observed higher bTB prevalence in these Prosopis areas (Chapter 4). Another study demonstrated that survival of lone star and American dog ticks was reduced by Japanese stiltgrass (*Microstegium vimineum*), an exotic annual grass invasive to eastern North America by lower densities of host and therefore reduced human disease risk (Civitello et al., 2008). Malmstrom et al. (2005) found that that the presence of *Avena fatua*, exotic annual grasses, increase incidence of infection by barley and cereal yellow dwarf viruses in native perennial bunchgrasses such as *Elymus glaucus*, as aphids consistently preferred exotic annuals as hosts. Hence, land use as a result of the introduction of the non-native plant species can alter the prevalence of both directly transmitted and vector-borne diseases through altering the host species community composition, density and mobility of hosts, but its net effect on disease risk depends on the species of invasive plant, the disease, and its interactions with hosts, vectors and pathogens.

Geographical distribution of bTB over the study area

There was a large geographical variation in prevalence of bTB from 0-13%. High prevalence of bTB occurred in the southern parts of the study area. The prevalence of bTB was generally lower in the far western part of the region, where agro-pastoralism is the dominant production system (Figure 6.1). A high prevalence of bTB was reported in pastoral cattle, where high numbers of cattle are kept under traditional husbandry practices (Kazwala et al., 2001; Bugwesa et al., 2013), relying on movement of livestock following grazing and water resources over considerable distances under seasonal changes. In Uganda, bTB prevalence was higher in agro-pastoral production systems (Faye et al., 2005). Other studies showed that bTB occurs in both agro-pastoral and pastoral farming systems with no distinct differences in prevalence (Kazwala et al., 2001; Munyeme et al., 2010). In line with these latter studies, my study showed that the prevalence of bTB in cattle was not significantly different among pastoral production and agro-pastoral production system (Chapter 2). Transhumant movements are more intensive during the

dry season, during these moments there is a lot of interaction between agro-pastoral and the pastoral cattle when transhumant herds graze in farmlands or on cotton farms, might resulting in a similar infection prevalence. Areas with high prevalence of bTB (Figure 6.1) were associated with Prosopis invasion (Figure 4.1), which have caused a decrease in host species evenness and an increase in cattle movement (Chapter 4). This suggests that the spatial distribution of bTB is determined by a combination of social and biological factors.

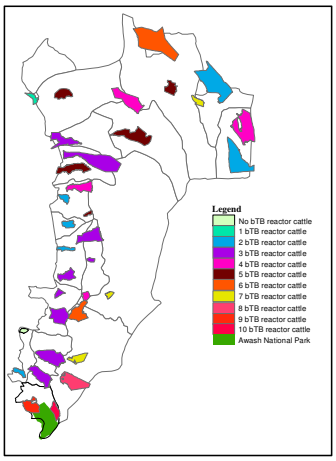

Figure 6.1. Geographical distribution of number of bTB positive cattle over the study area, results from the cross-sectional surveys conducted on bTB. Different colour indicate the number of bTB positive cattle (n=75) in the sub-district.

Part II. Land use as ecological driver of disease risk

As the human population increases worldwide, landscapes are changing at an unprecedented rate. Even though land use changes are common in many areas of sub-Saharan Africa, the rate of conversion is more rapid in arid and semi-arid pastoral areas, where vast rangelands are being transformed into irrigated agricultural lands (Barrett et al., 2001; Reid et al., 2004). Land use changes include deforestation, introduction of invasive species, large scale agricultural encroachment, and urbanization, which leads not only to habitat fragmentation and loss of biodiversity (Fahrig, 2003; Foley et al., 2005; Tischendorf et al., 2005; Devictor et al., 2008), but also to alteration of ecological processes (Ryall and Fahrig, 2006; Malanson et al., 2007), including the interaction between hosts and parasites. For instance, McGinnis and Kerans (2013) found that a low proportion of riparian forest likely increases stream sedimentation, creates more favourable habitat for *Tubifex tubifex*, and influences the interaction between hosts and parasite. Those changes can potentially influence the transmission of diseases depending on the availability

of their host, and in some cases facilitate the introduction of novel pathogens to these changing environments (Patz et al., 2000). There are different mechanisms through which land use changes can affect transmission of zoonotic diseases. For instance, a recent study found that habitat modification reduces mammalian species diversity, and thereby increases hantavirus prevalence in deer mice (*Peromyscus maniculatus*) by increasing intraspecific and decreasing interspecific contact rates (Dearing and Dizney, 2010). Therefore, analysing the role of land use change and identifying the underlying mechanisms in pathogen transmission is critical for predicting disease dynamics in the context of global land use change, and may provide valuable insights into disease interventions and control measures. Hereunder I discuss the evidence on how the changes in land use, caused by invasive species, can influence disease transmission.

Invasive species as land use change driver and its impact on host community composition

Land use changes due to the introduction of non-native plant species may have profound effects on the host community structure these systems hold (Ehrenfeld, 2010). *Prosopis* spp. are one of the most highly invasive plant species in the world (Gallahar and Merlin, 2010; Wakie et al., 2014). The species are rapidly invading several parts of southern and eastern African countries. Over four million hectares of land has been invaded by the species, threatening crop and range production, reducing water resources, and causing loss of native fauna and flora (Witt, 2010; Schachtschneider and February, 2013). The species are having dramatic ecological impacts at multiple spatial scales and levels of organization, such as the loss of native species (Schachtschneider and February, 2013). *P. juliflora* was introduced in the study area during the 1970s as a measure to control desertification (Haregeweyn et al., 2013; Wakie et al., 2014). Currently, the species is widely considered as a powerful invader and is becoming a problematic weed, invading rangelands and riverbanks (Haregeweyn et al., 2013; Mehari, 2015). The invasion of ecosystems by non-indigenous species is one of the greatest threats to biodiversity, with major impacts on community structure (Gurevitch and Padilla, 2004; Vitousek, et al., 1996). Invasions of Prosopis suppress the growth of grass and other herbaceous species and reduce species diversity (Getachew et al., 2012; El-Keblawy and Al-Rawai, 2007; Haregeweyn et al., 2013). The influence of Prosopis on the palatable herbaceous species in highly invaded areas may contribute to the reduction in mammal species evenness that I observed, as abundance and distribution of mammal species is partly determined by the availability and quality of palatable plant species (Haregeweyn et al., 2013; Treydte et al., 2013; Young et al., 2013; Mehari, 2015). To the best of

our knowledge, these correlation findings are the first demonstration that land use caused by Prosopis invasion can indirectly influence pathogen transmission.

