Risk factors associated with bovine tuberculosis and molecular characterization of *Mycobacterium bovis* strains in urban settings in Niger

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† This paper is dedicated to the memory of Francine Matthys who was the former medical director of MSF Belgium and a prominent public health researcher in the control of human tuberculosis in developing countries.

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Abstract

A retrospective and a longitudinal survey were carried out at the abattoir of Niamey. Results showed a highly significant difference of suspected tuberculosis (TB) gross lesions among different animals species (P <0.0001). The proportion of carcasses with TB-like lesions was 0.19% among cattle, 0.11% among camels, 0.001% among sheep and 0.0006% among goats. In cattle, cows are significantly more affected than the other categories (P<0.001). Also in cattle, TB-like lesions are mostly localized in the lungs (92.77%) followed by the lymph nodes (50.87%) and the liver (32.40%). The prevalence of gross lesions compatible with bovine TB (BTB) is strongly influenced by the season (P<0.0001), closely correlated with the origin of the animals (P<0.001), and has a negative impact on the weight of affected animals (P <0.0001). Sixty two samples of suspected TB gross lesions were subject to microbiological analysis and molecular typing of strains. *Mycobacterium bovis* was identified in 18 animals showing 5 different spoligotypes, belonging to type "African 1" (Af1) previously identified in Central and West Africa. In addition, a profile (SB1982) not previously reported distinguished by the absence of spacers 3, 4, 9, 16, 22, 30 and 39-43 has been characterized in this study. To assess risk factors for BTB transmission, a questionnaire on animal husbandry practices, food habits, and clinical signs of TB in animals and humans was submitted to the heads of 1,131 randomly selected households. The main risk factors identified are: consumption of unpasteurized milk (91%) and lack of hygiene within households (32 to 74%). Clinical signs that could be attributed to TB were also reported both in humans and in animals of the households.

Key words: *Mycobacterium bovis*, bovine tuberculosis, risk factors, livestock, urban settings

1. INTRODUCTION

Tuberculosis due to *Mycobacterium bovis* is often neglected as zoonotic disease in developing countries (Acha and Szyfres, 2005). In sub-Saharan Africa (SSA), bovine tuberculosis (BTB) is a serious threat for the economy but also for public health and animal health (Cosivi et al., 1998; Michel et al., 2006; Cleaveland et al., 2007; Humblet et al., 2009). BTB is widely distributed in SSA, where 85% of the herds and 82% of the human population live in areas where the disease has been reported (Cosivi et al., 1998). In most African countries, BTB control measures are not applied (OIE, 2007). Additionally, there are very complex interactions between the rural pastoral livestock systems and the semi-intensive system practiced in and around urban settings (Thys et al., 2006; Boukary et al., 2007). These interactions and inadequate sanitation measures are important risk factors favouring endemicity of zoonotic tuberculosis (Sidibé et al., 2003; Mfinanga et al., 2003; Cleaveland et al., 2007). The nutritional habits of the population consuming unpasteurized milk also endorse infection with *M. bovis* (Kang'ethe et al., 2007). However, TB due to *M. bovis* has hardly been studied in the sub-Saharan context where epidemiologic aspects of the disease remain largely unknown (Cosivi et al., 1998). Whether in animals or humans, the pathogen itself, *M. bovis,* is rarely studied (Thoen and Bloom, 1995). The Interafrican Bureau for Animal Resources (AU/IBAR, 2006) indicates that in 2006 only 9 of 53 African countries reported cases with a total of 176 outbreaks of BTB. Mostly cattle are affected representing 98.9% of reported animal cases.

Use of *in vitro* culture and subsequent molecular typing enabled progress in the characterization of *M. bovis* strains in some African countries (Rigouts et al., 1996; Portaels et al., 2001; Haddad et al., 2004). Molecular typing of *M. bovis* isolates from Tanzania (Daborn et al., 1997) has shown two lineages of *M. bovis:* an aboriginal lineage with atypical properties and a lineage imported from Europe displaying the classical spoligotyping profile. Subsequent work in Central and West Africa performed by Njanpop-Lafourcade et al. (2001), Diguimbaye et al. (2006), Schelling et al. (2005), Cadmus et al. (2006) and Müller et al. (2008; 2009) revealed a predominant characteristic *M. bovis* spoligotype in Central Africa and West Africa, called Af1 type.

Data on the importance of BTB in Niger are particularly scarce and not updated. Investigations of Alambedji (1984) and Bloch and Diallo (1991) by single intradermal skin test gave low prevalence rates varying from 1.56 to 3.20% among cattle.

The present work aims to determine the prevalence of BTB suspected gross lesions at the abattoir of Niamey, to contribute to the knowledge of current circulating *M. bovis* strains in Niger, and to identify risk factors for transmission of BTB from animal to human.

2. MATERIALS AND METHODS

2.1. Survey site and animal husbandry systems

The survey was carried out at the abattoir of Niamey located in the industrial area of the city on the banks of the Niger River. The "Abattoir de Niamey" is a semi-autonomous company which was founded in 1967. It's an old structure and the entire infrastructure is aging. At the moment of the study, the staff was composed of 60 technicians including 10 permanent staff inspectors working under the supervision of a sworn veterinarian. This staff is assisted by more than 300 temporary workers and butchers. Slaughter and carcass inspection is done at night between 10 PM and 5 AM.

The Niamey abattoir is in charge of animal slaughtering and refrigeration, and has a capacity of 10,000 tons per year. It is supplied by animals from the city livestock market (called Tourakou) and from rural markets, mainly those located in the areas of Torodi, Tera, Ayorou, Balleyara, Kollo and Boubon in the surrounding of Niamey (**Figure 1**). In rural areas (Ru), animals are managed under a traditional husbandry system (extensive/transhumant)) depending entirely on natural pastures and farm by-products without extra feed supplements or adequate health services. Cattle and small ruminants are usually driven to pasture together. Camels are generally led in separate herds and their feeding is mainly based on tree fodder. In the urban (Ur) and periurban (Pu) areas of Niamey city the livestock system is directed towards dairy production and animals are more fed with supplements, including agro-industrial by-products and kitchen waste.

