

Zoonotic *Mycobacterium* species in fresh cow milk and fresh skimmed, unpasteurised market milk (nono) in Makurdi, Nigeria: implications for public health

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SUMMARY

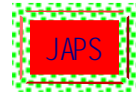
A study was carried out to determine the presence of and characterize *Mycobacterium* species in freshly drawn cow milk and nono in markets in Makurdi town, Nigeria, with a view to highlighting implications for public health. Two hundred and eighty five cows out of 590 cattle in three locations, and 90 'nono' samples from 3 markets were studied, using acid-fast microscopy, culture and biochemical tests, from December 2005 to May 2006. Four (1.4%) of the 285 freshly drawn milk and 2 (2.2%) of the 90 'nono' samples were *Mycobacterium bovis* positive. *Mycobacterium africanum* was detected in 1 (1.1%) of the nono samples. *Mycobacterium bovis* was detected in cows in the three settlements, and in milk samples from 2 of the 3 markets. February, March, April and May were the months with most positive samples. The presence of the zoonotic *Mycobacterium* species in cow milk presents a health hazard as it could be a source of extrapulmonary tuberculosis. Public health education, proper handling and processing of milk and milk products, and vaccination of cattle are recommended to prevent pathogen transmission.

1 INTRODUCTION

Zoonotic tuberculosis is a chronic, infectious, contagious debilitating disease caused by *Mycobacterium bovis* that has become a resurgent problem in animals and humans in Nigeria (Anon, 1990; Cadmus, 2002; WHO, 2002; Ofukwu, 2006). Incidence rates in cattle have continued to increase from 0.3% in 1976 to as high as 7.3% in 2003 (Ofukwu, 2006). In cattle this may be primarily due to a move towards sedentarism, importation of infected foreign breeds, and lack of sustained preventive measures (Cosivi *et al.*, 1995; Voctermeier *et al.*, 2001). The Nigerian human population also carries the largest tuberculosis burden in Africa, the fourth largest in the world, with an estimated 390,000 active infections and 107, 000

deaths per year (WHO, 2002). The emergence of drug-resistant strains of *Mycobacterium* species, the rise and synergism of HIV/AIDS infection with tuberculosis, poverty and neglect of tuberculosis control programs have contributed immensely to the resurgence of TB (WHO, 2002).

While *Mycobacterium bovis* is a major cause of pulmonary tuberculosis in cattle, it is also the primary cause of extra-pulmonary tuberculosis in humans (Cotter *et al.*, 1996; Cousins & Dawson, 1999) where cow milk is usually consumed fresh and unpasteurised. The clinical signs of this infection in humans are hardly differentiated from those caused by the classical



human type and are often difficult to assess clinically (Dawson, 1999).

Despite this resurgence of TB in animals and man, and the fact that large quantities of fresh cow milk and nono are consumed in Benue State, no effort has been made to estimate the gravity of the risk and its threat to public health. This trend is worrying considering

2 MATERIALS AND METHODS

2.1 Sample collection: Five cattle herds in three temporary settlements of the nomadic Fulani community at Agan, Apir and Fidi wards of Makurdi Local Government Area were randomly identified out of a total of seven of such peri-urban locations. The populations of the herds for each settlement were 281, 103 and 206 for Agan, Apir and Fidi, respectively. In each herd, the lactating cows were marked and selected using systematic random sampling method. Average cow herd was 118 heads of cattle. Each herd was visited twice a month for 6 months, from December 2005 to May 2006. During each visit, 50 ml of milk was expressed from each cow early in the morning into sterile pre-cooled McCartney bottles. At least 5 cows were sampled from a herd during each visit. The samples were transported to the laboratory and stored at 4°C until analysis.

Samples of nono hawked by Fulani women were obtained from Wadata, Wurukum and Makurdi International markets in Makurdi. In each market, 5 samples of 50 ml each were randomly collected from different hawkers once a month over a period of 6 months. These were put into sterile pre-cooled McCartney bottles and taken to the laboratory and refrigerated at 4°C until analyses. A total of 90 samples, 30 for each market were collected.

2.2 Sample analysis: The culture and mycobacterial identification was done as described by Cruickshank *et al.* (1975). Forty milliliters of each milk specimen was centrifuged in a 50 ml screw capped tube at 3000 RCF for 20 min. The supernatant was discarded into 10% formalin and the sediment re-suspended in 20 ml of 2% NaOH, containing phenol red indicator. The tube was allowed to stand for 30 min after which it was slowly

that in Nigeria, Benue State has the second highest prevalence rate (13.0%) of HIV/, a synergist of tuberculosis. This study was carried out to determine the presence of and characterize *Mycobacterium* species in freshly drawn cow milk and nono sold at markets in Makurdi with a view to highlighting the risk of consuming unwholesome market milk.

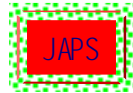
neutralized with 6N ammonium chloride indicated by colour change from purple to pink. After the neutralization, it was again centrifuged at 3000 RCF for 20 min, after which all but 2 ml of the supernatant was discarded. The sediment was then mixed thoroughly with the supernatant using sterile cotton tipped applicator. This was then used for staining, culturing and biochemical tests.

