Bovine tuberculosis in Rwanda: Prevalence and economic impact evaluation by meat inspection at Société des Abattoirs de Nyabugogo-Nyabugogo Abattoir, Kigali

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Despite the significant public health burden of bovine tuberculosis (bTB) in Rwanda, the prevalence of bTB is poorly documented. This study was conducted to estimate the prevalence of bTB in cattle using gross examination of granulomatous lesions, to identify mycobacteria species in suspected samples, and to evaluate the economic impact of meat condemnation based on bTB-like lesions in the meat industry in Rwanda. Routine meat inspection was conducted at Société des Abattoirs de Nyabugogo (SABAN)-Nyabugogo Abattoir. Tissue samples including 31 lymph nodes, 3 lungs and 2 livers were obtained from cattle of different ages with gross tuberculous lesions. *Mycobacterium bovis* was identified using microscopy with Kinyoun staining and isolation of mycobacterial species in culture on Löwenstein–Jensen and Colestos media, further identified using biochemical tests. Our findings, based on culture and postmortem results, show that the prevalence of bTB is 0.5% (0.587*148/16753), with an overall gross tuberculous lesion prevalence of 0.9% (148/16753). The presence of lesions were higher in cattle aged 2 years and older (1.6% vs. 0.6%, $p < 0.05$) and higher in females than in males (1.4% vs. 0.6%, $p < 0.05$). Of the 36 samples tested, 26 (72.2%) were positive by microscopic examination with Kinyoun staining while *M. bovis* was culture-confirmed in 21 (58.7%) cases. Bovine tuberculosis caused condemnation of 1683.5 kg of meat, resulting in an estimated loss of $4810. Our findings indicate that the prevalence of bTB in Rwanda is significant, and that bTB is a major cause of meat condemnation requiring continued implementation of surveillance and control measures. Furthermore, the results from this study also show important variations in sensitivity of the different tests that were used to determine the prevalence of bTB in cattle in Rwanda.

Introduction

Tuberculosis (TB) is an infectious and contagious disease common to human and many animal species (Ayele *et al.* 2004; Palmer, Waters & Whipple 2004) and remains a major health threat to both humans and animals throughout the world (Cosivi *et al.* 1995, 1998; Michel 2002). For this reason, TB is both an economic and public health threat (Biet *et al.* 2004; Haddad & Durand 2001; Michel *et al.* 2007). Laboratory diagnosis of *Mycobacterium tuberculosis* in humans and *Mycobacterium bovis* in cattle is based on biochemical identification, following isolation in culture, and molecular identification of the causative agent. However, in developing countries, bovine tuberculosis (bTB) diagnosis in cattle relies on postmortem examination by meat inspection (Shitaye *et al.* 2006).

In developed countries, (bTB eradication programmes based on screening and slaughtering of infected animals as well as milk pasteurisation have been successful (Cosivi *et al.* 1998; Franck *et al.* 2005).

In Rwanda, TB and bTB remains a significant public health threat, with an average of 91.3 per 100 000 populations per year of new human cases (all forms) reported from 2005 through 2007 (Minisante 2007). It is estimated that 5% of the cattle population in the world react positively to a tuberculin test; that is approximately 50 million of cattle that might be infected by bTB (Chantal 2001). Moreover, cattle remain the most important source of *M. bovis* infections to humans and other animals but are also at risk as cattle can be reinfected with *M. bovis* by contact with humans (Aranaz *et al.* 2004; Krauss *et al.* 2003).

The objectives of this study were to determine the prevalence of bTB by evaluating the presence of granulomatous lesions in cattle at a Kigali abattoir, to identify different mycobacteria species

Note: This article was republished with the correct spelling of Société des Abattoirs de Nyabugogo.

that are responsible for lesions resembling tuberculosis and evaluate the economic impact of bTB on the beef industry in Rwanda.

Materials and methods
Geographical study area
The study was carried out at Société des Abattoirs de Nyabugogo (SABAN)-Nyabugogo Abattoir in Kigali on slaughtered animals from four provinces, namely eastern, northern, southern and western Rwanda, and Kigali city (Table 1). Laboratory examinations were performed at the microbiological laboratory of the Higher Institute of Agriculture and Animal Husbandry (ISAE).

Meat inspection and sample collection
Routine meat inspection was performed by meat inspectors under the supervision of veterinarians from the Rwanda Agriculture Board (RAB). The study was conducted on 16 753 animals slaughtered at SABAN-Nyabugogo Abattoir in Kigali, Rwanda over a period of 11 months (January–November 2009) to estimate the prevalence of bTB by gross inspection of lesions resembling tuberculosis (Table 1). In this study, a lesion resembling bTB was defined as any grossly discernible raised lump with a diameter of 5 mm to > 25 mm showing, mostly, greyish white colour with a fluctuant or granular consistency. Bovine TB-like lesions could also be inspissated with a lamellar appearance on cut surface. An additional 36 tissue samples were obtained from the condemned organs of several animals. The samples comprised 31 lymph nodes (retropharyngeal, mediastinal and tracheobronchial), three lung samples and two liver samples. Suspected organs and tissues were dissected and inspected. Samples were collected into sterile plastic bags and placed in a cooler box with ice to maintain the cold chain and eliminate sample deterioration before transportation to the laboratory, where they were stored at 4 °C.