2.2. Characterization of suspected BTB infection at the abattoir of Niamey

Determination of the prevalence of TB-like lesions by retrospective data collection: Data on 432,764 cattle, 696,663 sheep, 145,898 goats and 18,754 camels slaughtered at the abattoir of Niamey from January 2003 to December 2008 were collected from the official records and analysed (**Table 1**). Carcasses underwent a standard meat inspection including the examination of the following lymph nodes: parotid, retropharyngeal, mediastinal, tracheobronchial, mesenteric, submaxillary, iliac, precrural, prescapular, supra-mammary, inguinal, apical, ischiatic and portal nodes. Organs/tissues including lungs, liver, kidneys, mammary glands, intestines, heart, abdominal and thoracic cavities, cerebral membranes and bones (ribs and vertebrae) were also thoroughly examined. Organs showing gross visible lesions compatible with BTB were confiscated. According to the regulation at the abattoir of

Niamey, suspected cases of active or stabilized tuberculosis that are confirmed by the veterinary inspector are subject to total or partial condemnation and destruction.

Characterization of suspected tuberculosis gross lesions by a longitudinal survey: An additional one-year longitudinal survey was implemented independently during standard meat inspection at the abattoir from July 2007 to June 2008. For all the cases of condemnation due to suspected TB data related to the animals (age, breed, sex, geographic origin and clinical history), the production and marketing system (including the various stakeholders), the affected organs and the description of the observed lesions were collected and analysed.

Bacteriology and molecular characterization of mycobacteria

A total of 147 samples were collected from 140 carcasses of animals (130 cattle, 3 sheep and 7 camels) with suspected BTB lesions. The frequent power cuts and difficulties in maintaining the cold chain during the survey period did not allow a good preservation of the specimens. At the end, only 62 samples from 60 animals were available for analysis; these samples covered the entire period studied and were subjected to laboratory-based analyses. They were packed in 1.2 mL Eppendorf® tubes containing semi-solid transport medium (Portaels et al., 2001) and sent at room temperature to the Mycobacteriology Unit of the Institute of Tropical Medicine, Antwerp, Belgium. Acid fast bacilli (AFB) were detected by microscopy using the Ziehl-Neelsen (ZN) technique applying the ATS scale to quantify the bacterial load (American Thoracic Society, 1999). *In vitro* culture on Löwenstein-Jensen and Stonebrink medium was carried out after decontamination using the inverted Petroff method (Durnez et al., 2008). Spoligotyping was carried out to identify *M. bovis* in all isolates (Kamerbeek et al., 1997). In addition, PCR targeting the 16S rRNA gene to detect *M. tuberculosis*-complex (Durnez et al., 2008) was used for 30 specimens of animals that yielded contaminated or negative (sub-) cultures, but yet positive smears in order to maximize recovery of probable *M. bovis*. Subsequently, PCR-positive samples were subjected directly to spoligotyping.

All spoligotypes were identified using the database on www.Mbovis.org (Smith and Upton, 2011). Presence or absence of the RDAf1 region was determined for all identified *M. bovis* cases as described (Müller et al., 2009).

2.3. Determination of risk factors for BTB transmission

From July 2007 to March 2008, a cross sectional household survey was conducted in the Pu zone from July to September 2007, in the Ru zone from October to December 2007 as well as in the Ur zone of Niamey city from January to March 2008. In total, 1,131 households (399 in Ur, 400 in Pu and 332 in Ru) were randomly selected from an up-to-date census database. 62% of household heads surveyed are Fulani ethnic, 20% are Zarma and 11% are Tuareg. The Fulani are traditionally nomadic herders and their presence in Ur and Pu areas is quite recent and due to climate changes observed in recent decades.

The questionnaire used in the face-to-face interview of the household heads included questions related to animal husbandry practices, food habits and the presence of clinical signs of TB both in animals and humans.

2.4. Statistical analysis

Descriptive statistics and comparisons were carried out using Intercooled Stata 9.2 for Windows (StataCorp LP, USA). Generalised linear models (Poisson regression and logistic regression) were employed to examine the effects of different risk factors: Poisson regressions yielded incidence rate ratios (IRR), logistic regressions resulted in odds ratios. Welch's test was used to appreciate the weight difference between the carcasses carrying TB-like lesions and healthy carcasses.

3. RESULTS

3.1. Determination of the prevalence of suspected TB gross lesions by retrospective data collection

Based on data recorded over the 6 years, it appears that the demand for meat consumption had remained fairly constant. The average number of animals slaughtered per month at the Niamey abattoir during this period was: 6011 (±315) cattle, 9676 (±970) sheep and 2026 (±361) goats. The survey conducted between July 2007 and June 2008 showed that the daily workload for meat inspectors does not seem to be a factor affecting their performance. However, they complain of poor equipment and poor working conditions.

At the Niamey abattoir, seizures of carcasses for suspicion of TB-like lesions were rare. Indeed, out of a 1,294,079 animals slaughtered during the 6-years period, condemnations based on suspected TB gross visible lesions included 847 carcasses (**Table 1**). During the entire period, the maximum daily record of suspected carcasses in cattle was 4. The average apparent prevalence of TB-like lesions was 0.19% (95% CI: 0.18 - 0.20) for cattle and 0.11% (95% CI: 0.07 - 0.17) for camels. Regarding small ruminants only 7 cases were reported in sheep and one in goats.

