Spillover risks of emerging (zoonotic) diseases at the human-livestock-wildlife interface: the case of bovine tuberculosis in cattle around Akagera National Park in Rwanda.

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Abstract

Worldwide, the contact between wildlife, domestic animals and humans increases, resulting in increased risk of disease spillover from one species to the next. This is especially a big problem in many sub-Saharan African countries, like Rwanda, where many high-impact potentially zoonotic diseases are present, like bovine tuberculosis (bTB). We use bTB in Rwanda as a case study to map the many problems and challenges in characterising disease spill-over in such a high-risk setting. Bovine tuberculosis is an important zoonotic disease in cattle, that has a wide host-range in wildlife and is underdiagnosed in many countries. We conducted a study at the human-livestock-wildlife interface in the Kayonza district in Rwanda, next to the Akagera National Park (NP), where the bTB maintenance host, the African buffalo, and many potential spillover-host-species live. We tested 150 cattle in 15 farms for bovine tuberculosis, using the single intradermal comparative cervical tuberculin (SICCT) test, and did interviews to determine the presence of risk factors. The farms were all within 2.5 km from the fence of the park and divided over three sectors. We found an animal-based apparent prevalence of 2% (95% CI: 0.52-6.2) and a herd apparent prevalence of 20% (95% CI: 5.3-49%), for cut-off value CIDT >4 mm. Risk factors for bTB present in this area are between-herd movements, shared water points, introduction of new animals, mixed rearing cattle and goats, nutritional stress, old age and contact with wildlife reservoirs. No statistically significant correlations were found between these risk factors and infection based on this dataset. Bovine tuberculosis poses a risk for public health, due to the consumption of raw milk on 40% of the test farms and close contact with animals. Other important cattle diseases that are present in the research area are trypanosomiasis, tick-borne diseases (especially East Coast fever or theileriosis and anaplasmosis), brucellosis, foot and mouth disease, Rift Valley fever and anthrax, among others. Through the questionnaires we showed that many wildlife species, from in- and outside of the park, visit the farms and have direct contact with the domestic animals. Among these are buffaloes, different antelope species, primates, hippopotami, warthogs and carnivores. In addition, there is an abundance of vector species like tsetse flies and ticks. These data together make spillover of diseases between domestic animals and wildlife highly likely. We recommend to further investigate the risk of bovine tuberculosis and other high-impact diseases at the domestic-wildlife interface near Akagera NP, by doing targeted disease monitoring in domestic animals and in wildlife, especially in buffaloes as they play an important role in diseases like bTB, brucellosis, tick-borne diseases and trypanosomiasis. One Health collaboration of different stakeholders is key in effectively protecting wildlife, humans and domestic animals.

1. Introduction

Contact between wildlife and humans with their livestock increases, due to a growing human world population and loss of natural habitats. This results in a higher chance of disease spillover from one species to the next, from wildlife to domestic species and to humans; and the other way around. The consequences of this can range all the way from mild to devastating in the context of animal health, public health and wildlife conservation [1].

Local community members, who often depend on livestock, can suffer social and economic losses, due to disease spillover and the resulting death and disease of their animals. Furthermore, the local people are at risk for zoonotic diseases, coming from wildlife through livestock. Disease in these people can be a possible starting point for bigger outbreaks among the population.

From a conservation perspective, disease spillover from livestock and humans to wildlife can result in the decline of already endangered species [2,3] and can disturb the balance of ecosystems.

Spillover of diseases is a risk world-wide, but especially relevant in many sub-Saharan African countries, because of the endemicity of many high-impact (zoonotic) diseases, many people living in proximity of wildlife habitats, and less resources for eradication programmes [4]. Rwanda is a country with a large human and livestock population, with many living in proximity of wildlife in national parks, like the Akagera National Park (NP) in the east of the country, bordering to Tanzania.

Preventing or controlling disease spillover is desired, but in many African countries little information is available about the prevalence and risks of diseases in wildlife and livestock. There are many high-impact emerging (zoonotic) animal diseases, but the research reported on in this master thesis has its focus on assessing risks of one disease in a specific area: bovine tuberculosis (bTB) in cattle (*Bos taurus*) herds living in proximity of Akagera NP (within 2.5 kilometres of the park fence) in the Kayonza district in Rwanda. Bovine tuberculosis is an example of a high-impact zoonotic disease in livestock that has a wide host-range in wildlife [4] and is underdiagnosed in many countries [5]. Many reservoir and spillover host species are present in Akagera NP, and there is a possibility to test for bTB in cattle, as well as enough available knowledge about the disease from other countries. It is also a non-vector disease, making the epidemiology and its related risk factors relatively accessible. By determining the prevalence of bovine tuberculosis and identifying the risks of transmission in cattle herds living in proximity of Akagera NP, in Kayonza district in Rwanda, we aim to give more insight into general disease spillover risks and risk assessment in this situation. The results can contribute to the information needed for future specific targeted measures to protect wildlife populations and humans with their livestock.

The following research questions are formulated:

1. What are risk factors for spillover of emerging (zoonotic) diseases between wildlife and livestock around Akagera National Park in Rwanda?
2. What are risk factors for contracting bovine tuberculosis for livestock around Akagera National Park in Rwanda?
3. What is the prevalence of bovine tuberculosis in cattle herds living in proximity of Akagera National Park, in the Kayonza district in Rwanda?
4. Is there a relation between the prevalence of bovine tuberculosis in the cattle herds and the present risk factors?

Before continuing with the practical research part, more context is provided to get a better idea of the complexity of the problem, starting with the bigger picture in Chapter 2 by giving an overview of hazardous diseases in Eastern Africa. In Chapter 3 we describe the situation of the specific region of our case study, and Chapter 4 provides a basic understanding about bovine tuberculosis.

2. Hazardous diseases at the domestic-wildlife interface in Eastern Africa

There are many places in Eastern Africa where people and domestic animals live in proximity of wildlife. In these areas there is a bigger risk for spillover of diseases. Some diseases can bring a big risk to public or animal health or have a big socio-economic impact. Spillover can occur via various ways: direct or indirect contact, droppings in shared pastures (faecal-oral route), air-borne or vector-borne. Table 2.1 shows some high-impact diseases present in Eastern Africa, with their host range. Many of these diseases can infect wildlife, and many of them are zoonotic or have zoonotic potential. Some diseases, like brucellosis and bTB, have multiple transmission routes, but they are organised by their main route of transmission between animals.

Main route transmission between animals	Disease	Domestic animals	East African wildlife	Zoo-notic	Source
Airborne	Bovine tuberculosis	Many, cattle most important	Many, buffalo most important		[5,6]
	Contagious bovine pleuropneumonia	Cattle			[7]
	Q-fever	Ruminants			[8]
	Canine distemper virus	Dog	Carnivores like lion and hyena		[3,9]
	Swine influenza	Swine			[10]
(In)direct contact	Anthrax	Many, mostly herbivores	Many, mostly herbivores		[11]
	Brucellosis	Many: ruminants, swine, etc	Many: buffalo, carnivores, antelopes, etc		[12,13]
	Foot and mouth disease	Ruminants and swine	Cloven-footed mammals		[14,15]
	Peste des petits ruminants	Mostly small ruminants	Artiodactyls: antelopes, buffalo, etc		[16,17]
	Leptospirosis	All animals	All animals		[18]
	Rabies	Many, dog most important	Many: bats, carnivores, primates, etc.		[2,19]
	Nipah virus encephalitis	Many, swine most important	Fruit bat		[20]
Faecal-oral	Toxoplasmosis	Cat as definitive and all others as intermediate hosts	Wild felids as definitive and all other animals as intermediate hosts		[21]
	Trichinellosis	Many, most important swine	Wild carnivores and omnivores		[22,23]
	Echinococcosis	Carnivore as definitive and all others as intermediate hosts	Wild carnivores as definitive and some species as intermediate hosts		[24,25]
	Taeniasis	Carnivore as definitive and swine, cattle and sheep as intermediate hosts	Wild carnivores as definitive hosts		[26]
Tick-borne	East Coast fever/theileriosis	Cattle	Buffalo		[27]
	Bovine babesiosis	Cattle			[28]
	Bovine anaplasmosis	Cattle	Buffalo		[29,30]
	Crimean-Congo haemorrhagic fever virus	Ruminants	Herbivores		[31]
	Ehrlichiosis/heartwater	Ruminants			[32]
Vector other than ticks	Rift Valley fever virus	Ruminants	Herbivores: buffalo, rhino, antelopes, etc		[33,34]
	Trypanosomiasis	All domestic animals, cattle most important	Many: buffalo, lion, elephant, warthog, antelopes, rhino, etc		[35]
	Lumpy skin disease	Cattle			[36]
	Bluetongue	Ruminants	Ruminants		[37]
	Leishmaniosis	Dog and cat	Jackal		[38]
	Epizootic haemorrhagic disease	Cattle	Ruminants		[39]

Table 2.1 High-impact diseases in Eastern Africa with their host-range

All diseases in Table 2.1 are WOAH listed, except for canine distemper virus, swine influenza, leptospirosis and toxoplasmosis. There are many other emerging WOAH-listed diseases, like Aujeszky, BVD, tularemia and African Swine Fever.

Spillover of infectious diseases can be devastating for wildlife species that already face extinction from various threats. Rabies is an emerging infectious disease that not only causes tens of thousands of deaths every year in humans and domestic dogs (*Canis lupis familiaris*) worldwide but is also a threat to wildlife. It has caused severe decreases in population sizes and even local extinction of the African wild dog (*Lycaon pictus*) and Ethiopian wolf (*Canis simensis*) [2]. Especially in areas at the domestic-wildlife interface where dogs are used for livestock herding or protection, there is a high chance of contact between dogs and wild predators, possibly resulting in disease spillover. There are more examples of disease outbreaks that led to a wildlife population decline, like in the case of canine distemper virus spillover to African lions (*Panthera leo*) in the Serengeti [3].

The major issue for our context, however, is spillover in the opposite direction, with diseases having a reservoir in wildlife species and transmitting to domestic animals and humans. There is often little disease surveillance in wildlife, resulting in undetected disease prevalence in wildlife species. Examples of diseases that show the importance of wildlife disease surveillance are anthrax and Rift Valley fever.
Anthrax (*Bacillus anthracis*) is a disease that can result in big losses of domestic and wild mammals and is present in many wildlife species in Eastern Africa, causing outbreaks mostly in herbivores [40]. It is also a dangerous zoonosis. When wildlife and domestic animals can come into direct contact with an infected carcass, there is a big risk of transmission.
Rift Valley fever (RVF) is also an important zoonosis at the human-livestock-wildlife interface, causing abortion and perinatal mortality in livestock and acute fever in humans. It is found in many wildlife species [34].

Other important diseases are bovine tuberculosis, brucellosis, foot and mouth disease, trypanosomiasis and several tick-borne diseases. They will be discussed in Chapters 3 and 4.

3. The situation of Akagera National Park and Kayonza district in Rwanda

In this chapter we focus on spillover of diseases in the area of interest: the human-livestock-wildlife interface next to Akagera NP in the Kayonza district in Rwanda (see Figure 3.1 for a map of Rwanda). First, context will be given of Akagera NP and cattle systems in Kayonza district and then some important diseases in this area will be discussed.

Figure 3.1 Map of Rwanda. The Kayonza district is in the Eastern Province, containing a big part of Akagera National park (green on the map), and bordering the country of Tanzania. (Rwanda map with districts. 2024; Available at: https://maps-rwanda.com/rwanda-map-with-districts)

3.1. Akagera National Park

Akagera National Park (NP) is a protected savannah habitat of approximately 1120 km^2, with wetlands making 30% of the surface; and grasslands, woodlands and forests making the remaining 70% [41]. It is home to many wildlife species, including endangered species like the black rhinoceros (*Diceros bicornis*), savanna elephant (*Loxodonta africana*) and Masai giraffe (*Giraffa camelopardalis* ssp. *tippelskirchi*). Many local communities with their domestic animals live near the border of the park, in proximity of these wildlife species. This proximity can result in disease spillover, with consequences for local communities and wildlife populations.

In 2013 an electric fence (120 km long and 2.5 m high) was constructed around Akagera NP, to protect local communities from wildlife destruction and to protect the park from cattle and poacher encroachment. A study from 2010 to 2015 in the Gatsibo and Nyagatare districts on the border of the park [42] assessed population densities of wildlife and domestic livestock, before and after fencing. Continuously high cattle densities, decreasing densities of zebra (*Equus quagga*), decreasing encounter frequencies of waterbuck (*Kobus ellipsiprymnus*), stable encounter frequencies of topi (*Damaliscus lunatus jimela*) and stable densities of impala (*Aepyceros melampus*) were found. Impala can jump fences up to three metres high and topi can crawl under them. Reduced predation can attract them to go outside the park [42].

Before the fencing cattle grazed and shared waterpoints with wild herbivores. Spotted hyenas still cross the fence from Akagera NP to cattle farms. Movement of carnivores feeding on aborted tissue and live calves and goats in both park and cattle farms may play a role in transmission of zoonotic diseases between wildlife and livestock and vice-versa [43].

3.2. Cattle systems near Akagera National Park in the Kayonza district

Livestock plays an important part in food security in Rwanda and development in livestock production is one of the most important strategies used for reducing poverty [44]. The Eastern Province of Rwanda contains the largest area of farmlands of the country. Livestock keeping has undergone many changes since 1994, the year of the genocide in Rwanda, due to livestock losses in the war and post-genocide disease epidemics following the return of refugees with their livestock. Akagera NP has lost part of its original area in order to create more grazing areas [44].

Nowadays, a big part of the area next to the Akagera NP in the Kayonza district is occupied by cattle farms. The herd sizes range from around ten to over 100 animals. The majority of the cattle are crossbreeds, but there are also some exotic breeds (mainly Holstein Friesians) and local breeds (mainly Ankole). Most cattle farms are extensive and traditional [45], with cattle occupying a big area (fenced by *Euphorbia* bushes) for grazing and cowboys staying close to the herd for protection and milking in the field. Most farms have an enclosure (made of wood and bushes) where the animals stay overnight. There are some modern farms where cattle are held in an outdoor stable for most of the time.

