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Prevalence of Zoonotic bovine tuberculosis and associated risk factors among cattle herds in North Central Nigeria

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A B S T R A C T

Prevalence of Bovine Tuberculosis (BTB) was investigated among 600 lactating cows in 48 herds in North Central Nigeria using tuberculin skin test, culture, acid fast stain and biochemical characterization, well structured questionnaire was administered to 200 cattle farmers and non cattle farmers respectively to determine risk factors associated with BTB in the study area. Forty five (7.5%) lactating cows tested positive for tuberculin skin test, 11 (1.8%) were inconclusive reactors, 544 (90.6%) tested negative, 30 (5%) milk samples from tuberculin positive cows were cultured on Lowenstein-Jensen media, while 33 (5.5%) were acid-fast positive. Biochemical test identified 19 isolates of *M. bovis* indicating prevalence of 3.2% for *M. bovis* in the study area. Cattle farmers indicated close association with cattle and consumed unpasteurized cow milk for years, while 42 non cattle farmers consumed unpasteurized cow milk. Milking maids processed cow milk by boiling under uncontrolled temperatures and sometimes sell raw cow milk to the public. Biochemical test identified 3.2% prevalence for Bovine Tuberculosis while close association with cattle and prolonged consumption of unpasteurized milk were identified as risk factors. Further studies using molecular test to identify *M. bovis* strains is recommended where similar risk factors exist.

Introduction

Bovine tuberculosis (BTB) is an infectious disease of cattle mainly caused by *Mycobacterium bovis* and characterized by progressive development of tubercles in any tissue or organ of the body, it is a major infectious disease among cattle in many countries; infected cattle may carry and shed the organism for many years without showing any sign of the disease (Clarke, 1998). Although cattle are the main host and reservoir of this chronic infection, other mammals, including humans, are also susceptible to *Mycobacterium bovis* (Mcllroy *et al.*, 1986). Bovine tuberculosis is becoming increasingly important due to the susceptibility of humans to the disease

caused by *M. bovis* (Huard *et al.,* 2003) and there is increasing evidence that *M. bovis* infections may be much more significant than generally considered (Kleeberg,1984). Tuberculosis due to *M. bovis* is often reported where bovine disease is poorly controlled, with high incidence amongst cattle herdsmen, abattoir workers and people who work directly with cattle, they may acquire the disease by inhaling cough spray from infected animals, consumption of unpasteurized milk, improperly cooked infected meat and through close contact with infected animals over a period of time (Ayele *et al.,* 2004, Cosivi *et al.,* 1998. O'Reilly and Daborn, 1995, Francis, 1971).

M. bovis has been isolated in unpasteurized fresh and soured cattle milk and abattoir samples in Nigeria (Ofukwu *et al.,* 2008a, Abubakar, 2007a, Cadmus *et al.,* 2006), the consumption of unpasteurized milk is a common practice in many parts of Nigeria (Abubakar, 2007a). TB is a major public health problem in Nigeria, as the country ranks 5th amongst the 22 high TB burden countries which collectively bear 80% of the global TB burden. Previous reports indicated 14% of all human tuberculosis in Nigeria was associated with *M. bovis* (Abubakar, 2007a). Earlier studies have confirmed existence of BTB in different cattle herds in Nigeria (Ofukwu *et al.,* 2008a, Abubakar, 2007a, Cadmus *et al.,* 2006, Alhaji, 1976, Anywale, 1984, Dusai and Abdullahi, 1994, Cadmus *et al.,* 1999) with an average prevalence of about 6.5%. Both *M. tuberculosis and M. bovis* have been isolated in infected human sputum samples and unpasteurized cow milk commonly consumed by humans in Nigeria (Idigbe *et al.,* 1986, Idrisu and Schnurrenberger, 1977).

The presence of these opportunistic, pathogenic bacteria in bovine milk has emerged as a public-health concern, especially among individuals who consume raw milk and related dairy products, while many epidemiologic and Public Health aspect of infection remain largely unknown (Cosivi *et al.,* 1998, Collins and Grange, 1983) posing major public health threat especially with the potent impact of the epidemic of human immunodeficiency virus infection. The current increasing incidence of tuberculosis in humans, particularly in immunocompromised persons, is posing a serious public health threat to human population and has given rise to a renewed interest in the zoonotic importance of *M. bovis*, especially in developing countries (Radostits, 2000). Hence, the need to further investigates the prevalence of Bovine tuberculosis infection in dairy cattle herds in Nigeria. This study investigated the prevalence of bovine tuberculosis among the nomadic Fulani herds in the North Central zone of Nigeria. An accurate knowledge of the prevalence of zoonotic BTB is essential for appropriate intervention strategies.

