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Bacteriological Studies of Bovine Granulomatous Lesions in Cross River State Abattoirs

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ABSTRACT

Abattoir survey was conducted on cattle slaughtered in three major abattoirs (Ogoja, Ikom and Calabar) in Cross River State within a 12-month study period April, 2002 – March, 2003. Mycobacteriological study of bovine granulomatous lesions obtained from these abattoirs was carried out. The diagnostic tools used for this study are gross pathology, acid fast microscopy and culture. By systematic (1 in 2) random sampling, 60 out of 120 laboratory confirmed organ specimens were cultured. The results of growth in Lowestein Jensen (LJ) media and reaction to antimycobacterial agents are summarized. Of the 60 organ specimens cultured, 38 (63.3%) produced growth. Biochemical characterization revealed that 26 (68.4%) of the isolates were *Mycobacterium bovis*, 2 (5.3%) M. *tuberculosis* and 10 (26.3%) other uncertain species of acid-fast bacilli.

Key words: Tuberculosis, slaughter cattle. Cross River State, Nigeria

INTRODUCTION

Tuberculosis is a chronic slowly progressive wasting disease, which affects both man and animals and is caused by the tubercle bacilli, mycobacterium species. In cattle, man and birds the disease is caused by *Mycobacterium bovis*, *M. tuberculosis* and *M. avium,* respectively. In addition to these three, *M. leprae* is among the four most pathogenic species often referred to as tubercle bacilli (Ayanwale, 19880. Tuberculosis is characterized by progressive development of tubercle in any of the organs, in most species abscess formation with resultant caseation and calcification, cachexiation and high fatality (Blood and Radostits, 19890.

Examinations of samples for Mycobacterium started in Nigeria in the early 1940s. Lindley, 1949 at the Federal Veterinary Research Laboratory Vom, reported 107 of 221 samples for tuberculosis diagnosis from the Eastern Regions were positive on laboratory examination. Other tuberculosis diagnostic works were carried out in Birnin Kebbi, Kaduna and Maiduguri (North-West state and North-East State tuberculosis files, 1963 – 64). The method of diagnosis of Mycobacterium employed was basically Ziehl-Neelsen acid-fast staining method. Culture and laboratory animal inoculation suing guinea pigs were rarely performed.

Today, the use of laboratory animal inoculation, culture and biochemical characterization of the isolates are oftentimes used. In Sokoto a biochemical test conducted on 72 positive organ specimen yielded 50 (69%) *M. bovis*, 13 (18%) *M. tuberculosis* and 9 (13%) a typical *Mycobacterium* (Garba, 2002). In Ibadan, a survey conducted on food animals namely cattle, sheep, goats and pigs in two resident cattle herds and a major abattoir revealed that 18 (8.3%) out of the 216 animals examined were positive for tuberculosis (Cadmus, 2003). The concern for bovine granulomatous lesions in Cross River State is because of the increased consumption of meat in the state, due to increased livestock farming and reactivation of Obudu Cattle Ranch. These factors have increased the exposure risk to zoonotic infections.

This paper reports the characterization of *Mycobacterium* species isolated from organs specimens in slaughtered cattle from abattoirs in Cross River State of Nigeria.

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MATERIALS AND METHODS

Collection of tuberculosis specimens at the abattoirs

Identification of tuberculosis cases was based on presence of typical tubercular granulomatous and caeseated lesions. Specimens of suspected tuberculosis lungs, liver, intestine, spleen, and lymph-nodes namely mediastinal, bronchial, retropharyneal and mesenteric were collected from the three major abattoirs in, Ogoja, Ikom and Bakoko (Calabar) in the three senatorial zones of Cross River State. The specimens from the tuberculosis organs were refrigerated overnight and then carried to the Department of Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, where they were frozen and kept at 20^{o}C until processed. Specimens were collected at each zonal abattoir once weekly for 12 months (April, 2002 - March, 2003).