The climate in the Eastern Province is warm and dry, with an annual rainfall of 700-1100 mm (most falling in the two rain seasons), with a mean annual temperature of 20-22°C [46]. The farmlands are highly vulnerable to increasing drought. The vegetation of the area next to Akagera NP is grassland with low inclined hills and an altitude of between 1400-1600 metres.

3.3. Diseases on the human-livestock-wildlife interface around Akagera National Park

In and around Akagera National Park, some studies have been conducted concerning animal diseases at the human-livestock-wildlife interface. The diseases that have been assessed are foot and mouth disease virus in cattle, goats (*Capra hircus*) and African buffaloes (*Syncerus caffer)* [47], bovine brucellosis in cattle [43] and trypanosome infections in cattle and glossina flies [41,48]. These are common cattle diseases in this area, especially trypanosomiasis. In addition, tick-borne diseases are very common for cattle around Akagera NP, mostly East Coast Fever (ECF or theileriosis) and anaplasmosis.

3.3.1. Trypanosomiasis

In Akagera NP there is an abundance of *Glossina* (tsetse flies), who biologically transmit trypanosomes that can infect humans and animals. A study conducted in and around Akagera NP [41,48] found *Glossina pallidipes* in higher numbers than other species, therefore the most important vector of trypanosomiasis in this area. The distribution of *Glossina* is limited to the protected Akagera NP and a narrow band in its surroundings. Cattle who graze just outside the fence are therefore vulnerable. [41]
Some of the flies were infected with trypanosomes, 13.9% in the head and proboscis and 24.3% in the thorax and abdomen. Eight species of trypanosomes were identified, several which can affect cattle. No evidence of human-infective *T. Brucei rhodesiense* (sleeping sickness) was found. The most frequent hosts for the flies were found to be the African buffalo and the rhinoceros. Most trypanosome infections were associated with a buffalo blood meal, meaning buffaloes are an important reservoir of tsetse-transmitted trypanosomes in the area [48].

3.3.2. Tick-borne diseases

After trypanosomiasis, tick-borne diseases are the most common in cattle around Akagera NP, especially ECF and bovine anaplasmosis, but bovine babesiosis and ehrlichiosis (heartwater) are also present.
Most of these tick-borne diseases can have transmission between livestock and wildlife [30]. The risk for spillover around Akagera NP is bigger for vector-borne diseases than diseases that require direct or close contact, because the fence does limit animal movement, but it does not stop ticks or flies from passing through. Like with many other important cattle diseases at the wildlife interface, buffalo play an important role as a host in ECF and anaplasmosis [30,49].

3.3.3. Brucellosis

Brucellosis (*Brucella spp.*), also called undulant fever, is a zoonotic disease that can cause severe disease in humans. People are at risk of infection with bovine brucellosis (*B. abortus*) by drinking raw milk and during procedures which require close contact with cattle, like assisting in calving without biosafety protection and manual milking [43]. The birth fluids of an infected animal contain high levels of bacteria, which can survive in the environment for several months [12].
Many wildlife species are sensitive for brucellosis, like the African buffalo, antelope species and carnivores among others. Buffaloes seem to play a role as a reservoir species. Studies in Africa have documented brucellosis seroprevalence in African Buffalo and the pooled prevalence is 13.7% [13]. Cattle herd seroprevalence is high at livestock-wildlife interfaces in East Africa, ranging from 26.7-68.7% [43].

A study from 2020 showed a brucellosis endemicity in cattle farmed close to wildlife in Rwanda, see Figure 3.2 [43]. At the human-livestock-wildlife interface in districts around Akagera NP bovine brucellosis seroprevalences of 8.3% on an animal level and 30.9% on a herd-level were found. In peri-urban areas (not at the wildlife interface) the seroprevalence was 0,0%. A herd was considered positive if at least one animal tested positive. Potential risk factors were determined to be old age (more than five years), cattle farmed close to wildlife, herds of cattle together with small ruminants, history of abortions and replacement of animals. These were significantly associated with testing positive for brucellosis [43].

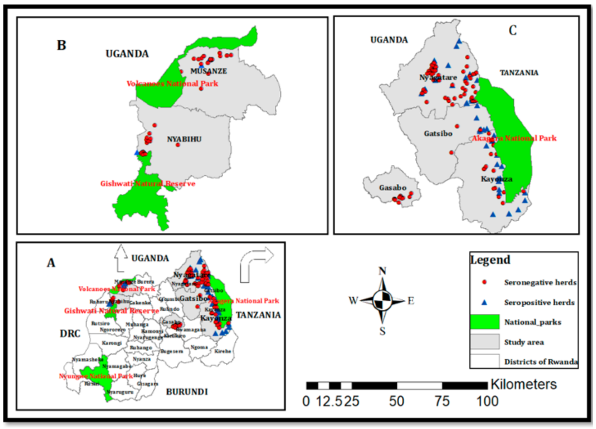

Figure 3.2 Maps of (A) Rwanda with different districts. (B) the Musanze and Nyabihu districts border the Virunga and Gishwati national parks, respectively, and (C) the Nyagatare, Gatsibo, and Kayonza districts border Akagera National Park, and Gasabo is an urban district with peri-urban areas. Red circles and blue triangles indicate seronegative and seropositive herds found in this study. (Ntivuguruzwa JB, Kolo FB, Gashururu RS, Umurerwa L, Byaruhanga C, Van Heerden H. Seroprevalence and associated risk factors of bovine brucellosis at the wildlife-livestock-human interface in Rwanda. Microorganisms 2020;8(10):1553)

3.3.4. Foot and mouth disease

Fout and mouth disease (FMD) is endemic in Rwanda and is the disease with the highest economic impact. In the beginning of 2024, there was an outbreak of FMD in the Kayonza district near the Akagera NP, suspected to be caused by the introduction of new animals. The infected animals were removed, and the healthy animals are now receiving yearly vaccination.

A study from 2021 sampled 823 cattle, 188 goats and seven African buffaloes. They found a seroprevalence of 9.36% for cattle and 2.65% for goats in the Eastern province. The oropharyngeal fluids from seven mature African buffaloes from inside the park did not show FMD, but several other pathogens. The sample size was too small to exclude the possibility of buffaloes playing a role. However, they are not likely to be the origin of the outbreaks, especially because of the fence around the park [47].

There is always a possibility of increased fence permeability, because of damage by large mammals, weather conditions or human activity, like was the case in Kruger NP where five outbreaks of FMD occurred in the beginning of the 21st century [15], despite an electric fence between cattle herds outside and buffaloes inside the park. In addition, there are other wildlife host species to take into consideration, like impalas who are highly susceptible for FMD and can jump fences up to three metres high [15].

4. Bovine tuberculosis

Mycobacterium bovis is the etiological agent of bovine tuberculosis (bTB) and a member of the *Mycobacterium tuberculosis* complex (MTC). *M. bovis* has a wide host range, contrary to *M. tuberculosis,* and infects domestic animals, wildlife and humans. Cattle is the most important host. It is a challenging endemic disease to control and eradicate, partly due to its complex epidemiology. In the right climate and conditions, the bacteria can persist in the environment [13].

Bovine tuberculosis is a chronic disease, and various species can develop tuberculous granulomas in lungs, lymph nodes, intestines, liver, kidneys and other organs. Clinical signs are non-specific, like emaciation, coughing, weakness, anorexia – and depend on the route of infection. Many animals can remain asymptomatic [13]. It is categorised as a listed disease by WOAH and classified as a major zoonosis and public health concern [6]. Furthermore, it has a big socio-economic impact due to decreased beef and milk production and carcass condemnation [13].

This chapter aims to provide a general understanding of bTB, relying on different chapters of the book 'Tuberculosis in Animals: An African Perspective' as an important source.

4.1. Wildlife

In many African countries, transmission of bTB to different wildlife species is common. Bovine tuberculosis has a wide host range, from antelopes and other herbivores, to predators, and primates and other omnivores [13]. It has been confirmed in at least 29 free ranging African wildlife species [5]. Maintenance hosts are greater kudus, Kafue lechwe and most important the African buffalo. African buffaloes can get persistently infected and play an important role as a reservoir species in bTB epidemiology in countries like South Africa, Zimbabwe, Tanzania and Uganda and they are present in large numbers in Akagera NP. Warthogs (*Phacochoerus africanus*) and lions, both present in Akagera NP, are potential maintenance hosts, and many other species are spillover hosts [5]. See Appendix I for the list of mammal species in Akagera NP, with their susceptibility for bTB.

Bovine tuberculosis is underdiagnosed in wildlife, even more than in cattle and in many countries, it is not obligated to report bTB when found in wildlife. Without this disease surveillance information, it is hard to predict or prevent spillover from wildlife to livestock [5].

4.2. Zoonotic tuberculosis

Mycobacterium bovis is a public health risk because it can cause zoonotic tuberculosis (zTB) in humans. People can get infected by consuming raw milk (products) and raw or undercooked meat of an infected animal, resulting in extra-pulmonary TB (often intestinal problems and tuberculous lymphadenitis); or by having close contact with infected animals, most likely resulting in pulmonary TB. The disease in humans is clinically and pathologically indistinguishable from *M. tuberculosis*. Most tuberculosis cases are caused by *M. tuberculosis* and this remains the larger risk, but zTB is underdiagnosed and most likely underestimated, resulting in little knowledge about the contribution of *M. bovis* in tuberculosis disease around the world. Not knowing if a tuberculosis infection is caused by *M. bovis* is a risk due to all *M. bovis* strains being resistant to pyrazinamide, one of the antibiotics used in treating human TB. This resistance to therapies is a risk, especially in areas with a high prevalence of HIV, because TB is one of the leading causes of death among people with HIV [50,51].

4.3. Epidemiology

Bovine tuberculosis is not easy to control due to its chronic nature. Infected cattle can survive for several months or years, many remain asymptomatic, and it can take over a year after infection for the first disease symptoms to be visible. Furthermore, what complicates it, is that mycobacteria are naturally resistant to drying, acidity and alkalinity and to many antibiotics [52].

The primary route for infection in cattle and wildlife is respiratory, through aerosol transmission, resulting in tuberculous lesions in the lungs and associated lymph nodes. The oral route of infection happens during predator/prey encounters, through feed and water and vertical transmission through milk, but this route is negligible. As a third, percutaneous infection has been documented in greater kudus [52].

There are multiple risks involved in contracting bTB. Risk factors on the herd-level are large herds, high cattle densities (more likely found in intensive husbandry systems), between-herd movements (during trading or communal grazing for example), mixed rearing of cattle with other stock (especially goats or pigs) and contact with wildlife reservoirs. The prevalence of bTB is normally low under extensive farming conditions where cattle live in the open. On an animal-level predisposing factors determining susceptibility of cattle to bTB are age (six years and older) and malnutrition or nutritional stress. Lastly, water points with shaded places give a higher risk of contracting the disease [52].

4.4. Diagnosis

Diagnosing bTB in cattle based on clinical signs is hard, because the infection can remain subclinical for months to years (and more than 90% of cattle in a herd may remain asymptomatic) and the clinical signs are not specific: respiratory problems, emaciation, lethargy, lymphadenitis, fluctuating fever, anorexia and death. These could also be the symptoms of other diseases like trypanosomiasis and CBPP among others [53].
In most wildlife species bTB also results in emaciation, lethargy, coughing or death. Over 75% of buffaloes don't show any signs and only coughing in a herd might be noticed.

That is why other diagnostic methods are needed for detecting *M. bovis*. All indirect detection methods are herd tests, not individual tests, due to a lack of sensitivity and specificity. The different diagnostic possibilities are the tuberculin skin test, interferon gamma assay (IFN-y), serology, abattoir meat inspection, histopathology, microscopic examination, culture and PCR. Two of these methods have been accepted by WOAH (formerly OIE) for bTB surveillance and eradication: the tuberculin skin test and IFN-y assay. Both are based on a delayed hypersensitivity reaction of the immune system [53].

There are three types of tuberculin skin tests (TST): the single intradermal tuberculin (SIT), single intradermal comparative cervical tuberculin (SICCT) and caudal fold tuberculin (CFT) tests.
TST works by injecting purified protein derivate (PPD), prepared from heat-killed cultures of *M. bovis*, intradermally. If the animal has been infected with *M. bovis* there will be a local cell-mediated hypersensitivity response and infiltration of T-lymphocytes, resulting in local oedema and fibrin around collagen. The reaction can be seen as swelling, heat, pain, necrosis, adhesion and oedema. The skin thickness must be compared between before and 72 hours after administration of the tuberculin. The test result is based on the degree of the increase. In the comparative test (SICCT) *M. avium* PPD is injected simultaneously in a different site and the test result is based on the difference between the two increases in skin thickness. The sensitivity of the SICCT is 68-95% and the specificity is 96-99.9%. As said before, due to the lack of sensitivity, this is a herd test [53]. Also, there is a possibility of false positivity caused by cross-reactions if animals have been exposed to other mycobacteria. After infection with *M. bovis* it takes three to six weeks to develop a cell-mediated immune response that can be detected in the tuberculin test, making TST not a suitable test for very young animals [54].

4.5. Rwanda

Bovine tuberculosis is endemic in Rwanda but poorly investigated. The data is based on meat inspection in abattoirs. Prevalence seems to be low based on this data, but commonly when doing postmortem inspections 50% of bTB cases are not detected [50]. The prevalence is likely to be higher than found in the abattoirs, especially because in 2012 in bordering country Tanzania the animal prevalence ranged from 0.2-13.3% in different regions [55] and a study from 2014 found a herd prevalence of 14,5% in an area in Uganda that is bordering Rwanda [56]; and there is no specific prevention and control programme present in Rwanda [45].
Bovine tuberculosis is a significant threat to animal production in Rwanda: socio-economically (with livestock production being a central asset in reducing poverty) and affecting the health of livestock, humans and ecosystems. It is also likely to increase in the presence of diseases like HIV, especially if there is no control program implemented [45,51].
It is likely that bTB occurs in wildlife species in Akagera National Park. Intermingling of livestock and wildlife at the livestock-wildlife interface increases likelihood of disease transmission [45]. Furthermore, bTB has been found in both cattle and wildlife in neighbouring countries Uganda and Tanzania [5].