Based on the observation of TB-like lesions there is a highly significant difference in susceptibility to infection among different species ($P<0.001$). Camels and cattle are species at significantly high risk compared to goats and to sheep. There is no significant difference in susceptibility to infection between sheep and goats.

Insert Table 1

Condemnations due to suspected TB lesions mostly concerned cattle with 819 cases on a total of 847 for all included species. Of these 819 cases, only 747 cases (91.2 %) could be described, whereas for the remaining detailed descriptions were lacking in the registers. Data collected were related to the affected organs, types of lesions observed and the weight of carcasses.

In cattle, the majority of the TB-like lesions were detected in the lungs (92.77%). Lung lesions were in most cases accompanied by reactions in the lymph nodes (50.87%) and lesions in the liver (32.40%). Other affected organs were: the heart (0.80%), the kidneys (0.54%) and the spleen (0.27%). About one tenth (9.77%) of the cases were suspected of generalized miliary TB.

The apparent prevalence of TB-like lesions in cattle at the abattoir in Niamey is strongly influenced by the season ($P<0.0001$). The Incidence Rate Ratio (IRR), of suspected BTB shows two peaks (**Figure 2**). The first slight peak is observed during the hot dry season in April ($P<0.05$) and two strong peaks are observed during the first half of the rainy season in July ($P<0.001$) and in August ($P<0.01$).

Insert Figure 2

3.2. Characterization of suspected TB gross lesions by a longitudinal survey

A total of 71,373 cattle, 124,759 sheep, 19,731 goats and 2,604 camels were slaughtered at the abattoir of Niamey during the one-year survey. The 71,373 cattle included 4,086 oxen, 14,630 bulls, 31,190 cows and 21,467 calves (animals younger than 4 years are considered as calves at the abattoir of Niamey). A total of 130 cattle, 3 sheep and 7 camels presented gross visible lesions compatible with BTB.

Experts of the abattoir consider that 50% of animals slaughtered are from the Tourakou market in Niamey, 30% come from the Torodi area and 20% from other rural markets.

Data collected by this independent tracking confirms the picture observed in cattle during the six-year period. Out of the 130 BTB suspected cases in cattle, organs and tissues most affected were lungs, lymph nodes, liver and to a lesser extent kidneys and heart (**Table 2**).

The presence of macroscopic lesions is closely correlated with the origin of the animals ($P<0.001$). Over half (56.2%) of the cases were from the rural zone of Torodi, 22.3% from the urban community of Niamey and 20.5% from the other rural areas. Compared to urban markets, the risk of having animals suspected of TB is statistically higher in Torodi (OR=4.2; 95% CI: 2.7 - 6.5) and in other rural markets (OR=2.4; 95% CI: 1.4 - 4.1).

In cattle, there was a significant difference in sensitivity to infection between the different categories (bulls, oxen, cows and calves) (df=3; Chi2=84 $P<0.001$). Cows are significantly more sensitive to infection than the other categories (OR=9.4; 95% CI: 4.4 - 20.2).

A significant negative relationship was found between the presence of TB-like lesions on the carcasses and the weight of affected animals. Overall, we found that the carcasses of cattle suspected for BTB are significantly lighter than those of healthy animals (Welch's test $P<0.0001$). The difference of weight between healthy carcass and those with gross visible lesions is on average 14 kg (95% CI: 8 - 20).

Insert Table 2

3.3. Molecular characterisation of mycobacteria

Acid-fast bacilli were detected by smear microscopy in 31 (50.0%) of the 62 post-mortem samples. Overall, we observed a high contamination rate (25.8%) for culture, most probably due to suboptimal storage conditions. Thirteen samples were positive for culture among which 7 were clearly identified as *M. bovis* type Af1 by the non-appearance of spacer 30 and the Af1-specific deletion (Table 3). Five isolates were identified as non-tuberculous mycobacteria (NTM); the latter were not further identified to the species level. The remaining culture (BK 090046) yielded a weak spoligotype result and failed in subculture; most probably it was *M. bovis* as spacers 39-43 were clearly missing, and the Af1 specific deletion. However, the spoligotyping reactions were too weak to determine the exact *M. bovis* spoligotype (SB type). In addition, in 11 animals that yielded negative or contaminated culture results but a MTB-complex positive PCR, *M. bovis* type Af1 was clearly identified by spoligotyping and the RDAf1 PCR directly in the specimen. For 6 more specimens we noted a weak spoligotype reaction even though the MTB-complex-specific PCR turned positive; again most probably the latter are *M. bovis* as spacers 39-43 were clearly missing for all of them; five showed the Af1-specific deletion, whereas one failed in this assay, probably due to the low DNA content. Spoligotypes were too weak to determine the exact SB type in the seven specimens.

So, *M. bovis* type Af1 was clearly identified in 18 animals (16 bovines, 1 camel and 1 sheep), and 5 different spoligotypes were observed (**Table 3**). SB0944 profile was the most abundant being identified in 1 sheep (Bali-Bali race) and 12 bovines (11 belonging to the Djeli and 1 to the Mbororo breed). SB0300 was found in two Djeli cows, SB1440 in one Djeli cow, SB1433 in one camel. Furthermore a new strain not yet reported to the *M. bovis* database prior to this study was found in a Djeli cow. Hence, it has now been reported to the database, and designated as SB1982.

Insert Table 3

3.4. Determination of risk factors for BTB transmission

Table 4 shows that, in most cases, mating was not controlled, especially in urban areas. Eighty-five to 94% of the households consumed fresh milk or products derived from unpasteurized milk. In Pu and Ru zones, poor hygiene was scored in 74% of the households and in 32% in Ur zones. On average, only 1% of households employed disinfectants for cleaning kitchen tools used to prepare food from animal products.

