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Research Article

Mycobacteria in Terrestrial Small Mammals on Cattle Farms in Tanzania

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The control of bovine tuberculosis and atypical mycobacterioses in cattle in developing countries is important but difficult because of the existence of wildlife reservoirs. In cattle farms in Tanzania, mycobacteria were detected in 7.3% of 645 small mammals and in cow's milk. The cattle farms were divided into "reacting" and "nonreacting" farms, based on tuberculin tests, and more mycobacteria were present in insectivores collected in reacting farms as compared to nonreacting farms. More mycobacteria were also present in insectivores as compared to rodents. All mycobacteria detected by culture and PCR in the small mammals were atypical mycobacteria. Analysis of the presence of mycobacteria in relation to the reactor status of the cattle farms does not exclude transmission between small mammals and cattle but indicates that transmission to cattle from another source of infection is more likely. However, because of the high prevalence of mycobacteria in some small mammal species, these infected animals can pose a risk to humans, especially in areas with a high HIV-prevalence as is the case in Tanzania.

1. Introduction

The genus *Mycobacterium* comprises more than 140 named species recognized currently [1], of which several are pathogenic; most of them are environmental mycobacteria that may cause opportunistic infections. The pathogenic species are responsible for some important diseases in humans and animals in the developed world as well as in developing countries, namely, tuberculosis (TB), leprosy, and Buruli ulcer [2]. Susceptibility to mycobacterial infections can be higher in patients with underlying conditions such as human immunodeficiency virus-acquired immunodeficiency syndrome (HIV-AIDS), sarcoidosis, silicosis, or emphysema. With the rising number of HIV-AIDS patients in Africa, TB and in some extent other mycobacterial diseases, caused by, for example, *M. avium* complex, are an important cause of morbidity and mortality [3]. Mycobacterial diseases in cattle such as bovine tuberculosis (BTB), caused by *Mycobacterium bovis*, and atypical mycobacterioses (e.g., paratuberculosis caused by *M. avium* subsp. *paratuberculosis*) can also have serious implications on public health and on economy [4–6]. Therefore, the control of BTB and atypical mycobacterioses is important. In countries with a wildlife reservoir of *M. bovis*, BTB in cattle is more difficult to control. In the UK, New Zealand, the United States, and Africa, a number of animals have been found to be infected with and act as a reservoir for *M. bovis*, namely, the European badger (*Meles meles*), brushtail possums (*Trichosurus vulpecula*), white-tailed deer (*Odocoileus virginianus*) and bison (*Bison bison*), and the African buffalo (*Syncerus caffer*), respectively [7].

Not much research has been conducted on the wildlife reservoir of *M. bovis* in sub-Saharan Africa and research has focused mainly on the presence of *M. bovis* in South Africa [8–11]. In Tanzania in 2005, *M. bovis* was demonstrated in free-ranging wildlife, namely, in wildebeest, topi, the lesser kudu, and lions in the Serengeti national park, the Tarangire National Park, and the Ngorongoro crater in Tanzania [12]. More information is present on the prevalence of *M. bovis*-infection in cattle in Tanzania. This prevalence ranges between 0.2% and 14% and that of atypical mycobacterioses in cattle between 0% and 13.0% [6, 13–17].

Also, other pathogenic mycobacterial species including *M. microti*, *M. avium*, and *M. marinum* have been found in wild animals [18–21]. Although these mycobacteria are less virulent for humans, they do cause infections or diseases in humans, especially when humans are immunocompromized [3, 22, 23]. As such, animals could transmit mycobacteria to humans [24].

Until present, little research is performed on the reservoir status of rodents and insectivores for mycobacteria [19, 21, 25–27] although they are hosts for pathogens causing diseases in humans and livestock [28, 29] such as leptospirosis, plague, and toxoplasmosis [30]. Cattle farms can be prone to rodent infestations because of the abundant amounts of shelter, water, and food [31], thus augmenting the rate of direct or indirect contact between rodents and cattle and the risk of disease transmission. Moreover, rodents are sensitive to experimental infection with mycobacteria that can cause disease in cattle, for example, *M. bovis* and *M. avium* subsp. *paratuberculosis* [32, 33] and recent studies have shown that African rodents and insectivores can carry mycobacteria of the *M. avium*-complex [21, 26]. In developed areas (UK and New-Zealand), rodents and insectivores were found to carry *M. bovis* [29, 34, 35] but with a low estimated transmission risk [20, 36]. However, in Africa, other rodent and insectivore species are present and the risks of rodent-borne diseases (both for humans and cattle) are higher because of a higher contact rate between humans, cattle, and rodents.

Therefore, the aim of this study was to evaluate the possibility of a rodent/insectivore reservoir for *M. bovis* and other mycobacteria in Tanzania, from which cattle (and humans) could be infected. We have collected small mammals in cattle farms with a known tuberculin status. The single comparative intradermal tuberculin test (SCITT) can detect cattle exposed to *M. bovis* as well as atypical mycobacteria [37, 38]. A positive SCITT test can thus serve as an indication for exposure to mycobacteria. By targeting the small mammal collection in cattle farms housing animals with known reactor status, we can get an indication of the transmission direction or the involvement of other source(s) of infection as summarized in Table 1.