4.6. Prevalence in other countries around National Parks

Several studies have been conducted on the prevalence of bovine tuberculosis in cattle living in proximity of wildlife. In 2022 in South and East Cameroon an apparent animal prevalence of 1.8% (based on SICCT threshold value 4 mm) was found and significant risk factors associated with bTB were herd size, cattle age and contact between wildlife and livestock [57]. In 2011 in the Serengeti (in Tanzania, east of Lake Victoria – 500 km from Akagera NP), where livestock and wildlife have much interaction, they found an apparent animal prevalence of 2.4% (95% CI: 1.7-3.5%) and a herd prevalence of 50% also using the SICCT test, with a sample size of 1103 cattle in 32 herds [58]. In the same year in Botswana prevalences were found in cattle in the Okavango Delta and Chobe NP of respectively 0.7% and 2.4% using the IFN-y assay [59].

Figure 4.1 shows the distribution of bTB in cattle and wildlife in Africa during 1996-2011 [4].

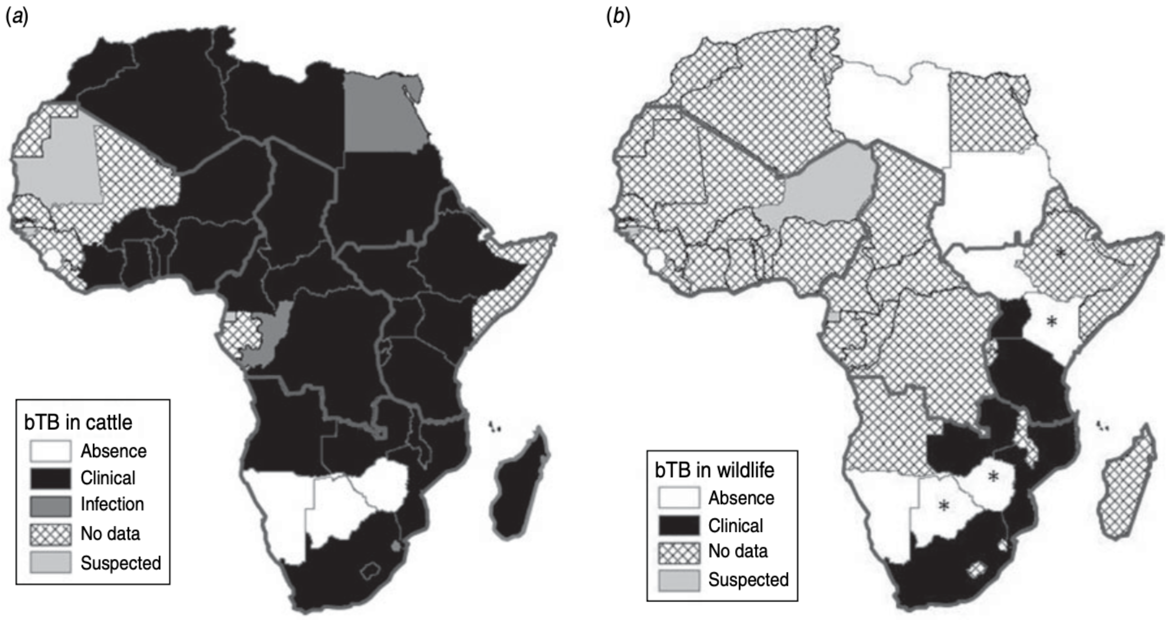

Figure 4.1 Distribution map of bovine tuberculosis in Africa during 1996–2011. (a) Cattle status at country level; (b) wildlife status at country level. Asterisk (*) indicates countries (i.e. Botswana, Ethiopia, Kenya, Zimbabwe) where suspected and confirmed cases have been detected but not yet reported to OIE. No additional information (e.g. species) was available for suspected cases reported in wildlife for Niger, Equatorial Guinea and Guinea–Bissau and confirmed cases in wildlife in Mozambique. Data compiled from World Animal Health Information databases/OIE. (De Garine-Wichatitsky M, Caron A, Kock R, Tschopp R, Munyeme M, Hofmeyr M, et al. A review of bovine tuberculosis at the wildlife–livestock–human interface in sub-Saharan Africa. Epidemiology & Infection 2013;141(7):1342–1356.)

5. Methods for the bTB case study

We visited 15 farms in total, in three sectors bordering Akagera NP (Gahini, Mwiri and Murundi), five farms in each sector (see Figure 5.1). These farms were all within a range of 2.5 kilometres from the fence of Akagera NP. Nine of them (60%) had grazing areas next to the fence. The herd sizes ranged from 15 to 97 animals, with an average herd size of 35. In all farms we did an interview with the farm manager and tested ten animals for bovine tuberculosis using the tuberculin comparative skin test. In one farm nine and in another farm 11 animals were tested due to practical considerations. The research team consisted of the researcher, a VSF (Vétérinaires Sans Frontières) representative, a private veterinarian and a sector animal resources officer (government veterinarian). Permission to do our research activities and to use the gathered information was asked to the farmers and given. Every farm was given a small financial compensation for their help and their time: this was neither announced nor given beforehand, but at the end of the visit.

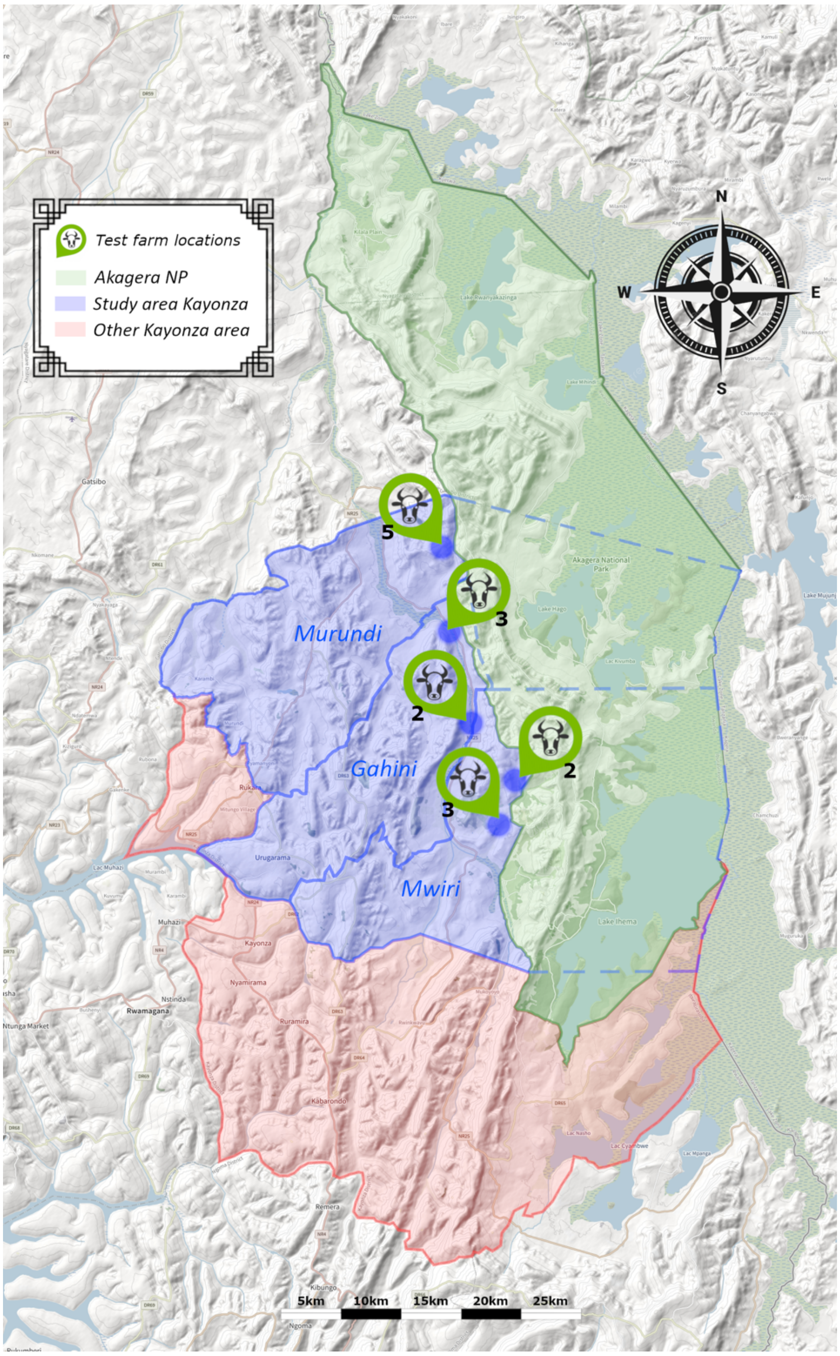

Figure 5.1 Study area within the Kayonza district, with five farms in each of three sectors: Gahini, Mwiri and Murundi. Blue: study area, green: Akagera NP, red: other Kayonza district area. The numbers indicate the number of test farms in that location.

5.1. Questionnaires

The interviews with the farm managers were held using questionnaires. The questionnaire consisted of 25 questions with sub questions, about general cattle management, wildlife seen in and outside of the park, contact with other animals (domestic and wild), animal purchase, animal health and public health (see Appendix II). The questions were in English and translated on the spot to Kinyarwanda by a veterinarian with Kinyarwanda as native language and good proficiency in English. The answers were then translated back to English and written down by the researcher. Sometimes further questions were asked for clarification. During the interview, the GPS location of the farm (cattle night enclosure) was saved in OsmAnd application. This location was used to determine the distance from the fence of Akagera NP to the farm.

Apart from the farm manager questionnaire, each sector animal resources officer was given a list of diseases and asked to fill out the presence, occurrence and year of last case or outbreak for each of them. This list contains important diseases that can affect domestic animals and wildlife in East Africa (see Appendix III).

5.2. Bovine tuberculosis testing

Within a herd ten cattle were tested for bovine tuberculosis using the single intradermal comparative cervical tuberculin (SICCT) test, preferably a mix of ages and sex. From each cow the following information was noted on the day of testing (day zero or D0): identification (ear tag or name), breed, age, sex, body condition score (BCS), body temperature, skin thickness on the two testing sites, and any additional notes concerning health or identification (see Appendix IV).

On D0 two areas on the left side of the neck were shaved of approximately four by four centimetres, with at least 12.5 centimetres distance between them (which is considered sufficient), and cleaned with water or alcohol, depending on the availability. Then the skin thickness was measured of these two testing sites using an electronic calliper and noted in millimetres with one decimal. After the measurement and the skin had dried, the bovine tuberculin was injected intradermally in the caudal site and the avian tuberculin in the cranial site (with a HSW HENKE-JECT® TBC syringe). Then we checked if a palpable nodule was present in the skin at each injection site. In very young animals the bovine site would be on the right site of the neck, to prevent the distance between bovine and avian injection sites from being too small.

72 hours (plus or minus four hours) later, on day three (D3), we returned to the tested herd for reading the results. After identification of the animal, the skin thickness was measured again on both testing sites using an electronic calliper and noted in millimetres with one decimal. If the swelling on the bovine site had an increase of more than 2 mm compared to the avian increase, additional information was noted about the swelling: the appearance (diffuse or circumscribed), consistency (hard, soft, oedematous) and clinical observations (necrosis, exudation, heat, pain, tenderness or adhesions). The presence of clinical signs and a swelling that is diffuse and soft or oedematous indicate bovine reactivity, while a circumscribed and hard swelling indicates cross reactivity. Also, the body temperature and any additional notes were written down.

See Appendix V for the materials used during testing.

The interpretation of the results was done using Table 5.1:

Difference bovine and avian increase in skin thickness (CIDT): B(D3-D0) – A(D3-D0)	**Diagnosis**
>4 mm	Positive
2-4 mm	Inconclusive
<2 mm	Negative

Table 5.1 Interpretation of SICCT test results

Two alternative interpretations were done based on different cut-off values. The first alternative differs slightly from Table 5.1 with a result being positive when CIDT >3 mm. The second alternative was used for data analysis and is described in paragraph 5.3.

5.3. Data analysis

The collected data (questionnaire results, individual cow information and tuberculin skin test results) were entered in Microsoft Excel.

The animal-level prevalence of bTB was determined as the proportion of positive cases in the total of 150 cattle. The bTB herd prevalence was determined as the proportion of positive herds (at least one positive animal in a herd) in the total of 15 herds. Following that, the 95% confidence intervals of these proportions were determined using RStudio [60]. The true prevalence was estimated based on the proportion of positive animals and the sensitivity (ranging from 68 to 95%) and specificity (ranging from 96 to 99.9%) of the SICCT test using Epitools [61].

To see if there was a correlation between infected animals and risk factors in our collected data, logistic regression was performed in RStudio. Then Odds ratios with their confidence intervals were determined. Statistical significance was set at p-value <0.05. A p-value between 0.05 and 0.1 was considered a trend result. The dependent variable was 'infected animals', where the inconclusive animals were considered as positive for analytical purposes (positive and inconclusive = 1, negative = 0). The independent variables were breed, age, sex, BCS, herd size, distance to the park, grazing next to fence, contact with other herds, contact with goats, wildlife on farm (relevant species based on interviews), direct contact with wildlife, cattle origin outside district and introduction of new animals in the herd last year. Two different data sets were used for this logistic regression, different in interpretation of the results: one considered results positive, inconclusive or negative based on Table 5.1. In the other dataset we defined animals positive with CIDT >3 mm and inconclusive with CIDT 2-3 mm, but only if the bovine increase was at least 4 mm.

Additionally, an ANOVA was performed in RStudio with as response variable the continuous results of the difference between bovine and avian increase in skin thickness (CIDT). This increase was compensated in the analysis for the base values of the bovine site skin thickness.

6. Results

6.1. Questionnaires

6.1.1. Farm managers

All farms were within a range of 2.5 kilometres from the fence of Akagera NP, were fenced with *Euphorbia* bushes and except for one modern farm they all had an extensive traditional farming system. Of all farms, 60% had grazing areas next to the fence. The herd sizes ranged from 15 to 97 animals. All herds consisted of cross breeds, two had exotic breeds (mainly Holstein Friesian) and ten had local breeds (mainly Ankole). On all farms most animals were born there, but 53-60% have animals originating from the community or local markets and 27% of the farms have animals originating from other districts (Bugesera, Kirehe, Gicumbi, Nyagatare and Gatsibo). In the past year, 33% had introduced new cattle. See Appendix VI for the questionnaire results per herd.