Sample processing and laboratory examination
Collected samples were analysed either the same day or the day following arrival at the laboratory. Microscopy with Kinyoun staining technique, culture on Löwenstein–Jensen and Colestos media and biochemical tests for species identification were used to confirm suspected cases of bTB.

The presence of acid-fast bacteria (AFB) was confirmed by Kinyoun staining of an impression smear made directly from a tissue sample. A positive control for Kinyoun staining was also used as previously described (Nolte & Metchock 1995).

Stained microscopic slides were observed under microscope with objectives × 100 (oil immersion objective). Samples to be cultured were prepared according to World Organisation for Animal Health (OIE) guidelines (OIE 2009). Using sterile scissors, excess fat was removed and tissue was carefully cut into small pieces to avoid incising the lesion. Cut pieces were then homogenised in a motor with sea sand and a sterile solution of sodium chloride (NaCl 0.85%). A pestle was used for grinding. The ground material was homogenised with vortex and then 7 mL was withdrawn and centrifuged in a test tube. An additional 5 mL of sodium hydroxide (NaOH) 0.5 N was added, and after 10 min the mixture was neutralised by adding a few drops of 6 N hydrogen chloride solution until the colour changed to yellow. The pH was lowered by adding a few drops of 1 N sodium hydroxide until the appearance changed to a pale pink colour. The suspension was then centrifuged at 3000 rpm for 15 min. The supernatant was discarded and the medium was inoculated with 200 μL of the pellet per tube. For culturing purposes, two culture media were used: Colestos containing glycerol, which was ready to use, and Löwenstein–Jensen without glycerol, which was prepared before use. Each sample was inoculated into two test tubes containing Löwenstein–Jensen and Colestos media, respectively. After inoculation, the tubes were incubated at 37 °C in an inclined position and unscrewed for 4 days to allow the condensation to evaporate. The incubated tubes were checked once a week. The incubation was maintained for 16 weeks. After isolation of mycobacteria, identification by biochemical tests was performed. Two tests were used, namely a urea/indole test and a catalase test.

Record on meat condemnation at the abattoir
From January to November 2009, the condemned carcasses and organs (head, liver, lungs, intestines, abomasum and kidneys) were recorded and weighed daily. The economic losses were estimated at prevailing market values.

Data analysis
Demographic and epidemiological characteristics were analysed using descriptive statistics. Categorical variables were described with the use of percentages. Bivariate analysis was performed using χ^2 and Fischer's exact tests with p-values ≤ 0.05 considered significant. Data analyses were performed using Excel and STATA 12.1.

Ethical considerations
Ethical approval for this study protocol was obtained from the Institutional Review Board of the Faculty of Veterinary

TABLE 1: Origin of slaughtered animals.

Origin	Month											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Total
East	1677	1705	770	1030	1240	1825	1176	346	290	526	475	11 060
North	151	164	55	188	327	330	226	222	777	975	928	4343
South	29	19	29	-	29	22	65	92	152	64	80	581
West	19	-	-	-	-	-	14	91	141	235	237	737
Kigali City	19	-	-	-	-	-	-	-	-	8	5	32
Total	**1895**	**1888**	**854**	**1218**	**1596**	**2177**	**1481**	**751**	**1360**	**1808**	**1725**	**16 753**

Medicine, College of Agriculture and Veterinary Sciences, University of Rwanda. The sample collection was performed by a qualified veterinarian assisted by meat inspectors through routine antemortem and postmortem inspection to ensure personnel safety. Abattoir owners were informed of the study purpose and procedures and provided written consent prior to study procedures and sample collection from their animals.

Results

Estimation of losses as a result of meat condemnation as a result of bovine tuberculosis

Before suspected organs were discarded, they were weighed and the monetary loss was estimated. A total of 1683.5 kg of meat was condemned and the loss as a result of the presence of bTB lesions was estimated to be RWF3 030 300 ($4810). The pathomorphological examination during meat inspection showed that 148 (0.9%) of the 16 753 slaughtered cattle had tuberculous lesions. It was also revealed that females had higher lesion prevalence than males (1.4% vs. 0.6%, $p < 0.05$, $n = 16\,753$). Cattle aged > 2 years had higher lesion prevalence than those < 2 years (1.6% vs. 0.6%, $p < 0.05$, $n = 16\,753$). The head was the most predominant of the condemned organs, with 65 (43%) condemned heads (Table 2), because of caseous necrosis lesions in the lymph nodes. This was significantly higher ($p < 0.05$, $n = 148$) than in other organs, except the liver, which also had a higher occurrence of bTB lesions (33.8%). A monthly trend analysis indicated that all the values recorded in the period between June and August were significantly higher ($p < 0.05$) than the rest of the year, except the month of February (Table 2).