Methods and Materials

Study area

The study was conducted in the North Central Zone of Nigeria. The North Central zone of Nigeria consists of six states and Abuja the Federal Capital Territory (Benue state, Kogi state, Kwara state. Nasarawa state, Niger state, Plateau and Abuja, the Federal Capital Territory of Nigeria) with a population of about 20,266, 257 people and over 8, 000,000 heads of cattle, situated geographically in the middle belt region of the country, spanning from the west, around the confluence of the River Niger and the River Benue. The region itself is rich in natural land features, and boasts some of Nigeria's most exciting scenery. The region is also home to many historical and colonial relics (Wikipedia, 2015).

Study design

The prevalence of BTB was investigated among 600 lactating cows from 48 Nomadic Fulani cattle herds in 3 states randomly selected and Abuja, in the North Central Zone of Nigeria, using screening test and laboratory analysis while a well questionnaire was administered to determine the risk factors. Data obtained were subject to simple descriptive statistical analysis to determine the prevalence rate.

Administration of questionnaire

A well structured questionnaire was administered to voluntary participants among the Fulani cattle farmers and non cattle farmers' after an informed consent. The questionnaire was administered to two hundred Fulani cattle farmers and 200 non cattle farmers in the study area randomly selected. Information sought include names, sex, ages, address, occupation, BCG vaccination history, history of respiratory tract related diseases, evidence of coughing in the past or present, consumption of unpasteurized milk, duration of association with cattle, knowledge of zoonotic diseases, knowledge of tuberculosis, history of TB test or treatment, types of animals kept and breeds of cattle, system of husbandry, reasons for keeping animals and methods of processing cow milk.

Tuberculin skin test

Six hundred lactating cows made up of Bunaji (White Fulani), Sokoto Gudali (Bokolooji); Rahaji and Adamawa Gudali breeds from 48 nomadic Fulani cattle herds in the North Central zone of Nigeria randomly selected were screened for tuberculosis using the single intra-dermal comparative tuberculin test (SICTT) using standard method (Monaghan *et al*,. 1994, Shirima *et al*,. 2003). Tuberculin test was conducted using bovine and avian purified protein derivative (PPD) from Veterinary Laboratory Weybridge, UK. Both sites on the neck were shaved; skin thickness was measured using the Vernier caliper prior to injecting. 0.1 ml equivalent to 2,500 IU of avian tuberculin was injected intra-dermally on the right side and an equivalent dose of bovine tuberculin was injected into the left side. Skin fold thicknesses of both sites were measured and recorded 72hrs post-injection and results interpreted as described by (Monaghan *et al*,. 1994).

Collection of milk sample for demonstration of tubercle bacilli

Milk samples were collected from positive tuberculin reactors. Milk samples were collected into sterile screw-capped universal containers and labeled properly. All samples were conveyed to the laboratory immediately after collection on wet ice packs and stored properly until processed.

Staining and microscopic examination

Acid-Fast /Zeihl-Neelsen (ZN) Stain

Processed milk samples were stained using Ziehl-Neelsen staining technique in accordance with standard protocol (24). Examination under a microscope with oil immersion lens x I00 reveals acid-fast bacilli (AFB). The bacilli (positive) were stained red, straight or slightly curved rods occurring either singly or in small groups while non acid-fast microorganisms (negative) stained blue (Kazwala *et al*).

Culturing

Specimens of milk samples processed, was inoculated onto 2 slants of Lowenstein-Jensen media (glycerol and pyruvate

enriched) before incubating at 37°c for a minimum of 8 weeks. Culture procedures were carried out according to standard methods as described in the CRC manual of clinical laboratory procedures (Anonymous, 1970). Isolates of mycobacterium were identified to the species level by their growth rate, colony morphology, pigmentation and conventional biochemical tests, such as the para-nitrobenzoic acid (PNB), thiophen-2-carboxylic acid hydrazide (TCH), niacin test, nitrate reduction test, urease, semi-quantitative catalase, Tween 80 hydrolysis, NaCl tolerance (Quinn *et al*,. 1994, Vestal, 1977). A combination of negative activity for niacin, nitrate reduction, catalase at 68^{o}C, Tween 80 hydrolysis, arylsulphatase and TCH were considered as characteristics of *M. bovis* (Vestal, 1977).