Intra- and interspecies contact

Of the herds, 67% had contact with other herds, due to shared water points, shared enclosures for spraying against ectoparasites or vaccination and communal grazing in the case of two herds.
On 87% of the farms the cattle had contact with dogs or goats. Other domestic animals, like chicken (*Gallus domesticus*), sheep (*Ovis aries*) and cats (*Felis catus*), were also present on some farms.

Buffaloes were seen on 40% of the farms. Hyenas and antelope species like topi, bushbuck and impala were seen on 87% of the farms and primates like vervet monkeys (*Chlorocebus pygerythrus*) and olive baboons (*Papio anubis*) on 80%. In the Gahini district hippopotami (*Hippopotamus amphibius*) were seen on four out of five farms. Other wildlife species that were seen on some farms are warthogs, leopards (*Panthera pardus*), zebras and bushpigs (*Potamochoerus larvatus*). Wildlife seen on the other side of the fence (inside the park) are the above-mentioned species and lions, elephants and giraffes in addition.
Most of the farmers (87%) said they saw direct contact between the herd and wildlife, mainly with antelope species and primates, but also with buffaloes, hippopotami, warthogs and others.

Public health

On 40% of the farms there was raw milk consumption, mostly by cowboys in the field. There was no blood or raw meat consumption on any of the farms.

Animal health

Of the farm managers, 93% said there were many tsetse flies and ticks. All farms had cases of trypanosomiasis and tick-borne diseases (East Coast fever, anaplasmosis, babesiosis and/or heartwater), 53% had an outbreak of FMD and 47% had brucellosis cases in the past years. Two farms mentioned Rift Valley fever (RVF) and one had an anthrax case five years ago.
All farms had seen respiratory, digestive and chronic symptoms in the past year. Many mentioned nutritional stress due to the dry season. Some had many sudden deaths in the herd, swelling of the head or blood in urine/faeces. One mentioned a carcass with much bleeding.
Most farms vaccinated for FMD, RVF, lumpy skin disease, black quarter and anthrax. Some farms vaccinated for brucellosis, contagious bovine pleuropneumonia and/or ECF as well. All farms sprayed one or two times per week for ectoparasites, with ashimetrin, rabitraz or nortraz, used preventative treatment for trypanosomiasis, dewormed one to four times a year and treated regularly with ivermectin. Veterinarians are called for dystocia, diseases, reproduction issues, vaccination, et cetera.

6.1.2. Sector animal resources officers

Common diseases in all three sectors were found to be brucellosis, East Coast Fever, anaplasmosis and trypanosomiasis, the latter being the most common disease of cattle in the area. Peste des petits ruminants and taeniasis were common in two sectors. Other diseases that were found present but less common in the sectors are anthrax, FMD, babesiosis, heartwater, RVF, rabies, leptospirosis and bovine tuberculosis.
See Appendix VII for the complete list.

6.2. Presence of bovine tuberculosis in cattle

A total of 150 cattle from 15 herds in three sectors were tested for bTB using the SICCT test (see Appendix VIII for all individual results). Ages were ranging from three months to 11 years, 85% of the cattle was female and 71% was cross, 3% exotic and 26% local breed. Three cows were found positive for bTB, in three different herds. This gives an animal-based apparent prevalence of 2% (95% CI: 0.52-6.2%) and a herd-level apparent prevalence of 20% (95% CI: 5.3-49%) in the research area, for cut-off value CIDT >4 mm. The true prevalence on an animal-level is estimated to be between 0 and 2.8% (based on range in test sensitivity and specificity).

Ten herds had cows that tested inconclusive (ranging from one to six inconclusive results in the herd). Two herds did not have any positive or inconclusive test results, so they tested negative. Tables 6.1 and 6.2 display the number of animals and herds that tested positive, negative and inconclusive (based on cut-off values in Table 5.1). Figure 6.1 shows the bTB herd-level results on a map of the research area.

Sector	+	+/-	-	Animals
Gahini	2	5	43	50
Mwili	1	10	39	50
Murundi	0	4	46	50
Total (%)	**3 (2%)**	**19 (13%)**	**128 (85%)**	**150 (100%)**

Table 6.1 bTB results on the animal level

Table 6.2 bTB results on the herd level

Sector	+	+/-	-	Herds
Gahini	2	2	1	5
Mwili	1	4	0	5
Murundi	0	4	1	5
Total (%)	**3 (20%)**	**10 (67%)**	**2 (13%)**	**15 (100%)**

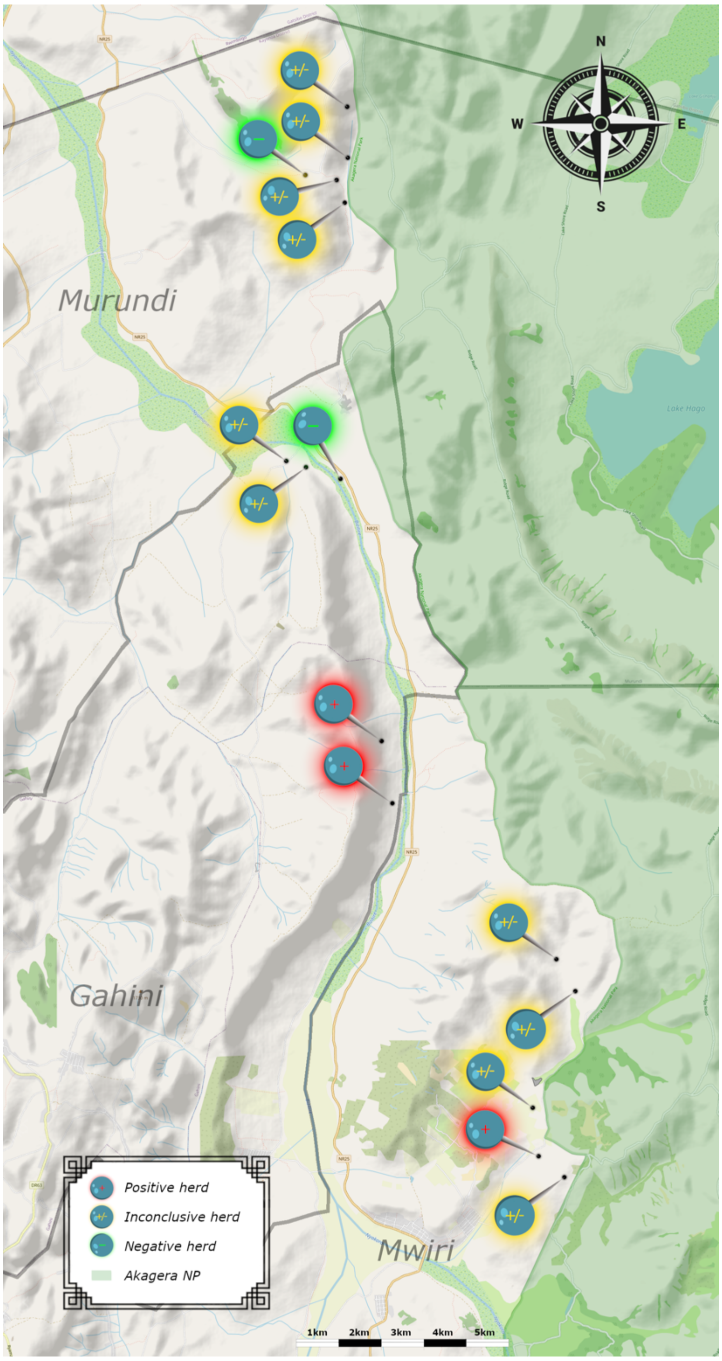

Figure 6.1 Map of the research area showing bTB herd results (CIDT >4 mm). Three farms are positive (red +), two are negative (green -), the rest is inconclusive (yellow +/-).

Using the alternative interpretation, with cut-off value CIDT >3 mm, eight animals were found positive for bTB in six different herds (two in each sector), giving an animal-based apparent prevalence of 5.3% (95% CI: 2.5-11%) and a herd-level apparent prevalence of 40% (95% CI: 17-67%). The true prevalence on an animal level is between 1.5 and 7.7% for cut-off value CIDT >3 mm.

Avian reactors (as an indication of cross-reactivity due to the presence of environmental mycobacteria) were mostly found in the Gahini sector, with a mean avian increase of 3.1 mm (with a spread of 0 to 6.8 mm) compared to an overall mean of 2.1 mm (see Appendix VIII)

The injection areas did not show clear clinical signs like necrosis, exudation, heat, pain, tenderness or adhesions. The measured temperatures were often inconsistent within the same animal on D0 and D3. The inconclusive results could not be considered negative or positive based on clinical observations (see Appendix VIII).

6.3. Present risk factors for contracting bovine tuberculosis

Risk factors for bTB found in literature, that are found to be present in the research area, are: between-herd movements, shared water points, introduction of new animals, mixed rearing of domestic stock (goats), nutritional stress, age (six years and older) and contact with wildlife reservoirs.

On two positive and three inconclusive tested farms there was raw milk consumption.

The two farms in Gahini sector that both tested positive, are further from the park than the other farms, but close to both is a hippopotamus herd that came from inside the park a few years ago and settled in a dam next to the village. The hippopotami visit these farms almost every night.
Of the five farms that had an introduction of cattle last year, three were found positive and two inconclusive. The introduced cattle came from the community or local market on four farms and from Kirehe district on one farm.

6.4. Statistical results on correlation bovine tuberculosis in herds and risk factors

Based on the p-values, no statistically significant correlation has been found between positive/inconclusive cases and the independent variables listed in Table 6.3. So, none of the independent variables seem to have an influence on a cow being tested positive or inconclusive, based on this data set. The factor with the smallest p-value was 'Age', with a p-value of 0.09, an estimate of -0.23 and an Odds ratio interval of 0.59-1.0.
When doing logistic regression with exclusively 'Age' as independent value, no trend was found:
Estimate -0.057 P-value 0.547 OR 0.94 OR interval 0.78 – 1.13

Dependent variable: Infected (positive or inconclusive based on inclusion criteria Table 5.1)					
Independent variables	**Odds ratio**	**Odds ratio confidence intervals**		**Estimate**	**P-value**
		2.5%	**97,5%**		
Exotic breed	2.2 e-08	NA	5.5 e+248	-17.7	0.997
Local breed	0.74	0.18	2.9	-0.297	0.669
Age	**0.80**	**0.59**	**1.0**	**-0.229**	**0.094**
Male	8.8 e-09	9.7 e-257	8.5 e+36	-18.5	0.993
BCS	1.3	0.30	5.1	0.234	0.739
Distance to park	2.5 e-05	1.9 e-21	3.4 e+11	-10.6	0.995
Grazing next to fence	4.8 e+04	6.1 e-122	2.0 e+134	10.8	0.999
Herd size	0.36	0.0031	36	-1.03	0.996
Contact other herds	0.0015	9.4 e-149	3.5 e+20	-6.49	0.995
Contact goats	4.0 e-09	NA	9.2 e+78	-19.3	0.995
Buffaloes on farm	0.0069	3.3 e-07	130	-4.98	0.991
Warthogs on farm	5.0	4.7 e-27	3.1 e+26	1.60	0.999
Antelopes on farm	15	1.9 e-17	6.2 e+18	2.73	0.999
Bushbuck on farm	1.9 e+08	4.5 e-42	8.5 e+57	19.1	0.997
Primates on farm	2.5 e-27	2.4 e-175	3.6 e+118	-61.3	0.997
Hippopotami on farm	0.061	8.8 e-39	2.7 e+35	-2.80	0.999
Direct contact wildlife	3.4 e-10	1.1 e-128	2.6 e+102	-21.8	0.999
Cattle origin outside district	4.6 e+04	2.1 e-24	1.8 e+112	10.7	0.993
Introduction last year	5.1 e+04	1.4 e-17	1.4 e+26	10.8	0.996

Table 6.3 Results linear regression and Odds ratios

The logistic regression performed on the dataset with different inclusion criteria (positive with CIDT >3 mm and inconclusive with CIDT 2-3 mm, with a bovine increase of at least 4 mm) and the ANOVA test using a continuous response variable (CIDT), also showed no relation between the independent variables and cattle being positive or inconclusive.

7. Discussion

7.1. Summary of results

This study found bTB-positive cattle living at the human-livestock-wildlife interface next to Akagera NP in the Kayonza district, using the SICCT test. We found an apparent animal-prevalence of 2% (95% CI: 0.52-6.2) and a herd-prevalence of 20% (95% CI: 5.3-49%), for cut-off value CIDT >4 mm. Possible sources for bovine tuberculosis in this area could be the introduction of new animals or contact with wildlife reservoirs. On 40% of the farms, including two positive farms, there was raw milk consumption, emphasising the zoonotic risk of bTB in this area. Risk factors for cattle contracting bovine tuberculosis that are present in this area are contact between different herds, shared water points, mixed rearing of cattle and goats, introduction of new animals, nutritional stress, many animals of six years and older and contact with wildlife reservoirs.
This study suggests, based on results from questionnaires, that many wildlife species, from in- and outside of the park, visit farms and have direct contact with the domestic animals. Among these are buffaloes and different antelope species, that share many diseases with our domestic ruminants. A herd of hippopotami that is originally from Akagera NP, visits two bTB-positive farms almost every night. Furthermore, primates, warthogs and carnivores are frequently seen on farms and many of these species are hosts of bTB. Although most are bTB spillover hosts and the risk associated with spillover hosts is lower than with maintenance hosts, they can still be a potential source of infection. This proximity and close contact give a high probability of direct and indirect transmission of diseases between livestock and wildlife. Also, the abundance of vector species like tsetse flies and ticks make it likely for spillover to take place.
African buffalo are seen on 40% of the farms we visited. Buffaloes are not only a reservoir species for bovine tuberculosis but also play an important role in other cattle diseases, like brucellosis, tick-borne diseases and trypanosomiasis.
There are many zoonotic diseases present in this area and people are at risk of contracting these due to close contact with livestock, unhygienic milk procedures and consumption of uncooked milk.