Implication for control of invasive species as a disease management strategy

The invasion of ecosystems by Prosopis species is one of the greatest threats to biodiversity and community structure. Currently, Prosopis poses a threat to indigenous biodiversity in the study area in particular because of its weedy and invasive nature (Mehari, 2015), causing a loss of wild animal species (Haregeweyn et al., 2013). I did not found species like *Oryx beisa*, *Gazella soemmeringi, Equus grevyi* and *Tragelaphus scriptus* in Prosopis infested areas. Haregeweyn et al. (2013) noticed that *O. gazelle*, *E. grevyi*, *Madoqua saltiana* and *Kobus ellipsiprymnus* were more abundant in other areas because of the dense thicket of *P. juliflora*. Thus, I argue that control or removal of Prosopis could decrease the loss of mammalian host species evenness, and thereby increases the diversity of mammalian hosts (Figure 6.2), which could have the potential to decrease transmission and risk of exposure to zoonotic diseases, as mammalian species diversity is thought to be an explanatory factor to decrease the risk of bTB transmission (Chapter 3;4). In pastoral areas of Africa, Odadi et al. (2007) found that the preference of foraging habitat for cattle was lower in the presence of wild grazers (Odadi et al., 2007). The presence of non-competent or spillover mammalian species might act as barriers to cattle herd movement and distribution, and reduce encounter rates among cattle herds by changing the grazing behaviour and habitat preference (e.g., avoidance of sites contaminated by faeces or different preferences for feeding patches). Such an "encounter reduction" (Keesing et al., 2006) might lead to reduce the probabilities of bTB transmission. Similarly other studies demonstrated that the presence of non-competent species can have profound effects on the behaviour of deer mice, reduce encounter rates among deer mice by shifting microhabitat use, thereby reducing the prevalence of Sin Nombre virus in deer mice (Clay et al., 2009; Dizney and Ruedas, 2009). It is also possible that the presence of other host species in the community that are less competent reservoirs for bTB i.e., transmitting the pathogen ineffectively, and their encounters with cattle may not result in successful transmission events and decreasing bTB transmission rates among competent hosts through encounter reduction as described by Keesing et al. (2006). Due to the presence of those less competent reservoirs host, contacts between bTB wildlife reservoir hosts and cattle might also become infrequent, which would decrease the prevalence of bTB. Similarly, rodent hosts on more diverse area are more likely to come in contact with hetero-specific mammals and less likely to come in contact with conspecifics, reducing the probability of transmission of hantavirus (Clay et al., 2009). On the other hand, control or removal of Prosopis may increase species evenness

with a high reservoir competence, transmitting the pathogen efficiently, such that contacts between hosts become more frequent and bTB prevalence subsequently increases. However, a recent study showed that spillback of bTB from a wildlife maintenance host to a domestic host is also possible (Munyeme and Munangandu, 2011; Musoke et al., 2013), but seems to be rare in Africa (de Garine-Wichatitksy et al., 2013). My study was a field study on free-ranging animals, and it is hard to differentiate among the underlying causal mechanisms by which control or removal of Prosopis modulates bTB transmission from these results. More experimental studies including for instance surveys of host communities, host behaviour change and contact rates among hosts in relation to invasive species, coupled with a Prosopis removal experiment are needed to test the underlying mechanisms by which host-environmental interactions influence disease risk. I argue that removal or control of the invader in Africa could mitigate disease risk by increases the diversity of non-competent or spill-over mammalian hosts, coupled with the benefits of invasive plant removal to wildlife communities, suggesting a potential win-win scenario for wildlife conservation and disease control at the wildlife–livestock interface.

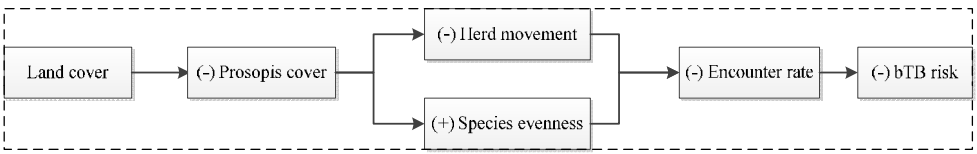

Figure 6.2. Schematic diagram showing control or removal of invasive species as a disease control strategy. Arrows indicate the direction of the paths and the signs indicate (+) increase or (-) decrease associated with the factor.

The invasion by Prosopis reduces availability of palatable herbaceous species (Mehari, 2015), which could increase the movement of cattle herds in search for pasture. The cattle herds moved more and grazed in larger areas, hence the probability of contact with either infected domestic or infected wildlife hosts increased (Chapter 4). Such high encounter rates might lead to an increase in the probability of pathogen transmission. Ndhlovu et al. (2016) experiment showed that canopy cover was significantly different in Prosopis cleared and uninvaded sites for the annual and perennial grasses. Hence, control or removal of Prosopis invasion might increase availability of palatable plant species, and thereby decrease long distance herd mobility, which might reduce encounter rate between livestock herds (Figure 6.2), which could serve as bTB control strategy.

Part III. Conflict of social practices and disease risk

Pastoral communities in arid and semi-arid lands of Africa depend on livestock for their livelihood. The area is are characterized by high temporal and spatial climatic variation, creating an uneven distribution of the availability of resources. There are two contrasting, but often co-

existing, interpretations of key problems in disease control strategies in this pastoral area of Africa. Common social practices, such as having a large herd size, with large herd mobility and using livestock transfers, are key production methods used by pastoralists to efficiently utilize available resources and to adapt to uncertainties in rainfall, diseases, and raid losses (Getachew, 2001; Davies and Bennet, 2007; Moritz et al., 2011). However, I identified herd movement, herd size and animal exchange as a risk factor for transmission of bTB (Chapter 2;5; Figure 6.3). Thus, the traditional social practices to cope with the production risks in arid and semi-arid areas might become a maladaptation in the modern era with larger risks associated with disease transmission. From a bTB point of view, these two narratives are both vital in formulating workable disease control strategies across African pastoral communities. In this part of the synthesis I attempt to synthesize the debate by highlighting key pastoral practices to manage resource variability and uncertainty and maladaptation of those traditional adaptation practices in relation with the risks of bTB transmission. First I review discussions around social practices among African pastoralists, and then highlight the important tensions between those practices and the risk of disease transmission. Then I discuss the debates to bring these tensions, trade-offs and choices together, to balance costs against the benefits of minimizing those risks.

