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DETECTION OF MYCOBACTERIUM BOVIS INFECTION IN GOATS AND SHEEP SLAUGHTERED AT FOUR ABATTOIRS IN JIGAWA STATE, NIGERIA

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ABSTRACT

The study was carried out in the four abattoirs (Dutse, Gumel, Hadejia, and Kazaure) in Jigawa State Nigeria to determine the seroprevalence of *Mycobacterium bovis* infection in slaughtered goats and sheep and also assess the knowledge of butchers on tuberculosis and its zoonotic potential. A total of 176 blood samples were collected from 100 sheep and 76 goats and the plasma were screened using ID vet sandwich ELISA. Close-ended questionnaires were administered to butchers to assess their knowledge of *Mycobacterium bovis* infection and tuberculosis. From the results, the seroprevalence of *Mycobacterium bovis* in sheep was found to be 0.01% (1/100) with no positive reactors recorded in goats. *Mycobacterium bovis* was detected only in sheep slaughtered at the Hadejia abattoir with a prevalence of 3.4% (1/29) with no positive reactors recorded in goats and sheep slaughtered at other abattoirs. Prevalence based on sex indicated positive reactor in males (1.2%: 1/86) with no positive reactors in females. Sheep in the age group 2½ -3 years were found to be positive at 2.9% (1/34). Analysis of the questionnaires indicated that butchers have a low level of knowledge on zoonotic potential and ways of transmission and prevention of tuberculosis. The study has demonstrated the presence of *M. bovis* infection in sheep at the Hadejia abattoir with no positive reactors among the goats selected in the study. It has also indicated that butchers in the study areas have poor knowledge of *M. bovis* infection and the various ways of transmission and prevention. The detection of *Mycobacterium bovis* infection in sheep is suggestive of potential transmission to humans and calls for further investigation into the role of small ruminants in the epidemiology of human tuberculosis in the location. It is hereby recommended that ruminants (sheep and goats) should be screened for *M. bovis* infection at the abattoirs and butchers should be educated on the risk of the zoonotic potential of tuberculosis and its various ways of transmission and prevention.

Keywords: *Mycobacterium bovis, Sheep, Goats, ELISA, Prevalence, Jigawa State*

INTRODUCTION

Livestock in Jigawa State is mainly kept by small to medium scale farmers and the production sectors are defined in three categories small, ranging from 1 to 30 animals, medium 31 to 50 animals and large, more than 50 animals based on a total number of cattle, goats, and sheep (1). Goats and sheep contribute significantly to the economy and food security of the farmers in the State and are mainly utilized for skin and meat production which serves as a source of income for the owners (2). Several documented bacterial, viral, and parasitic diseases which are directly and indirectly transmitted occur commonly and are poorly controlled in both livestock and human populations (3). These diseases have a great socio-economic impact such as low production due to abortion, reduced milk production, and loss of draft power, which hurt cash income. However, despite their economic and social importance, livestock management as well as programmes to control infectious diseases in Africa including tuberculosis and brucellosis has declined due to decreasing government support, particularly for operational costs of disease control (3). There is a lack of good data on the occurrence and impact of zoonotic infections including tuberculosis in many developing countries (3).

Tuberculosis is a chronic contagious disease in both animals and humans (4, 5). The disease is caused by members of the *Mycobacterium* tuberculosis complex (6). The *Mycobacterium* Tuberculosis Complex (MTC) consists of the following members: *Mycobacterium*, *africanum*, *Mycobacterium bovis*, *Mycobacterium canettii, and Mycobacterium tuberculosis* (6). They are known pathogens of humans, wild and domesticated species of mammals (7, 8). They are obligate pathogens and the etiologic agents of tuberculosis, so named because of the nodular lesions observed in the lungs, which are termed tubercles (9). These organisms can be visualized using acid-fast stains such as the Ziehl–Neelsen, Fite, and Kinyon stains, which fail to decolorize with weak acids due to the organism's wax-like cell wall. Performing Gram stain on the organism will show weakly Gram-positive or negative. *Mycobacterium tuberculosis* and *M. bovis* ar e the two most common species in the MTC to be reported in Old World primates and can cause sporadic or epizootic disease of high morbidity and mortality in these species. Infection with other members of the

MTC (*M. africanum*) is less commonly reported (6, 10).

Tuberculosis in goats and sheep is caused predominantly by *Mycobacterium bovis* and *Mycobacterium caprae* (7) and few are caused by *Mycobacterium tuberculosis* (8). Epidemiological studies indicated that tuberculosis in goats and sheep has a wide global distribution and has been reported in various countries, including New Zealand, Sudan, Spain, Nigeria, the United Kingdom, Italy, Algeria, and Ethiopia (4). The disease is characterized by the formation of granulomas in tissues and organs, more significantly in the lungs, lymph nodes, intestines, liver, and kidneys (8, 11). *Mycobacterium bovis* has the widest host range among all *Mycobacterium* species (4). Cattle, goats, and pigs are most susceptible to *M. bovis*, with sheep and horses showing a higher natural resistance (8, 12). The existence of natural reservoir hosts and asymptomatic carriers contributes to the persistence of this disease in the environment (8).