7.2. Study limitations

The results of this research are interesting on a qualitive level, but the sample size of 15 farms for the interviews was not sufficient to make conclusions quantitatively about risk factors in relation to infection with bTB. Also the number of positive cases was too low for a good statistical analysis. Defining the inconclusive cases as positive for analysis purposes can be risky, leading to false correlations, as many inconclusive individuals are likely to have had a cross-reaction and in reality could be negative for *M. bovis*. This could have been the reason for almost having a trend result between younger age and infection with *M. bovis*, while in literature it states that old age (six years and older) is a risk factor for contracting bTB, not young age.

Furthermore, all farms shared many of the same risk factors, making it hard to compare between them. For example, even though we made two different groups (grazing next to the fence of the park or not), they were all in close proximity to Akagera NP and all had wildlife species coming on their land. To make a good comparison, it would have been better to have tested a second group of farms, much further away from the park, where there are no buffaloes, antelopes, hippopotami, etc. This group would have to be comparable concerning herd sizes and farming system. The problem is that it is hard to find the type of farms present next to Akagera NP, in areas further away from the park, because there is less space for extensive cattle farming, grazing and larger herds. Farms further away from the park generically have few cows and they are often kept inside.

The animal-prevalence of 2% (95% CI: 0.52-6.2) is corresponding to the animal-prevalence found in cattle in the Serengeti in 2011 of 2.4% (95% CI: 1.7-3.5%). The confidence interval of our study is wider, caused by the smaller sample size: 150 versus 1103 cattle.

The tuberculin skin test is a herd test, so after finding one positive we considered the farm as positive. Specificity is not 100% so there is always a chance of getting a result that is a false positive, especially due to cross-reactivity to environmental mycobacteria. From the positive farms, two farms had inconclusive(s) result(s) in addition to the positive result, but in one farm the other cows were found negative. Another farm had six inconclusive results, of which three would be considered positive with the other inclusion criteria (CIDT >3 mm instead of >4 mm). It could be more likely for that last farm to be infected with *M. bovis* than the farm with exclusively one positive result. These two herds were actually in contact with each other through a shared enclosure and water point. It is hard to draw conclusions, also, because the sensitivity of the test is relatively low (68-95%), which can result in many false negatives.

It is possible to say for an inconclusive result if it more likely positive or negative, based on clinical signs, like the consistency of the skin reaction, the appearance of the swelling and clinical observations like necrosis, heat, pain, etc. Because we lacked that knowledge at first (not described in the documentation of the tests), we did not look for these clinical signs in the first 80 cows, of which three were positive and 13 inconclusive. After receiving verbal suggestions by professor Anita Michel, we did do these observation for the last 70 cows, but we were not sufficiently experienced to be able to draw conclusions for the remaining six inconclusive results.

We took temperature measurements with the idea to prevent false positives due to illness, or to see if there would be a rise in temperature alongside a positive or inconclusive test. But temperature is very dependent on weather conditions, especially for these extensively held cattle herds, who sometimes have to walk for multiple kilometres in the middle of the day to go to the water point. On sunny and warm days, we often found temperatures above 39 and even reaching 41 degrees Celsius, while on cooler and rainy days the temperatures (in the same herd) were within normal range.

Ideally we would have conducted the research in collaboration with Akagera NP, to get insight into health monitoring results and wildlife populations and distribution, and in the best situation we could have been testing wildlife, especially buffalo, for bTB. Unfortunately, this collaboration could not take place due to refusal of the park authority.

Lastly, as bovine tuberculosis is a zoonosis, it would have been interesting to have asked local hospitals for information about tuberculosis disease burden in the population of the three sectors and specifically for the area next to the park.

In conclusion, this research has much room for improvement, and should be seen as a pilot study. The local research infrastructure was limited, there was a limited time frame, limited (financial) resources, there are many challenges when doing research abroad (not speaking the local language, depending on many different people and availabilities, field conditions, etc.) and it was the first time conducting a field research for the student.

7.3. Recommendations

There is a high risk of disease spillover between species at the human-livestock-wildlife interface next to Akagera NP, due to proximity and (in)direct contact of livestock, wildlife and humans. Monitoring of diseases is extra important here, to be able to intervene at an early stage.

To further identify the risk of bovine tuberculosis at the human-livestock-wildlife interface, wildlife should be tested for bTB, especially buffaloes, in and outside of Akagera NP. Furthermore, the prevalence of bovine tuberculosis in cattle should be determined in the area bordering the north of Akagera NP, in Nyagatare district, as large herds of buffalo can be found in the northern plains of the park.
To be able to quantitively determine a relation between risk factors and bTB prevalence, the sample size of these studies should be bigger than in this study.

It is important to create awareness about zoonotic diseases among the local population and emphasize on boiling milk and ensuring hygiene during milking procedures and close contact with animals, due to the presence of bovine tuberculosis and other diseases like brucellosis. Especially since we found bTB-positive cows on farms where people consume raw milk.

To effectively protect wildlife, humans and domestic animals in the Kayonza district, a One Health collaboration should be established which should certainly include Akagera NP (managed by African Parks). By sharing disease monitoring results, parties like VSF, local authorities, Akagera NP and local hospitals, can work together on preventing outbreaks. Akagera NP could start doing more disease monitoring for some important diseases, especially in reservoir species like buffalo and endangered species like the black rhino and elephant.

With this research we have shown that it is not easy to quantify the risk factors of diseases in a complex system, even if it is a specific disease in a specific area of which there is relatively much knowledge, local awareness, and a rich scientific literature. Despite that, this research underscores the risks of high-impact (zoonotic) diseases at the human-livestock-wildlife interface and the need for a control strategy. Making a risk assessment for multiple diseases requires time since much information is needed and it is a complex system with many different factors playing a role in epidemiology. It would be relevant to create a risk score in order to take specific measures to protect humans, domestic animals and wildlife. These specific measures could include mass vaccination of domestic animals or wildlife, enhancing biosecurity in specific animal husbandry practices and expanding surveillance for specific pathogens.

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Disclaimer

Performing diagnostics for bovine tuberculosis during this research were done for academic purposes. It is not part of a government monitoring program, but the results have been shared with the Rwanda Agricultural Board. The results are not confirmed with further diagnostics and there are no consequences for the farms based on these results.

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Appendix I: mammal species in Akagera national park

Group	**Species**	**IUCN status** LC = least concern NT = near threatened VU = vulnerable EN = endangered CR = critically endangered	**bTB susceptibility** Red = maintenance host Yellow = possible maintenance host Green = spillover host
Large mammals	Cape buffalo (*Syncerus caffer)* [5]	NT	
	Savanna elephant (*Loxodonta africana*) [62]	EN	
	Hippopotamus (*Hippopotamus amphibius*) [63]	VU	
	Masai giraffe (*Giraffa camelopardalis* ssp. *tippelskirchi*) [5]	EN	
	Plains zebra (*Equus quagga*)	NT	
	Rhinoceros Black (*Diceros bicornis*) [5]	CR	
	Rhinoceros White (*Ceratotherium simum*) [5]	NT	
Antelopes	Bohor reedbuck (*Redunca redunca*)	LC	
	Bushbuck (*Tragelaphus scriptus*) [5]	LC	
	Cape eland (*Tragelaphus oryx*) [5]	LC	
	Common duiker (*Sylvicapra grimmia*) [5]	LC	
	Defassa waterbuck (*Kobus ellipsiprymnus* ssp. *defassa*)	NT	
	Impala (*Aepyceros melampus*) [5]	LC	
	Klipspringer (*Oreotragus oreotragus*)	LC	
	Oribi (*Ourebia ourebi*)	LC	
	Roan (*Hippotragus equinus*)	LC	
	Sitatunga (*Tragelaphus spekii*)	LC	
	Topi (*Damaliscus lunatus*) [5]	LC	
Primates	Blue monkey (*Cercopithecus mitis*)	LC	
	Northern lesser bushbaby (*Galago senegalensis*)	LC	
	Olive baboon (*Papio anubis*) [5]	LC	
	Thick-tailed greater bushbaby (*Otolemur crassicaudatus*)	LC	
	Vervet monkey (*Chlorocebus pygerythrus*) [5]	LC	
Suids	Bushpig (*Potamochoerus larvatus*) [5]	LC	
	Warthog (*Phacochoerus africanus*) [5]	LC	
Big carnivores	Leopard (*Panthera pardus*) [5]	VU	
	Lion (*Panthera leo*) [5]	VU	
	Serval (*Leptailurus serval*)	LC	
	Side-striped jackal (*Lupulella adustus*)	LC	
	Spotted hyena (*Crocuta crocuta*)	LC	
Others	Aardvark (*Orycteropus afer*)	LC	
	African civet (*Civettictis civetta*) [5]	LC	
	Banded mongoose (*Mungus mungo*) [5]	LC	
	Greater ground pangolin (*Smutsia gigantea*)	EN	
	Honey badger (*Mellivora capensis*) [5]	LC	
	Large spotted genet (*Genetta maculata*) [5]	LC	
	Porcupine (*Hystrix spp.*)	LC	
	Scrub hare (*Lepus saxatilis*)	LC	
	Spotted-necked otter (*Hydrictis maculicollis*)	NT	
	Temminck's pangolin (*Smutsia temminckii*)	VU	

Appendix II: questionnaire farm manager

Many questions were based on a bovine tuberculosis study in Botswana [59]

General cattle management	
1. Are you the main person who looks after the cows?	Yes ☐ No ☐
2. How many cattle (bulls, cows and calves) do you own?	Total number: Bulls: Cows: Calves:
3. How many of them are older than 42 days (1,5 months)?	Number:
4. What breed?	Local breed ☐ cross breed ☐ exotic breed ☐
5. Do your cattle graze?	Yes ☐ No ☐ If yes: fenced area ☐ communal land ☐ Does it change depending on the season? Do they graze with other stock? How far do your cattle move for grazing?
Contacts with other animals	
6. Can you describe the places where your cattle graze/live? Can you see the fence?	Description: Yes ☐ No ☐ Which wildlife do you see on the other side of the fence?
7. Can your cattle reach the fence of the National Park?	Yes ☐ No ☐
8. Have you seen wildlife outside the park, on your land?	Yes ☐ No ☐ If yes: which wild animals do you most frequently see? How often does it happen? How long do they stay?
9. Have you seen direct contact between wildlife from the park and the herd?	Yes ☐ No ☐ If yes: how often?
10. Is there direct contact between your herd and other herds?	Yes ☐ No ☐
11. Is there direct contact between your herd and other domestic animals (like goats/dogs)?	Yes ☐ No ☐ If yes, specify what animals:
Animal purchase	
12. Did you inherit your cattle or buy them outside?	
13. Where do you buy your cattle from?	Local market ☐ community ☐ outside ☐
14. Have you introduced new cattle into your herd since last year?	Yes ☐ No ☐ If yes: where from?

Animal Health	
15. Are your cattle vaccinated for any diseases?	Yes ☐ No ☐ If yes: for what diseases:
16. Are there tsetse flies and/or ticks surrounding your cattle?	Yes ☐ No ☐ If yes, how many?
17. Do you spray your cattle against ectoparasites like ticks?	Yes ☐ No ☐ If yes, how often and what drugs?
18. Did your cattle get treatment for parasites like ticks?	Yes ☐ No ☐
19. How often do you deworm your cattle?	
20. Have your cattle shown any of these symptoms in the past year?	☐ Respiratory (coughing, sneezing, dyspnea, nasal discharge) ☐ Digestive (diarrhea, weight loss, no appetite) ☐ Chronic (low milk yield, growth retardation, rough coat) ☐ Other (fever, anemia, skin problems, lameness, sudden death, etc.)
21. Have your cattle been suffering from these diseases (as far as you know)?	☐ Trypanosomiasis ☐ East Coast Fever/ theileriosis ☐ Anaplasmosis ☐ Babesiosis ☐ Ehrlichiosis/ heartwater ☐ Foot & mouth disease (FMD) ☐ Brucellosis ☐ Rift Valley fever ☐ Anthrax ☐ Bovine tuberculosis
22. Did you encounter health problems for which you called a vet or animal health worker?	Yes ☐ No ☐ If yes, what problems?
Public health	
23. Do you drink raw milk from your cattle?	Yes ☐ No ☐
24. Do you drink blood from your cattle?	Yes ☐ No ☐ If yes: do you boil it first? Yes ☐ No ☐
25. Do you eat raw meat from your cattle?	Yes ☐ No ☐

Appendix III: questionnaire sector animal resources officers

Sector: Murundi ☐ Gahini ☐ Mwiri ☐

Disease	Present?	Occurrence	Last outbreak/case
Bovine tuberculosis	☐Yes ☐No ☐Don't know	☐Common ☐Not common	
Anthrax	☐Yes ☐No ☐Don't know	☐Common ☐Not common	
Brucellosis	☐Yes ☐No ☐Don't know	☐Common ☐Not common	
Foot & mouth disease	☐Yes ☐No ☐Don't know	☐Common ☐Not common	
East Coast fever / theileriosis	☐Yes ☐No ☐Don't know	☐Common ☐Not common	
Babesiosis	☐Yes ☐No ☐Don't know	☐Common ☐Not common	
Anaplasmosis	☐Yes ☐No ☐Don't know	☐Common ☐Not common	
Crimean-Congo hemorrhagic fever	☐Yes ☐No ☐Don't know	☐Common ☐Not common	
Ehrlichiosis / heartwater	☐Yes ☐No ☐Don't know	☐Common ☐Not common	
Rift Valley fever virus	☐Yes ☐No ☐Don't know	☐Common ☐Not common	
Trypanosomiasis	☐Yes ☐No ☐Don't know	☐Common ☐Not common	
Bluetongue	☐Yes ☐No ☐Don't know	☐Common ☐Not common	
Peste des petits ruminants	☐Yes ☐No ☐Don't know	☐Common ☐Not common	
Rabies	☐Yes ☐No ☐Don't know	☐Common ☐Not common	
Nipah virus	☐Yes ☐No ☐Don't know	☐Common ☐Not common	
Trichinellosis	☐Yes ☐No ☐Don't know	☐Common ☐Not common	
Echinococcosis	☐Yes ☐No ☐Don't know	☐Common ☐Not common	
Taeniasis (*T. solium, saginata & ovis*)	☐Yes ☐No ☐Don't know	☐Common ☐Not common	
Leptospirosis	☐Yes ☐No ☐Don't know	☐Common ☐Not common	
Toxoplasmosis	☐Yes ☐No ☐Don't know	☐Common ☐Not common	