Large proportions of livestock keepers observed severe weight loss in their animals (48.6% in Ur, 58.8% in Pu and 51.8% in Ru) despite provision of additional feed. 18%, 27% and 25% of household's heads interviewed respectively in Ur, Pu and Ru zones reported animal death casualties due to chronic cough.

Regarding the disease in humans, 43.1% of the interviewed household's heads in Ru stated to have observed in their vicinity clinical signs that could be related to TB. Some stated to suffer themselves from persistent cough or knew people with similar symptoms. Chronic cough in humans was also reported to have been observed by respondents in Ur (22.8%) and in Pu (30.5%). In the rural zone of Torodi, a significant relationship (OR=5; 95% CI: 3.0 - 26.5) was found between the presence of people with severe chronic cough in households and the presence of animals suffering from chronic cough within the same household.

Insert Table 4

4. DISCUSSION

4.1. Determination of the prevalence of suspected tuberculosis gross lesions by retrospective data collection

In SSA, research on animal TB mainly focused on cattle. Data on other domestic species are particularly scarce (Cosivi et al., 1998; Razanamparany et al., 2006; Diguimbaye et al., 2006) and should be further investigated in the future.

Our results showed the presence of TB-like lesions in all animal species slaughtered at the Niamey abattoir, but the risk of suspected BTB infection is significantly higher in cattle and camels compared with goats and sheep that are less susceptible to *M. bovis*. Nevertheless, the apparent prevalence of TB-like lesions (0.19%) observed in cattle slaughtered at the abattoir of Niamey is among the lowest recorded on the African continent. It varies in West African abattoirs from 1 to 8.8% (Dao, 2005, Diguimbaye et al., 2006, Njanpop-Lafourcade et al., 2001; Cadmus et al., 2006; Müller et al., 2008). Our finding is also lower than the 7.9 to 19.8% prevalence record in EasternEastern Africa (Regassa et al. 2008; Cleaveland et al. 2007; Biffa et al., 2011).

The low prevalence observed in small ruminants compared to cattle and camels can be explained partly by the difference in sensitivity of a physiopathological point of view between these species. Indeed, sheep and goats are inherently more resistant to contracting the disease, i.e. they require a much higher infective dose than cattle before infection can become established (Allen, 1988; WHO, 2001). According to some authors, BTB infection in sheep and goats is a sign of infection in other in-contact species (Allen, 1988; Ayele et al. 2004).

The disease does not spread easily between small ruminants (Allen, 1988). When exposure to infection is high, there is no doubt that small ruminants can become infected, and display lesion morphology and distribution in the body similar to cattle (Fischer et al., 2009). In Niger cattle and small ruminants normally graze together, and this practice could constitute a higher risk for transmission of bovine TB among these animals.

The probability of lesions to be detected at slaughter is difficult to assess (Müller et al., 2008). The very low figures at Niamey could be due to underestimation, which in turn can be attributed i.a. to the lack of rigor in the veterinary inspection. According to Assaged et al. (2004), meat inspection at the abattoir can detect only 55% of infected animals with confirmed visible lesions. The low number of carcasses condemned for BTB reasons may also be linked to the high incidence of illegal slaughtering. Indeed, at the abattoir in Niamey, any animal found with BTB lesions is subjected to total or partial condemnation. This urges butchers to clandestine slaughtering. As a result, there may be TB-infected meat on the market and an increased risk of transmission to people (Asiimwe et al., 2009).

Analysis of data recorded from the abattoir during the 6-years period shows a strong seasonal variability of condemnation due to BTB ($P<0.001$). The frequency of gross lesions is higher in the beginning of the rainy season (July-August). This may be explained by the massive destocking of animals by farmers. Indeed, this period concurs with the return of animals from transhumance. Sick animals and those with poor general condition are usually sold or culled.

Lung lesions are the major cause of condemnation due to BTB in SSA (Dao, 2005, Diguimbaye et al. 2006; Njanpop-Lafourcade et al., 2001; Cadmus et al., 2006; Müller et al., 2008; Asiimwe et al., 2009). Our results show that 92.77% of the 747 detected gross lesions are in the lungs with 28.65% of animals presenting lung lesions only, whereas the remaining presented multiple lesions involving the lungs and other organs. This suggests that in this setting the lungs are the entry port of *M. bovis* infection. This is in agreement with the findings of Müller et al. (2008) showing that the presence of pulmonary lesions was closely associated with *M. bovis* infection. Lymph node reactions (50.87%) are also characteristic for BTB. They are the tissues that are investigated primarily by meat inspectors. Lesions in the liver are frequent too (32.40%). This organ contains usually caseous nodules which can reflect chronic infection (Thorel, 2003).

4.2. Characterization of suspected tuberculosis gross lesions by longitudinal survey

It should be noted that so far, no serious investigation has been conducted on this topic in Niger and control of TB is limited to meat inspections in abattoirs. Cows are significantly more at risk to BTB infection than bulls, oxes and calves.

The high prevalence of TB-like lesions observed in cows may be explained by the fact that they remain longer in the herds for milk production while the other categories are slaughtered or sold earlier. It has been shown that the prevalence of bovine tuberculosis increases with age of animals especially in regions where infection is endemic (Blancou et al., 1971; Sidibé et al., 2003, Cleaveland et al., 2007; Humblet et al., 2009). Indeed, in endemic situations, older animals are more likely to be exposed and to develop the disease. Moreover, under the effect of stress or age, latent infections are reactivated resulting in a higher prevalence in older animals (Pollock and Neill, 2002).

In this survey, the presence of TB-like lesions in cattle is closely related to the origin of the animals ($P<0.001$). Rural areas that supply Niamey in cattle are the most affected. Compared to urban markets, the risk of having animals with gross visible lesions is statistically higher in Torodi and other rural markets. Indeed, 70.77% of animals with gross lesions were from Ru with 56.15% of them originating from the Torodi area, which is located in the far west of Niger at the border with Mali, Burkina Faso and Benin. Our results corroborate those found by Alambedji (1984) who reported that 46% of cattle suspected of BTB in the Niamey abattoir are from Torodi.