Routine screening of small ruminants for *Mycobacterium bovis* infection at abattoirs in Nigeria and Jigawa state is not adequate (13). Thus, the epidemiology and zoonotic potential of tuberculosis in small ruminants in Jigawa State is largely unknown. The only documented information on *Mycobacterium bovis* infection in the state was in cattle where a prevalence of 1.08% was reported (13, 14). There is a dearth of information on the status of *Mycobacterium bovis* infection in small ruminants and its public health significance in the four abattoirs in Jigawa State (1). This study was carried out to determine the seroprevalence of *M. bovis* infection in small ruminants slaughtered in the four major abattoirs in the State and also make recommendations on strategies for control and prevention.

MATERIALS AND METHODS

Study Area

Jigawa State lies between latitude 10°57′ and 13°28′N and longitude 8°08′ and 10°37′E with a total land area of about 22,410 km^2. The State has 27 Local Government Areas (LGAs) with an estimated small ruminant population of about 6,787,000 (2). The climate is semi-arid, characterized by a long dry season and variable rainy seasons which vary considerably over the years and are erratic. The mean annual temperature is about 25^0C in the coolest month and 39^0C in the hottest month (Metrological Unit, Jigawa Research Institute, Kazaure, 2001). The study was conducted in the four abattoirs in Jigawa State that are located in the four agricultural

zones of the State which include Zone 1 (Dutse kudu), Zone 2 (Gumel), and Zone 3 (Hadejia) and Zone 4 (Kazaure).

Study Animals

Small ruminants presented for slaughter were selected using systematic random sampling and information on each sheep and goat such as age and sex was recorded.

Questionnaire Survey

Before sample collection, closed-ended questionnaires were administered to 150 consenting butchers to assess their knowledge of tuberculosis, its zoonotic implication, and its various ways of transmission and prevention

Samples Collection and Processing

Five (5) milliliters of blood were collected from each slaughtered sheep and goat in sterile EDTA universal sample bottles and labelled according to specie age and sex. A total of 176 blood samples from 100 sheep and 76 goats were collected. The samples were transported on ice to the laboratory and centrifuged at 1000 rpm for 1 minute to obtain the plasma which was immediately transferred into test tubes and labelled.

Principle of the Serological Assay

A serological test was carried out using ID-vet screening sandwich ELISA (ID-vet Innovative Diagnostics Montpellier France). The assay is designed to detect native bovine, caprine, and ovine interferon gamma (anti-IFN-γ) in plasma or culture supernatant. It uses two different monoclonal antibodies against ruminant IFN-γ and incorporates native ruminant IFN-γ as a positive control/standard. Results are expressed concerning a standardized, freeze-dried positive reference control.

Serological Assay Procedure

The plasma samples and controls were added to the microwells coated with an anti-IFN-γ monoclonal antibody (Mab). The wells were washed and an anti- IFN-γ Mab–HRP conjugate was added, forming a Mab-antigen-Mab-HRP complex. After another wash to eliminate the excess conjugate, the substrate solution (TMB) was added. A blue solution appeared which becomes yellow after the addition of the stop solution. Twenty-five microliter (25µl) of dilution buffer 1 and 25 µl of the negative control was added to wells A1 and B1. Twenty-five (25 µl) of the Positive Control was added to wells C1 and D1 and 25 µl of each sample to be tested (activated and control samples) was added to the remaining wells. The plate was gently agitated for 2 min at 25°C and covered with a plastic sheet and incubated for 1 hour at 37°C. Each well was washed six (6) times with approximately 300 µl of the Wash Solution. A 1X conjugate solution

was prepared by diluting the Conjugate 10X to 1/10 in Dilution Buffer 1. One hundred microliters (100 µl) of the 1X Conjugate was added to each well. The plate was covered with a plastic foil and incubated for 1 hour at 37°C. Each well was washed six (6) times with 300 µl of the Wash Solution and 100 µl of the Substrate Solution was added to each well and incubated for 15 min at 21°C in the dark. Exactly 100 µl of the Stop Solution was added to each well to stop the reaction. Finally, the microplate was read using an ELISA reader at 450nm.

Result Interpretation

For each sample, the sample-to-positive ratio (S/P) was calculated, expressing the level of interferon production in the percentage of the Positive Control as follows:

$$S/P = \frac{\text{OD activated sample} - \text{OD control sample}}{OD_{PC} - OD_{NC}} \times 100$$

Samples with an S/P % less than 15 % were considered negative (no IFN-γ production induced by the antigen tested), while those with S/P % greater than or equal to 15 % were considered positive (specific IFN-γ production induced by the antigen tested).

Data Analysis

The results of the screening test and questionnaires were analyzed using Statistical Package for Social Scientist (SPSS) version 15. Prevalence of *M. bovis* infection in sheep and goats and awareness of butchers on tuberculosis was analyzed using descriptive statistics.