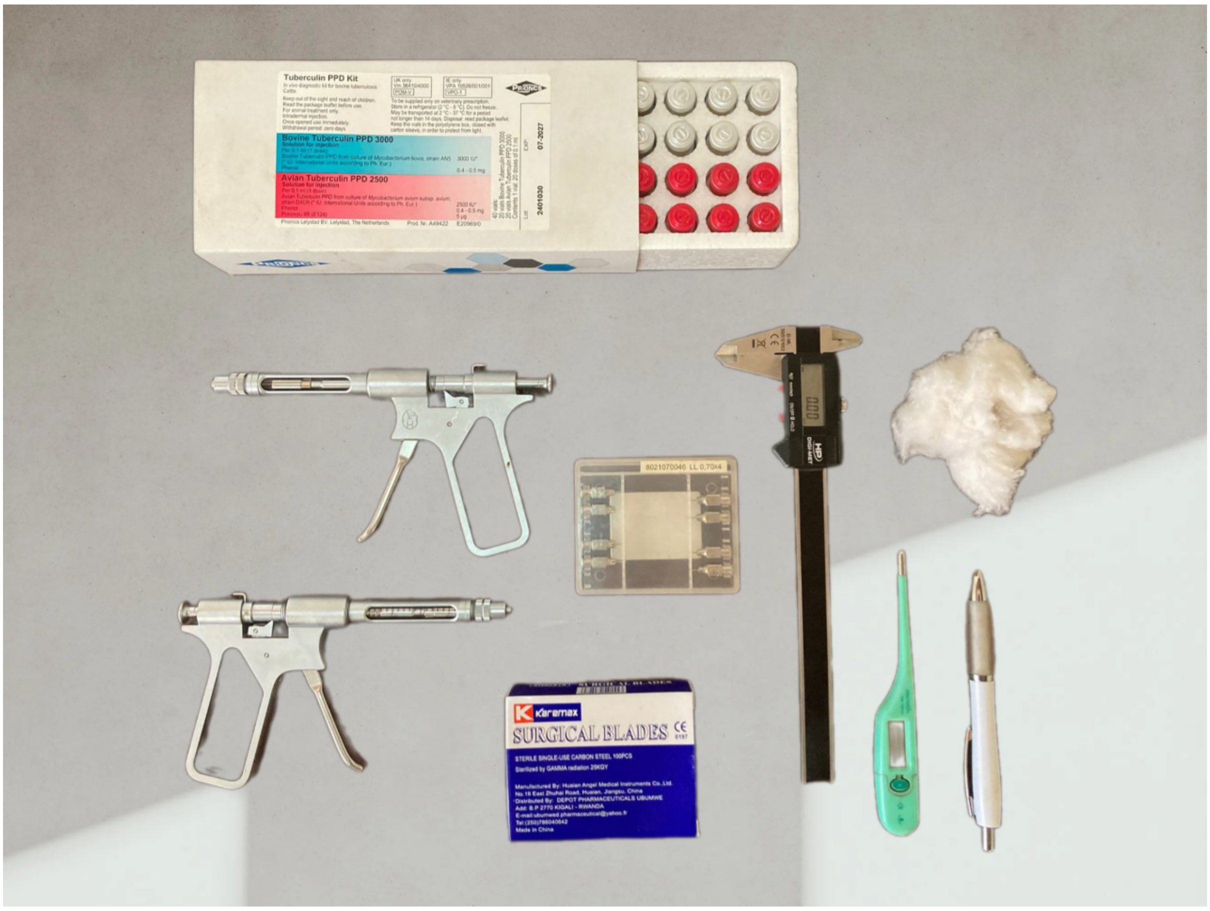
Appendix IV: information to be noted during testing

Cow (nr)	Herd (nr)	Breed	Age (years)	Sex	BCS	Additional notes	Temp.	T0 skin (mm)	72h skin (mm)	Swelling	Consistency (hard/soft/oedema	Appearance (circum/diffuse/flat)	Clinical observations (necrosis, exudation, heat, pain, tender, adhesions, lymph nodes)
								A: B:	A: B:				
								A: B:	A: B:				
								A: B:	A: B:				
								A: B:	A: B:				
								A: B:	A: B:				
								A: B:	A: B:				
								A: B:	A: B:				
								A: B:	A: B:				
								A: B:	A: B:				
								A: B:	A: B:				

Appendix V: materials needed for testing

Materials:

- Bovine tuberculin PPD 3000 (0.1 ml per test)
- Avian tuberculin PPD 2500 (0.1 ml per test)
- 2 HSW HENKE-JECT® TBC syringes
- Needles (0,70 x 4mm)
- Calliper
- Surgical blades for shaving
- Gloves
- Cotton wool
- Thermometer
- Questionnaires (Appendix II)
- Notepaper (Appendix IV)
- Pen



Appendix VI: results questionnaires farm managers

General farm information:

Herd	Can reach fence while grazing	Herd size	Breed	Grazing place	Cattle origin	Introduction cattle last year
1	No	47	Cross	Fenced area	Inherited, local market	No
2	Yes	97	Cross	Fenced area	Inherited, outside (Bugesera)	No
3	No	38	Cross	Fenced area, communal land	Inherited, local market	No
4	No	20	Cross, exotic	Fenced area	Inherited, community, outside (Kirehe & Gicumbi)	Yes: outside district (Kirehe)
5	No	27	Cross, local	Fenced area	Inherited, local market, community	Yes: local market, community
6	Yes	30	Cross, local	Fenced area	Inherited	No
7	No	28	Cross, local	Fenced area	Inherited, community	Yes: community
8	No	39	Cross, local	Fenced area	Inherited, community, outside (Nyagatare)	No
9	Yes	15	Cross, local	Fenced area	Inherited, local market, community	Yes: community
10	Yes	30	Cross, local	Fenced area, communal land	Inherited, local market, community	No
11	Yes	29	Cross, local	Fenced area	Inherited, local market	Yes: local market
12	Yes	20	Cross, local	Fenced area	Inherited, community	No
13	Yes	42	Cross, local	Fenced area	Inherited, local market, community	No
14	Yes	38	Cross, exotic	Fenced area	Inherited, outside (Gatsibo)	No
15	Yes	28	Cross, local	Fenced area	Inherited, local market, community	No

Intra- and interspecies contact (antelope = topi):

Herd	Stay in farm for drinking & spraying	Contact with other herds	Contact with other domestic animals	Wildlife seen in park	Wildlife seen on farm	Direct contact cattle with wildlife
1	Yes	No	Dogs, goats	Buffalo, warthog, zebra	Buffalo, antelope, hippo, hyena	Yes: antelope
2	Yes	No	Dogs, goats	Buffalo, warthog, lion, antelope, impala, elephant, zebra	Antelope, impala, zebra, hippo, hyena	Yes: hippo
3	No	Yes	Dogs, goats	Buffalo, impala, zebra, hyena	Buffalo, hyena	Yes
4	Yes	No	Dogs, goats, chicken	No	Antelope, bushbuck, monkey, hippo, hyena	Yes: bushbuck, monkey
5	No	Yes	Dogs	No	Antelope, bushbuck, monkey, hippo, hyena	Yes: bushbuck, monkey
6	No	Yes	Dogs, goats, sheep, chicken	Buffalo, warthog, impala, bushbuck, monkey, giraffe, elephant	Warthog, bushbuck, baboon, monkey, hyena	Yes: monkey
7	No	Yes	Dogs, goats, chicken	Buffalo, warthog, topi, impala, bushbuck, monkey, baboon, zebra, elephant	Monkey, baboon	No
8	No	Yes	Dogs, goats, sheep, chicken	Buffalo, warthog, lion, topi, impala, bushbuck, monkey, baboon, zebra	Bushbuck, monkey, baboon, hyena	No
9	Yes	Yes	Dogs, goats, chicken, cats	Buffalo, warthog, antelope, monkey, baboon, hyena	Buffalo, warthog, antelope, monkey, baboon, hyena	Yes: warthog, antelope, monkey, baboon, hyena

10	No	Yes	No	Antelope, baboon, hyena	Antelope, monkey, baboon, hyena	Yes: antelope, monkey, baboon, hyena
11	Yes	No	Dogs, goats, sheep, chicken	Buffalo, warthog, antelope, impala, baboon, zebra, giraffe, hyena	Antelope, impala, baboon, hyena	Yes
12	Yes	No	Goats	Buffalo, warthog, antelope, impala, monkey, baboon, zebra, giraffe, hyena	Antelope, monkey, baboon, hyena, leopard	Yes: monkey, hyena
13	Yes	Yes	Dogs, goats, sheep, chicken	Buffalo, warthog, lion, antelope, impala, monkey, baboon, zebra, giraffe, hyena	Buffalo, warthog, antelope, impala, bushbuck, baboon, zebra, hyena, leopard	Yes: antelope, bushbuck, zebra
14	No	Yes	Dogs, goats, chicken, cats	Buffalo, warthog, lion, antelope, impala, monkey, baboon, zebra, giraffe, hyena	Buffalo, bushbuck, impala, monkey, baboon, bushpig, zebra	Yes: buffalo, impala, monkey, baboon, zebra
15	No	Yes	Dogs, goats, chicken, cats	Buffalo, warthog, lion, antelope, impala, monkey, baboon, zebra, giraffe, hyena	Buffalo, warthog, antelope, impala, bushbuck, baboon, zebra, hyena, leopard	Yes: buffalo, impala, bushbuck, monkey, baboon, zebra, hyena

Animal health:

Herd	**Vaccinations**	**Presence of tsetse flies & ticks**	**Spraying frequency**	**Spraying drugs**	**Treatment ectoparasites**	**Deworming**
1	Anthrax, brucellosis, RVF, FMD, bq	Many	2 times a week	Ashimetrin or permapy	Yes: ivermectin	4 times a year
2	RVF, FMD, bq, LSD	Many	2 times a week		Yes: ivermectin	4 times a year
3	FMD, LSD, CBPP	Many	1 time a week		Yes	1 time a year
4	Anthrax, RVF, ECF, FMD, bq, LSD	Many	2 times a week		Yes: ivermectin	2 times a year
5	Anthrax, RVF, ECF, FMD, bq, LSD	Many	2 times a week	Ashimetrin	Yes: ivermectin	2 times a year
6	Anthrax, brucellosis, RVF, FMD, bq, LSD	Many	1 time a week	Ashimetrin (or rabitraz?)	Yes: ivermectin	2 times a year
7	Anthrax, brucellosis, RVF, FMD, bq, LSD	Many	2 times a week	Ashimetrin (or rabitraz?)	Yes: ivermectin	2 times a year
8	Anthrax, brucellosis, RVF, FMD, bq, LSD	Many	1 time a week	Rabitraz	Yes: ivermectin	2 times a year
9	Anthrax, brucellosis, RVF, FMD, bq, LSD	Many	1 time a week	Rabitraz	Yes: ivermectin	2 times a year
10	Anthrax, RVF, FMD, LSD, CBPP, bq	Many	1 time a week	Rabitraz	Yes: ivermectin	2 times a year
11	RVF, FMD, LSD	Many	1 time a week	Rabitraz	Yes: ivermectin, trypashish	2 times a year
12	Anthrax, RVF, FMD, LSD, CBPP, bq	Not many	1 time a week	Rabitraz	Yes: ivermectin, trypashish	2 times a year
13	Anthrax, RVF, FMD, LSD, bq	Many	1 time a week	Nortraz	Yes: ivermectin, trypashish	2 times a year
14	Anthrax, RVF, FMD, LSD, bq	Many	2 times a week	Nortraz	Yes: ivermectin, trypashish	2 times a year
15	Anthrax, RVF, FMD, LSD, bq	Many	1 time a week	Nortraz	Yes: ivermectin, trypashish	2 times a year

Animal health and public health:

Herd	Disease symptoms past year	Diseases in herd	Health problems requiring a vet	Raw milk consumption	Blood or raw meat consumption
1	Respiratory, digestive, chronic, other	Trypanosomiasis, ECF, anaplasmosis, babesiosis, heartwater, brucellosis	Dystocia, disease, injury	No	No
2	Respiratory, digestive, chronic, other, pain in chest and go in coma	Trypanosomiasis, ECF, anaplasmosis, babesiosis, heartwater, FMD, brucellosis	Dystocia	Yes	No
3	Respiratory, digestive, chronic, other	Trypanosomiasis, ECF, anaplasmosis, FMD	Dystocia, disease	Yes	No
4	Respiratory, digestive, chronic, other	Trypanosomiasis, ECF, anaplasmosis, babesiosis, heartwater, FMD, RVF	Dystocia, disease	Yes	No
5	Respiratory, digestive, chronic, other	Trypanosomiasis, ECF, anaplasmosis, babesiosis, heartwater, FMD	Dystocia, disease, AI	Yes	No
6	Respiratory, digestive, chronic, other	Trypanosomiasis, ECF, anaplasmosis, babesiosis, heartwater, brucellosis	Dystocia, disease	No	No
7	Respiratory, digestive, chronic, other	Trypanosomiasis, ECF, anaplasmosis, babesiosis, heartwater	Disease, injury, placenta retention, snake bite, AI	No	No
8	Respiratory, digestive, chronic, other, swelling neck	Trypanosomiasis, ECF, anaplasmosis, babesiosis, heartwater	Dystocia, disease	Yes	No
9	Respiratory, digestive, chronic, other	Trypanosomiasis, ECF, anaplasmosis, babesiosis, heartwater, brucellosis	C-section, disease	No	No
10	Respiratory, digestive, chronic, other, many sudden deaths, tremor, polyuria, hematuria	Trypanosomiasis, ECF, anaplasmosis, babesiosis, heartwater, FMD, brucellosis, RVF, anthrax 5 years ago	Dystocia, disease, vaccination	Yes	No
11	Respiratory, digestive, chronic, other, swelling head, hematuria, lacrimation	Trypanosomiasis, ECF, anaplasmosis, FMD in 2021	Dystocia, vaccination	No	No
12	Respiratory, digestive, chronic, other, swelling nose, carcass with bleeding	Trypanosomiasis, ECF, anaplasmosis, babesiosis	C-section, disease	No	No
13	Respiratory, digestive, chronic, other, dry feces with blood	Trypanosomiasis, ECF, anaplasmosis, babesiosis, heartwater, FMD, brucellosis	Disease	No	No
14	Respiratory, digestive, chronic, other (but no sudden death)	Trypanosomiasis, ECF, anaplasmosis, babesiosis, heartwater, FMD many years back, brucellosis many years back	Dystocia, disease, placenta retention	No	No
15	Respiratory, digestive, chronic, other (sudden death very common)	Trypanosomiasis, ECF, anaplasmosis, babesiosis, heartwater, FMD in 2023, brucellosis	Dystocia	No	No

