Preliminary results of the study on zoonotic brucellosis and tuberculosis in Niamey

Abdou Razac Boukary[1]; Claude Saegerman[2]; Leen Rigouts[3]; Francine Matthys[4]; Dirk Berkvens[5]; **Eric Thys[5*]**
1. Department Animal Health and Livestock promotion, ONG Karkara, Niamey, Niger
2. Department Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, Université de Liège, Liège, Belgium
3. Department of Microbiology, Institute of Tropical Medicine, Antwerp, Belgium
4. Department of Public Health, Institute of Tropical Medicine, Antwerp, Belgium
5. Department of Animal Health, Institute of Tropical Medicine, Antwerp, Belgium
* Corresponding author
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Introduction

In sub-Saharan Africa, tuberculosis (TB) and brucellosis represent a major challenge for development. Indeed, these diseases can cause important economic losses through decreased animal productivity and cause morbidity in man with increasing costs in the delivery of human health services (1). Increasing rural poverty urges African dwellers to move into urban areas where livestock plays a substantial role in subsistence with subsequent impact on health and environmental welfare. Indeed, the lack of effective sanitation measures, animal overload and close contact between humans and animals represent risk factors that may promote the transmission of TB and brucellosis from animal to man in these settings (2). The risk of transmission of these diseases can be important in a city like Niamey, where also the consumption of unpasteurized dairy products is very common (3). However, zoonotic brucellosis and TB have hardly been little studied in the context of sub-Saharan Africa (4). In Niger, prevalence data of these two zoonoses in man and animal are almost inexistent. The objective of this study is to investigate the presence of certain risk factors for transmission in urban and suburban areas and to compare them with the situation in rural areas; to estimate seroprevalence of brucellosis in animals and to study the presence of tuberculosis in animals slaughtered at the slaughterhouse in Niamey.

Materials and methods

Determination of risk factors: A cross sectional household survey was conducted in the urban (Ur) and periurban (PU) zones of Niamey city as well as in the rural areas (Ru). In total, 1,131 households (399 in Ur, 400 in PU and 332 in Ru) were randomly selected from an actualised census database. The questionnaire used in the face-to-face interview with the head of the household included questions related to animal husbandry practices and food habits. Data were captured in SPSS software. Descriptive statistics and parametric comparisons were performed with STATA.8 software. Pearson's chi-square test was used to estimate relations between categorical variables.

Determination of animal brucellosis seroprevalence: In the three strata, 5,272 blood samples (3,239 cattle, 1,189 sheep and 844 goats) were collected in vacutainer tubes from the jugular vein. These tubes were labelled according to the zone and the herd of origin and transported in cool boxes to the Central Veterinary Laboratory in Niamey. Serum was tested for *Brucella* antibodies with the Rose Bengal test (RBT) as described by OIE (5).

Determination of the bovine TB (BTB) prevalence by retrospective data collection: Data of 432,764 cattle slaughtered between January 2003 and December 2008 at the abattoir of Niamey. All the cases of condemnation for reasons of TB during standard meat inspection were analyzed. Parameters related to: animal (age, breed, sex, geographic origin, clinical history), the production and marketing system (including the various stakeholders), the organs affected by BTB and description of the observed lesion were analysed.

Determination of the BTB prevalence by post-mortem inspection and laboratory analyses (transversal survey): sixty-two samples of lesions were packed in 1.2 mL Eppendorf® tubes containing semi-solid transport medium (6) 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. In vitro culture was carried out after decontamination using the inverted Petroff method (6). Spoligotyping was used to identify *M. bovis* for all isolates (7). PCR was used for 20 specimens that yielded contaminated cultures. Spoligotypes were identified by the database on www.Mbovis.org.

Results

Study of risk factors

Results of the interviews show (table 1) that, in most households animal reproduction is uncontrolled, especially in urban areas (table 1). Concerning the disposal of dead animals, a large proportion (37 to 87%) of the respondents throws the carcasses in the trash. Depending on the strata, 40 to 65% of the respondents

observed abortions in their herds and in 40 to 84% of the cases, abortion material was said to be dumped. Results show also that 85 to 91% of the households consume fresh milk or products made from unpasteurized milk. In PU and Ru zones, poor hygiene was scored for 74% of the households and 32% in Ur zones. On average, only 1% of households employed disinfectants for cleaning kitchen tools used to prepare food from animal products.

