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# THE PRESENCE OF *MYCOBACTERIUM* SPECIES IN RAW MILK OBTAINED FROM LACTATING COWS IN LEWA AND DUDA OF VIMTIM WARDS, MUBI NORTH

**DOMO GA[1*] and ISHAKU Japhet[2]**

1- Biological Sciences Department
2- Adamawa State University, P.M.B. 025 Mubi

***Corresponding author*:** DOMO GA

**ABSTRACT:** A study was carried out to detect the presence of Mycobacterium species in 10 dairy herds in Lewa and Duda of Vimtim ward Mubi North Local Government Area, consisting of 100 lactating cows. The cows were mostly local breeds and some few white Fulani (Bunagi) breed. All herds were semi-intensively managed and produce milk for public consumption. Cold Rapid Test (CRT) strip and Ziehl-Neelsen's stain (ZNS) specific for detection of Mycobacterium antibodies in any fluid (e.g. milk) were used. The result showed that 2% (2/100) positive for antibodies of Mycobacterium species (suspected to be M. bovis). Fresh raw milk samples were collected from cows and subjected to Ziehl-Neelsen's Stain (ZNS) in order to detect the presence of bacilli in the milk. Thus, only 1 sample was found positive. The result obtained showed that CRT was sensitive in detecting Mycobacterium antibodies.

***Keywords*:** PRESENCE, MYCOBACTERIUM, MILK, COWS, VIMTIM WARDS.

## INTRODUCTION

Tuberculosis is an infectious disease that is caused by a bacterium called Mycobacterium tuberculosis. Tuberculosis primarily affects the lungs, but can also affect organs in the central nervous system, lymphatic system and circulatory system among others. The disease was called "consumption" in the past because of the way it will consume from within anyone who become infected with. According to Medilexicon's medical dictionary, tuberculosis is "a specific disease caused by infection with Mycobacterium tuberculosis, the tubercle bacillus which can affect almost any tissue or organ of the body, the most common site of the disease being the lungs (Konstantinos, 2010).

There are two forms of tuberculosis that cause significant disease in mammals. Human tuberculosis, sometimes acute –brief and severe –but much more commonly a chronic lung infection is caused by the bacterium Mycobacterium tuberculosis. The chronic lung infection is usually more severe and long duration ( compared to acute tuberculosis ) and is said to be transmitted via bacteria contained in water droplets, usually from the cough of a person with active tuberculosis ( a chronic bacterial infection caused by Mycobacterium tuberculosis). Most people who are infected with tuberculosis harbor the tuberculosis bacterium without any tuberculosis symptoms. This is known as latent tuberculosis –a condition in which the body is able to fight the tuberculosis bacteria and stop them from growing. And about one in ten latent infections eventually progresses to active disease, kills more than 50% of those infected (Konstatinos, 2010). Mycobacterium bovis is a slow-growing (16 to 20 hours generation time) aerobic bacterium and the causative agent of tuberculosis in cattle (known as bovine tuberculosis). Related to M. tuberculosis –the bacterium which causes tuberculosis in humans –M. bovis can also jump the specie barrier and cause tuberculosis in humans (Grainge, 1996). M. bovis is the causative agent of tuberculosis in a range of animal species and man with worldwide annual losses to agriculture of $3 billion (Thierry, 2003). M. bovis is the agent responsible for bovine tuberculosis however it can also cause the disease in humans if there is consumption of infected materials. Tuberculosis has affected millions of organisms for thousands of years and studies of early human skeletons show

tuberculosis lesions dating back to the Neolithic period. Tuberculosis in humans is caused by either M. bovis or M. tuberculosis. It is possible to extract surviving ancient mycobacterial DNA and amplify it by PCR. Using this technique, a laboratory performed a study of five Iron Age individuals from Aymyrlyg, South Siberia to determine the type of tuberculosis infection (Michael, 2007).