Statistical analysis

Data obtained were analyzed using simple descriptive statistics to determine the prevalence of bovine tuberculosis among lactating cows.

Results and Discussion

A total of 200 cattle farmers and 200 non cattle farmers were administered questionnaire in this study. Their ages ranged between 15 – 65 years, all nomadic Fulani cattle farmers indicated close association with cattle and consumed raw unpasteurized cow milk for over ten years, while 42 non cattle farmers also consumed unpasteurized cow milk. All respondents consumed only cooked meat and were aware of zoonotic diseases with little knowledge on their mode of transmission. Seventy two cattle farmers and 15 non cattle farmers' revealed history of cough and other respiratory tract related illnesses and were never tested or treated for tuberculosis; two hundred and one respondents had received BCG in the past. Breeds of cattle reared in the study area include; Bunaji (White Fulani), Sokoto Gudali (Bokolooji), Rahaji and Adamawa Gudali. Cattle farming were the source of livelihood for the Fulani cattle farmers, they sell milk and matured animal to the public to earn income. The Fulani milking maids processed their milk by boiling under uncontrolled temperature and sometimes sell raw cow milk to the public. Forty five (7.5%) lactating cows reacted positive for tuberculin skin test, 11 (1.8%) were inconclusive reactors, 544 (90.6%) were negative (Table 1), 30 (5%) milk samples from tuberculin positive cows were cultured on LJ, while 33 (5.5%) were acid-fast positive (Table 2). Biochemical test on isolates from milk samples identified 19 isolates of *M. bovis* indicating the prevalence of 3.2% of Bovine Tuberculosis in cattle in this study (Table 1).

Tuberculin skin test detects the presence of *M. tuberculosis complex* in both man and animals. This test has traditionally been used to determine the prevalence of infection in animals and man using the purified protein derivative of Mycobacterium (PPD-tuberculin) (OIE, 2009). Acid-fast smears, cultures, use of direct smear microscopy is also an inexpensive, rapid test for diagnosis of tuberculosis (Araujo *et al*, 2014) which does not permit differentiation between species of *M. tuberculosis* complex.

This study observed *M. bovis* infection (Bovine tuberculosis) among lactating cows, most cattle farmers had close association with cattle for many years, consumed and sold unpasteurized or inadequate heat treated cow milk to the general public, resulting in the risk of milk contamination with *M. bovis* which is a potential health hazard to consumers while the cattle farmers' poor understanding of the epidemiology of BTB further exacerbates the situation. These findings are in

agreement with previous reports demonstrating a link between animal and human tuberculosis (Ayele *et al.*, 2004, Davies, 2006, Thoen *et al.*, 2009, Srivastava *et al.*, 2008, Leite *et al.*, 2003). The prevalence of *M. bovis* in cattle was observed to be 3.2% using biochemical test, this collaborates earlier reports in cattle herds and suspected cattle tissues from abattoirs in Nigeria (Cadmus *et al.*, 2006, 1999, Ofukwu, 2006, Abubakar *et al.*,b). Therefore, the spread of *M. bovis* infection from animal to man in Nigeria and other endemic countries remains a serious threat especially amongst occupationally exposed persons, people consuming unpasteurized milk and immunocompromised persons.

Conclusion

This study demonstrates the existence of *M. bovis* infection (Bovine tuberculosis) in cattle in the study area while cconsumption of raw milk and close association with cattle tends to potentiate animal to man transmission of *M. bovis*. Strategic surveillance and control of tuberculosis with particular attention to *M. bovis* and public enlightenment on dangers of consuming unpasteurized milk are recommended, further studies using molecular test to demonstrate *M. bovis* strains is also recommended for countries where similar risk factors exist.

Table.1 Biochemical characterization

Diagnostic Method	**Prevalence of *Mycobacterium bovis***
Biochemical characterization	3.2%

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