

Mycobacterium bovis, but also *M. africanum* present in raw milk of pastoral cattle in north-central Nigeria

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Abstract Using deletion typing technique, five mycobacteria isolated from unpasteurised milk samples from cows in north-central Nigeria were characterized as *Mycobacterium bovis* ($n=4$) and *M. africanum* ($n=1$). This report emphasizes that transmission between the animal and human reservoir is a serious threat in Nigeria.

Keywords *Mycobacterium bovis* · Pasteurization · Zoonosis · Milk · Nigeria

Abbreviations

MTC	<i>Mycobacterium tuberculosis</i> complex
NALC-NaOH	N-acetyl-L-cysteine Sodium hydroxide
NPC	National Population Commission
RD	Regions of difference
TB	Tuberculosis

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Introduction

In most African countries, consumption of unpasteurized milk is a regular practice (Ayele et al. 2004) leading to considerable risk of zoonotic infection with *Mycobacterium bovis* and other mycobacteria. *M. bovis* has been isolated earlier from unpasteurized milk and lesions of slaughtered cattle in Nigeria, as well as from patients with pulmonary and extrapulmonary tuberculosis (TB; Cadmus et al. 2006).

Most cow milk consumed in north-central Nigeria is from livestock owners who are unaware of the public health risks associated with bovine tuberculosis (BTB). To estimate the risk of zoonotic transmission of mycobacteria, we screened raw milk of pastoral cattle from north-central Nigeria for these bacteria and identified them using a molecular method.

Materials and methods

Study area The work was conducted at the South Agricultural Zone of Niger State, north-central Nigeria.

Sample collection About 20 ml of milk was drawn from the disinfected udder of selected cows into sterile universal bottles from each of the 400 out of a total of 4,023 cattle representing 10% of the 416 identified herds. Samples were selected using the systematic sampling technique while the herds were identified by physical randomization technique (Cameroon, 2003).

Isolation of mycobacteria This was done as described by Cadmus and Adesokan (2007), using Middlebrook 7H11 slopes and incubating at 37°C for 12 weeks.

Table 1 Result of deletion typing

Regions of differences (RD)	<i>M. bovis</i>	<i>M. africanum</i>
RD1	Present	Present
RD4	Absent	Present
RD9	Absent	Absent
RD12	Absent	Present

Deletion typing The RD9 deletion was used to distinguish *Mycobacterium tuberculosis* from other members of the *M. tuberculosis* complex (MTC; Warren et al. 2006). Isolates with a deletion in this region were further investigated with primers specific for RD4. This reaction distinguishes between *M. bovis*, *M. caprae*, and other MTC.

Results and discussion

Of the 400 milk samples, five (1.25%) were positive by culture and further molecular analysis identified four *M. bovis* and one *M. africanum* (Table 1). Herd and individual animal prevalence amounted to 10% (4/40) and 1.25% (5/400), respectively.

We report the first molecular characterisation of *M. bovis* and *M. africanum* from unpasteurised milk of pastoral cattle in north-central Nigeria. This may be an important finding considering that although people in this region suffer from tuberculosis, there are no documented literature to show a reliable identification of *Mycobacterium* humans are exposed to.

The findings indicate that there is high herd prevalence of BTB in this region, leading to serious public health implications. Therefore, since BTB is endemic in Nigeria, it becomes imperative to carry out surveillance programs to forestall zoonotic spread going by the reports of Hlavsa et al. (2008) and Ayele et al. (2004) that approximately 1.4% of human tuberculosis cases in the developed countries and 3.1% in the developing countries, respectively, could be attributed to *M. bovis*.

It is noticeable that not only *M. bovis*, but also *M. africanum* was isolated from milk of cows. It is known that the latter species is more related to *M. bovis* than *M. tuberculosis*, but it remains unclear whether it is also transmitted among cows, or whether this is an accidental transmission of *M. africanum* from humans to cows as reported several times for *M. tuberculosis*. However, the presence of *M. africanum* in milk hints at a disseminated

infection in the cow, and this is to our knowledge, not reported for this bacterium.

The limitations of this study (i.e., screening of limited numbers of animals and lack of collection of specimens from tuberculosis patients in adjoining hospitals to link them directly with the animals or consumption of milk products as recently done by Byarugaba et al. (2009) in Uganda) notwithstanding, the habit of pooling milk in this region poses great danger to people who consume them raw as fresh or fermented product.

In conclusion, there is the need for pasteurization of milk from pastoral herds in Nigeria to prevent zoonotic transmission of tuberculosis.

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