M. bovis is usually transmitted to humans via infected milk, although it can also spread via aerosol droplets. Actual infections in humans are rare, mostly due to pasteurization killing any bacteria in infected milk; as well, cattle are randomly tested for the disease and immediately culled if infected, but can still be used for human consumption. However, in areas of the developing world, pasteurization (a process of heating a food to a specific temperature for a definite length of time and then cooling immediately) is not routine. M. bovis is a relatively common cause of human tuberculosis (O'Reilly and Debora, 1995). Bovine tuberculosis is a chronic infectious disease which affects a broad range of mammalian host including humans, cattle, deer, pigs, domestic cats, wild carnivores (fox, coyotes, and badgers), possums, and rodents. It rarely affects equids or sheep (Delahay, 2002). The disease can be transmitted in several ways, for example: badgers excrete M. bovis in exhaled air, sputum, faeces and pus, so the disease can be transmitted by direct contact with the excreta of an infected animal, or inhalation of aerosols depending on the species involved (Phillip, 2001).

## SIGNIFICANCE OF THE STUDY

This research work is significant because the number of people consuming milk and milk products are increasing almost every day, as such increasing the chances of hazard because it can lead to disease (tuberculosis) outbreak. For this reason, it has become necessary for this research to be conducted to investigate the presence of the *Mycobacterium* species in raw milk obtained from lactating cows in these metropolitan areas.

### *STATEMENT OF THE PROBLEM*

Bacteriological study of food is of utmost importance before food could be considered to be of good quality for human consumption or sold on commercial basis. Therefore the unhygienic selling and buying of raw milk which could come from unknown infected cows (cows infected with *M. bovis*) found in some villages (Duda and Lewa) needs to be assessed and that is what this project work is out to study.

### *AIMS AND OBJECTIVES*

The aim of this research work is to determine the presence of Mycobacterium bovis in raw milk, which is to be achieved through the following objectives:

- To detect the presence of Mycobacterium bovis in milk using Ziehl Neelsen's staining and tuberculosis Cold Rapid Test (strip method).

To evaluate the high risk involved in consuming unpasteurized milk and milk products that some rural people consume.

## MATERIALS AND METHODS

### *The study area*

Herdsmen's settlements are within Mubi North local government area– which include Lewa and Duda of Vimtim ward. These areas were selected using simple sampling method and are located at the northern region of Mubi, and the approximate distance between Lewa and Duda is about 1mile. Mubi is located in the northern part of Adamawa state in the North-East zone of Nigeria. It shares national border with Cameroon in the north (Adebayo and Tukur, 1999). The major ethnic groups in Mubi metropolis include Fulani, Gude, and Fali, while in the study area (Duda and Lewa), 75% of the people are Fali. The minors are Margi and Kilba. The economic activities of the people in these areas include: food and cash crops, irrigation agriculture, livestock farming, traditional industries, transportation, and marketing. However, the emphasis in this research work is on raw milk gotten from livestock farming. Adamawa state is one of the states of the federation with very high concentration of wide variety of livestock. The variety of livestock in the state and Mubi in particular include cattle, sheep, goats, and pigs. Of the general livestock estimated of 7.5 to 8.3 million cattle are the most significant. The major breed of cattle type found in the state are the Adamawa Gudali, which are found all over the state but generally in the hands of settled pastoralists under extensive management system. There are also the Sokoto Gudali or Bokoloji which are mainly in the hands of the more nomadic Fulbe Bororo and those practicing intensive management in the urban areas. Bunaji or Yakanaji generally called white Fulani, are also found in some parts of the State (Adebayo and Tukur, 1999).

### *METHOD OF SAMPLE COLLECTION*

100 samples of fresh raw milk were collected separately from ten different herds of cattle. Different sterile sampling bottles (2ml) were distributed to herdsmen in Lewa and Duda of Vimtim ward. The collected samples were labeled accordingly and kept in a refrigerator to prevent any further microbial activities.

### *METHODS OF SAMPLE ANALYSIS*

There are different methods of milk analysis, however for the purpose of this research, Ziehl Neelsen's staining (acid fast stain) and strip methods were applied for detection of the tubercle bacilli or its antibodies. The statistical analysis of results used was simple percentage. Ziehl Neelsen's method was selected because it identifies and differentiates acid fast bacilli such as *Mycobacterium tuberculosis* from other non-acid fast bacilli (Gaffa and Zoro, 2005).