Due to its geographical position, Torodi constitutes a crossroad for trade and transit of cattle. The complex interactions between transhumant livestock system, the semi-intensive system practiced by farmers and the presence of an important livestock market in this area promote contact between animals from different regions with a significant risk of spread of BTB. In addition, it should be noted that Torodi is located at the edge of the natural park that is shared by Niger, Benin and Burkina Faso. The proximity of this natural reserve leads necessarily to close contact between domestic animals and wildlife which can be a source of transmission of *M. bovis* (Zieger et al., 1998). The role of wildlife in transmission or recurrence of BTB in domestic animals has been well documented (Michel et al., 2006; 2008). In South Africa, for example, BTB is now a serious problem in the Kruger National Park where the disease was first diagnosed in buffalo in 1996 (Bengis et al. 1996). Even in countries where BTB is eradicated, wildlife remains a risk of transmission to domestic animals through the parks. Woodford (1982) found *M. bovis* in warthogs (*Phacochoerus aethiopicus*) and buffaloes *Syncerus caffer* (Sparrman, 1779) in the Ruwenzori National Park in Uganda. Keet et al. (1996) also reported bovine TB in other wildlife at the same park.

Bovine tuberculosis can be a serious threat for the economy in sub-Saharan Africa (Cosivi et al., 1998). Indeed BTB causes a decrease in financial capital and an increase of production costs for the farmers. The disease also causes indirect losses in agricultural productivity, due to the loss of animal traction and manure. We observed significant differences in body weight ($P < 0.0001$) between healthy carcasses and those with gross visible lesions; a decrease of 14 kg in average weight in cattle presenting TB-like lesions was seen. Similarly, Blancou and Cheneau (1974) found weight losses ranging from 3.1 to 9.7 kg in Malagasy zebu carcasses with gross visible lesions. The loss of weight due to suspected BTB varies depending on the disease status and the farming system and is higher as the lesions caused by the pathogen are more severe (Blancou and Cheneau, 1974). In contrast, Biffa et al. (2011) found no significant correlation between body condition and infection with bovine tuberculosis in cattle in Ethiopia and suggested that body condition may not be considered a reliable predictor of TB under Ethiopian conditions.

4.3. Molecular characterisation of mycobacteria

As far as we know, this is the first study conducted on molecular characterization of *M. bovis* isolates from slaughter animals in Niger. However, the low number of samples analyzed (62/147) due to frequent power cuts hampers the study. Freeze-thaw cycles and frequent transfers of samples from one location to another due to load shedding of electricity were the basis for the loss of a number of samples. Müller et al. (2008) were confronted to the same situation in a similar study conducted at the abattoir of Bamako in Mali.

Several mycobacterium were isolated from 50% (n=62 samples) of the 60 carcasses with characteristic TB-like lesions. This is relatively higher than the 35.0% (n=60) and the 31.2% (n=105) respectively from cattle carcasses in Mali and in Ethiopia (Müller et al., 2008; Biffa et al., 2011), but still lower than expected. As suggested by some authors (Cleaveland et al., 2007; Müller et al., 2008; Biffa et al., 2011), we agree in our case that the relatively low isolation frequency could be due to reduced sensitivity of culture arising from prolonged storage of specimens. The low recovery of bacteria could also be explained by the high amount of completely calcified lesions without viable tubercle bacilli (Müller et al. 2008).

We clearly identified *M. bovis* in 18 animals (n=60) and NTM were found in 7 animals. It's well established that in sub-Saharan Africa, TB-like lesions may be caused by an array of pathogens amongst which NTM could play a crucial role (Müller et al. 2009). This suggestion is strengthen by the findings of a previous study in some African countries where NTM such as *M. fortuitum*, *M. kansasii*, *M. aquae* and *M. smegmatis* were cited as causative agents of TB-like lesions in domestic animals (Diguimbaye et al., 2006; Müller et al., 2008; Sahraoui et al., 2009 ; Biffa et al., 2010; Mamo et al., 2011)

Among the spoligotypes identified in Niger, SB0944 is present in 13 (72%) of the positive samples, which confirms the predominance of this strain in Central and West African regions. Indeed, this strain represents a significant proportion of spoligotypes identified in Nigeria (46.1%), in Cameroon (62.7%), in Chad (40%) and in Mali (9%) (Njanpop-Lafourcade et al., 2001; Cadmus et al., 2006; Diguimbaye et al., 2006; Müller et al., 2008). Based on the similarity of the SB0944 pattern with the BCG-like profile commonly seen in strains from France, an influence of the French colonial history on the West African *M. bovis* population has been suggested (Njanpop-Lafourcade et al., 2001). Our discovery of this strain in Niger confirms its regional coverage.

Up to now, The SB1433 and SB1440 strains have been shown in Nigeria and the SB0300 strain in Mali. The presence of these three strains in Niger suggests that this country should play a central role through its particular geographical position. Indeed, Niger is a country of important transboundary transhumance and a major exporter of cattle to other countries (MRA, 2001). A feature common to all these strains identified in Cameroon, Chad, Nigeria, Mali and now in Niger is the lack of the spacer 30 typical for the Af1 *M. bovis* type (Müller et al., 2009). This suggests a close relationship between strains from Niger and those other countries. In addition to the absence of spacer 30, SB0300 also lacks spacer 6, a characteristic not seen in the strains from Central African countries (Njanpop-Lafourcade et al., 2001; Diguimbaye et al., 2006). Müller et al. (2008) suggest that spoligotype pattern SB0300 may have evolved from strains with spoligotype pattern SB0944 either by drift or a selective sweep. Similarly, the new strain (SB1982) might have been imported from neighbouring countries, where it remained undetected due to non-systematic isolation and/or typing of *M. bovis*, or it might be the result of ongoing evolution cattle and/or other animal species (e.g., wildlife animals), reflecting the long-term presence of *M. bovis* in the country. Indeed, this strain lacks spacer 6 and 22 in addition.