Laboratory analysis

Kinyoun staining and microscopic examination

Of the 36 samples that were microscopically examined, 26 (72.2%) were positive to Kinyoun staining. Amongst the 26 samples, there were 22 lymph nodes from different organs, two liver specimens and two lung specimens (Table 3).

Isolation and identification

A total of 36 samples (both negative and positive to Kinyoun staining) were cultured and mycobacteria were isolated in 23 (63.9%) of them (Table 4). With the exception of two

samples for which colonies appeared on the 9th day, the first colonies appeared on the 24th day. Based on results of biochemical test for identification, *M. bovis* was present in 91.3% ($n = 23$) of the samples and atypical mycobacteria in 8.7% ($n = 23$) (Table 5).

Estimating bovine tuberculosis prevalence

AFB were microscopically recognised and confirmed in 26 (72.2%) samples of the total 36 specimens examined by the Kinyoun staining technique. *Mycobacterium bovis* was only confirmed in 21 (58.7%) samples by culture. As AFB culture is a relatively confirmatory method compared to the Kinyoun staining technique for *M. bovis*, the overall postmortem prevalence of *M. bovis* using culture was estimated to be 0.5% (0.587*148/16753).

Discussion

Bovine TB is a chronic disease of animals caused by the bacterium *M. bovis*, which is closely related to the bacterium *M. tuberculosis*, the causative agent of human tuberculosis. Humans are susceptible to bTB and can be infected by both by drinking raw milk from infected cattle or by inhaling infective droplets. It is estimated in some countries that up to 10% of human tuberculosis can be ascribed to bTB (OIE 2012). Diagnosis of bTB in cattle is by the tuberculin skin test (Cosivi *et al*. 1998; Palmer *et al*. 2004), by which a small amount of antigen is injected into the skin and the immune reaction is measured. Culturing the bacteria in the laboratory for about 8 weeks is used to confirm diagnosis. This study is the first reported to describe the prevalence of bTB in cattle in Rwanda

TABLE 3: Results of Kinyoun staining.

Organ	Number of samples	Kinyoun positive	Percentage (%)
Lymph nodes	31	22	70.8
Lungs	3	2	66.6
Liver	2	2	100
Total	**36**	**26**	**72.2**

TABLE 4: Positive cultures from samples.

Kinyoun	Negative cultures	Positive cultures	Total	Percentage (%)
Kinyoun (-)	8	2	10	27.8
Kinyoun (+)	5	21	26	72.2
Total	**13**	**23**	**36**	**100**
Percentage (%)	**36.1**	**63.9**	**100**	**-**

TABLE 2: Condemned organs according to month.

Organ	Month											Total	Percentage (%)
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.		
Liver	-	-	1	2	2	5	16	18	5	-	1	50	33.8
Lungs	5	2	2	1	1	4	3	-	1	-	-	19	12.8
Intestine	-	2	-	1	2	3	-	-	-	-	-	8	5.4
Kidney	-	-	-	-	-	-	-	-	2	-	-	2	1.3
Head	3	13	8	3	7	11	2	-	6	7	5	65	43.9
Whole carcass	-	-	1	-	-	1	-	-	-	-	-	2	1.3
Abomasum	-	1	-	-	-	-	-	-	-	-	-	1	0.6
Total condemnation	-	1	-	-	-	-	-	-	-	-	-	1	0.6
Total	**8**	**19**	**12**	**7**	**12**	**24**	**21**	**18**	**14**	**7**	**6**	**148**	**100**
Percentage (%)	**5.4**	**12.8**	**8.1**	**4.7**	**8.1**	**16.2**	**14.1**	**12.1**	**9.4**	**4.7**	**4.0**	**100**	**-**

TABLE 5: Summary of results of Kinyoun staining versus culture.