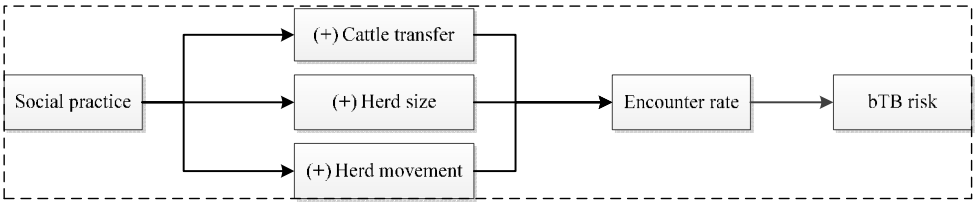

Figure 6.3. Schematic diagram showing social practices among African pastoralists as a risk factor for transmission of bTB. Arrows indicate the direction of the paths and the signs indicate (+) increase or (-) decrease associated with the factor.

Diseases in context: epidemiological realities and scientific uncertainties

Over the past several decades, the pastoral area of eastern Africa has experienced tremendous changes and transformations. In the area, the distribution of grazing resources is uneven, with many pastoralists concentrated in key areas along rivers or on hilltops, and grazing resources are highly variable, determined by episodic and uncertain rainfall (Kaimba et al., 2011). The region's pastoralist systems evolved to incorporate adaptive mechanisms for managing resource variability and uncertainty. Livestock herd mobility is critical for making efficient use of grazing and water resources (Alerstam et al., 2003, Manzano-Baena and Casas, 2010; Oteros-Rozas et al., 2013) by daily and weekly changes in grazing ranges, including seasonal migrations over large landscapes. Movement of cattle herds for searching for water and pasture was recently shown to be a critical

risk factor for transmission of bTB due to higher chances of coming in contact with infected animals (Chapter 2).

Large herd sizes are common in pastoral areas of Africa as a kind of "bank account" adaptation to uncertainty in rainfall, diseases, and raid losses (Mace and Houston, 1989). Moreover, wealth and status in pastoral societies are typically dependent on the size of one's herd, to help ensure a reliable flow of livestock products (Livingstone, 1991; Sivakumar et al., 2005; Moritz, 2008). Traditionally, pastoralists keeping extra animals as insurance for when conditions deteriorated (Livingstone, 1991). However, in African pastoral areas herd size has an influence on the prevalence of bTB (Chapter 2). The more cattle there are on a farm, the greater the probability that one of them will acquire an infection. Large herds generally graze on a larger area, with a higher probability to have more contiguous herds, thus increasing the risk of disease spread (Chapter 2).

Exchange of livestock within and between clans is a common practice in pastoral areas of east Africa (Getachew, 2001; Davies and Bennet, 2007; Moritz et al., 2011) to spread risks and build supportive relationships. My network analysis showed that a greater level of livestock transfer network contacts by receiving cattle from others was identified as an important risk for positive herd tuberculin reactivity (Chapter 5). However, the current scientific knowledge and status of disease control efforts in this part of Africa is not clearly defined and illustrates a dilemma between existing social practices and scientifically identified risk factor for disease transmission.

The need for disease control

Nowadays, rangeland fragmentation, through processes of large scale agricultural expansion, colonization, nation formation, population growth, social and economic modernization, and the imposition of statutory land tenure systems have frequently impinged on the current pastoralist ways of life (Galaty 2013; Nunow, 2013; Tache, 2013). These factors have tended to decrease pastoralists' capacity for customary governance and grazing management and to affect their traditional strategies for coping with disturbances such as drought (Fratkin and Mearns, 2003; Catley et al., 2013), failing to meet households' livelihood needs and maintain ecological resources (Galvin 2008; Kaye-Zwiebel and King, 2014). For instance, in 2009-2010, the Maasai in Kenya suffered due to drought, in which pastoralists lost 64% of cattle herds and 62% of sheep (Zwaagstra et al., 2010). Today, the traditional pastoralism activities are still based on large herd size, long distance movements and maintaining a network of bond friendships through which to exchange livestock as the basis for mitigating production risks. In arid and semi-arid rangelands

of Africa, those activities have been practiced more than ever before as key resource areas are fenced and set aside for non-livestock uses (Homann et al., 2008). However, those traditional adaptation practices become maladapted to risks associated with current zoonotic disease transmission.

The World organisation for animal health (OIE) estimates that morbidity and mortality due to animal diseases causes the loss of at least 20% of livestock production globally. This represents at least 60 million tonnes of meat and 150 million tonnes of milk with a value of approximately USD 300 billion per year (OIE, 2015). Animal diseases are widespread and endemic in many pastoralist areas of Africa, and have been identified as limiting factors to pastoralist livestock production (Racloz et al., 2013; Gustafson et al., 2015). The loss of livestock production due to zoonotic disease infection is significant in pastoralist herds, and restricts possible export of livestock and livestock products (Pearson 2006; Rushton, 2009; Thornton, 2010). Tanzania in 1999 quantified the impact of bTB and found a significant difference in milk production between TB reactor and non-reactor cattle (DFID, 1999). Studies conducted in other countries showed that bTB infection in cattle was associated with a 18% decrease in milk production in Bangladesh (Rahman and Samad, 2008) and 4% in USA (Hermandez and Baca, 1998). bTB is also an economical and financial burden to society (Zinsstag et al., 2006) but its cost is largely unknown for Africa. In sub-Saharan Africa nearly 2 million human TB cases occur each year. As a zoonotic disease, there is substantive evidence of incidence of human TB due to *M. bovis* in pastoral communities (Ayele et al., 2004). In Nigeria, Idigbe et al. (1986) found *M. bovis* in 4% of patients with lower respiratory tract symptoms. Hoffner et al. (1993) reported isolation and biochemical characterisation of *M. tuberculosis* and *M. bovis* in humans in Guinea-Bissau. Rigouts et al. (1996) isolated *M. bovis* in 38% of clinically suspected bovines. In Madagascar, a proportion of *M. bovis* (1.25%) was observed among human TB positive patients (Rasolofo-Razanamparany et al., 1999). Kazwala et al. (1998) also isolated mycobacterial species from the raw milk of pastoral cattle in the Southern Highlands of Tanzania. Thus, consumption of raw animal products from infected cows and close human-to-livestock contact are the main factors that expose the pastoralists to different zoonotic diseases (Zinsstag et al., 2006). Therefore, due to the risk of infection to the human population, loss in productivity due to infected animals and animal market restrictions, controlling disease transmission risks in pastoralists livestock should be a priority for improving pastoral livelihood, as a key to moving out of poverty.