The fact that the strain SB1433 was only found in camel can be explained by the separate husbandry of camels in Niger making direct contact with other species rare. However, this hypothesis has to be verified as the animal in which this strain was isolated is certainly not representative of the entire camel husbandry system. We also know that this animal came from the livestock market of Tourakou, but we had no information on its exact origin. Some authors (Wernery et al. 2007; Mamo et al., 2011) reported that BTB occurs more frequently in camels when they are kept close to other camels or in close contact with cattle. Cases of contamination of camels by wildlife (gazelles) have also been reported in the Arabian Peninsula (Ostrowski et al., 1998). Further investigations with a larger number of animals are therefore needed to better understand the epidemiology of tuberculosis in camels in Niger.

4.4. Determination of risk factors for BTB transmission

One of the characteristics of animal husbandry in SSA, whatever the livestock system considered, is the close proximity between humans and animals (Cosivi et al. 1998). In pastoral areas, humans and animals share the same microenvironment and water sources especially during the hot and dry seasons, which is a high potential risk of transmission of *M.*

bovis (Cosivi et al., 1998; Mfinanga et al., 2003; Ameni et al., 2006). In urban and suburban areas, the closeness is such that humans and animal reservoirs live confined in unsanitary and poorly ventilated places (Sidibé et al., 2003; Boukary et al., 2007; Regassa et al., 2008). Our results show the existence of BTB in Niger in all animal husbandry systems, since *M. bovis* has been identified in animals from urban, periurban and rural areas. During the survey in those three areas, some livestock keepers declared to have observed clinical signs characteristic for BTB in their animals.

Poor farming practices, consumption of unpasteurized milk and poor food hygiene conditions are important risk factors for transmission of *M. bovis* from animals to humans (Kazwala et al., 1998; Sidibé et al., 2003; Ameni et al., 2006; Cleaveland et al., 2007; Kang'ethe et al., 2007; Humblet et al., 2009). *M. bovis* is usually transmitted by ingestion of unpasteurized milk and causes extrapulmonary tuberculosis especially in children (Kleeberg, 1984; Dankner and Davis, 2000). The proportion of tuberculosis due to *M. bovis* is currently not known with precision in developing countries (Cosivi et al., 1998) however it would be, according to some authors (Collins and Grange, 1983; Dankner and Davis, 2000), comparable to that existing in 1945 in Great Britain, where 30% of cases of tuberculosis among children under 5 years were due to *M. bovis*. Our study revealed that the potential risk of contamination of humans with TB is high, as respectively 22.8, 30.5 and 43.1% of the respondents in Ur, Pu and the Ru said they knew people with symptoms that can be related to tuberculosis. Risk factors for transmission of *M. bovis* to humans was higher in the rural zone of Torodi where a significant interaction (P=0.01) was found between the records of people suffering of severe chronic cough and the presence of animals suffering also from chronic cough within the same household. This results corroborate those found by Adamou (2005) which estimated the prevalence of human TB pulmonary smear positive cases to 144 per 100,000 inhabitants in the rural area of Gaya, Niger. This prevalence is higher than the national average of Niger, which is 77 TB pulmonary smear positive cases per 100,000 inhabitants in 2007 (WHO, 2011). More investigations in the rural area should be performed in order to confirm its probable role as an epidemiological outbreak of BTB in Niger.

It should be noted that in Niger, as in other countries of SSA, cultures and customs play an important role in the persistence and spread of TB (Mfinanga al., 2003; Ayele et al., 2004). In many African cultures TB is stigmatised which generally pushing patients to hide their illness for fear of the discrimination they can suffer from that (Edginton et al., 2002).

5. CONCLUSION

Our results corroborate the work of several authors who have shown the existence of BTB in sub-Saharan Africa. Indeed, we isolated five different *M. bovis* strains with one new strain designated as SB1982 from samples coming from the abattoir of Niamey. The existence of strains common to several countries in West and Central Africa suggests that transboundary movements of livestock is a major risk factor for transmission of BTB between animals in this geographical area. It also highlighted factors that expose humans to infection with *M. bovis*: eating habits, husbandry practices, and lack of hygiene and sanitation.

For a better control and eventual eradication of BTB in Niger and SSA, a better understanding of the epidemiology and dynamics of circulating *M. bovis* strains is imperative. Our recommendations are to:

- Evaluate the actual prevalence and economic impact of BTB in different geographical areas and different farming systems, especially in areas of high suspicion, like the region of Torodi;

- Further investigate on husbandry practices in relation to the forest environment for a better understanding of the interaction between domestic animals and wildlife and the potential role of wildlife in the maintenance and transmission of BTB;
- Implement measures to control risk factors related to the transmission of the disease from animal to human in different husbandry systems, especially in urban and suburban livestock systems;
- Implement coordinated actions stimulating a synergy among researchers and institutions in human medicine and animal sciences (Zinsstag et al., 2005).

TABLES AND FIGURES

Table 1: Different animal species slaughtered at the abattoir of Niamey and cases of condemnation due to BTB in 2003-2008 (retrospective survey)

Table 2: Characterization of BTB macroscopic lesions observed in cattle slaughtered at the abattoir of Niamey from July 2007 to June 2008

Legend: * Livestock market of the urban community of Niamey

Table 3: Samples yielding positive culture and/or PCR-based results for the detection/identification of mycobacteria

Legend: F= female; M= male; Pos = culture positive without culture number; Neg = remained negative in culture; Cont = contaminated culture; NT = Not tested; M.sp = mycobacterial species different from *M. tuberculosis*-complex; Spoligotypes were obtained from isolates in case of successful culture and from the decontaminated biopsies in case culture failed.