Appendix VII: results questionnaires sector animal resources officers

Green: present, common
Yellow: present, not common
Grey: don't know
White: not present

Disease	Murundi	Gahini	Mwiri
Bovine tuberculosis			2018
Anthrax	2023		2019
Brucellosis	2024		2024
Foot and mouth disease	2024		2023
East Coast Fever/ theileriosis	2024		2024
Babesiosis	2024		2023
Anaplasmosis	2024		2024
Crimean-Congo hemorrhagic fever			
Ehrlichiosis/heartwater	2024		2021
Rift Valley fever virus	2022		2022
Trypanosomiasis	2024		2024
Bluetongue			
Peste des petits ruminants	2022		2023
Rabies	2023		2023
Nipah virus			
Trichinellosis			
Echinococcosis			
Taeniasis (solium, saginata & ovis)	Ovis, 2024		2021
Leptospirosis			2022
Toxoplasmosis			

Appendix VIII: animal results bTB testing

Herd	Breed	Age (years)	Sex	BCS (../5)	Temperature T0	Temperature 72h	Additional notes
1	Cross	6	Female	2.5	38.2	38.5	
1	Cross	3	Female	3	38.7	38.6	rough haircoat, treated for trypanosomiasis
1	Cross	3	Female	3	38.5	38.4	
1	Cross	4	Female	3	38	38.5	
1	Cross	7	Female	3	38.7	38.7	
1	Cross	4	Female	2.5	38.6	38.6	
1	Cross	8	Female	3	38.5	38.3	
1	Cross	5	Female	3	38.6	39.7	
1	Cross	4	Female	3	38	38.5	
2	Cross	5	Female	3	38.4	38.4	
2	Cross	6	Female	3	38.3	38.5	
2	Cross	5	Female	4	39.7	39.1	
2	Cross	7	Female	2.5	38.2	38.6	
2	Cross	6.5	Female	3	38.6	38.7	
2	Cross	0.33	Male	3	39.6	39.4	
2	Cross	2	Female	3	39.3	38.6	used to have LSD
2	Cross	3	Female	3	39.3	38.5	
2	Cross	3	Female	3	39.6	39.9	rough haircoat
2	Cross	4	Female	3	39.2	38.5	
3	Cross	4	Female	2.5	39.4	39.6	
3	Cross	8	Female	2.5	39.1	38.8	
3	Cross	3	Female	3	39.1	38.5	
3	Cross	1	Female	3	39.1	38.8	
3	Cross	0.67	Male	3	40	39.4	
3	Cross	3	Female	2.5	39.3	39.1	
3	Cross	6	Female	2	39.2	38.6	empty rumen
3	Cross	3	Female	2	39.8	38.4	
3	Cross	5	Female	2.5	39.2	38.7	
3	Cross	2	Female	3.5	39.3	38.2	pregnant
3	Cross	3	Female	3	39.2	39	
4	Cross	6	Female	2	38.3	38.7	
4	Cross	8	Female	2	38.8	38.7	
4	Cross	6	Female	4	38.7	39.1	pregnant
4	Exotic	2	Male	3	38.8	38.5	rough haircoat, treated for trypanosomiasis
4	Exotic	7	Female	2	38.1	37.8	
4	Exotic	5	Female	2.5	38.2	38.3	
4	Cross	4	Female	3	38.5	37.5	
4	Cross	6	Female	2.5	38.6	38.2	introduced two years back
4	Cross	1.5	Female	3	38.4	38.6	lethargic look at D3
4	Exotic	0.58	Male	2.5	39.1	38.7	
5	Local	6	Female	2.5	38.9	38.9	
5	Cross	0.67	Female	3	39.3	39.5	
5	Cross	1	Female	3.5	38.8	39.2	
5	Cross	0.58	Male	3	39.6	40.4	wound on leg
5	Cross	1	Male	2	39.8	40.2	trypanosomiasis treatment ends today (D0)
5	Cross	8	Female	2	38.5	38.7	
5	Cross	0.25	Female	2	39.4	39.3	
5	Cross	0.5	Male	2	39.9	39.5	
5	Cross	1	Male	2.5	39.6	40	dermatomycosis around eye
5	Cross	2	Female	3.5	39.1	39	
6	Local	3	Female	2	38.7	39.9	
6	Local	2.5	Female	2.5	38.5	38.4	
6	Local	7	Female	2	38.7	38.2	
6	Local	4	Female	2.5	38.3	38.6	
6	Cross	3	Female	2.5	38.9	38.4	
6	Local	8	Female	3	39.1	38.4	
6	Cross	3	Female	2.5	38.8	38.2	rough haircoat
6	Local	2	Female	3.5	39	38.5	
6	Cross	7	Female	2	39	38.6	
6	Cross	5	Female	2.5	39.7	38.2	
7	Local	3	Female	2	38.8	39	
7	Local	3	Female	2	38.9	38.7	
7	Cross	1.5	Male	3	40.3	40	
7	Local	1.5	Female	3	39.1	41.2	
7	Local	5	Female	2	39.6	38.2	
7	Cross	1.5	Female	2.5	39.6	39.7	
7	Local	3	Female	2	39.2	39.2	
7	Cross	5	Female	2	38.9	38.9	
7	Local	10	Female	2	39.1	39.2	
7	Cross	3	Female	2	38.9	38.4	
8	Cross	5	Female	2.5	38.5	38.4	
8	Cross	8	Female	2.5	39.1	38.7	
8	Cross	6	Female	2.5	39.5	38.7	
8	Local	3	Female	3	39.5	37.6	

8	Local	6	Female	3	39.4	38.3	
8	Local	11	Female	2	38.8	38.3	
8	Local	1.5	Female	3	39.3	38.7	
8	Cross	1	Male	3	40.3	39.1	
8	Local	3	Female	2.5	38.6	38.2	
8	Cross	0.75	Female	3	39.2	38.9	
9	Cross	3	Female	2.5	38.6	38.7	
9	Local	3	Female	3	38.7	38.6	
9	Local	0.5	Female	3	39	39.4	dermatomycosis around eye
9	Cross	6	Female	3	38.7	38.7	
9	Cross	0.5	Female	2.5	39.1	39	
9	Cross	0.5	Female	3	38.8	39	under trypanosomiasis treatment & injection site left neck
9	Local	0.5	Female	3	39.3	39	
9	Local	8	Female	2.5	38.1	38.4	
9	Cross	2	Male	3	38.2	39.3	
9	Cross	3	Female	2.5	38.8	38.8	
10	Local	2	Male	3	39.1	38.6	
10	Local	3	Female	3	38.3	37.7	
10	Cross	8	Female	2.5	39.4	38.5	
10	Local	7	Female	2	39.1	38.6	
10	Local	4	Female	2.5	39.1	37.9	
10	Local	1	Male	3	39.5	38.8	
10	Local	0.58	Male	2	40.3	39.2	
10	Cross	3	Female	2.5	39.3	38.1	
10	Local	5	Female	3	39.4	38.9	
10	Cross	0.42	Female	2.5	40.8	39.4	
11	Cross	0.58	Female	3	39.6	37.7	
11	Cross	2	Male	2.5	38.6	38.2	
11	Local	8	Female	2	38.6	38.1	
11	Cross	8	Female	2	38.4	38.2	
11	Cross	4	Female	2.5	38	37.9	
11	Local	6	Female	2.5	38.4	38.1	
11	Cross	3	Male	3	38.3	37.7	
11	Cross	0.5	Male	3	39.6	38.2	
11	Cross	5	Female	2	38	37	
11	Cross	3	Female	2.5	38.9	38.2	
12	Cross	6	Female	3	38.5	39.1	
12	Local	1.5	Female	3	38.8	41.5	
12	Local	6	Female	2.5	38.7	38.6	
12	Cross	8	Female	2	38.1	37.7	pregnant
12	Cross	1	Female	3	39.6	38.6	
12	Cross	0.42	Female	3	39.3	39.3	
12	Cross	1	Male	3.5	39.3	38.9	
12	Cross	1	Male	3	39	39.1	
12	Local	2	Female	3	39.2	39	
12	Local	2	Female	3	38.9	38.9	
13	Cross	1	Female	3	38.9	39.1	rough haircoat
13	Cross	1	Male	3	38.6	39.2	rough haircoat
13	Cross	1.25	Male	2.5	39.9	38.2	
13	Cross	1.42	Female	3.5	38	38.3	
13	Cross	0.75	Female	2.5	39.3	38.7	
13	Cross	2.25	Male	2.5	38.5	38	
13	Cross	1	Female	2.5	39.1	38.7	dermatomycosis
13	Cross	1.42	Female	2.5	39	38.4	
13	Cross	1.25	Female	3	38.3	38	
13	Cross	1.58	Female	3.5	39.3	38.7	
14	Cross	5	Female	2.5	38.2	38	
14	Cross	9	Female	3	38.2	37.9	
14	Cross	7	Female	2.5	39	37.2	
14	Cross	8	Female	2	38.9	37.3	many lumps on skin
14	Cross	5	Female	2.5	38.3	37.5	under trypanosomiasis treatment
14	Cross	3	Female	2.5	39.1	38.1	scabies on the back
14	Cross	1.42	Female	2.5	39.1	37.7	
14	Cross	5	Female	2.5	39.1	38	
14	Cross	0.33	Female	3	39.9	38.6	received injection fenylbutazol
14	Cross	0.33	Female	2.5	40	38.6	
15	Cross	3	Female	2.5	38.6	38.9	
15	Local	5	Female	2.5	40.8	39.4	
15	Cross	1.5	Female	3	39.3	38.6	
15	Cross	5	Female	2	38.4	37.4	
15	Local	7	Female	3	39.4	38.6	
15	Cross	2	Female	3.5	39.9	39.2	
15	Local	2.42	Female	3	39.6	39.2	
15	Cross	4	Female	2.5	38.9	38.5	
15	Cross	2.42	Female	2.5	41.9	38	
15	Local	1.42	Male	2.5	39.6	39	