Despite the increased challenges to pastoralist communities, there are a number of factors that may promote improvement of their livelihoods. The demand for meat and other livestock

products is increasing rapidly due to population growth and rising incomes, particularly in developing countries, leading some to label the trend a 'livestock revolution' (Delgado et al., 2001; Rosegrant et al., 2001). Yet, ironically, there is a widening gap between production and consumption of animal products. Existing and projected market demands create opportunities for pastoralists to meet this demand by supplying products of animal origin to the region's expanding urban populations and support their livelihood through involving in to the markets (Cartín-Rojas, 2012). Access to trade in livestock and their products is increasingly accepted as an important contribution to a strategy to mitigate and reduce poverty, and support the livelihood of the poorest communities (Moll, 2005; Rushton 2009; Cartín-Rojas, 2012). Surprisingly, while there has been considerable research in pastoral areas during the past three decades, much of it highlighted poverty as a key issue, but systematic analyses on the application of disease control strategies as a key to moving out of poverty in pastoral area of Africa are limited.

Implication for development of disease control strategies

Currently, control of livestock diseases in these pastoralist communities is challenging not only because of the number and complexity of risk factors involved, but also because these risk factors are tightly linked and often inherent to the farming and social practices and lack of knowledge about transmission of bTB and other zoonotic and livestock diseases. The effective control and eradication of bTB in livestock depend on identifying and isolating potential sources and risk factors of infection (Ayele et al., 2004). Pastoral practices have been identified as risk factor for diseases transmission (Chapter 2;5), which can have devastating impacts on the livelihood of pastoralists as the disease compromises their sustainable food supply and income. However, disease control measures that focus only on herd movement, herd size and livestock transfer schemes to control disease in cattle populations might not be acceptable due to cultural practises and hard to change. For examples of such cultural practices include the belief that vaccination causes livestock abortion, and prevents the acceptance of brucellosis control activities in pastoral area (Smits, 2013). Rather than neat win-win solutions, there are always trade-offs and so the costs and benefits of control strategies of diseases in pastoral areas of Africa should be considered to come up with strategies to minimize risks associated with disease transmission which are accepted by the society. Models of livestock systems in northern Tanzania suggest that diseases may limit livestock populations more than forage availability (Boone et al., 2002). For that reason, minimizing risks associated with disease transmission to improve the health of pastoralists and their livestock's health should be top priority. In addition to increasing livestock mortality, disease can reduce pastoralists' income due to lower market prices for sick animals,

quarantines, and loss of public trust in animal products (Barrett et al., 2003; Morton, 2007). Thus, I argue that benefits to human health, livestock productivity and expected positive impact on pastoral livelihood through trade of livestock and livestock products would outweigh the costs of practicing maladapted traditional adaptation strategies which are risks for bTB transmission .

The eradication of bTB from livestock is expensive since it requires intensive surveillance of the livestock, the slaughter of the infected animals that are detected, and the compensation of those who owned each slaughtered, infected animal. In industrialised countries, regular tuberculin skin testing and the elimination of infected animals have been successful in eradicating, or significantly reducing bTB from cattle herds. However, these control measures are not affordable and may not be practical in the traditional pastoral production systems of Africa. Therefore, complete elimination of the disease in this society is far more complex, necessitates greater commitment and more financial investment, and is probably not feasible in the immediate future. This is the virtue of the large numbers of livestock involved, the mobility of animals (pastoral production), lack of animal identification, lack of surveillance, weak veterinary services, limitations of diagnostic tests (concerning both sensitivity and specificity), and the social and economic factors involved (Ayele et al., 2004; Humblet et al., 2009). The need for implementing complex multi-species surveillance and control efforts using limited resources are other factors that limit the eradication of bTB. Therefore, I suggests that integrated bTB control approaches through identifying and isolating potential sources and risk factors of infection is needed to address those major obstacles.

Collection of detailed epidemiological data on bTB is a prerequisite before starting any large scale control program, as most African countries have little information on the prevalence of bTB, its geographical distribution, the major risk factors involved in transmission, circulation patterns among hosts, and the knowledge, attitudes and practices of livestock keepers. Based on the results of the epidemiological study, infection or disease free areas or landscapes can be identified and mapped (Figure 6.4). The concept of disease control based around geographical zones and compartments remain the key fundamentals to control bTB (Marcotty et al., 2009). In bTB free area, risk reduction based bTB control strategies such as livestock movement and exchange restriction from bTB presence area, as well as improving sanitary and hygienic standards is suggested to prevent the spread of bTB from an infected area to areas free of the disease. However in pastoral area, livestock movement controls may not be feasible. In this case, restriction of introduction of livestock, sharing of common grazing areas, and movement of

animals from bTB endemic areas to bTB free area should be implemented as a control strategy (Figure 6.4).

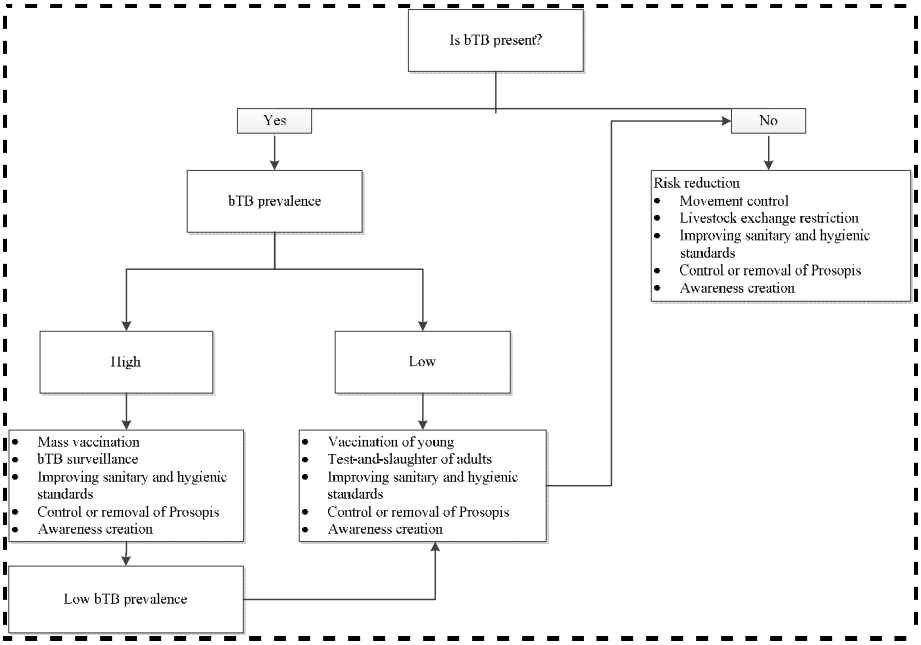

Figure 6.4. Decision making tree for controlling bTB.

