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Prevalence and associated risk factors of mycobacterial infections in slaughter pigs from Mubende district in Uganda

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Abstract To date, the public health relevance of mycobacterial infections in pigs is not well investigated despite high risk of infection. Recently, there has been a documented increase in opportunistic infections and risk of acquiring opportunistic mycobacterial infections in HIV/AIDS patients in Mubende district; unfortunately, there has been no published information on the epidemiology of mycobacterial infections in this area. This study was carried out between September 2008 and February 2009. Investigations were done to assess the prevalence and associated risk factors of mycobacterial infections in slaughtered pigs in Mubende district of Uganda. A total of 997 pigs (53.7% male and 46.3% female) from 31 different slaughterhouses were examined for the presence of lesions compatible with TB and mycobacterial infections. Pathologic tissue specimens were collected for culturing and isolation of mycobacteria. A cross-sectional technique was used based on convenient visits to slaughterhouses but random selection of individual slaughtered pigs for a detailed post-mortem inspection on a daily basis. The results reflected a 9.3% and 3.1% (95% CI) prevalence of *Mycobacterium* species based on necropsy examinations and culture isolation, respectively. The highest prevalence of mycobacterial infection was recorded in Buwekula County (the mixed agro-zone) whilst the lowest was in Kassanda County (pastoral zone). A multivariable logistical regression analysis identified age ($P \leq 0.001$) and sex ($P \leq 0.05$) as risk factors for mycobacterial infections in pigs. Post-estimation statistics of the regression model evaluation and validation fit it well into the data (HL, χ^2=5.9; P=0.69 for necropsy, HL χ^2=2.9; P=0.94 for culturing). This study documented a high prevalence of mycobacterial infections in slaughter pigs in Mubende district. The fact that pigs and human often

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share common housing and environment poses a high risk of zoonotic transmission. This then warrants further molecular investigation to identify the specific *Mycobacterium* species and their public health importance in this area.

Keywords Mycobacteria · Pigs · Risk factor · Uganda

Introduction

The public health importance of nontuberculous mycobacteria (NTM) has become a subject of renewed interest in the awake of increasing incidence of disseminated mycobacterial infections and antecedent pulmonary disease in HIV/AIDS-infected human populations globally (Phillips et al. 2005; Griffith et al. 2007; Huang et al. 2009). Unlike *Mycobacterium tuberculosis* complex, NTM has been given less priority up until recently. *M. tuberculosis* infects one third of the world's population and is the leading cause of death due to a single infectious agent worldwide. Most of the cases have been reported in developing countries (Anonymous 2009). Uganda ranks 15th on the list of 22 high-burden tuberculosis (TB) countries in the world with 60% TB patients also infected with HIV (Anonymous 2009). The Mubende district state of environmental report also showed an increase in opportunistic pulmonary infection in HIV/AIDS patients (Anonymous 2004a). NTM plays a key role in the aetiology of TB-like syndromes in industrialised countries; however, the contribution of NTM to such disease has been examined on a small scale in Africa (Buijtels et al. 2009).

Although there are many sources of NTM for humans, previous studies have shown that pigs are among the possible sources of mycobacterial infections for humans and animals (Komijn et al. 1999; Pavlik et al. 2000; Oloya et al. 2007; Johansen et al 2007).

The other concern especially in Africa has been the fact that NTM diminishes the efficacy of Bacillus Calmette-Guérin (BCG), the only available vaccine against tuberculosis. In a study done in Denmark, it was shown in a mouse model that prior exposure to the Kalonga environmental mycobacteria isolates resulted in reduction of efficacy or complete blockage of BCG activity (Brandt et al. 2002).

Economic losses due to swine mycobacteriosis range from mortality to morbidity as well as dissemination of nontuberculous *Mycobacterium* species which sensitise cattle, resulting in non-specific reaction to tuberculin skin test (Mohamed et al. 2009). Therefore, the false-positive reactors are rejected during selection for animal meat export, thus costing the farmers heavily and, in a wider context, the national economy.