2. Materials and Methods

2.1. Trapping Sites. A total of 26 cattle farms were chosen in and around Morogoro, a medium-sized city 200 km west of Dar es Salaam (37.26–37.49°E; 6.18–6.52°S). These farms can be divided into two reactor types based on the single comparative intradermal tuberculin test (SCITT) conducted in the cattle residing on the farms in 2005 and 2006 [14]. For all animals, the "current reactor status" at the moment the trapping took place was known. For the trapping period of 2005, the "future reactor status" of the farms was known (i.e., the SCITT-results of 2006). For the trapping period of 2006, the "past reactor status" of the farms was known (i.e., the SCITT-results of 2005). Other trapping sites included a grass field around the slaughterhouse in Morogoro, and a quarter in Morogoro where a high prevalence of mycobacteria in rodents and insectivores was observed in a previous study, namely, Mwembesongo [21].

The trapping took place in the wet and dry season of both 2005 and 2006.

2.2. Sample Collection. Three types of live traps were used: Sherman LFA Live Traps, Box traps, and big wire cage traps [21]. Peanut butter with maize bran and fresh maize cobs were used as bait [21].

The animals were processed in the laboratory following a standard protocol as described by Durnez et al. [21]. In brief, the animals were euthanized with chloroform, and external characteristics and measurements such as weight and head-body length were recorded. During necropsy, pieces of liver, spleen, lung, mesenteric lymph nodes, and external lesions if present were taken for detection of mycobacteria. The carcasses were kept in formalin and sent to the University of Antwerp for further identification to species level: primary identification was confirmed, and skulls were removed and cleaned to identify the animals to species level.

2.3. Pooling of Samples and Detection and Identification of Mycobacteria. The samples were pooled in a stratified way: the same organs were pooled per one to six individuals of the same species trapped at the same trapping site. A flow chart of the pooling procedure is given in Figure 1. The number of animals in a pool depended on the trapping number per species at a trapping site (resulting in 1 to 6 animals per pool). In this way, 645 individual animals were pooled into 307 groups of individuals. For each group, the four different organ homogenates collected from each animal were pooled separately, resulting in 1228 pools to be tested. A subset of samples was used to test whether pool screening and individual screening gave comparable prevalence estimations. In this subset of samples, the pooled results and the individual results were available.

The pools were analyzed for the presence of mycobacteria by culture and PCR as described before [21]. In short, the organs were homogenized and decontaminated to reduce overgrowth of nonmycobacterial organisms [39], before inoculating them on culture media (Löwenstein-Jensen, Stonebrink, and Löwenstein-paratuberculosis medium [40]) and performing DNA extraction (described in [41]) and PCR (described in [14]) with inhibition check.

Cultivation took place at 37 degrees C [39] for ten to twelve months.

The mycobacteria isolated on culture were checked for acid fastness using Ziehl-Neelsen staining (ZN) and

Table 1: Interpretation of possible differences in prevalences of mycobacteria in small mammals in relation to the reactor status of the farm on which the small mammals were collected.

Analysis in relation to	Possible difference in prevalence of mycobacteria in small mammals collected in reacting and nonreacting farms	Indication on transmission direction and the involvement of other source(s) of infection*
(1) Current reactor status	(a) No difference	Transmission between small mammals and cattle might occur, but cattle and small mammals probably have a different source of infection
	(b) Higher prevalence in currently reacting as compared to non reacting farms	Transmission between small mammals and cattle might occur, but common source of infection more probable.
	(c) Higher prevalence in currently nonreacting as compared to reacting farms	Transmission between small mammals and cattle might occur, but cattle and small mammals probably have a different source of infection
(2) Future reactor status	(a) No difference	Transmission from small mammals to cattle might occur, but cattle also has another source of infection
	(b) Higher prevalence in future reacting as compared to non reacting farms	Tranmission from small mammals to cattle may occur, either directly or indirectly
	(c) Higher prevalence in future nonreacting as compared to reacting farms	Transmission from small mammals to cattle might occur, but cattle has another, probably more important, source of infection
(3) Past reactor status	(a) No difference	Transmission from cattle to small mammals might occur, but small mammals also have another source of infection
	(b) Higher prevalence in past reacting as compared to non reacting farms	Transmission from cattle to small mammals may occur, either directly or indirectly
	(c) Higher prevalence in past nonreacting as compared to reacting farms	Transmission from cattle to small mammals might occur, but small mammals have another, probably more important, source of infection

* Another source of infection can be other wild or domestic animals, the environment, or humans.

identified to species-level by biochemical methods and by sequencing the 16 S rRNA gene [42].

2.4. Collection and Analysis of Milk Samples. Every trapping period, from every milking cow on the cattle farms where small mammals had been trapped, a milk sample (1 to 10 mL per cow) was collected. The milk samples were kept at −20°C and analyzed in Belgium by culture and PCR as described by Durnez et al. [14].