Prevalence at herd level is the requisites to decide on the strategy once the presence of bTB in the area has been established. Under conditions of high prevalence, I suggests mass vaccination of all animals as a tool for reducing the level of infection. When vaccination used exhaustively in the whole herd, the incidence greatly decreases. Daborn and Grange (1996) and Ayele et al. (2004) have also suggested vaccination of susceptible domestic animals in endemic areas as a feasible bTB control option for Africa, which is acceptable and practical measure. Skinner et al. (2003) reported that vaccination could potentially be used to control bTB in countries where wildlife reservoirs exist and in those that cannot afford conventional control procedures. I argue that mass vaccination as an initial approach might be more appropriate and practical to reduce the prevalence of bTB.

Once the herd prevalence has been reduced, more effective control of the disease may be achieved through vaccination of young replacement animals combined with test-and-slaughter of infected adults. A final step under very favourable conditions would be implementation of risk reduction based bTB control strategies (Figure 6.4). In the meantime, control or removal of Prosopis invasion is needed as a control strategy which might be important to increase availability

of palatable plant species and increase diversity of non-competent mammalian hosts, which might reduce encounter rate between livestock herds and reducing the probability of transmission of bTB. bTB wildlife reservoir hosts should be kept away to minimise contact between wildlife and livestock if it is suspected that contact may introduce infection.

From successful experience in many developed countries, bTB can be controlled only and effective implementation of these activities would be possible when there is a strong political and producer support, and an appropriate legal framework to enforce control measures. Moreover, control of bTB provides an ideal platform for the One Health approach, which can be operationalized through adapted approaches for reducing risks of disease transmission, improving surveillance and meat inspection, enhancing community awareness, promoting milk pasteurization at the community level, and strengthening inter-sectoral collaboration.

Nevertheless, control and eradication of bTB is a desirable objective, which could be possible, if movement of cattle is controlled, if there is regular compulsory testing of and removal or slaughter of positive reactors cattle, if compensation is provided to owners for all positive reactors, if compulsory identification is done, and if disease free area is established and maintained, and if sufficient resources to fulfil these tasks are provided.

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Summary

Bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, is an important zoonotic disease affecting many mammal species, and mainly spreads via aerosol transmission. Zoonotic tuberculosis is an important cause of concern for public health, veterinary services and conservationists, especially in developing countries, because of deficiencies in preventive and control measures. The disease is shared between multiple species and represent a potential burden, including direct losses to the livestock production sector through increased mortality and reduced productivity, and indirect losses associated with cost of control, loss of trade, decreased market values, and food insecurity. In Africa, the disease has a wide distribution with a high prevalence in both wild and domestic animals. Strikingly, risk factors for bTB transmission are not well known in developing countries, as most studies were conducted in developed countries where farming practices are more intensive, and control and eradication programmes have been implemented since decades. It is therefore necessary to explore the relationships between pastoral livestock production systems and bTB prevalence in a multiple host community to better understand the effects of interactions between ecological, social practices and animal management risk factors. Knowledge derived from these analyses can contribute to formulate disease control strategies to manage cattle in ecosystems characterized by seasonally limited resources and intense wildlife-livestock interactions.

The individual overall prevalence of bTB in cattle in the Afar Region, Ethiopia was 5.5%, with a herd prevalence of 46%. The older the age of the cattle and the lower the body condition the higher the chance of a positive bTB test result, but sex, lactation status and reproductive status were not correlated with bTB status at individual animal level (Chapter 2). Herd size, contact with wildlife, and the interaction of herd size and contact with wildlife were identified as significant risk factors for bTB prevalence in cattle. In a structural equation model I showed that the probability of contact with wildlife was influenced by herd size, through herd movement. As larger herds moved more and grazed in larger areas, the probability of grazing in an area with wildlife and contact with either infected cattle or infected wildlife hosts increased, and thereby also increased the chances for bTB infection (Chapter 2).

I detected a possible dilution effect in bTB, where higher mammal species evenness reduced the probability of bTB occurrence (Chapter 3). This dilution effect might be caused by an encounter reduction, in which the distribution of non-competent mammal species might act as barriers to herd movement of cattle and reduce encounter rates among herds, which leads to a decreased probability of bTB outbreaks. Besides, I demonstrated that the presence of greater kudu

(*Tragelaphus strepsiceros*), as a maintenance host for *M. bovis*, had a positive identity effect on the incidence of bTB.

I showed that bTB prevalence was positively associated with the invasion of the plant Prosopis (*Prosopis juliflora*). Similarly, in a regression tree model I identified the proportion of Prosopis in an area as an important land use predictor for the prevalence of bTB, with higher bTB prevalence values in areas with higher Prosopis cover. The invasive Prosopis, although only a minor proportion of the total area of the landscape, has invaded the majority of the dry season pastureland in the Awash River Basin, and thereby indirectly regulates host community composition, and other aspects of the pastoral production system. The loss in host species evenness and the increase in cattle herd movement as a consequence of the loss of palatable grass in Prosopis areas could be potential mechanisms accounting for the observed higher bTB prevalence in these areas (Chapter 4).

In chapter 5, I carried out a network analyses, studying the connections between herds, through the exchange of cattle. In such an analysis, a herd is defined as a nod, and the exchange of cattle is defined as the connection between two herd, the so-called edge. I found that herds with a greater in-degree (direct connections through cattle exchange) were positively correlated with the risk of being infected with bTB. Herds with a greater number of edges had more connections in the livestock transfer network, increasing the probability of becoming infected with bTB. Herds that fall close to the centre of the network (i.e., with higher closeness centrality scores) was positively associated with the risk of becoming infected with bTB. A herd with higher closeness centrality scores is not only more susceptible to becoming infected with bTB but also facilitate diseases transmission through networks, as these herds were tightly connected to the rest of the herds in the network. Betweenness centrality, the number of edges that pass from one herd to another, was also positively associated with the presence of bTB infection. There was a negative relationship between the fragmentation index and bTB infection, likely because a high fragmentation index was related to a decreased number of contacts with other nodes in the network (Chapter 5). The study clearly demonstrated that the extent to which a herd is connected within a network has significant implications both for its risk of becoming infected and, if infected, the probability that it will transmit the disease to other herds in the network.