In Uganda, pigs are predominantly raised by subsistent farmers as a source of household cash income generation and food supply. The animals are often provided with sub-optimal management systems with inadequate feed supply and veterinary care (Anonymous 2005). There is a close intimacy between people and pigs where both share a common ecosystem, and this is expected to pose a health risk to the people in terms of zoonotic transmission of infections. Due to little research done on pigs, there has been scarcity of information with regards to mycobacterial infections particularly in Uganda and Africa in general.

In light of the increasing evidence that NTM are pathogenic to human and pigs as well as the overwhelming economic losses to pig farmers, it is therefore necessary to have epidemiological information in order to set up appropriate control and prevention strategies.

This study was thus aimed at determining the prevalence mycobacterial-compatible lesions and associated viable mycobacteria in slaughter pigs in Mubende district, in addition, to investigate risk factors associated with the infection.

Material and methods

Study area and study animals

This study was carried out in Mubende district, Uganda. Mubende is located in the central region of Uganda bordering Mityana to the east and south east, Sembabule and Mpigi to the south, Kyenjonjo on the southwest, Kibaale on the North West and Kiboga to the north and northwest (Fig. 1). Administratively, Mubende compromises of two counties Buwekula and Kassanda. The counties are further subdivided into 11 sub-counties which include Bagezza, Butologo, Kasambya, Kitenga, Kiyuni, Madudu, Mubende