From the sediment of each sample, 4 smears were prepared, dried, slightly fixed over flame and stained with Ziehl-Neelsen (Acid fast) stain. Strongly stained acid fast rod appearing bright red with a blue background under oil immersion lens was identified as *Mycobacterium* species (Anon, 1978). Samples positive for presence of acid fast bacilli were cultured separately on slants of Lowenstein Jensen (L-J) medium with pyruvate and L-J medium with glycerol. These were done by smearing thick inoculums of sediments on the surfaces of the slants and incubating them at 37°C for 8 weeks. The slants were then examined every week after 4 weeks of incubation for growth. Lowenstein-Jensen slants with glycerol that showed dull, raised, rough, moist, convex light yellow growth within 2 – 8 weeks were presumptively regarded as *Mycobacterium tuberculosis* (Kent & Kubica, 1985). Slants with pyruvate that showed small, moist, smooth, flat growth after 4 weeks of incubation were presumed to be *Mycobacterium bovis* positive (Kent & Kubica, 1985). The presumed *Mycobacterium* isolates were further subjected to biochemical tests for characterization and identification as described by Lennette *et al.* (1975). Isolates that were niacin and nitrate positive were identified as *Mycobacterium tuberculosis* while those that were negative for both tests were identified as *Mycobacterium bovis*.

3 RESULTS

Twenty two (7.7%) of the 285 fresh milk and 12 (13.3%) of the 90 “nono” samples were positive for acid-fast bacilli. Cultures and biochemical tests

showed that only 4 (18.2%) of the 22 acid-fast bacilli positive fresh milk and 2 (16.7%) of the 12 “nono” samples were positive for *Mycobacterium bovis*. One



(8.3%) of the 12 positive nono samples was positive for *Mycobacterium africanum* species. All the isolates

produced white, moist, slightly rough and friable colonies (Table 1).

Table I: Mycobacteriological study of freshly drawn cow milk and fresh “nono” in Makurdi

No. isolates	Tests					Identity
	AFB	L-J P	L-J G	Nit	Nia	
Fresh cow milk						
4	+	+	—	—	—	<i>M. bovis</i>
18	+	—	—	—	—	Other AFB
Fresh “nono”						
2	+	+	—	—	—	<i>M. bovis</i>
1	+	—	+	+	—	<i>M. africanum</i>
9	+	—	—	—	—	Other AFB

Note: AFB = Acid-fast bacilli; L-J P = Lowenstein Jensen medium with pyruvate; L-J G = Lowenstein Jensen medium with glycerol; Nit = nitrate reduction; Nia = niacin production

The distribution of zoonotic *Mycobacterium* species in freshly drawn milk and ‘nono’ are shown in Tables 2 and 3, respectively. Four (1.4%) of the 285 freshly drawn cow milk samples from the 3 different settlements tested positive for *Mycobacterium*. *Mycobacterium* was detected in milk from 2 out of the

3 markets sampled (table 2). The result shows that 3 (3.3%) of the 90 “nono” samples from the various markets tested positive for *Mycobacterium*. Positive fresh milk samples were observed in the months of February, March and May while that of ‘nono’ were seen in March and April (Table 4).

Table 2: Incidence of *Mycobacterium bovis* contamination of fresh cow milk in sampled peri-urban areas of Makurdi town, Nigeria.

Settlement	No. sampled	No. positive (%)
Agan	122	1 (0.8)
Apir	59	1 (1.7)
Fidi	104	2 (1.9)
Total	285	4 (1.4)

The figures in parenthesis are percentages (%) of number examined that were positive.

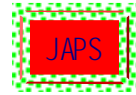
Table 3: Incidence of *Mycobacterium* contamination in “nono” in Makurdi town markets, Nigeria.

Market	No. sampled	No. positive (%)
Wadata	30	1 (3.3)
Wurukum	30	0
Makurdi International	30	2 (6.7)
Total	90	3 (3.3)

The figures in parenthesis are percentages (%) of number examined that were positive.

Table 4: Monthly incidence of *Mycobacterium* species in fresh milk and ‘nono’ in Markudi, Nigeria.

Months	Fresh cow milk sample from herds		Fresh “nono” sold in markets	
	No. examined	No. positives n(%)	No. examined	No. positive (%)
December	47	0	15	0
January	49	0	15	0
February	49	2 (4.1)	15	0
March	47	1 (2.1)	15	1
April	49	0	15	2
May	44	1 (2.3)	15	0
Total	285	4 (1.4)	90	3 (3.3)



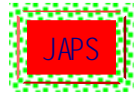
4 DISCUSSION

The detection of *Mycobacterium bovis* in freshly drawn cow milk samples (1.4%) and “nono” (2.2%) in Makurdi markets corroborated the findings of other workers (Alhaji, 1976; Idrisu & Schurrenberger, 1977; Ijaiya, 1980; Shehu, 1991; Kolo, 1992). This confirms that the presence of zoonotic *Mycobacterium bovis* in milk in Nigeria has persisted over time. Considering the recent report that *Mycobacterium bovis* infection accounted for 1.6% of cases of tuberculosis in HIV patients globally, the continued detection of this pathogen in cow milk is worrisome as cow milk forms the bulk of animal protein that is normally recommended for immunocompromised persons.

The presence of *Mycobacterium africanum* in one of the “nono” samples may be due to post milking contamination from humans, and thus it indicates the unhygienic practices that are associated with processing of milk in Nigeria (Alhaji, 1976; Shehu, 1992; Kolo, 1992). Detection of significant numbers of other acid-fast bacilli, 6.3 and 10% of the total samples for fresh milk and “nono”, respectively also corroborated the work of Alhaji (1976) who reported that a minimum of 6.4% of such isolates from market milk represented Runyon groups of mycobacteria and other acid-fast bacteria. These Runyon groups are, however, regarded as contaminants (Chapman *et al.*, 1965; Chapman & Speight, 1968) and are often of no clinical value.

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