2.5. Data Analysis. For the results of the pooled samples, the data analysis was based on the use of likelihood ratio tests (LRTs) in the usual parametric model for pool testing. The pool screening sampling model is basically that of a Bernoulli trial with success probability $\theta = [1 - (1 - p)^n]$, where n is the pool size and p is the infection rate in the population of interest. The random variable which is denoted by X is the result of the testing of the pool and has value 1 if the pool is positive and 0 if the pool is negative.

Thus, the probability model describing the sampling is given by the probability mass function

$$f_x(x \mid n, p) = \left[1 - (1 - p)^n\right]^x \left[(1 - p)^n\right]^{1-x}, \quad x \in \{0, 1\}. \tag{1}$$

This model has a long history in the statistics literature [43–47] and is described in detail in all of these papers. The investigator collects pools of various sizes, n_i, and after testing the pool knows the value of the result, denoted by x_i. Thus, for any pool, given the pair (n_i, x_i), the only unknown quantity in the model is the value of p. The standard method for estimating p is the method of maximum likelihood [48]. This depends on the likelihood function which in this case is

$$L(p) = \prod_{j=1}^{m} \left[1 - (1 - p)^{n_j}\right]^{x_j} \left[(1 - p)^{n_j}\right]^{1-x_j}, \tag{2}$$

where m is the number of pools tested. The maximum likelihood estimate (MLE) is found by maximizing $L(p)$ as a function of p. We note that no special adjustments are required for the inequality of the pool sizes, because this is a feature which is part of the model. This sampling model is the basis for constructing any standard likelihood ratio type test. Tests for a one way or two way design are constructed by replacing p by a linear model in the factors of interest. In this case, a coding scheme analogous to cell mean coding in standard analysis of variance is convenient. Likelihood ratio test methods are a standard technique in statistics. Details of the actual implementation of such tests in

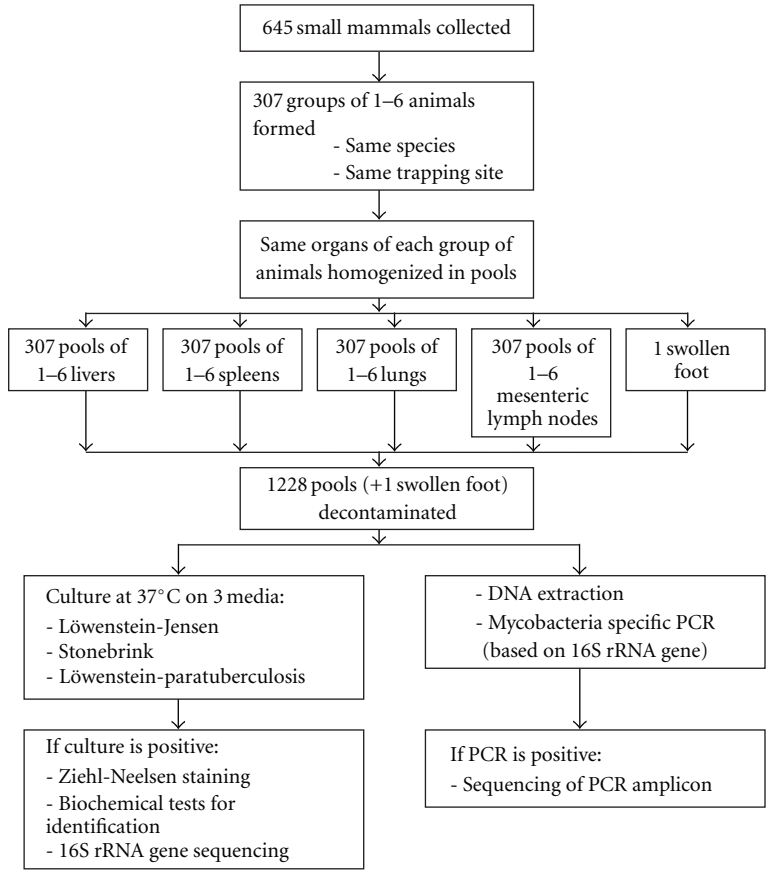

Figure 1: Flow chart of pooling procedure.

this case are computationally complex and requires extensive mathematics but would add nothing to the subject matter of this paper. The calculations were done using a custom FORTRAN program running under MS Windows developed by one of the authors (C. R. Katholi). The core of the estimation is the optimization software used, in this case the program NLPQLP [49–51].

A kappa-test (using SPSS 16.0) was used to compare the results of the individual versus pooled samples analysis.

3. Results

3.1. Trapping Results. The number of animals trapped in this study are listed per species in Table 2.

3.2. Mycobacterial Results

3.2.1. Comparison of Pool Prevalence Estimation versus Individual Prevalence Estimation. For culture and PCR, the respective Kappa-values, comparing the results of the pooled samples and the individual samples, were 0.691 and 0.876, showing a high concordance. For culture, the estimated prevalence with pool prevalence estimation was 6.8% (95% CI: 2.8%–13.3%), while with individual tests the prevalence was estimated to be 7.6% (95% CI: 3.5%–14.0%). For PCR, these estimated prevalences were respectively 3.3% (95% CI: 0.8%–8.2%) and 3.8% (95% CI: 1.2%–8.8%).