Finally, I combined these findings and reviewed the evidence in order to gain a better understanding of the mechanisms and generality of the ecological risk factors associated with zoonotic disease transmission, to provide new insights with regard to interventions and disease control measures. I discuss the conflicts between common social practices in pastoral areas of

Africa to manage resource variability and uncertainty, and the potential maladaptations of those traditional adaptation practices in relation with risk reduction of bTB transmission. I conclude by formulating socially acceptable and workable disease control strategies across African pastoral communities (Chapter 6).

Samenvatting

Rundertuberculose (TBC), veroorzaakt door de bacterie *Mycobacterium bovis*, is een belangrijke ziekte die kan worden overgedragen van dieren naar mensen, een zogenaamde zoönose. TBC wordt vooral via de lucht verspreid en kan vele zoogdiersoorten, inclusief de mens, infecteren. Daarom is TBC een belangrijke ziekte voor zowel de mens als landbouwhuisdieren en wilde dieren, vooral in ontwikkelingslanden waar problemen vaak worden veroorzaakt door het ontbreken van efficiënte preventie- en controlemaatregelen. De ziekte heeft zowel directe als indirecte gevolgen voor boeren, zoals sterfte en een verlaagde productiviteit van vee (direct) en kosten van controlemaatregelen, verminderde handel in dieren, lagere marktwaarde voor dieren en een verlaagde voedselzekerheid (indirect).

TBC komt in heel Afrika voor en zowel wilde als landbouwhuisdieren kunnen een hoge besmettingsgraad bereiken. Opvallend genoeg is er weinig bekend over de risicofactoren voor TBC transmissie in ontwikkelingslanden, omdat veel onderzoek is gedaan in ontwikkelde landen waar de veehouderij veel meer is geïntensiveerd en waar al decennia lang controle- en uitroeiingsprogramma's worden uitgevoerd. Het is daarom noodzakelijk om de relatie tussen weideveeteelt en het voorkomen van TBC te onderzoeken in een diverse gastheergemeenschap om een beter inzicht te krijgen in de rol van ecologie, sociale structuur en diermanagement in het bepalen van het TBC risico in ontwikkelingslanden. De kennis die voortkomt uit een dergelijke analyse zal bijdragen aan de ontwikkeling van ziektebestrijdingsstrategieën voor rundvee in ecosystemen die worden gekarakteriseerd door voedselbeperking in droge seizoenen en veelvoudig contact tussen wilde dieren en vee. In mijn proefschrift heb ik een dergelijke analyse gedaan voor de Afar regio in Ethiopië.

5.5% van de individuele runderen en 46% van de rundveekuddes in de Afar regio in Ethiopië was geïnfecteerd met TBC. Oudere runderen en runderen met een lage conditie score waren vaker geïnfecteerd dan jongere dieren of dieren in goede conditie. Er was geen correlatie tussen TBC infectie en geslacht, lactatie-status of reproductieve van het individu (Hoofdstuk 2). De grootte van de kudde, contact met wilde dieren en de interactie tussen deze twee factoren waren ook gecorreleerd met infectiestatus voor TBC. Met een structural equation model vond ik dat de kans op contact met wilde dieren indirect gecorreleerd was met kuddegrootte, via de ruimtelijke bewegingen van de kuddes. Omdat grotere kuddes grotere afstanden afleggen en in grotere gebieden graasden was de kans op contact tussen vee en geïnfecteerde wilde dieren of geïnfecteerd vee waarschijnlijk groter, waardoor de infectiegraad met TBC hoger is in grotere kuddes (Hoofdstuk 2).

Ik vond bewijs voor een mogelijk verdunningseffect van soortenrijkdom op TBC omdat een hogere gelijkheid in de soortensamenstelling van wilde zoogdieren in het gebied negatief gecorreleerd was met de aanwezigheid van TBC (Hoofdstuk 3). Dit verdunningseffect wordt mogelijk veroorzaakt door een vermindering in contact tussen geïnfecteerde en niet geïnfecteerde dieren. Zoogdieren die TBC niet kunnen overdragen zouden kunnen werken als een barrière voor kuddes, waardoor de kuddes minder grote afstanden afleggen en ze minder met elkaar in contact komen. Hierdoor wordt de kans op een TBC uitbraak kleiner. Daarnaast vond ik een positief identiteitseffect van de grote koedoe (*Tragelaphus strepsiceros*), as belangrijke gastheer voor *M. bovis*, op het voorkomen van TBC in rundveekuddes.

Ik vond een positieve correlatie tussen de invasie van de plant Prosopis (*Prosopis juliflora*) en het voorkomen van TBC. In een regressiemodel vond ik dat de bedekkingsgraad met Prosopis in een gebied een belangrijke factor was die het voorkomen van TBC in dat gebied bepaalde, waarbij er vaker TBC werd gevonden in gebieden met meer Prosopis. De invasieve Prosopis boom groeit maar op een klein deel van het totale landoppervlakte, in het stroomgebied van de Awash rivier. Toch heeft het een grote invloed op het weideveeteeltsysteem omdat het belangrijke graslanden die worden gebruikt voor beweiding met vee in het droge seizoen heeft overwoekerd. De hoge prevalentie van TBC in deze gebieden zou kunnen worden verklaard door een verlies in de gelijkheid van de soortensamenstelling van wilde zoogdieren, of door een toename in de afstanden die rundveekuddes afleggen als gevolg van een verlies aan gebieden met geschikte grassoorten (Hoofdstuk 4).

In hoofdstuk 5 heb ik een netwerkanalyse uitgevoerd om te onderzoeken hoe connecties tussen kuddes invloed hebben op het voorkomen van TBC, via de uitwisseling van vee. Ik vond dat kuddes met meer directe connecties een hogere kans hadden om geïnfecteerd te zijn met TBC. Kuddes waar meer dieren binnenkomen via uitwisseling van vee hadden meer connecties en daardoor een grotere kans hadden om geïnfecteerd te zijn. Ook kuddes die in het midden van het sociale netwerk stonden hadden een hogere kans om geïnfecteerd te zijn met TBC. Deze kuddes hebben niet alleen een hogere kans om een infectie binnen te krijgen, maar ze zijn ook van belang voor de verdere verspreiding van TBC omdat ze in contact staan met een groot deel van de andere kuddes. Kuddes die tussen veel verschillende andere kuddes inlagen hadden ook een grotere kans om besmet te zijn met TBC. Kuddes met een laag aantal uitwisselingen van vee met andere kuddes hadden echter een lagere kans om besmet te zijn met TBC (Hoofdstuk 5). Uit deze resultaten concludeer ik dat de plaats van een kudde in het sociale netwerk heel erg

belangrijk is in het beïnvloeden van de kans dat een kudde geïnfecteerd wordt met TBC, en de kans dat de TBC infectie daarna wordt overgedragen naar andere kuddes.