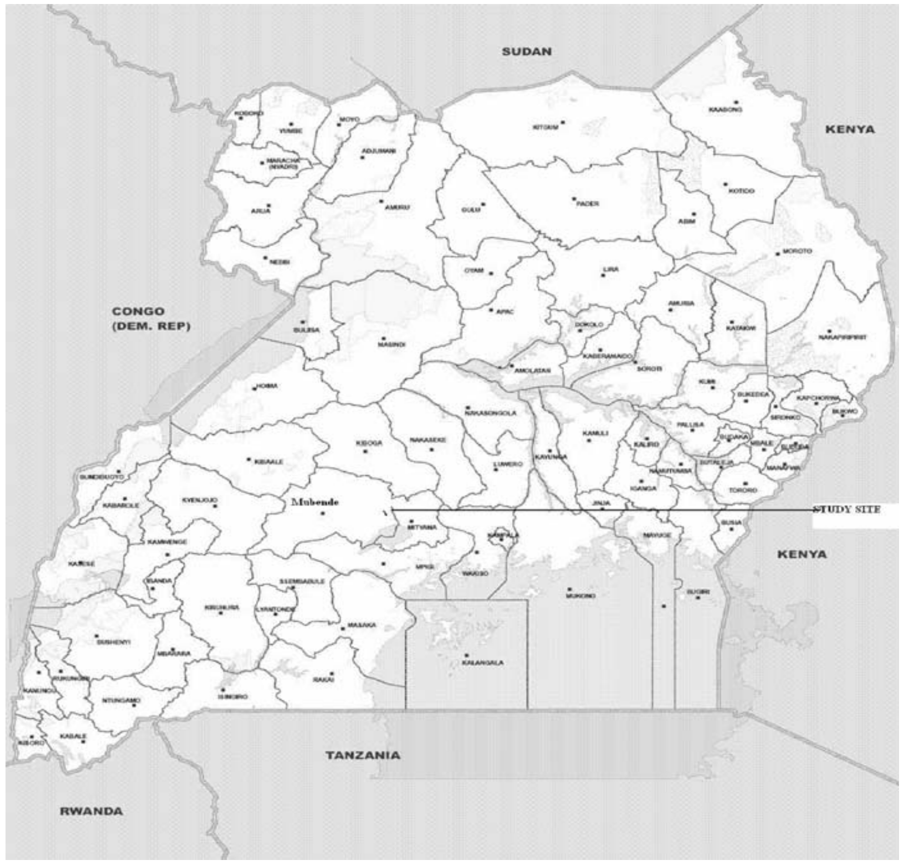

Fig. 1 The map of Uganda showing the study district

town council, Bukuya, Kassanda, Kiganda and Myanzi. Mubende lies at an altitude between 1,372–1,448 m above sea level and with a remarkably low rainfall distribution with peaks in April and October to November. However, January 2009 showed unseasonal rain triggered by tropical cyclone activity in the Mozambique Channel (Magezi 2009). The northern half of the district lies within the agro-livestock basin while the southern part falls under the Ugandan cattle/pastoral corridor. Mubende is the poorest district in central Uganda today, with more than 64% of its population falling below the poverty line (Anonymous 2005). Much of the population in the district earn their household subsistence economic income from agricultural activities based on traditional farming style such as animal herding and crop production. The district has a considerably high animal population, estimated at 35,195 cattle, 80,000 pigs, 12,238 goats, 5,955 sheep and 360,157 chickens (Anonymous 2007). Majority of the farmers keep their pigs for subsistence purposes; backyard and free-range is the most common style of pig rearing, and most of the pigs are fed on swill and crop residues. It is also generally known that veterinary and extension services are limited in this district. In such a livestock-dependent lifestyle, close physical contact between animals, environment and humans is expected to constitute a significant risk factor for transmission of various disease agents among the hosts.

Study design

Initially, a cross-sectional multi-stage sampling technique was planned that would involve a systematic random selection of pigs with stratification at county, sub-county and slaughter house level between August and December 2008. However, due to some difficulties encountered in the field study, including inaccessible roads, irregularity in pig slaughtering and heavy rainy seasons, we were obliged to make modification of the initial sampling approach. To that effect, a convenient-random sampling strategy was adopted with convenient selection of slaughterhouses and random selection of individual pigs among the daily slaughter population.

Selection of 31 slaughterhouses was done based on the following criteria: Human and animal population in the area, terrain of the area, agricultural practices, vegetation cover and type of water source (these would range from swamps, valley dams, boreholes, stream water, ponds, rivers and lakes), all of which were deemed to affect the prevalence of mycobacteria.

The sample size determination

The sample size estimation was calculated based on the following assumptions:

1. We assumed 33.2% as the mean expected prevalence of mycobacterial infections (based on studies from a similar environment in Cameroon (Awa et al. 1999). To date, this is the only available statistics on the prevalence of mycobacterial lesions in pigs in east and central Africa.
2. The total population of pigs in Mubende district is estimated to be 80,000 according to District Information Book (Anonymous 2007).
3. The desired of precision ($\acute{\alpha}$) was set at 0.05 and the detection power (β) of the test at 0.95.

With these assumptions, the minimum sample size required to detect relative infection prevalence patterns in the pigs slaughtered in Mubende district was 340 pigs. This sample size was divided among the 11 sub-counties which required that at least 31 pigs be sampled from each of the sub-counties. Given that the study was carried out in a time frame much longer than initially proposed, we were able to inspect as many as 997 with an average of 50 carcasses per sub-county. The inspected sample size would be expected to give a more precise value of the parameters estimated.

Isolation of mycobacteria

Specimens from 93 lymph nodes with gross pathological characteristics were cultured on appropriate media for detection of growth of viable mycobacteria. The technique is briefly described as follows: Fat and connective tissue was neatly dissected away from the lymph node; then, 3 g of the tissue was cut into small pieces using a sterile scalpel blade. This was then ground with sterile sand in a sterile porcelain dish (mortar) to facilitate grinding. Then, 5 ml of sterile physiological saline was added and further ground, and decontamination was done by adding 25 ml of 5% oxalic acid. This was then left to stand for 5 min at room temperature. The sample was then decanted in a new sterile labelled tube. The sample was then left to stand at room temperature with intermittent shaking for further 20 min. Additional centrifugation was done at 3,000 rpm/s for 7 min, and the resultant pellet then suspended in 2.5 ml sterile physiological saline and shaken. Finally, 2–3 drops were transferred to both Stone-brink and Middlebrook media and incubated for 8 weeks at 37°C (Valheim et al. 2001). The media were monitored weekly for any growth.