3.2.2. Mycobacteria Detected in Rodents and Insectivores. The number of positive groups for mycobacteria and the estimated prevalence are listed in Table 2, the identification of the mycobacteria in Table 3. A total of 44 groups out of 307 tested positive for mycobacteria in culture or PCR, which makes a total estimated prevalence of 7.3%. The estimated prevalence of mycobacteria in *C. gambianus* was higher than in *M. natalensis* ($P = .011$) and in *R. rattus* ($P < .001$), while no significant difference was observed with *C. hirta* ($P = .123$). *C. hirta* also carried more mycobacteria than *R. rattus* ($P < .001$), while no difference was observed with *M. natalensis* ($P = .164$). *M. natalensis* carried significantly more mycobacteria than *R. rattus* ($P = .028$).

When testing for a difference between organs, the liver was found to be the least infested with mycobacteria, while the lung was most prone to contain mycobacteria (Page's test statistic $L = 115.5$; $\alpha < 0.01$). The positivity of mycobacteria in the different organs per animals species is given in Table 4.

A total of 33 groups out of 233 groups of animals trapped on cattle farms tested positive for mycobacteria, with an estimated prevalence of 7.0%. Since we were interested in the relation between the SCITT reactor status of the farm (current, past, or future) and the mycobacterial presence in

Table 2: Rodents and insectivores trapped in and around Morogoro and the prevalence of mycobacteria in the different animal species.

Animal species	Total number of animals trapped[a]	Number of groups analyzed for mycobacteria	Number of groups positive for mycobacteria	Estimated mycobacterial prevalence (95% confidence interval)	95% confidence intervals for zeroestimates of *M. bovis* and *M. avium* subsp. *Paratuberculosis* prevalence
Rodents					
Rattus rattus (Linnaeus, 1758)	268 (216/1/51)	94	7	2.8% (1.0–5.7%)	0–0.71%
Mastomys natalensis (Smith, 1834)	165 (142/23/0)	91	12	7.5% (3.7–13.1%)	0–1.15%
Cricetomys gambianus Waterhouse, 1840	36 (12/2/22)	32	8	23.9% (10.3–42.7%)	0–5.19%
Mus spp.	29 (2/1/26)	22	0	0% (0–6.4%)	0–6.4%
Grammomys surdaster Mathey, 1971	3 (3/0/0)	3	0	0% (0–47.3%)	0–47.3%
Gerbilliscus vicina (Matschie 1911)	2 (0/2/0)	2	0	0% (0-61.7%)	0-61.7%
Squirrel (not identified)	1 (1/0/0)	1	0	0% (0–85.3%)	0–85.3%
Insectivores					
Crocidura hirta Peters, 1852	137 (127/9/1)	58	15	12.5% (6.8–20.4%)	0–3.3%
Atelerix albiventris Wagner, 1841	4 (1/3/0)	4	2	50% (7.7–92.3%)	0–38.1%

[a](on cattle farms/around slaughterhouse/in Mwembesongo).

rodents and insectivores, the analyses were performed for the current, past, and future reactor status of the farms, when enough data were available. Results are summarized in Table 5.

Additionally, two-way ANOVA analyses revealed no effect of season (dry or wet) on the prevalence of mycobacteria in rodents and insectivores in RR and NR farms (data not shown). One-way ANOVA analyses showed that there was no difference in the prevalence of mycobacteria in farms that changed reactor status (NR to RR or RR to NR) during the study as compared to farms of which the reactor status remained the same (NR or RR) (data not shown).

The prevalence of mycobacteria in rodents and insectivores trapped around the slaughterhouse and in Mwembesongo is listed in Table 5. No significant difference was found in the prevalence between rodents and insectivores trapped on these sites. When comparing the prevalence of mycobacteria in the animals trapped around the slaughterhouse and trapped in the NR and RR farms (current reactor status), a significant difference was observed ($P = .001$) with a significant interaction in the two-way ANOVA ($P = .04$). For insectivores, a significantly higher prevalence was observed in slaughterhouse as compared to NR farms ($P = .007$), while no difference was observed with RR farms ($P = .280$). No difference was observed for the past or future reactor status of the farms. Also, no difference was observed between the prevalence of mycobacteria in rodents trapped in Mwembesongo and in the NR or RR farms.

Out of 226 milk samples collected on the same farms where animals had been trapped, 6 (2.7%) were positive for mycobacteria by culture and 12 (5.3%) by PCR. The identifications of the mycobacteria are listed in Table 6.