Table 1: Exploratory variables in the three strata (%)

Factor	Ur	PU	Ru
Uncontrolled mating	92.1	75.3	65.7
Dumping of dead animals	81.0	74.4	37.1
Dumping of abortion material	77.0	84.2	40. 0
Consumption of unpasteurized milk	85.5	93.7	90.9
Households using disinfectants	1.4	0.5	0.7

Seroprevalence of animal brucellosis

RBT detected brucellosis in the animals of the three areas. In sheep and goats the overall prevalence was respectively 2.4% and 3.7%. Among cattle, the prevalence was significantly higher in Ru (21.3%) than in PU (5.07%) and Ur (3.25%) zones. The prevalence of brucellosis was significantly higher among older animals ($p < 0.001$). No difference was found related to sex.

Characterization of BTB in the abattoir of Niamey

From the 432,764 cattle carcasses, 0.19% showed large visible lesions compatible with BTB (apparent lesion prevalence). BTB lesions (fig. 1) were mostly detected in lungs (94.5%) and 7.95% were found in the liver.

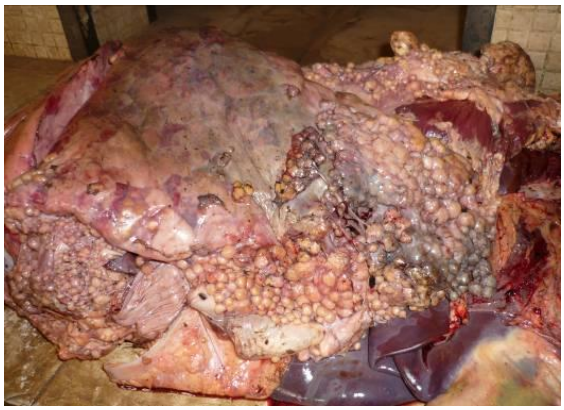

Figure 1: BTB lung lesions observed in the Niamey abattoir, Niger

Acid fast bacilli were detected in 48.3% of the 62 post-mortem samples sent to Antwerp. Twenty-tree samples (37.1%) were positive for culture. After molecular typing of the isolates we noted 5 weak reactions and 3 samples showing profiles that do not corresponding to *M. tuberculosis* complex. Finally, *M. bovis* was isolated from 15 (13 cattle, 1 camel and 1 sheep) and 4 different spoligotypes were observed among these 15 isolates (table 2). Eleven of the strains showed the SB0944 profile. SB0300 was found in two Djeli cows and one camel presented the SB1433 profile. Finally, an unknown profile has been identified in a Djeli cow.

Table 2: Different *M. bovis* profiles identified

Spoligotype	Number of animals				
	Total	Bovine		Sheep	Camel
		Djeli	Mbororo		
SB0300	2	2			
SB0944	11	7	3	1	
SB1433	1				1
Unknown profile	1	1			

Discussion

This study confirms that animal brucellosis and tuberculosis exists in Niger, in urban as well as in periurban and rural production systems. Food habits and breeding techniques indicated that the risk of transmission of brucellosis and BTB are high. The high consumption of unpasteurized dairy products (85-91%) and poor hygiene are risk factors favouring human contamination through food (8). Uncontrolled mating promotes the transmission of the diseases from infected to healthy animals (9). Dumping of carcasses and aborted material contributes to spread germs in the environment (10).

The prevalence of bovine brucellosis in Ur and PU (3.25 – 5.07%) are similar to those found in the periurban area of Abidjan, Ivory Coast (11). In the Ru zones, the prevalence of bovine brucellosis (21.3%) was significantly higher than in Ur and PU areas. This can be explained by the presence of large cattle population and mixed herds in rural areas (12). Low prevalence in sheep and goats was also observed in similar systems with small flocks (4). The significantly higher prevalence of brucellosis among older animals may reflect the longer exposition period of those animals compared to the younger ones (13).

The apparent prevalence of 0.26% gross visible lesions in Niamey slaughter cattle was surprisingly very low compared to results published in other African countries (14, 15, 16, 17). This suggests an underestimation of the true BTB prevalence; which can be attributed i.a. to the lack of rigor in the veterinary inspection at the slaughterhouse or clandestine slaughtering. Among the spoligotypes identified in Niger, the SB0944 is present in 73% of the samples, which confirms the predominance of this strain in West African region. Indeed, this strain represented a significant proportion of spoligotypes identified in Nigeria (46.1%), in Cameroon (62.7%), in

Chad (40%) and in Mali (9%) (14, 15, 16, 17). SB1433 and SB0300 were also reported in Nigeria (17) and Mali (14). A feature common to all these strains identified in West Africa is the lack of the spacer 30 (18) typical for the AF1 *M. bovis* type. In Cameroon, because of the similarity to patterns of strains isolated in France it was suggested that *M. bovis* could have been imported to this region during the French colonial period (16).

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