RESULTS

The prevalence of *M. bovis* infection in sheep was found to be 3.4% (1/29) at the Hadejia abattoir with an overall prevalence of 0.01% (1/100). There were no positive reactors in goats in the four abattoirs (Table 1). Prevalence of *M. bovis* based on sex showed 1.2% (1/86) positive reactor in males with no positive reactors in females. The only positive reactor was recorded among sheep who were in the age group 2 ½ - 3 years old (2.9%: 1/34) (Table 2).

Analysis of the questionnaires indicated that the butchers enrolled in the study had knowledge of tuberculosis as a disease of animal origin but a low level of knowledge on zoonotic potential and ways of transmission and prevention (Table 3).

Table 1: Prevalence of *M. bovis* infection in sheep in four abattoirs in Jigawa State

Abattoir Location	Sheep Tested	Number Positive	Prevalence (%)
Dutse	27	0	0
Gumel	23	0	0
Hadejia	29	1	3.4
Kazaure	21	0	0
Total	**100**	**1**	**0.01**

Table 2: Prevalence of *M. bovis* infection based on Age and Sex

Variable	Number Tested	Number Positive (%)	Prevalence (%)
Sex			
Male	86	1	1.2
Female	14	0	0
Age group			
6 mnts - 1year	12	0	0
1½ -2 years	23	0	0
2 ½ - 3 years	34	1	2.9
3½ - 4 years	31	0	0

Table 3: Butchers' knowledge and awareness of tuberculosis (Tb) in the four abattoirs

Variable	Yes (%)	No(%)
Knowledge of the zoonotic potential of Tb	30 (20)	120 (80)
Knowledge of Tb transmission	25 (16.7)	125 (80.3)
Knowledge of Tb prevention	35 (23.3)	115 (76.7)
Knowledge of Tb as a disease of animal origin	116 (77.3)	34 (22.7)

DISCUSSION

In this study, the prevalence of *M. bovis* infection in sheep was found to be 3.4% at the Hadejia abattoir and an overall prevalence of 0.01% in the four abattoirs using the ID vet sandwich ELISA. This prevalence is low when compared to the 4.3% prevalence previously reported in abattoirs using the postmortem technique (14) and the seropositive sheep can serve as a source of infection to humans and other animals most especially cattle in the area because of close contact and husbandry practice. The immunological assay has also demonstrated it is sensitive and specific and is capable of detecting positive cases of *M. bovis* infection in very early stages thus enhancing the accuracy of diagnosis (15).

Only one sheep was found to be positive for *M. bovis* infection in the Hadejia abattoir and no positive reactors were recorded in goats in all the four abattoirs. The sheep that was found positive at the Hadejia abattoir could be an asymptomatic carrier sheep harboring and coming to the abattoir with the infection from an unknown source and was not presenting any clinical signs at ante mortem inspection. It might also be an animal that co-mingled with infected cattle at communal grazing and water drinking points since it is common husbandry practiced by most pastoralist herders (13, 16). It could also be a sheep that had acquired the infection from the livestock market due to contact or airborne transmission (8, 13). Sheep have been reported to have more contact with cattle compared to goats thus putting them at risk (10, 12, 17). Goats are mainly kept by crop farmers (18, 19) and mostly by women in backyard farming and are not usually herded together with cattle which limits their

exposure to potentially infected cattle which have been reported to be the natural host of the bacterial pathogen (11, 14).

The only positive reactor was recorded in males in the study and could be attributed to the large number of males slaughtered in the abattoirs compared to females. It has been shown that, the larger the sample size, the more likely chances of getting a positive case (20). It has also been reported that female animals are mostly kept by farmers for reproductive purposes (2) and they hardly go for slaughter unless they are aging, or not reproducing (1). However, studies have shown that there is no difference in susceptibility to *M. bovis* infection for the sexes as both stands an equal chance of acquiring *M. bovis* infection (16). The only positive sheep from the screening were within the age group 2½ - 3 years old and this age has been identified as a risk factor for acquiring the infection (8). The older the animal gets the higher its chances of acquiring the infection due to reduced immunity (8).

Analyses of the questionnaires indicated that the butchers interviewed in the study lack the knowledge of the zoonotic potential of tuberculosis, and its various ways of transmission and prevention, but are aware that tuberculosis is a disease that can be acquired from animals. This finding could be due to their poor knowledge and understanding of the disease and also a lack of public education about the disease from various stakeholders involved in disease prevention and control. These findings are in agreement with previous reports (13, 19).

CONCLUSION

The study has demonstrated the presence of *M. bovis* infection in sheep at the Hadejia abattoir with no positive reactors among the goats selected in the study. These can serve as a potential source of transmission to humans and other small ruminants in the location. It has also indicated that butchers in the study areas have poor knowledge of *M. bovis* infection and the various ways of transmission and prevention. It is hereby recommended that small ruminants (sheep and goats) should be screened for *M. bovis* infection at the selected abattoirs and butchers should be educated on the risk of the zoonotic potential of Tb and its various ways of transmission and prevention. Further studies on the role of small ruminants in the epidemiology of tuberculosis in the study areas is also recommended

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CONFLICT OF INTEREST

The authors hereby declare that there is no conflict of interest

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