Organ	Number of samples	Kinyoun (+)	Kinyoun (-)	Culture (+), Kinyoun (-)	Culture (-), Kinyoun (+)	Total *Mycobacterium bovis*	Percentage (%)
Lymph nodes	31	22	9	2	4	17	54.7
Lungs	3	2	1	0	1	2	66.7
Liver	2	2	0	0	0	2	100
Total	**36**	**26**	**10**	**2**	**5**	**21**	**58.7**

using both routine meat inspection and confirmation by laboratory diagnosis of *M. bovis*. Kinyoun staining allowed the identification of AFB resistant to alcohol from 72.2% (26/36) of suspect samples. However, this technique proved inadequate for the presumptive diagnosis of bTB because it yielded many false negatives. This might be because of the nature of tuberculous lesions and their stage of evolution (Vestal 1975). The sensitivity of Kinyoun staining compared to culture was 91.3%, while the specificity was 61.5%. Biochemical tests were successful in identifying and characterising 21 isolates of mycobacteria as *M. bovis* but failed to characterise two atypical mycobacteria. The choice of staining as a method of presumptive diagnosis of bTB was motivated by its speed, simplicity, cost and value (Thorel 1997). Amongst 26 Kinyoun positive samples, five yielded negative cultures. This could be explained by the inactivation or death of mycobacteria during the process of decontamination before culture (Mahjoubi 2006). The fact that *M. bovis* was isolated from two Kinyoun negative samples by culture illustrates the sensitivity of this technique. Also, this could be ascribed to paucibacillary samples, to insufficient microscopic observation with fewer microscopic fields examined, or to the heterogeneity of the distribution of mycobacteria in the sample (Thorel 1997). Atypical mycobacteria were isolated from two (8.7%) samples. This proportion is slightly high compared to 2.3% reported by L'Kassmi *et al.* (1998).

The study showed that the gross lesion prevalence of bTB was slightly higher in females (1.4%) than in males (0.6%). This could probably be ascribed to the stressful nature of the females' husbandry (housing and extended confinement); the stress of lactation and gestation make them more vulnerable to infection. In addition, contact with humans with TB during milking might be another reason (Imtiaz *et al.* 2008; Skuce, Allen & McDowell 2011; Walter, Guilherme & Leila 2007).

Animals older than 2 years had a higher gross lesion prevalence (1.6%) than those younger than 2 years (0.6%). Older cattle have had a longer overall exposure to tuberculosis, but calves can become infected in the uterus or by drinking milk from a tuberculous cow. This contradicts other reports (Blood, Henderson & Radostitis 2000; Mohammed, Hailu & Gebreyesus 2012) which indicated that young animals are less resistant than adults. Furthermore, as the current study was based on gross lesions, the bacteria might have been missed in the incubation stages in younger animals (Brooks-Pollock *et al.* 2013). The macroscopic lesions appear at advanced stages of infection (Awah-Ndukum *et al.* 2012; Imtiaz *et al.* 2008; Skuce *et al.* 2011).

The higher figure observed between June and August coincided with the dry season. During this time farmers are forced to sell all animals in poor body condition to reduce losses as a result of deaths and hence more animals presented for slaughter are likely to be infected.

Although the overall prevalence of bTB was 0.5% (which is not alarming in a large-scale study), the zoonotic status of the disease warrants implementation of appropriate control strategies. This prevalence is lower than the 1.33%, 1.67% and 2.12% reported by El Abrak, Lhor and Zrira (2001) in Morocco in 1998, 1999 and 2000, respectively. However, this prevalence is higher than the 0.03% reported by Shitaye *et al.* (2006) in Ethiopia.

Limitations to this study include the lack of appropriate facilities to perform more sensitive and specific diagnostic methods, such as polymerase chain reaction (PCR) assay; a limited data collection period of only 11 months; and a sample limited to only culled animals.

Conclusion

The study findings indicate that routine meat inspection protocols utilised in Rwandan abattoirs are able to detect 0.9% of bTB lesions at the gross level. Laboratory culture and microscopic examination were demonstrated to improve the detection level as reference tests, even though they failed to detect a small number of carcasses that presented with grossly visible lesions of bTB. The findings of this study have managed to establish a baseline prevalence of bTB in Rwanda at 0.5%. There is potential for further research to establish the true prevalence of bTB using high-sensitivity, high-specificity techniques such as PCR assay, performed over a longer period of time using randomly selected animals in the field rather than at an abattoir.

Given that many urban families in Kigali and other large cities keep some dairy animals in their backyards (and the trend is increasing), the human–animal interface continues to expand. The true prevalence of bTB and human TB must be determined as such information is of the utmost importance to city health authorities in order for surveillance and control measures to be applied accurately. The relationship between human TB and bTB at the human–animal interface in Rwanda can only be established if the true prevalence of both conditions is known. Finally, this study shows that bTB is an important cause of beef meat condemnation, leading to significant economic losses for farmers.

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Competing interests

The authors declare that they have no financial or personal relationship(s) which would have inappropriately influenced the writing of this article.

Authors' contributions

G.H. (University of Rwanda) was responsible for the fieldwork, prepared the samples and performed most of the experiments. J.R. (United States Centers for Disease Control and Prevention) was the supervisor and contributed to project design. M.O.N. (University of Nairobi) and B.M. (University of Fort Hare) contributed to the writing and editing of the manuscript.

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