Ziehl–Neelsen staining

Smears were made from colonies that fitted the typical *Mycobacterium* colony morphology. Carbol fuschsin was added to the slide with intermittent heating for 5 min, followed by rinsing with water and thereafter, a decolouriser (3% alcohol) was added until the slide was clear. The slide was then rinsed with water and methylene blue added for 1 min, after which a final rinsing and drying was done. The slide was then observed under a microscope (×100 with oil) for acid-fast bacilli (Pfyffer et al. 1997; Valheim et al. 2001).

Data collection and analysis

During the period of September 2008 to February 2009, a total of 997 pigs from 31 slaughterhouses were examined with routine meat inspection procedure. Pathological changes observed on lymph nodes were recorded in detail. Additional information included sex, age (the age was obtained from slaughterhouse records), breed (local, cross and exotic breed), weight of the pig and source and the type of slaughter house and study month were also recorded.

The raw data obtained for the individual study unit, slaughterhouse details and study site data from the record sheet were assembled and validated using Microsoft Excel® 2003. The data were then exported to Stata (Stata/SE 10 for Windows, StataCorp, College Station, TX) for appropriate statistical analysis.

The survey–data-analysis procedure was used for estimating prevalence of grossly visible lesions and viable mycobacteria and its univariate association (odds ratio with 95% CI) with individual exposure variables, considering individual pigs as primary sampling unit. Multivariable regression analysis was used to identify risk factors associated with prevalence of the infection. Considering their biological plausibility, in addition to their statistical relevance, we decided to include all the recorded variables into the model. Finally, a multivariable logistic regression model analysis was built with backward elimination procedure to include variables in the model (inclusion criteria of $P \leq 0.25$). Model validity was assessed using the Hosmer–Lemeshow goodness-of-fit test by default approach of categorising the data set into ten groups. Model reliability was assessed using receiver operating characteristic curve (ROC) curve and model sensitivity and sensitivity.

Results

A total of 997 pigs were inspected from 31 slaughter houses in Mubende district. Ninety-three (9.3%) of the slaughtered pigs had mycobacterial-compatible lesions. Prevalence and its univariate association with breed, sex, age, geographic localities and month of study period is shown in Table 1.

The prevalence of lesions is significantly high in old pigs with 21% of sampled old animals having lesions, with eight times more likely to developing

Table 1 Univariate association between prevalence of grossly visible and recorded exposure variables

Exposure variable	Label	No carcasses inspected	Necropsy-positive	Percentage (%)	Odds ratio (95% CI)	*P* value
Total animals	Sampled pigs	997	93	9.3	–	–
Sex	Male	535	43	8.03	1	
	Female	462	50	10.83	1.4 (.92–2.17)	0.11
Breed	Local	846	76	8.99	1	
	Cross	129	15	11.63	1.31 (0.73–2.36)	0.36
	Exotic	22	2	9.09	0.99 (0.23–4.35)	0.99
Age	Infant	310	9	2.90	1	
	Adult	304	11	3.62	1.25 (0.51–3.06)	0.62
	Old	351	74	21.10	8.200 (4.03–16.67)	0.00
Location county	Sub-county					
Buwekula	Madudu	113	15	13.27	1	
	Kasambya	62	7	10.14	0.74 (0.28–1.91)	0.53
	Town council	116	13	11.21	0.82 (0.37–1.82)	0.63
	Kiyuni	64	1	1.56	0.10 (0.01–0.80)	0.03
	Kitenga	79	6	7.59	0.54 (0.20–1.45)	0.22
	Bagezza	26	5	19.23	1.55 (0.51–4.75)	0.44
	Butologo	69	12	17.65	1.38 (0.60–3.14)	0.45
Kassanda	Bukuya	236	17	7.00	0.54 (0.26–1.11)	0.09
	Myanzi	110	9	8.18	0.58 (0.24–1.39)	0.22
	kiganda	52	2	3.84	0.73 (0.28–1.91)	0.53
	Kasanda	63	6	9.52	0.68 (0.25–1.87)	0.46
Months						
	September	168	18	10.70	1	
	October	186	11	5.91	0.52 (0.24–1.14)	0.11
	November	248	16	6.50	0.57 (0.28–1.16)	0.12
	December	167	12	7.20	0.65 (0.30–1.40)	0.26
	January	112	18	16.10	1.60 (0.80–3.22)	0.19
	February	116	19	16.40	1.63 (0.82–3.26)	0.16