4. Discussion

This study is the first to investigate mycobacteria present in rodents and insectivores collected on the farms in relation to the tuberculin reactor status of the cattle residing on these farms. The rationale for this study was that pathogens that infect more than one host species, as is the case for many pathogenic mycobacteria, are likely to be encountered in several host populations, some of which may constitute infection reservoirs [53]. However, this means that the presence of infection in a wild animal population does not prove that the animal species is a reservoir of the infectious agent [4]. To get more information on the possible reservoir status of a certain host, the data of the presence of infection in that host should, therefore, be analysed in relation to data of infections in the target population. In this respect, it is important to acknowledge the existence of different host types; that is, a maintenance host, in which infection can persist by intraspecies transmission alone, and a spillover host, in which infection will not persist indefinitely unless there is reinfection from another species. Both maintenance and spillover hosts may transmit infection to other species, but this difference is important when control of a host species is considered [4].

In general, rodents and insectivores can come into contact with mycobacteria through the environment by feeding, contact with soil, and contact with the feces of wild and domestic animals or humans. These mycobacteria can pass through the stomach of these animals without being digested, since they are resistant to acid. Pathogenic and opportunistic mycobacteria can pass through the stomach and can survive in tissues and organs. In this way, they can

Table 3: Mycobacteria detected in rodent and insectivores in and around Morogoro, Tanzania.

Mycobacteria[a]	Small mammal species	Detected by PCR or culture
Human risk group 1[c]		
*M. duvalii***	*C. gambianus*	Culture
M. gordonae	*A. albiventris*	PCR
M. gordonae-like	*C. gambianus*	PCR
M. gordonae-like**	*C. hirta*	Culture
M. gordonae-like	*C. hirta*	PCR
M. gordonae-like	*M. natalensis*	PCR
*M. moriokaense**	*R. rattus*	Culture
M. mucogenicum	*M. natalensis*	PCR
M. nonchromogenicum	*C. hirta*	Culture
*M. nonchromogenicum**[b]	*R. rattus*	Culture
M. nonchromogenicum-like	*M. natalensis*	Culture
M. nonchromogenicum-like	*R. rattus*	PCR
M. sphagni-like	*R. rattus*	PCR
M. terrae	*C. hirta*	Culture
*M. terrae****	*C. gambianus*	Culture
M. terrae	*R. rattus*	Culture
Human risk group 2[c]		
M. chelonae var. *niacinogenes*	*M. natalensis*	PCR and culture
M. genavense-like	*C. hirta*	PCR
*M. intracellulare****	*C. gambianus*	Culture
M. intracellulare	*C. hirta*	Culture
M. intracellulare	*C. hirta*	PCR and culture
M. intracellulare	*C. hirta*	Culture
M. intracellulare	*M. natalensis*	PCR and culture
M. intracellulare-like	*C. gambianus*	Culture
M. intracellulare-like	*C. gambianus*	Culture
M. intracellulare-like	*C. hirta*	Culture
M. scrofulaceum-like	*C. gambianus*	Culture
M. szulgai	*M. natalensis*	PCR
MAIS	*C. gambianus*	PCR and culture
MAIS	*C. gambianus*	Culture
MAIS	*C. hirta*	PCR and culture
Recently described species, not yet classified[c]		
M. alsiensis	*M. natalensis*	PCR and culture
M. chimaera	*C. hirta*	Culture
M. chimaera-like	*C. hirta*	Culture
M. colombiense	*C. hirta*	PCR and culture
M. frederiksbergense-like	*M. natalensis*	PCR
*M. goodii**[b]	*R. rattus*	Culture
M. immunogenum	*R. rattus*	PCR
M. septicum	*A. albiventris*	PCR
M. septicum	*M. natalensis*	PCR
M. septicum	*M. natalensis*	PCR

[a] *, ** and *** point out mycobacteria detected in the same group of animals but in different organs.
[b]These mycobacteria were first detected in 2005 in *R. rattus* trapped on a farm and were later detected in 2006 in the milk of cattle residing on the same farm (see Table 6).
[c]The classification in human risk groups is based on the clinical point of view in which human risk group 1 contain species that never or with extreme rarity cause disease. Human risk group 2 are species that normally live freely in the environment but also cause opportunistic infections in humans. Human risk group 3 are the obligate pathogens (*M. tuberculosis* complex and *M. leprae*) [52].

Table 4: Positivity of mycobacteria (in %) in different organs for all animals and for the main animal species collected.

	Liver	Spleen	Lung	Mesenteric lymph nodes
All animals	1.6 (0.8–2.7)	2.1 (1.1–3.4)	3.2 (2.0–4.8)	1.9 (1.0–3.2)
C. gambianus	5.9 (1.0–17.4)	5.9 (9.0–17.4)	8.9 (2.2–21.8)	8.3 (2.0–20.4)
C. hirta	2.2 (0.6–5.7)	5.5 (2.4–10.5)	5.3 (2.3–10.1)	3.9 (1.4–8.3)
M. natalensis	1.2 (0.2–3.7)	1.2 (1.2–3.7)	3.7 (1.5–7.4)	3.7 (1.5–7.4)
R. rattus	0.38 (0.020–1.7)	0.38 (0.020–1.7)	1.6 (0.48–3.6)	0.76 (0.13–2.3)

Page's test for order tests the following hypothesis:
H_0: liver = spleen = lymph = lung; H_a: liver < spleen < lymph < lung.
Test statistic L = 115,5; $\alpha < 0.01$.