### *ZIEHL NEELSEN'S STAINING (ACID FAST STAIN) TECHNIQUE*

Ziehl Neelsen's staining method is used for the identification, and differentiation of acid fast bacilli such as *Mycobacterium tuberculosis* from other non-acid fast bacilli (Gaffa and Zoro, 2005).

### *STERILIZATION OF MATERIALS*

The apparatus used (glass bottles, slides, Pasteur pipette) were sterilized in a hot air oven for a required period of time, and the sample (raw milk) was kept in a refrigerator, under low temperature ($4^0$c) to inhibit growth of microorganisms and other foreign bodies (Gaffa and Zoro, 2005)

### *COLD RAPID TEST (CRT) METHOD*

### *PROCEDURE*:

The rapid bovine tuberculosis antibodies test strip specific for *Mycobscterium tuberculosis* antibodies containing the test devices procured from the manufacturer (Myun) South Korea were used in detecting *M. bovis* antibodies in the raw milk collected. The milk samples were taken out of the freezer and allowed to attain room temperature (15-30$^o$C) before use as described by Gaffa and Zoro (2005).

## RESULTS AND DISCUSSION

### *RESULTS*

The presence of Mycobacterium bovis in raw milk collected from some villages within Mubi North local government Area of Adamawa State was tested using various methods and the results obtained are displayed in the following tables:

Table1. Dairy herd composition and farm management

| Dairy farm No. | Location (village) | Herd composition (breed of cattle) | Farm management system | No. of lactating cows on farm | No. of lactating cows sampled |
|---|---|---|---|---|---|
| A | Duda | Local breed | Semi-intensive | 9 | 9 |
| B | Duda | Local breed | Semi-intensive | 11 | 11 |
| C | Duda | Local breed | Semi-intensive | 10 | 10 |
| D | Duda | Local breed | Semi-intensive | 15 | 15 |
| E | Duda | Local breed | Semi-intensive | 17 | 17 |
| F | Duda | Local breed | Semi-intensive | 7 | 7 |
| G | Lewa | Local breed /white Fulani | Semi-intensive | 13 | 13 |
| H | Lewa | Local breed | Semi-intensive | 6 | 6 |
| I | Lewa | Local breed | Semi-intensive | 4 | 4 |
| J | Lewa | Local breed | Semi-intensive | 8 | 8 |
| Total: 10 | | | | 100 | 100 |

Table 2. Detection of antibodies to M. bovis in 10 dairy farms using CRT

| Dairy farm | | No. of cows tested | No. of cows positive | Percentage of cows positive |
|---|---|---|---|---|
| A | | 9 | - | - |
| B | | 11 | - | - |
| C | | 10 | - | - |
| D | | 15 | - | - |
| E | | 17 | 1 | 1.0 |
| F | | 7 | - | - |
| G | | 13 | 1 | 1.0 |
| H | | 6 | - | - |
| I | | 4 | - | - |
| J | | 8 | - | - |
| Total | 10 | 100 | 2 | 2% |

Table 3. Detection of Mycobacterium bovis in fresh raw milk using Ziehl-Neelsen's stain in 10 dairy farms

| Dairy farm | | No. of cows tested | No. of cows positive | Percentage of cows positive |
|---|---|---|---|---|
| A | | 9 | - | - |
| B | | 11 | - | - |
| C | | 10 | - | - |
| D | | 15 | - | - |
| E | | 17 | 1 | 1.0 |
| F | | 7 | - | - |
| G | | 13 | - | - |
| H | | 6 | - | - |
| I | | 4 | - | - |
| J | | 8 | - | - |
| Total | 10 | 100 | 1 | 1% |

Table 4. A comparison of sensitivity to M. bovis across farms using CRT and ZNS techniques

| Dairy farm | | Percentage of cows positive to CRT | Percentage of cows positive to ZNS |
|---|---|---|---|
| A | | 0.0 | 0.0 |
| B | | 0.0 | 0.0 |
| C | | 0.0 | 0.0 |
| D | | 0.0 | 0.0 |
| E | | 1.0 | 0.0 |
| F | | 0.0 | 0.0 |
| G | | 1.0 | 1.0 |
| H | | 0.0 | 0.0 |
| I | | 0.0 | 0.0 |
| J | | 0.0 | 0.0 |
| Total | 10 | 2% | 1% |