Table 4: Exploratory variables in the three strata (%)

Figure 1: View of the study zone and location of livestock markets (including that of Torodi) witch supply Niamey abattoir

Figure 2: Monthly incidence rate ratio (IRR) for bovine carcasses in the abattoir of Niamey, 2003-2008

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Table 1: Different animal species slaughtered at the abattoir of Niamey and cases of condemnation due to BTB in 2003-2008 (retrospective survey)

		Carcasses confiscated for TB lesions		
Species	**Number of animals slaughtered**	**Number**	**Proportion (%)**	**95% CI**
Cattle	432,764	819	0.19	0.18-0.20
Sheep	696,663	7	0.001	0.0004-0.002
Goats	145,898	1	0.0006	0.00002-0.004
Camels	18,754	20	0.11	0.067-0.165
Ruminants (total)	1,294,079	847	0.065	0.061-0.070

Table 2: Characterization of BTB macroscopic lesions observed in cattle slaughtered at the abattoir of Niamey from July 2007 to June 2008

		Breed			Organ/tissue affected					Sex	
Location	Zone	Azawak	Mbororo	Djeli	Lung	Nodes	Liver	Kidney	Heart	Female	Male
Balleyara	Ru	1	1		2	-	-	-	-	1	1
Kollo	Ru	-	2	7	6	1	-	-	-	6	3
Say	Ru	-	4	4	8	5	-	-	-	3	5
Tera	Ru	1	4	4	9	4	1	-	-	4	5
Torodi	Ru	-	11	62	73	40	7	1	1	34	39
Tourakou*	Ur	3	14	12	29	16	4	-	-	16	13
Total		**5**	**36**	**89**	**127**	**66**	**12**	**1**	**1**	**64**	**66**

Legend table 2:

* Livestock market of the urban community of Niamey.

Table 3: Samples yielding positive culture and/or PCR-based results for the detection/identification of mycobacteria

ITM specimen number	Animal							Microscopy (ATS scale)	Culture	MTBc-specific PCR	Identification by spolygotyping	RDAf1
	Species	Breed	Sex	Age	Origin	Organ	Type of lesion					
BK091163	Sheep	Bali-bali	F	5	Torodi	Lung	Caseous tubercles	1+	Pos	NT	*M. bovis* SB0944	Deletion
BK090032	Bovine	Djeli	M	7	Torodi	Lung	Caseous tubercles	4+	Pos	NT	*M. bovis* SB0944	Deletion
BK090037	Bovine	Djeli	M	6	Torodi	Lung	Gray nodules	2+	Pos	NT	*M. bovis* SB0944	Deletion
BK091153	Bovine	Djeli	F	6	Torodi	Prescapular node	Caseous-calcified tubercles	1+	Pos	NT	*M. bovis* SB0944	Deletion
BK091154	Bovine	Djeli	F	15	Torodi	Lung	Caseous tubercles	1+	Pos	NT	*M. bovis* SB0944	Deletion
BK091155	Bovine	Djeli	F	7	Torodi	Liver	Caseous-calcified tubercles	2+	Pos	NT	*M. bovis* SB0944	Deletion
BK090048	Bovine	Mbororo	M	6	Balleyara	Liver	Caseous tubercles	1+	Pos	NT	*M. bovis* SB0944	Deletion
BK093747	Bovine	Djeli	F	5	Tera	Lung	Caseous tubercles	1+	Cont	Pos	*M. bovis* SB0944	Deletion
BK097348	Bovine	Djeli	F	4	Tourakou	Lung	Miliary tubercles	4+	Cont	Pos	*M. bovis* SB0944	Deletion
BK097352	Bovine	Djeli	F	11	Torodi	Lung	Caseous tubercles	4+	Neg	Pos	*M. bovis* SB0944	Deletion
BK090030	Bovine	Djeli	F	15	N'dounga	Lung	Lesoins	2+	Neg	Pos	*M. bovis* SB0944	Deletion
BK090035	Bovine	Djeli	F	4	Torodi	Lymph node	Lesoins	4+	Neg	Pos	*M. bovis* SB0944	Deletion
BK091156	Bovine	Djeli	F	6	Torodi	Lung	Caseous tubercles	4+	Neg	Pos	*M. bovis* SB0944	Deletion
BK097355	Bovine	Djeli	F	8	Torodi	Lung	Caseous tubercles	4+	Neg	Pos	*M. bovis* SB0300	Deletion
BK097356	Bovine	Djeli	F	7	Tourakou	Prescapular node	Caseous tubercles	4+	Neg	Pos	*M. bovis* SB0300	Deletion
BK097346	Camel	Dromedary	M	-	Tourakou	Lung	Gray nodules	1+	Cont	Pos	*M. bovis* SB1433	Deletion
BK091158	Bovine	Djeli	F	9	Tourakou	Lung	Miliary tubercles	2+	Neg	Pos	*M. bovis* SB1440	Deletion
BK097342	Bovine	Djeli	F	10	Tourakou	Lung	Caseous -calcified tubercles	4+	Cont	Pos	*M. bovis* SB1982	Deletion
BK090046	Bovine	Mbororo	F	10	Tourakou	Lung	Caseous tubercles	1+	Pos	Pos	*M. bovis?*	Deletion
BK090034	Bovine	Djeli	F	4	Torodi	Diaphragm	Lesoins	1+	Neg	Pos	*M. bovis?*	Deletion
BK090036	Bovine	Djeli	F	4	Torodi	Lung	Caseous tubercles	1+	Neg	Pos	*M. bovis?*	Deletion
BK097339	Bovine	Djeli	F	7	Torodi	Lung	Miliary tubercles	4+	Cont	Pos	*M. bovis?*	Deletion
BK097350	Bovine	Djeli	F	6	Tourakou	Liver	Caseous tubercles	1+	Cont	Pos	*M. bovis?*	Deletion
BK097357	Bovine	Mbororo	F	8	Tourakou	Lung	Gray nodules	1+	Neg	Pos	*M. bovis?*	PCR negative
BK097341	Bovine	Crossbred	F	8	Tourakou	Prescapular node	Caseous -calcified tubercles	1+	Cont	Pos	*M. bovis?*	PCR negative