Table 2 Univariate association between occurrence of viable mycobacteria and the recorded exposure variables

Variable	Label	No carcasses inspected	ZN test-positives	Percentage (%)	Odds ratio (95% CI)	*P* value
Total animal	Sampled pigs	997	32	3.21	–	–
sex	Male	535	14	2.61	1	
	Female	462	18	3.90	1.51 (0.73–3.10)	0.25
Breed	Local	846	23	2.72	1	
	Cross	129	8	6.20	2.36 (1.03–5.40)	0.04
	Exotic	22	1	4.54	1.70 (0.22–13.22)	0.61
Age	Infant	310	2	6.25	1	
	Adult	304	3	9.38	1.53 (0.25–9.22)	0.64
	Old	351	27	84.38	11.92 (2.81–50.54)	0.00
Location county	Sub-county					
Buwekula	Madudu	113	6	5.31	1	
	Kasambya	62	1	1.62	0.26 (0.03–2.22)	0.22
	Town council	116	7	6.03	1.15 (0.37–3.52)	0.81
	Kiyuni	64	1	1.56	0.28 (0.33–2.51)	0.25
	Kitenga	79	1	1.27	0.22 (0.03–1.94)	0.18
	Bagezza	26	0	-	–	–
	Butologo	69	4	5.80	1.09 (0.30–4.03)	0.89
Kassanda	Bukuya	236	8	3.40	0.63 (0.21–1.85)	0.22
	Myanzi	110	2	1.81	0.33 (0.06–1.67)	0.18
	Kiganda	52	1	1.92	0.35 (0.04–2.98)	0.33
	Kasanda	63	1	1.59	0.29 (0.33–2.44)	0.25
Months	September	168	9	5.40	1	
	October	186	3	1.61	0.29 (0.07–1.08)	0.06
	November	248	4	1.16	0.29 (0.08–0.95)	0.04
	December	167	7	4.20	0.77 (0.28–2.13)	0.62
	January	112	4	3.60	0.65 (0.19–2.20)	0.49
	February	116	5	4.31	0.80 (0.26–2.44)	0.69

lesions than the infant group (OR=8.2; $P \leq 0.001$).The results also show that cross-breed pigs are 1.3 times more likely to have lesions than the local breeds. Spatially, Bagezza and Butologo had the highest prevalence of lesions. The temporal distribution of lesions is highest in January and February.

The association between breed, sex, age, location and month of study period with the prevalence of *Mycobacterium* species is shown in Table 2. The prevalence shows a clear variation between the age groups, with the old pigs being almost 12 times more infectious than the infant (OR=11.9; P=0.001). Likewise, the cross-breeds are twice more likely to harbour *Mycobacterium* species than the local-breed pigs (OR=2.3; P=0.041). Spatially, although Bagezza and Butologo have high prevalence of pigs with *Mycobacterium* species, there is no statistical associ-