***DISCUSSION***

The percentage of positive cases of sampled dairy cow milk to CRT across farms was 2% out of 100 sampled animals. Farm 'G' at Lewa and farm 'E' at Duda had 1 positive case each [1/13(7.69%), 1/17(5.88%)] respectively. Tubercle bacilli were not detected in other eight dairy farms. A comparison of the percentage exposure of the sampled dairy cows to M. bovis using Cold Rapid Test strip and Ziehl-Neelsen's stain techniques across farms were 2% and 1% for farms G and E respectively (Table 4). The percentage of lactating cows in the farms investigated that reacted positively to CRT was lower than the 17.5% reported in a similar study by Danbirni et al, (2010) in Kaduna State, using IQRT (Immunochromatographic Qualitative Rapid Test). The difference in the percentages obtained may be due to the difference in the type of test used. IQRT is more specific for detection of any Mycobacterium species (e.g M. bovis). Thus the 2% prevalence obtained in this study might not be a true representation of the herds' prevalence status to M. bovis. Also the percentage prevalence obtained in this study is less than 5% reported by Okaiyeto (2008) in a study carried out on dairy farm in Kaduna State using PPD, lending further credence to the low sensitivity of PPD compared to IQRT as pointed out earlier. The reason for the variation in the percentage reactivity across farms could be because farm G had a mixed local breed with some white Fulani breed (Table 1). Though the white Fulani breed is more susceptible compared to the local breed and the closeness of the animals to each other thus, could be facilitating the transmission of Mycobacterium of infection by inhalation within the pen. This finding based on the type of management practice agrees with the report of Machntyre and Plant (1998) that the risk of acquiring tuberculosis in both animals and humans increases with closeness. In addition, the only watering trough for the animals in these farms takes several days before being washed; there by providing a potential source of acquiring the infection as all animals drink from one and only drinking trough. This may agree with the finding of Radostits, (2003) which stated that stagnant drinking water may cause infection up to 18 days after its last use by an infected animal. Farm E was

not truly negative for M. bovis using Ziehl-Neelsen's stain, though the cow in this farm previously tested positive to M. bovis with CRT (table 3), but it indicated that it was not properly shedding the bacilli in milk (M. bovis may be in a dormant form). These animals if allowed to stay long in the farm particularly under stressful conditions could eventually start shedding the bacilli because Bovine tuberculosis is a chronic and progressive disease (Cadmus, 2004). This study showed that ZNS was less potentially sensitive for early detection of M. bovis exposed animals before the animal starts shedding the Mycobacterium in milk. This may indicate that all the cows exposed to M .bovis were infected and were shedding the bacilli in milk a potential danger to any individual consuming raw or inadequately pasteurized milk. Farm E and G showed that the cows were not only infected but were shedding M. bovis in milk (Table 3) even though the cows appeared apparently healthy. This could be a potential danger to calves and the public consuming unpasteurized milk. This result agrees with those of Corner(1994) and Kazwala(1998), that apparently healthy cows may shed Mycobacterium in milk without showing clinical signs of the disease.

## CONCLUSION

In this study, apparently healthy lactating cows may contain viable Mycobacterium species (which may likely be M. bovis) in milk, thus, may cause a serious health problem for man (especially the herdsmen) and calves, hence, not advisable for public consumption –either for nutritional or medicinal purposes.

The tuberculosis Cold Rapid Test strip has been found to be more sensitive for the detection of earlier exposure of cows to M. bovis in the rural areas, and comparison with other test would be required to appreciate its sensitivity and specificity. Ziehl-Neelsen's staining technique though less sensitive in detecting early infected animals with M. bovis was also found to be important in determining the status of one cow that was not apparently infected but was shedding the bacilli in milk.

### *RECOMMENDATIONS*

Some possible health measures against humans and calves infected with Mycobacterium species from consumption of contaminated raw milk include:

1. Herdsmen and their cattle should be routinely treated for lung infections including tuberculosis.
2. Consumers of raw milk obtained from lactating cows should ensure proper boiling before use.
3. Infected persons with tuberculosis should be reported to available medical centers for appropriate treatment.

Further studies should be conducted using other methods.

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