Herd	T0 skin avian (mm)	72h skin avian (mm)	Increase avian (mm)	T0 skin bovine (mm)	72h skin bovine (mm)	Increase bovine (mm)	CIDT (mm)	CIDT >4 result	CIDT >3 result	CIDT >3 and bovine increase >4	Notes
1	9.9	14.5	4.6	9.8	12.3	2.5	-2.1	Negative	Negative	Negative	
1	11.8	12	0.2	8	8.8	0.8	0.6	Negative	Negative	Negative	
1	12.2	14.9	2.7	12.5	13	0.5	-2.2	Negative	Negative	Negative	
1	12.6	19.4	6.8	11.2	16.7	5.5	-1.3	Negative	Negative	Negative	
1	11.1	12.3	1.2	7.6	10.8	3.2	2	Inconclusive	Inconclusive	Negative	
1	9.4	10.7	1.3	8.2	11	2.8	1.5	Negative	Negative	Negative	
1	11.8	18.2	6.4	13.7	16.4	2.7	-3.7	Negative	Negative	Negative	
1	13.5	18.2	4.7	11.4	12.3	0.9	-3.8	Negative	Negative	Negative	
1	10.7	14.3	3.6	9.9	9.6	0	-3.6	Negative	Negative	Negative	
2	13.1	19.3	6.2	8.8	15.8	7	0.8	Negative	Negative	Negative	
2	15	18.9	3.9	9.9	13.1	3.2	-0.7	Negative	Negative	Negative	
2	16.1	25	8.9	14.6	20.5	5.9	-3	Negative	Negative	Negative	
2	13.6	20.3	6.7	12.6	15.4	2.8	-3.9	Negative	Negative	Negative	
2	10.2	13.6	3.4	8.9	13.5	4.6	1.2	Negative	Negative	Negative	
2	7.8	9.9	2.1	7.7	11.5	3.8	1.7	Negative	Negative	Negative	
2	8.9	7.5	0	7.7	7.5	0	0	Negative	Negative	Negative	
2	14.1	20.3	6.2	13.5	17.5	4	-2.2	Negative	Negative	Negative	
2	11.3	14.3	3	8	12.4	4.4	1.4	Negative	Negative	Negative	
2	9.8	13.5	3.7	7.5	9.3	1.8	-1.9	Negative	Negative	Negative	
3	14.4	19.5	5.1	11.6	13.1	1.5	-3.6	Negative	Negative	Negative	
3	22.8	29.2	6.4	15.4	20.3	4.9	-1.5	Negative	Negative	Negative	
3	19.7	24.9	5.2	19.5	22.3	2.8	-2.4	Negative	Negative	Negative	
3	15.3	18.2	2.9	12.5	15.6	3.1	0.2	Negative	Negative	Negative	
3	5.1	11	5.9	5.4	7	1.6	-4.3	Negative	Negative	Negative	
3	15	18.5	3.5	11.8	16	4.2	0.7	Negative	Negative	Negative	
3	14.6	17.9	3.3	12.3	17.3	5	1.7	Negative	Negative	Negative	
3	15.4	15.9	0.5	11.5	12.3	0.8	0.3	Negative	Negative	Negative	
3	17.2	21	3.8	12.4	14.4	2	-1.8	Negative	Negative	Negative	
3	18.7	24	5.3	13.6	17.6	4	-1.3	Negative	Negative	Negative	
3	20.7	24	3.3	17.5	23	5.5	2.2	Inconclusive	Inconclusive	Inconclusive	
4	8.3	10.1	1.8	6.7	8.6	1.9	0.1	Negative	Negative	Negative	
4	7.7	9.9	2.2	6.8	6.4	0	-2.2	Negative	Negative	Negative	
4	9.1	10.8	1.7	10.1	9.2	0	-1.7	Negative	Negative	Negative	
4	10.8	10.8	0	6.8	6.9	0.1	0.1	Negative	Negative	Negative	
4	9.8	13.8	4	7.1	11.4	4.3	0.3	Negative	Negative	Negative	
4	8.5	9.8	1.3	7.6	10.2	2.6	1.3	Negative	Negative	Negative	
4	9.2	7.5	0	7.9	6.9	0	0	Negative	Negative	Negative	
4	10.3	11.7	1.4	8	12.2	4.2	2.8	Inconclusive	Inconclusive	Inconclusive	
4	12.4	14.8	2.4	10.4	18	7.6	5.2	Positive	Positive	Positive	
4	8	7.2	0	4.8	5.4	0.6	0.6	Negative	Negative	Negative	
5	9.5	11	1.5	9.5	12	2.5	1	Negative	Negative	Negative	
5	6.8	7.8	1	5.9	9.7	3.8	2.8	Inconclusive	Inconclusive	Negative	
5	9.3	9.8	0.5	6.5	12.6	6.1	5.6	Positive	Positive	Positive	
5	7.8	9.1	1.3	7.9	6.6	0	-1.3	Negative	Negative	Negative	
5	11.4	14.4	3	7.4	11.3	3.9	0.9	Negative	Negative	Negative	
5	12.6	14.7	2.1	11	12.3	1.3	-0.8	Negative	Negative	Negative	
5	4.6	8.1	3.5	3	9.3	6.3	2.8	Inconclusive	Inconclusive	Inconclusive	
5	7.8	7.2	0	4.7	5.6	0.9	0.9	Negative	Negative	Negative	
5	5.6	11.1	5.5	5.5	10.2	4.7	-0.8	Negative	Negative	Negative	
5	15.4	14.3	0	11.8	13.5	1.7	1.7	Negative	Negative	Negative	
6	11.8	10.1	0	10.1	12.3	2.2	2.2	Inconclusive	Inconclusive	Negative	
6	15.2	21.4	6.2	10.4	20.1	9.7	3.5	Inconclusive	Positive	Positive	Less likely *M. bovis* because much avian increase
6	9.3	10.8	1.5	8.7	9	0.3	-1.2	Negative	Negative	Negative	
6	8.5	13.5	5	10	18.7	8.7	3.7	Inconclusive	Positive	Positive	Less likely *M. bovis* because much avian increase
6	11.7	13.6	1.9	7.5	9.2	1.7	-0.2	Negative	Negative	Negative	
6	10.3	11	0.7	9.9	12.1	2.2	1.5	Negative	Negative	Negative	
6	10.2	9.4	0	7.8	10	2.2	2.2	Inconclusive	Inconclusive	Negative	
6	12.5	14.4	1.9	8.7	13.9	5.2	3.3	Inconclusive	Positive	Positive	More likely *M. bovis* because little avian increase
6	12.6	13.2	0.6	10.2	12.4	2.2	1.6	Negative	Negative	Negative	
6	9.6	9.7	0.1	7.5	9.7	2.2	2.1	Inconclusive	Inconclusive	Negative	
7	11.9	13.3	1.4	9.6	11.8	2.2	0.8	Negative	Negative	Negative	
7	10.7	13.7	3	11.9	14.8	2.9	-0.1	Negative	Negative	Negative	
7	10.3	11.5	1.2	6.4	6.3	-0.1	-1.3	Negative	Negative	Negative	
7	11.7	15.4	3.7	10.3	15	4.7	1	Negative	Negative	Negative	
7	10.9	10.6	0	9	9.2	0.2	0.2	Negative	Negative	Negative	
7	9.1	11.5	2.4	8.1	8.7	0.6	-1.8	Negative	Negative	Negative	
7	9.5	10.7	1.2	8.1	10.8	2.7	1.5	Negative	Negative	Negative	
7	9.5	10.3	0.8	8	8.2	0.2	-0.6	Negative	Negative	Negative	
7	13.1	16.5	3.4	9	12.1	3.1	-0.3	Negative	Negative	Negative	
7	9.5	10.4	0.9	7.6	14.9	7.3	6.4	Positive	Positive	Positive	
8	5.5	5.7	0.2	5.5	7	1.5	1.3	Negative	Negative	Negative	
8	9.2	11.7	2.5	8.8	9.9	1.1	-1.4	Negative	Negative	Negative	
8	12.7	14.5	1.8	10	13.8	3.8	2	Inconclusive	Inconclusive	Negative	
8	11.9	12.8	0.9	10	13.5	3.5	2.6	Inconclusive	Inconclusive	Negative	
8	15.7	16.2	0.5	10.2	11.4	1.2	0.7	Negative	Negative	Negative	
8	11.9	17.7	5.8	11.1	13.4	2.3	-3.5	Negative	Negative	Negative	
8	19.7	17.7	0	10.8	12	1.2	1.2	Negative	Negative	Negative	

8	16.3	16.6	**0.3**	8.9	8.6	**0**	**-0.3**	Negative	Negative	Negative	
8	10.6	13.8	**3.2**	9.6	12.5	**2.9**	**-0.3**	Negative	Negative	Negative	
8	8.3	9	**0.7**	5.9	8.2	**2.3**	**1.6**	Negative	Negative	Negative	
9	13.8	15.7	**1.9**	12.9	14.8	**1.9**	**0**	Negative	Negative	Negative	
9	10.9	15.7	**4.8**	9.7	12.9	**3.2**	**-1.6**	Negative	Negative	Negative	
9	8.7	8.5	**0**	7.7	7.8	**0.1**	**0.1**	Negative	Negative	Negative	
9	15.6	18	**2.4**	12.8	13.3	**0.5**	**-1.9**	Negative	Negative	Negative	
9	6	6.1	**0.1**	6.2	6.9	**0.7**	**0.6**	Negative	Negative	Negative	
9	14.5	17.6	**3.1**	9.9	15.4	**5.5**	**2.4**	Inconclusive	Inconclusive	Inconclusive	Consistency: hard. Appearance: diffuse. Necrosis??
9	10.7	14.2	**3.5**	7.8	10.1	**2.3**	**-1.2**	Negative	Negative	Negative	
9	10.2	11.9	**1.7**	8.5	9	**0.5**	**-1.2**	Negative	Negative	Negative	
9	11.5	14.6	**3.1**	9.1	8.9	**0**	**-3.1**	Negative	Negative	Negative	
9	12.6	15.7	**3.1**	12.8	14	**1.2**	**-1.9**	Negative	Negative	Negative	
10	8.1	9.3	**1.2**	7.4	8.2	**0.8**	**-0.4**	Negative	Negative	Negative	
10	13	14	**1**	9.7	12.9	**3.2**	**2.2**	Inconclusive	Inconclusive	Negative	Consistency: hard. Appearance: diffuse.
10	9.1	10.1	**1**	9	9.1	**0.1**	**-0.9**	Negative	Negative	Negative	
10	8.8	8.2	**0**	9.3	8.7	**0**	**0**	Negative	Negative	Negative	
10	13.2	14	**0.8**	10.3	12	**1.7**	**0.9**	Negative	Negative	Negative	
10	13.2	15.3	**2.1**	11.9	14.9	**3**	**0.9**	Negative	Negative	Negative	
10	5.5	6.6	**1.1**	4.8	5.4	**0.6**	**-0.5**	Negative	Negative	Negative	
10	10.3	10.1	**0**	8.4	7.8	**0**	**0**	Negative	Negative	Negative	
10	11.8	12.3	**0.5**	8.5	10.6	**2.1**	**1.6**	Negative	Negative	Negative	
10	4.7	5.1	**0.4**	3.8	4.4	**0.6**	**0.2**	Negative	Negative	Negative	
11	5.5	6.5	**1**	6.4	6.6	**0.2**	**-0.8**	Negative	Negative	Negative	
11	18.5	16.1	**0**	12.1	12.3	**0.2**	**0.2**	Negative	Negative	Negative	
11	13.3	13.8	**0.5**	12.2	12.1	**0**	**-0.5**	Negative	Negative	Negative	
11	10	10.4	**0.4**	9.4	10	**0.6**	**0.2**	Negative	Negative	Negative	
11	12.3	12.4	**0.1**	10.9	12.4	**1.5**	**1.4**	Negative	Negative	Negative	
11	16.8	17.1	**0.3**	19	19.1	**0.1**	**-0.2**	Negative	Negative	Negative	
11	18.1	18.1	**0**	13.7	12.4	**0**	**0**	Negative	Negative	Negative	
11	4.4	5.5	**1.1**	5.8	5.1	**0**	**-1.1**	Negative	Negative	Negative	
11	14.5	13.9	**0**	8.9	11.1	**2.2**	**2.2**	Inconclusive	Inconclusive	Negative	Appearance: diffuse
11	13.1	14.6	**1.5**	13.3	13.6	**0.3**	**-1.2**	Negative	Negative	Negative	
12	13.3	15.7	**2.4**	13	13.1	**0.1**	**-2.3**	Negative	Negative	Negative	
12	12.7	13.1	**0.4**	12.4	13.1	**0.7**	**0.3**	Negative	Negative	Negative	
12	19.7	19.8	**0.1**	15.9	19.3	**3.4**	**3.3**	Inconclusive	Positive	Negative	Consistency: hard. Appearance: diffuse.
12	12.8	12.6	**0**	14	13.2	**0**	**0**	Negative	Negative	Negative	
12	9	8.5	**0**	6	6.1	**0.1**	**0.1**	Negative	Negative	Negative	
12	4.7	6.4	**1.7**	6	4.8	**0**	**-1.7**	Negative	Negative	Negative	
12	9.1	10.6	**1.5**	7.8	8.7	**0.9**	**-0.6**	Negative	Negative	Negative	
12	9.2	10.1	**0.9**	7.5	8.1	**0.6**	**-0.3**	Negative	Negative	Negative	
12	12.5	13.4	**0.9**	10.5	9.8	**0**	**-0.9**	Negative	Negative	Negative	
12	11.9	14	**2.1**	8.5	9.3	**0.8**	**-1.3**	Negative	Negative	Negative	
13	7.8	10.1	**2.3**	5.3	4.6	**0**	**-2.3**	Negative	Negative	Negative	
13	7.2	7.3	**0.1**	5.7	6	**0.3**	**0.2**	Negative	Negative	Negative	
13	5	6.5	**1.5**	3.9	4.4	**0.5**	**-1**	Negative	Negative	Negative	
13	7.9	6.4	**0**	6.3	5.2	**0**	**0**	Negative	Negative	Negative	
13	5.6	4.2	**0**	5	3.4	**0**	**0**	Negative	Negative	Negative	
13	15.4	24.4	**9**	12.2	17.7	**5.5**	**-3.5**	Negative	Negative	Negative	
13	11.9	19.8	**7.9**	8.9	12.8	**3.9**	**-4**	Negative	Negative	Negative	
13	7.6	11.4	**3.8**	7.4	6.8	**0**	**-3.8**	Negative	Negative	Negative	
13	10.6	11.7	**1.1**	7.1	8.1	**1**	**-0.1**	Negative	Negative	Negative	
13	5.2	5.6	**0.4**	4.2	5.1	**0.9**	**0.5**	Negative	Negative	Negative	
14	12.8	14.2	**1.4**	8.2	10.8	**2.6**	**1.2**	Negative	Negative	Negative	
14	8.4	8.3	**0**	6	6.6	**0.6**	**0.6**	Negative	Negative	Negative	
14	13.8	12.9	**0**	8.3	7.9	**0**	**0**	Negative	Negative	Negative	
14	11.3	11	**0**	7.2	8.7	**1.5**	**1.5**	Negative	Negative	Negative	
14	10.9	11.3	**0.4**	7.3	11.3	**4**	**3.6**	Inconclusive	Positive	Positive	Consistency: hard. Appearance: circumscribed.
14	8.1	9.9	**1.8**	6.2	9.5	**3.3**	**1.5**	Negative	Negative	Negative	
14	4.2	4.5	**0.3**	3.2	3.6	**0.4**	**0.1**	Negative	Negative	Negative	
14	8.3	11.1	**2.8**	6.1	8.7	**2.6**	**-0.2**	Negative	Negative	Negative	
14	7.3	12.5	**5.2**	5.7	8.1	**2.4**	**-2.8**	Negative	Negative	Negative	
14	7.4	11.8	**4.4**	3	3.6	**0.6**	**-3.8**	Negative	Negative	Negative	
15	11.1	12	**0.9**	9.7	10.1	**0.4**	**-0.5**	Negative	Negative	Negative	
15	10.6	11.9	**1.3**	8.4	10.3	**1.9**	**0.6**	Negative	Negative	Negative	
15	3.6	4	**0.4**	3.4	4	**0.6**	**0.2**	Negative	Negative	Negative	
15	8	10.4	**2.4**	7	8	**1**	**-1.4**	Negative	Negative	Negative	
15	12.4	12.7	**0.3**	10.2	11.3	**1.1**	**0.8**	Negative	Negative	Negative	
15	8.3	8.4	**0.1**	7	9.3	**2.3**	**2.2**	Inconclusive	Inconclusive	Negative	
15	7.8	12.1	**4.3**	7.4	12.7	**5.3**	**1**	Negative	Negative	Negative	
15	8.7	12.5	**3.8**	6.8	10	**3.2**	**-0.6**	Negative	Negative	Negative	
15	5.7	6.6	**0.9**	5.8	5.8	**0**	**-0.9**	Negative	Negative	Negative	
15	7.3	8.7	**1.4**	5.8	9	**3.2**	**1.8**	Negative	Negative	Negative	