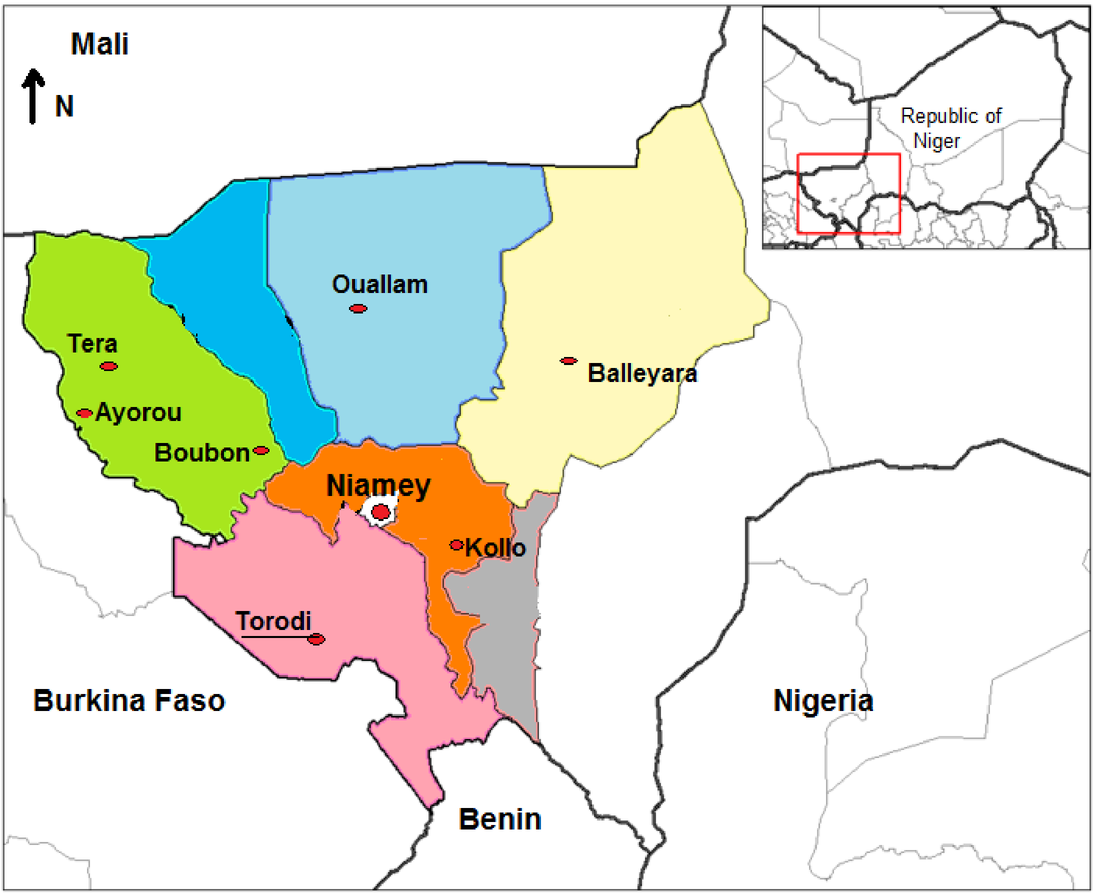
BK097344	Bovine	Crossbred	F	7	Tourakou	Lung	Caseous tubercles	1+	Cont	Neg	NTM	NT
BK090044	Bovine	Djeli	F	7	Tourakou	Liver	Caseous tubercles	1+	Pos	Neg	NTM	NT
BK090053	Bovine	Djeli	M	8	Torodi	Lung	Gray nodules	4+	Pos	NT	NTM	NT
BK091158	Bovine	Djeli	F	9	Tourakou	Lung	Miliary tubercles	2+	Pos	Neg	NTM	NT
BK090042	Bovine	Mbororo	M	6	Tera	Lung	Caseous tubercles	2+	Pos	Neg	NTM	NT
BK091152	Bovine	Mbororo	F	5	Tourakou	Prescapular node	Caseous -calcified tubercles	1+	Pos	Neg	NTM	NT

Legend table 3: F = female; M = male; Pos = culture positive without culture number; Neg = remained negative in culture; Cont = contaminated culture; NT = Not tested; NTM (non-tuberculous mycobacteria) = mycobacterial species different from *M. tuberculosis*-complex; microscopy scaling according to American Thoracic Society (ATS), 1999; RDAf1 = PCR to detect region of difference specific for *M. bovis* Af1 type; spoligotyping and RDAf1 results were obtained from isolates in case of successful culture and from the decontaminated biopsies in case culture failed BUT 16S-based PCR was positive for *M. tuberculosis*-complex.

Table 4: Exploratory variables in the three strata (%)

Factor	Ur	PU	Ru
Uncontrolled mating on animals	92.1	75.3	65.7
Consumption of unpasteurized milk	85.5	93.7	90.9
Households using disinfectants	1.4	0.5	0.7
Presence of animals with severe states of weight loss despite a good diet	48.6	58.8	51.8
Herds with animals died of persistent cough	18.0	27.0	25.0
People suffering from persistent cough	22.8	30.5	43.1

Fig. 1.


Mali
N
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Kollo
Torodi
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Benin