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Molecular identification of mycobacterium tuberculosis transmission between cattle and man: A case report

Abstract

This study described a case of transmission of *Mycobacterium tuberculosis* infection from a man to cattle confirmed by using Bioline® and multiplex PCR known as Genotype® MTBC Kit. A dairy farm was screened using caudal fold intradermal tuberculin test, and a heifer reacted positively to the bovine tuberculin but showed no gross pathological lesions at slaughter. The cattle attendant was also diagnosed with active pulmonary TB infection as a smear positive in the hospital. The attendant's sputum and the heifer's bronchial as well as mandibular and submandibular lymph nodes were collected, processed and cultured. The isolates obtained were analyzed using Bioline® as well as Genotype MTBC analyses. The results showed that both the attendant's sputum as well as the lymph nodes yielded *Mycobacterium tuberculosis*. This is the first case of human to cattle transmission of *Mycobacterium tuberculosis* in the study area as confirmed by using Bioline® techniques and Genotype® MTBC.

Keywords: *M. tuberculosis*, *M. bovis*, bioline, genotype MTBC, tuberculin test

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Introduction

Tuberculosis (TB) is among the diseases that have threatened the health and lives of people, and animals in the past century, causing mortality, morbidity and economic losses.[1,2] In Nigeria bTB (bovine tuberculosis) occurrence is on the increase as a result of lack of any National Control Program.[3]

Bovine tb (bTB) represents a major problem in terms of epidemiology, especially in countries where there is no control programs like Nigeria. It is a significant veterinary disease that can occasionally spread to humans.[4] In contrast, *Mycobacterium tuberculosis* is considered primarily a human pathogen.

Among domestic animals, infection with *Mycobacterium tuberculosis* has been most frequently identified in cattle.[5] According to published data, the prevalence of *Mycobacterium tuberculosis* infection in cattle herds did not exceed 1% in the majority of studies.[4,6,7] However, a few exceptions, like Algeria and Sudan with 6.2% and 7.4% prevalence, respectively, were also described[8] as a most probable consequence of the high prevalence of human TB in these two African countries.

Current knowledge has shown that, *Mycobacterium tuberculosis* does not appear to have an indigenous animal host of reservoir and the animals that become infected represent most probably accidental hosts.[7,5] Although, it has never been directly proved yet, human suffering from active TB are strongly believed to represent the main source of *Mycobacterium tuberculosis* in animal, including cattle.[5,7,9] To the best of our knowledge, we described in this report, the first transmission of *Mycobacterium tuberculosis* from human to cattle unequivocally confirmed by molecular analysis of isolates involved in the transmission. For the molecular analysis Bioline® was used, which has recently become a worldwide standard technique for the differentiation of the genus Mycobacteria into *Mycobacterium tuberculosis* complex and Non tuberculous Mycobacteria. Although *Mycobacterium tuberculosis* in cattle most frequently produces a quickly vanishing infection rather than a progressive disease, the infected animal do react positively when challenged with tuberculin.[10,11] The duration of sensitization to tuberculin is usually short, and the reactivity disappears when the infection source is removed.[12] Thus, when a tuberculin-positive animal is recognized during a routine tuberculin testing for the first time, and particularly when the tuberculin-positive animal is young, the possibility of human TB infection among farm workers or animal attendants should be considered. As reported by several authors,[13-15] animal attendants with active pulmonary TB represent an important source of *Mycobacterium tuberculosis* for animals, spreading the mycobacteria via urine, sputum, or feces.[5] As a result of such spreading, the classical form of TB occasionally develops in animals living in close contact with humans with active TB.[11]

In this report, we describe a case of *Mycobacterium tuberculosis* infection from human to cattle, confirmed by a modern molecular methods (Bioline® analysis), techniques that identify and differentiate the genus into *Mycobacterium tuberculosis* complex and Non tuberculous Mycobacteria and also the Genotype® MTBC analysis which differentiate the complex into various species.