Table 5: Prevalence of mycobacteria in rodents and insectivores trapped on cattle farms, around the slaughterhouse and in Mwembesongo. RR: positive tuberculin reactor status; NR: negative tuberculin reactor status. The *P* values given are significance values for the difference between RR and NR farms.

	Cattle farms									SH	MS
	Current reactor status			Past reactor status			Future reactor status				
	RR	NR	*P*	RR	NR	*p*	RR	NR	*P*		
Total	8.8%	2.9%	.014*	4.1%	8.9%	.495	8.3%	11.4%	.459	13.1%	6.1%
Rodents	6.2%	3.1%	.216	2.6%	0.0%	.438	9.1%	6.4%	.538	6.9%	6.2%
C. gambianus	12.5%	0.0%	.448	na	na	na	na	na	na	na	na
M. natalensis	9.7%	0.0%	.062	4.0%	0.0%	.673	10.4%	12.6%	.828	na	na
R. rattus	3.0%	4.4%	.644	1.1%	0.0%	.497	7.3%	5.6%	.703	na	na
Insectivores	18.2%	2.4%	.009*	9.8%	61.0%	.019*	9.4%	15.2%	.508	36.0%	na
C. hirta	18.2%	2.5%	.010*	10.0%	61.0%	.019*	9.4%	15.0%	.507	na	na

* The difference is statistically significant at $P < .05$.
na: not applicable because of insufficient or no data.
SH: Slaughterhouse.
MS: Mwembesongo.

be spread over long distances with the migration of these animals [27] and even if they are not part of the maintenance reservoir, they can play a role as transport host.

4.1. No Evidence for Rodents and Insectivores as Reservoir Hosts for M. bovis or M. avium Subsp. paratuberculosis. In previous studies in the UK and New Zealand, the prevalences of *M. bovis* in rodents and insectivores ranged from 0.4 to 2.8% and from 1.2 to 5%, respectively [20, 29, 34, 35, 54]. *M. avium* subsp. *paratuberculosis* was also found previously in rodents and insectivores in the Czech Republic and Greece at prevalences ranging from 1.3%, to 5.9% and from 1.7% to 2.6%, respectively [55, 56].

In the present study, rodents and insectivores were collected on cattle farms, some of which housed cattle infected with *M. bovis* and/or atypical mycobacterioses [14]. Although we have not detected *M. bovis* or *M. avium* subsp. *paratuberculosis* in the small mammals trapped on the cattle farms in Morogoro, African rodents or insectivores could still be a reservoir for these mycobacteria. As it has been the case in previous studies [20], for some species, not enough animals were trapped to definitely conclude that they do not carry *M. bovis* or *M. avium* subsp. *paratuberculosis*, as shown by the wide confidence intervals of the zero estimates for some species in Table 2. Moreover, although we have used different types and sizes of traps, some species of rodents and insectivores will not be caught in these traps because of their size. For example, most of the shrews species are too small to trigger the traps used in this study. Therefore, we could have missed some crucial species.

For the two species trapped in significant numbers, namely, *R. rattus* and *M. natalensis,* the confidence intervals show that they do not play a significant role as carriers of *M. bovis* or *M. avium* subsp. *paratuberculosis* in the studied area. A closely related species of *R. rattus*, *R. norvegicus*, is experimentally not sensitive to infection with *M. bovis* [32] although it has been found to carry *M. bovis* in the UK [34, 35] but at low prevalences (1.2–2.2%).

4.2. Rodents and Insectivores as Hosts for Other Mycobacteria. Tuberculin tests in cattle have revealed a high prevalence of atypical mycobacterioses in Tanzanian cattle [14]. Atypical mycobacteria, such as *M. avium* subsp. *paratuberculosis*, can also have an effect on the cattle farm production [5], providing an economic incentive to prevent them in cattle. Therefore, all rodent and insectivore samples collected in the present study were also analyzed for the presence of atypical mycobacteria.

Analysis of the prevalences of mycobacteria in rodents and insectivores in relation to the reactor status of the farm on which the small mammals were collected gave us an indication on whether transmission between small mammals

Table 6: Mycobacteria detected in cow milk on the cattle farms.

Mycobacteria	Detected by PCR or culture
Human risk group 1[b]	
M. neoaurum	PCR
M. nonchromogenicum	Culture
M. nonchromogenicum[a]	Culture
M. gordonae	PCR
Human risk group 2[b]	
M. asiaticum	Culture
M. szulgai-like	Culture
Recently described species, not yet classified [b]	
M. engbaekii	Culture
M. goodii[a]	PCR
M. lacticola	PCR
M. septicum	PCR

[a]These mycobacteria were first detected in 2005 in *R. rattus* trapped on a farm and were later detected in 2006 in the milk of cattle residing on the same farm (see Table 3).
[b]The classification in human risk groups is based on the clinical point of view in which human risk group 1 contain species that never or with extreme rarity cause disease. Human risk group 2 are species that normally live freely in the environment but also cause opportunistic infections in humans. Human risk group 3 are the obligate pathogens (*M. tuberculosis* complex and *M. leprae*) [52].

and cattle would be possible, or if another source of infection would have to be involved.