In het laatste hoofdstuk combineer ik deze resultaten om beter te begrijpen hoe verschillende ecologische risico-factoren invloed hebben op de ziektetransmissie van TBC. Sociale praktijken en productiemethodes van deze veehouders zijn misschien in eerste instantie gericht op het omgaan met de hoge onzekerheid van voedsel en water in deze relatief droge gebieden van Afrika, maar het kan zijn dat deze praktijken niet zijn aangepast om de transmissie van TBC te verminderen of voorkomen. Uiteindelijk presenteer ik sociaal accepteerbare en werkbare strategieën om het ziekterisico voor TBC te verminderen in de Afrikaanse weideveeteelt.

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Acknowledgements

PhD journey is an adventure. It would have not been possible without the support and involvement of many individuals and institutions. Here, I would like to express my thanks to all of them.

First and for most, I would like to acknowledge the School of Animal and Range Science at Haramaya University of Ethiopia and the Resource Ecology Group of Wageningen University for giving me the chance to study my PhD. NUFFIC provided me financial support through Wageningen University to pursue this PhD. In addition Haramaya University provided the necessary facilities during my field and laboratory work in Ethiopia. I extend my thanks to the Awash National Park through the Ethiopian Wildlife Conservation Authority and the Afar National State of Agriculture and Natural Resource office in Ethiopia for allowing me to conduct my field research.

I am very grateful to my promotor, Prof. Dr Herbert H.T. Prins, for accepting me as a PhD student and promoting my study. You really gave me lots of encouragement on both my research and my ideals. I thank you Herbert for your wonderful feedbacks, comments and suggestions for my proposals, manuscripts and thesis, and returned faster with very insightful comments and ideas. I would like to extend my gratitude and appreciation to my co-supervisor Dr Willem F. de Boer, for your responsible supervision and unreserved scientific guidance. Besides my PhD study, I greatly appreciate your helps on my adaptation to the new life abroad. I was always coming to your office whenever I faced difficulties but I never seen any kind of inconveniency from you. You are an amazing supervisor! I also would like to thank my second co-supervisor, Dr Ignas M.A. Heitkönig for your unreserved guidance and supervision from the inception of the proposal writing until visiting the PhD research in the field in Ethiopia, as well as write-up of the manuscripts and final thesis. I also appreciate your kindness. I always enjoyed the conversation and discussion with you. Other than the supervisors, I also thank the other co-authors of the chapters in this thesis. They include; Dr Tessema Z., Dr Fistum A., Dr Danel A., Dr Harm B., Mr. Zelelem E. and Mss. Eline van Elburg. Your contributions towards this thesis are very much appreciated.

PhD study is indeed a cooperative work. As much as it trains one to be an independent scientist, it also trains one to be a cooperative scientist. I would like to extend my thanks and appreciations to all other family members from REG who were always willing to help me out. Thank you David, Frank, Herman, Kevin, Milena, Ron, Patrick, Pim and Sip for your scientific comments

and suggestions! More specifically, I would like to appreciate Herman for your honest, willingness and assistance in every aspect of my request. Special thanks should go to Patricia, Gerda, Joke and Marion for the quality administrative and financial service including organising my travel back to the Netherlands and provided me with all the assistance I needed and ensured I am comfortable during my frequent visits to Resource Ecology Group – from providing office space to stationary and answering to any query from the beginning to the end of my PhD study. Thank you to all dear REG PhD students and postdocs: Audrie, Benson, Daniel, Edson, Eduardo, Helen, Henjo, Iris, Jasper, Jente, Joost, Kyle, Lennart, Martijn, Mikhail, Mina, Ntuthuko, Priya, Rafael, Robert, Shyam, Tim (in translating the summary of this thesis to Dutch), Vincent, Yanjie, Yin, Yingying, Yorick, Yussuf, Zhang and Zheng: for sharing ideas and individual stories, as well as for your support, encouragements and funs during coffee breaks and lunch time throughout my stay in The Netherlands. A special thank goes to Zheng who helps to let me quickly adapt to the new life here. I had the opportunity to supervise two MSc students during their thesis. Pauline and Rogier, thank you for being my students.

I wish to express my deepest appreciation to Prof. Dr Nigussie Dechassa, Vice- President for Academic Affairs of Haramaya University of Ethiopia for providing me the necessary facilities during my field and laboratory work in Ethiopia and his encouragement. My special thanks should go to Assefa Nigussu (driver) for your genuine support during my field work. I was very happy for the time that I spent with Ethiopian family members in Wageningen during holidays and in various occasions. The following people were of great help while I was conducting the various experiments in laboratory in Ethiopia: Solomon, Jenber, Tsige, Kebede and Meseret. Specifically, I would like to thank Shelemew, Wendimagen, Abiyot, Getachew, Kunte, Bekele, Ahemedin and Ahemed for their hard work during data collection in the harsh and very hot weather conditions in Awash National Park and Afar Regional State of Ethiopia for several months. I would also like to thank Dr Sisay M., Dr Firew M., Dr Kibebew K., Dr Mengistu U., Dr Bobe B., Dr Mengistu K., Dr Yosef T., Yonas H., Yonas M., Dawit T., Hiruit Y., Biazen A., Anteneh B., Araya S., Siferaw Y., Tessema T., Kiros, Bruh, Takele, Dr Mitku and Dr Mohammed for all their assistance and moral support during my study.

I would like to extend my appreciation and thanks to my family, more specifically to Mrs. Aberash Abebe and her family, supported me throughout the four years of my study.

To my Dad, Workeneh and Mom, Hageritu, special thanks go to you for consistently supporting me throughout my academic life. My brothers: Demeke and Wubshet, my sister: Kalkidan and

my entire extended family and friends provided unwavering support and constantly kept tab of my progress. To all I say, thank you!

And finally, I come to my wife Mesay Tekeste, my daughter: Bethelihem and my son: Eyoel, for your patience and strengthen during my absence while I was fully engaged in my PhD study in the field and abroad for the last four years. You were all my sources of inspiration, strength and hope during my PhD study. Thank you very much, I love you!

Last but not least, my apology should go to the unintentional omission of individuals and institutions, who have directly or indirectly contributed during my research and write-up of this thesis in particular and for successful completion of my PhD study in general.