Materials and methods

In April, 2010, during tuberculin skin testing in cattle which was part of the National Bovine tuberculin tuberculosis epidemiological survey, a 2 year old heifer from a dairy farm reacted strongly to bovine tuberculin test. In the farm, 15 animals were considered to have positive reactions, out of 350 cows, and 3 animals, including the said heifer that had the strongest reactivity to the tuberculin test were purchased and slaughtered. The submandibular, tracheobronchial, retropharyngeal, bronchial, mediasternal, portal and mesenteric lymph nodes were collected for bacteriological investigation. No gross pathological changes were visible in the lymph nodes and tissues

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inspected at slaughter. However, after 8–weeks of incubation colonies were observed on Lowenstein–Jensen slants and were Ziehl-Neelsen stained, and acid-fast culture isolates were obtained. Similarly, sputum samples from the farm attendant who reported to the hospital sick was also obtained, the 3-sputum samples were pooled together and were Z-N stained before being processed for culture. All Z-N positive isolates from the cow and the farm attendant were analyzed using Bioline analysis and the Hain Assay test as described in their HLEA manual (GenoType MTBC analysis).

Bacteriological investigation of the collected lymph nodes as well as the sputum was performed according to the protocol described by Kent and Kubica, 1985. Following homogenization, decontamination and concentration, the materials were inoculated on Lowestein-Jensen slants (with pyruvate and the other with Glycerol supplemented), which were checked for growth once a week for eight weeks. Identification of colonies– All suspected growths were subjected to Z-N staining and those who were positive were chosen and subjected to SD Bioline® analysis, which differentiate them into *Mycobacterium tuberculosis* complex and other environmental mycobacteria. A Genotype® MTBC for molecular genetic assay for differentiation of the *Mycobacterium tuberculosis* complex from cultured material was used (Hain Lifescience, Germany). The kit was used according to the manufacturer's instructions.

Results

All colonies which were Z-N positive were subjected to Bioline® analysis, which identified them as *Mycobacterium tuberculosis* complex. The *Mycobacterium tuberculosis* complex were then subjected to Hain Assay test (Hain Lifescience, Germany) HLEA Line probe assay technique using Genotype® MTBC kit in order to identify and separate the complex into their various species. It was observed that, the samples belonging to the heifer as well as the attendant were found to be *Mycobacterium tuberculosis* species.

Discussion

Mycobacterium tuberculosis have been considered primarily a human pathogen; it has been reported to be present in both wild and domesticated animals, especially those living in close contact with human.[6,16,17] Cattle have been found to be most frequently infected with *Mycobacterium tuberculosis* among all domestic animals.[7] It was observed that, cattle infected with *Mycobacterium tuberculosis* reacts to tuberculin challenge, even though it produces a quick vanishing reaction but the infected cattle reacts positively when challenged,[11] Lesslie[12] hypothesized that, when a tuberculin positive animal is recognized during routine tuberculin testing for the first time in a herd and particularly, when the tuberculin positive animal is young, the possibility of human TB infection among farm workers should be considered. In this reported case, a young heifer was affected, no TB-like characteristics pathological changes were detected and the heifer reacted strongly to skin tuberculin test, and the fact that, the farm attendant was infected with TB, strongly suggested the possibility of an anthropozoonotic transmission of *Mycobacterium tuberculosis* infection. Available literature suggests that, when humans are suffering from active TB, they tend to be the most probable source of *Mycobacterium tuberculosis* infections in cattle.[18] It was also reported by many authors that, an animal attendant with active pulmonary TB may represent an important source of *Mycobacterium tuberculosis* for animals, spreading the bacilli via sputum, urine or feces.[5,13,14,19]

In conclusion, it has been shown in this report that, humans suffering from active pulmonary TB are the most probable source of *Mycobacterium tuberculosis* in cattle even though indirect data. The source was used. We consider this report as the first unequivocal evidence of human-to-cattle transmission of *Mycobacterium tuberculosis* in the study area. Also, it is suggested that, in areas where bovine TB and human TB coexist, a detailed microbiological investigation of the specimens of slaughtered tuberculin positive animals should always be performed in order to discriminate between *Mycobacterium tuberculosis* and Mycobacterium bovis infections.

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None.

Conflicts of interest

Authors declare that there is no conflict of interest.

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