4.2.1. Rodents. For *M. natalensis* there was no difference in the prevalence of mycobacteria between the different farm types for the present, future, and past reactor status. Another source of infection should thus be available for cattle and *M. natalensis* to become infected with mycobacteria. This other source could be another wild or domestic animal, the environment, or humans.

Some of the isolated mycobacteria, namely, *M. chelonae*, *M. intracellulare,* and *M. szulgai,* are known pathogens to humans, causing pulmonary disease, soft skin, or disseminated infections in immunocompetent (*M. intracellulare*), immunocompromized, or predisposed patients (*M. chelonae* and *M. szulgai*) [52]. Their impact on the health of cattle or on their milk production is not clear, however, although they have been isolated from cattle in several studies [14, 57, 58]. Most of the mycobacteria detected in *M. natalensis* were detected in the lung or mesenteric lymph nodes, so they may potentially be excreted by the animals through feces or respiratory secretions. In the present study, no fecal specimens were examined, but we have shown in Benin that 15.5% of small mammal's feces contain mycobacteria [26].

For *R. rattus*, the detection rate of mycobacteria is low (2.8%), with no mycobacteria belonging to human risk group 2 (Table 3) and, similarly to *M. natalensis*, no difference between farm types. However, interestingly, *M. nonchromogenicum* and *M. goodii* were first detected in 2005 in *R. rattus* trapped on a farm and were later detected in 2006 in the milk of cattle residing on the same farm (Tables 3 and 6). This could mean that *R. rattus* excretes these mycobacteria, for example, as a transport host, that these mycobacteria are conserved very well in the environment or that these mycobacteria are maintained in another domestic or wildlife host, from which cattle and *R. rattus* can pick up the mycobacteria as spill-over hosts. For *M. nonchromogenicum*, the source of infection is probably the environment [52]. The status and natural reservoir of *M. goodii,* however, is not yet clear, but it causes infections in both humans [59] and wildlife [60].

In accordance with a previous study [21], the highest prevalence of mycobacteria was found in *C. gambianus* (23.9%) as compared to the other rodents, with *M. intracellulare* and related mycobacteria as main mycobacteria found in this species. The difference in prevalence of mycobacteria in this animal species between reacting and nonreacting farms was not significant, possibly because of the low number of *C. gambianus* trapped in these cattle farms ($n = 12$). The elevated prevalence may indicate a potential risk to humans, since most of the infected animals were trapped in or near human dwellings in Mwembesongo.

The large difference in prevalences between the different rodent species might be due to different behavior, habitat, and food preference. Table 7 summarizes the habitat and food preference of the main animal species trapped in this study. Although their habitat ranges differ, they were all collected in the same environment around cattle farms and human dwellings. *M. natalensis, R. rattus,* and *C. gambianus* are all omnivorous and will eat whatever they will find in a human-created environment. The main difference is that *C. gambianus* use their cheek pouches to carry food and bedding material and that they regularly perform coprophagy [61]; in that way, they can have more frequent encounters with mycobacteria.

4.2.2. Insectivores. A difference in prevalence of mycobacteria between rodents and insectivores was also observed similar to a previous study [21]. This difference was only observed in reacting farms and not in nonreacting farms. Insectivores probably pick up mycobacteria from the environment through their scavenging behaviour [27]. Their coprophagy and feeding on freshly killed animals (Table 7) are possible explanations for the elevated prevalence. A difference was also found in the mycobacterial prevalences in these animals between reacting and nonreacting farms: For the current reactor status, a higher prevalence was observed in the reacting as compared to the nonreacting farms, which is an argument for a common source of infection for insectivores and cattle. For the past reactor status, a higher prevalence was observed in the nonreacting as compared to the reacting farms, also indicating that cattle are probably not the source of infection for insectivores.

Most of the mycobacteria found in the insectivore *C. hirta* were potentially pathogenic for humans (Table 3), most of which were part of the *M. avium*-complex (*M. intracellulare*, *M. chimaera*, and *M. colombiense*) [52].

Table 7: Habitat and food preference of the main animals species collected according to Nowak [61].

Animal species	Habitat	Food preference
C. gambianus	Forests, and villages	Vegetables, insects, crabs, snails, palm fruits, and palm kernels Regular coprophagy They use their cheek pouches to carry food and store large amounts of food in their shelters
C. hirta	Damp and dry forests, grasslands Cultivated areas Occasionally human settlements and buildings	Invertebrates Bodies of freshly killed animals (including frogs, toads and lizards) Regular coprophagy
M. natalensis	Savannah Cultivated and abandoned fields Buildings and villages	Mainly grass and other seeds, insects when available Everything that people eat when available
R. rattus	Cities, villages Cultivated fields some natural habitats	Variety of plants and animal matter: seeds, grains, nuts, vegetables, fruits Everything that people eat

The other insectivores collected in this study, the hedgehogs, are also known carriers of pathogenic mycobacteria [29, 62, 63] although only European hedgehogs have been studied in this context. In the present study, 2 out of 4 hedgehogs (*A. albiventris*) carried mycobacteria, but those mycobacteria are probably not pathogenic.