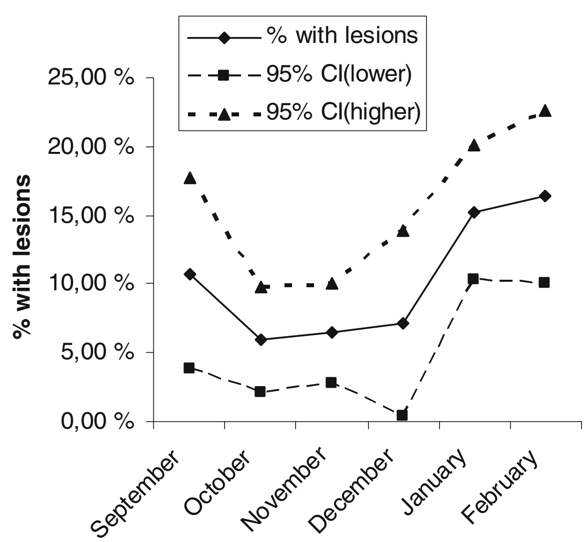

Fig. 2 Temporal occurrence of pathological lesions in pigs

Table 3 Multivariable logistic regression model for risk factors associated *Mycobacterium* sp-related lesions

Variables	Level	SE	Odds ratio	*P* value	95% CI
Months	September	–	1.0	–	–
	October	0.22	0.58	0.16	0.27–1.23
	January	1.82	5.15	0.00	2.58–10.29
	February	0.74	2.29	0.01	1.21–4.34
Sex	Female vs. male	0.42	1.68	0.04	1.03–2.73
Age	Infant	–	1	–	–
	Adult	0.91	1.84	0.22	0.70–4.90
	Old	6.60	16.40	0.00	7.50–36.01

ation with this distribution. Similarly, there is higher prevalence of *Mycobacterium* species in animals inspected in December, January and February, however; this bares no statistical significance as well.

Figure 2 indicates that there is seasonal variation in prevalence of lesions in pigs sampled in Mubende district. The highest increase was observed between December and February. This trend bears all the signs of seasonal changes, related to weather and movement of animal due to a fluctuating demand for pork.

In reference to variables in Table 1, sex, age and month of study period were included as the main explanatory factors for the prevalence of *Mycobacterium* species-related lesions in slaughtered pigs in the logistic regression (Table 3). The logistic regression fits the data (HL χ^2=5.9; P=0.69). The model shows that the female sex and old age are the biological explanatory factors for lesion prevalence, but the lesions were mostly encountered in January.

Including age, breed, type of slaughterhouse and month of study period as the main explanatory variables for the prevalence of *Mycobacterium* species in slaughtered pigs in the logistic regression, it fits the data as well (Hosmer–Lemeshow goodness-of-fit=2.9; P=0.94). Age and breed of the animal are good indicators of *Mycobacterium* species prevalence in slaughtered pigs in Mubende district. The older cross-breed pigs showed a higher prevalence of *Mycobacterium* species at slaughter (Table 4).

The evaluation of the models' reliability showed that the models were reliable, ROC=0.84 and ROC=0.86, respectively.

Discussion

The prevalence based on necropsy in pigs was found to be 9.3% (Table 1). This was lower than the 33.33% prevalence reported from northern Cameroon (Awa et al. 1999). However, based on culture growth and acid-fast test, 34.4% of the lesions were due to *Mycobacterium* species. Therefore, the confirmed relative prevalence of mycobacterial infection in pigs in this study is 3.21% in Mubende district (Table 2). In comparison to the findings in Netherlands and Finland where the prevalence was 0.34% and 0.5%, respectively (Komijn et al. 1999; Tirkkonen et al. 2007), the prevalence is considerably higher in Mubende. It was, however, lower than the 10% in Egypt and 15% in Germany (Tirkkonen et al. 2007;

Table 4 Multivariable logistic regression model identifying factors associated with occurrence of mycobacteria in tissues of TB-exposed pigs in Mubende district, Uganda

Variables	Level	SE	Odds ratio	*P* value	95% CI
Age	Infant	–	1	–	–
	Adult	2.86	3.03	0.24	0.48–19.20
	Old	16.21	21.30	0.00	4.78–94.76
Breed	Cross-breed vs. local	1.25	2.78	0.02	0.1.15–6.71
Type of slaughter house	Open vs. closed	0.07	0.18	0.00	0.08–0.40
Months	September	–	1	–	–
	October	0.15	0.24	0.02	0.06–0.86
	November	0.21	0.37	0.07	0.12–1.11

Mohamed et al. 2009). Our finding showed a remarkable difference with results obtained in South Africa's 5-year study on porcine lymph nodes which showed that 75% of the lesions positive were due to *Mycobacterium* species (Coetzer and Tustin 2004).