Curriculum Vitae

Sintayehu Workeneh Dejene was born on 27 September 1983 in Tepi, Ethiopia. After completing high school, he joined the Jimma University in 2002. He received his Becholar's degree in Biology in 2005, after which he stayed at Addis Ababa University and started his MSc degree in Dryland Biodiversity, which was completed in 2009. He did his MSc thesis research on "Ecology of small mammals and the impact of human interference on their abundance and diversity in Nechisar National Park, Ethiopia". After his MSc degree, Sintayehu was employed as a lecturer in the School of Animal and Range Sciences at Haramaya University of Ethiopia. Sintayehu has been worked as lecturer in the School of Animal and Range Sciences at Haramaya University of Ethiopia from June 2009 until he joined Wageningen University of the Netherlands for his PhD study in May 2013. Sintayehu has been teaching different courses for undergraduate students. Moreover, he has supervised several BSc thesis research in biodiversity assessment, wildlife ecology and conservation, and ecotourism at Haramaya University. Furthermore, Sintayehu has conducted and leaded several research project at Haramaya University of Ethiopia.

In May 2013, he joined the Resource Ecology Group of Wageningen University for a PhD study through NUFFIC Scholarship under the supervision of Prof. Dr H.H.T. Prins and co-supervision of Dr W.F. de Boer and Dr I.M.A. Heitkönig. His study focussed on the role of ecological and societal risk factors on the prevalence of bTB under the influence of wildlife-livestock interactions. The results of this research culminated to this Thesis.

List of Publications

Dejene, WS., Heitkönig, IMA., Prins, HHT., Kelkay, ZT. and de Boer, WF. (2017b). Effect of host diversity and species assemblage composition on bovine tuberculosis (bTB) risk in Ethiopian cattle. Parasitology: 144: 783–792.

Dejene, WS., Heitkönig, IMA., Prins, HHT. and de Boer WF. (2017a). Disease transmission in animal transfer networks. Preventive Veterinary Medicine, 137: 36–42.

Dejene, WS., Heitkönig, IMA, Prins, HHT, Fitsum, A., Daniel, A., Zelalem, E., Kelkay, ZT. and de Boer, WF. (2016). Risk factors for bovine tuberculosis (bTB) in cattle in Ethiopia. PLoS One, 11: e0159083. doi:10.1371.

Dejene, WS. and R.Uttama Reddy (2016). Species Composition and Habitat Association of Small Mammals in Nechisar National Park, Ethiopia. International Journal of Sciences: Basic and Applied Research, 26: 62-71.

Dejene, WS. (2016). The African Elephant (*Loxodonta africana*) in Ethiopia: A Review. European Journal of Biological Sciences 8: 8-13.

Dejene, WS., Henock Seyoum and Uttama RR. (2014). Ecotourism potential and its role for sustainable development and livelihood in Awash National Park, Ethiopia. International Journal of Science and Research, 3: 792-796.

Dejene, WS., Nigussie Dechassa and R. Uttama Reddy (2016). Coexistence of human and hyena and associated impacts in Haramaya district of eastern Ethiopia. International Journal of Biodiversity Conservation 8: 1-7.

Dejene, WS., Afework, B. and Balakrishnan, M. (2012). Species diversity and abundance of small mammals in Nechisar National Park, Ethiopia. African Journal of Ecology 50: 102–108.

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Chase, MJ., Schlossberg, S., Griffin, CR., Bouche, PJC., **Dejene WS**., Paul W. Elkan, Sam Ferreira, Falk Grossman, Edward Mtarima Kohi, Kelly Landen, Patrick Omondi, Alexis Peltier, SA. Jeanetta Selier and Robert Sutcliffe (2016). Continent-wide survey reveals massive decline in African savannah elephants. PeerJ, 4: e2354; DOI 10.7717/peerj.2354.

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (6 ECTS)

- Eco-epidemiology of Bovine Tuberculosis (bTB)

Writing of project proposal (4.5 ECTS)

- Eco-epidemiology of Bovine Tuberculosis (bTB) at the wildlife-livestock interface

Post-graduate courses (5.1 ECTS)

- Linear Models; PE&RC (2013)
- Introduction to R for statistical analysis; PE&RC (2013)
- Generalized Linear Models; PE&RC (2013)
- Mixed Linear Models; PE&RC (2013)
- Bioinformatics - A User's Approach; PE&RC (2013)

Laboratory training and working visits (1.5 ECTS)

- Application of GIS for Agriculture and related sciences

Invited review of (unpublished) journal manuscript (3 ECTS)

- African Journal of Ecology; AFJE-16-165 - Lion (*Panthera leo*) and spotted hyena (*Crocuta crocuta*) abundance in Bouba Ndjida National Park, Cameroon; trends over the last decade (2016)
- African Journal of Ecology; Diversity, Distribution and Relative abundance of Medium and Large sized Mammals in Kuni Muktar Mountain Nyala Sanctuary West Hararghe Zone, Eastern Ethiopia
- PLos One; PONE-D-16-39705 - A social-economic assessment of the impact of Prosopis Julifora on livestock production in the Afar region, Ethiopia

Deficiency, refresh, brush-up courses (3 ECTS)

- Ecological Methods; PE&RC (2014)

Competence strengthening / skills courses (1.9 ECTS)

- Data Management; PE&RC (2013)
- Project and Time Management; PE&RC (2016)

PE&RC Annual meetings, seminars and the PE&RC weekend (0.9 ECTS)

- PE&RC Day (2016)
- PE&RC weekend (2016)

Discussion groups / local seminars / other scientific meetings (6.5 ECTS)

- Wageningen Evolution and Ecology Seminars (2013-2016)
- Movement Ecology (2013-2016)
- Ecological Theory and Application (2013-2016)
- R Group (2013-2016)
- Conference on Camel Dairy Technologies (2016)
- 26th Ethiopian Biological Society meeting (2016)
- 27th Ethiopian Biological Society meeting (2017)
- Enhancing Pastoralists Livelihood and Resilience through Market expansion (2017)

International symposia, workshops and conferences (3 ECTS)

- 2016 African ESP Conference
- The Impact of El Niño on Biodiversity, Agriculture, and Food Security

Lecturing / supervision of practicals / tutorials (3 ECTS)

- Ecological Methods and Design; Haramaya University (2014-2016)
- Wildlife Ecology and Management; Haramaya University (2014-2016)

Supervision of a MSc student (3 ECTS)

- Abundance of parasites in dung in combination with the wildlife/cattle interaction in Awash National Park, Ethiopia
- Resource use overlap of between wildlife and cattle in Awash National Park, Ethiopia

The research described in this thesis was financially supported by Netherlands Fellowship Programme (NUFFIC) and Haramaya University.

Cover design, layout and photo: Sintayehu Workeneh Dejene

Printed by Digiforce B.V.