4.2.3. Organs. In general, the lung was the most prone to contain mycobacteria, followed by the mesenteric lymph nodes, the spleen, and the liver. This is consistent with transmission of mycobacteria through aerosols and through digestion. In two cases, mycobacteria were cultured from lesions: *M. terrae* from a swollen foot of *R. rattus* and a mycobacterium related to *M. intracellulare* from a swollen lymph node of *C. gambianus*. In the other animals presenting mycobacteria, no macroscopic pathomorphological lesions were observed and for most of the isolates only one colony was observed in culture, suggesting colonization rather than infection.

As mycobacteria were found in all four organs, and considering what is currently known about transmission of diseases by rodents and of diseases in general [64], several ways of transmission of mycobacteria are possible: through direct contact with rodent excreta, through ingestion of food or water contaminated with rodent excreta, through ingestion of the animal itself, through inhaling aerosolized rodent excreta, through rodent bites, or through ectoparasites.

4.3. Pool Screening Approach. The study used the pooled screening approach to save time and resources when analyzing the specimens. We have shown that although there is a slight underestimation of the prevalence when pool screening is used for mycobacterial detection, the 95% confidence intervals are as wide as with individual screening. This has been previously reported by Vansteelandt et al. [65] for viral detection. As the pooling was done in a stratified way, per habitat, per organ and per species, there was no loss of information since several hypotheses could still be tested. If pooling would have been done in a different way, for example, pooling all organs from the same animal as has been done by some researchers [20, 66], we would have lost information, about the site of infection or colonization. Therefore, we strongly recommend the stratified pool screening method in mycobacterial reservoir research.

4.4. PCR versus Culture for Detection of Mycobacteria. For only seven pools, PCR and culture results were consistent. This probably is due to a difference in sensitivity of the methods [67]: for detection of *M. tuberculosis* in clinical samples, PCR is less sensitive than culture [68]. This is also true for mycobacteria in general. Although a specific 16S rDNA PCR is very useful to detect mycobacteria in different samples, it is not as sensitive as culture, because of the fact that its target only occurs once or twice in the mycobacterial chromosome [69]. Indeed, five of the eight pools from which more than one colony grew in culture, were also positive for PCR (see Supplementary Table S1 that could be found at doi: 10.4061/2011/495074).

A second reason for the inconsistency is the fact that the methods target other mycobacteria; for example, not all mycobacteria grow at the temperature in which the inoculated media are kept [52], while PCR targets also the DNA of dead mycobacteria (killed either by the immune system of the animal, during the transport or storage, or during the decontamination) [70]. This inconsistency has been shown and discussed in previous studies as well [21, 26].

4.5. Risk of Transmission of Mycobacteria from Small Mammals to Humans. Little is known about the prevalence of atypical mycobacterioses in the human population in Tanzania. However, Kazwala et al. [71] reported that 16%

of the mycobacterial isolates from extrapulmonary human samples in Tanzania were *M. bovis* and 13.6% were atypical mycobacteria, whereas Mfinanga et al. [72] demonstrated that 10.8% of mycobacterial isolates from extrapulmonary human samples were *M. bovis* and 47.7% were atypical mycobacteria. This shows that although few data are available, human cases of both BTB and atypical mycobacterial disease are present in Tanzania. Recently, three additional cases of invasive atypical mycobacterial disease in HIV-positive patients in Tanzania were described caused by *M. sherrisii* and *M. avium*-complex [73]. At ITM, we have records of *M. intracellulare*, *M. terrae*, *M. arupense*, *M. colombiense*, and *M. kumamotonense* isolated from clinical samples in Tanzania although the clinical significance of these mycobacterial isolates is not known. Of the mycobacteria isolated in humans, *M. intracellulare*, *M. colombiense*, and *M. terrae* were detected in small mammals in the present study. In a previous study in the same region in Tanzania, we detected also *M. intracellulare* and *M. arupense* [21]. Although the HIV prevalence has been decreasing slowly in Tanzania, it still reached 6.2% and 6% in 2005 and 2006, respectively [74]. In 2009, the HIV prevalence has decreased to 5.6%, but this still means that a substantial proportion of the Tanzanian population is more sensitive to infections with these atypical mycobacteria. All rodents and insectivores in the present study were collected in close proximity to human dwellings, which means that infected animals could pose a risk to humans with a lowered immune system.

5. Conclusion

The present study is the first to investigate the presence of mycobacteria in rodents and insectivores in relation to the reactor status of the cattle farms on which they were collected. Analysis of the presence of mycobacteria in relation to the reactor status of the cattle farms does not exclude transmission between small mammals and cattle but indicates that transmission to cattle from another source of infection is more likely. However, because of the high prevalence of potentially pathogenic mycobacteria in some small mammal species, namely, in *C. gambianus* and *C. hirta*, the infected animals can pose a risk to humans, especially in areas with a high HIV-prevalence as is the case in Tanzania.

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