This study also showed no difference in prevalence of *Mycobacterium* species between urban and rural areas. This was noted discernibly by town council, Bagezza and Madudu, Butologo, respectively. Urban areas in Mubende have poor waste disposal systems characterised by open waste dumping sites which create very unsanitary conditions among households and alleys, giving fertile grounds for multiplication of all types of microorganisms (Anonymous 2004b). At the end of the rainy season, all these are washed into the surface and ground water sources. It should be noted that it is a common scene to see pigs, ruminants and other animals especially chicken roaming in Mubende town, thereby representing an additional source of contamination. It is important to note too that pigs from rural Mubende were transported and slaughtered in urban areas.

Pigs in the southern sub-counties namely Kasambya, Kitenga, Kiganda and Myanzi had a lower level of lesions and associated mycobacteria in comparison to the northern sub-counties. The phenomenon that water is the source of environmental mycobacteria could explain such a geographic difference. The southern part of the district is topographically more water-endowed than the northern half; water bodies like Lake Wamala, river Nabakazi, Kisojo and Katabalanga are present in the southern sub-counties. Ironically, the government has invested more water projects in the southern region than the north (Anonymous 2004b). Therefore, the northern half has had to resort to using water from swamps, water holes and valley dams. These water sources are mainly rain run-off water into valley areas, with a high probability of heavy bacterial contamination. The pigs in the northern part of the district therefore are most likely exposed to water with heavy contamination, especially after rainy seasons.

This study found no significant seasonal relationship with the prevalence of *Mycobacterium* species (Fig. 1). This is in agreement with previous studies where the seasonal relevance had not been observed in the epidemiology of environmental mycobacterial infections (Charette et al. 1989). However, there was seasonal incidence of lesion-positive animals. The trend follows the rainfall distribution especially the Mozambique Channel cyclone-related rainfall in January (Magezi 2009), but given the pathogenesis of *M. tuberculosis* complex (MTC) infections (chronicity). This rainfall pattern can only explain the lesion incidence of NTM infection in slaughter pigs, given the hilly terrain in the northern half of the district and that pig slurry is used as a fertiliser. It is possible that the slurry could easily be washed into the water sources after rain season giving rise to quick spread of mycobacterial infections, since faecal spread of *Mycobacterium* species is known to occur in pigs (Coetzer and Tustin 2004).

This study found that, regardless of fewer sows sampled compared to boars, the prevalence of lesion and *Mycobacterium* species recovery were relatively higher in sows. Generally, sows are kept longer than boars on farms for reproductive purposes, a phenomenon which gives *Mycobacterium* species more time to progress through the pathogenesis to the level of lesion development and appearance of overt clinical signs. This could explain why there is higher prevalence in sows in this study. Similarly, a study done in Croatia in 2006 showed sows to be more preponderant to *Mycobacterium caprae* infections that produced tuberculous lesion in both mammary glands and lymph nodes (Cvetnic et al. 2006).

The trend in molecular biological studies in recent years indicates the significance of pigs as source and reservoir of mycobacterial infection for humans (Komijn et al. 1999; Johansen et al. 2007), therefore, the presence of mycobacterial infection in pig herds should not be under-looked but rather be considered as a potential source to human. The poor livestock management practices in Mubende district, unreliable veterinary and extension services, inadequate poor water sources and unhygienic practices on the household level (Anonymous 2004a,b, 2007) simply increases the possibility of mycobacterial spread from both the environment and swine to human.

In conclusion, this study found a significantly high prevalence of lesions and *Mycobacterium* species in slaughter pigs in Mubende district. In order to determine the exact human exposure risk, molecular investigation is recommended in this regard.

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