Processing of specimens for staining

In the laboratory, the organs were processed according to the method described by Mycobateriology Unit, Veterinary Services Laboratories, United States Department of Agricultural, Ames, Lowa 1984.

Each tissue was decontaminated in 1:1000 sodium hypohloride for 4 hours. Fat was trimmed off and the tissue ground in sterile mortar two to three drops of phenol red was added as indicator. The tissue was then treated with equal volume of 2% NaOH for ten minutes and neutralized with HCl. The tissue was then centrifuged and the supernatant decanted.

Staining

Two smears of the homogenates of each specimen were made and stained by Ziehl-Neelsen (Z-N) method as described by Elmer (1992). The slides were examined meticulously under oil immersion objective lens for the presence of typical acid-fast bacilli with characteristic cellular morphology. These samples that were negative were subjected to concentration technique after which those still testing negative were discarded.

Culture

Portions of the samples that were decontaminated and concentrated were inoculated into 5 ml slant preparations of l-J medium in Bijou bottles. One bijou slant contained the medium with glycerol, the other contained pyruvate. A duplicate of each type was made inoculated. The inoculation was done using sterile pasture pipette to put two drops of the sample into each medium in duplicate (Kolo, 1991). The slants were incubated at 37^{o}C. Carbon dioxide was introduced into the incubator using the candle flame in order to stimulate the growth of Mycabacteria. The tubes were kept in the incubator in a horizontal position to maintain the proper spread of the inoculate at a temperature of 37^{o}C and observed for growth at 3 day interval for the first one week and then weekly for another 7 weeks.

Biochemical characterization of isolates

For the purpose of the work, three biochemical tests namely pigmentation, niacin production and nitrate reduction tests were carried out for speciation of the isolates. Growth rate of cultures in Lowestein-Jensen media was also recorded.

Pigmentation

Test for pigmentation was used as described by Kubica (1963). Primary isolates were sub-cultured on three tubes of L-J medium. Two tubes were wrapped with aluminium foil and the third left unwrapped control. Both wrapped and unwrapped were incubated for 2-3 weeks at 37^{o}C. When growth was indicated in the wrapped control tube, one of the wrapped tubes was then exposed for three to five hours to strong light using 100 watts bulb with the cap of the tubes loose. The three tubes were then re-incubated and all 3 tubes were examined at 24,48 and 72 hours for pigmentation. After the three days observations, the isolates were interpreted as follows:

I. These with yellow pigments both in the wrapped and exposed tubes are classified as scotochromogens.
II. Those that showed yellow to orange pigments only in those exposed tubes are classified as photochromogens.
III. Those that produced no pigments either in the dark or after exposure period of 72 hours are the achromogens.

Niacin production test

The detection of niacin was done according to the method, employed by Lennette *et al.* (1985). One milliliter (ml) of distilled water as aseptically added to a three week old culture slant of Lowestein-Jensen medium. The medium was kept for 15 minutes in a horizontal position for niacin extraction. Half of a milliliter of the fluid (extract) was added to a clean screw-capped test-tube containing half milliliter each of 4% aniline and 10% cyanogens bromide. Yellow colouration was interpreted as positive for niacin production.

Nitrate reduction test

Two to three drops of sterile distilled water was placed in a screw-capped tube. A loop of the mycobacterium colony was emulsified in the water and 2ml of sodium nitrate solution added. The tube was shaken and incubated at 37°C for 2 hours. One drop of 50% hydrocarbonic acid, two drops of 0.2% sulfanilamide and two drops of 0.15 N-naphthyethylenediamine dihydrochloride were respectively added to the culture medium. The tube was then examined immediately for a pink to red colour, which was interpreted as positive for nitrate reduction (Kolo, 1991). Tubes containing sodium nitrate that were not incubated with the organism were used as control. Also whether the reduction had progressed beyond nitrate to nitrogen gas and water. The presence of nitrate was detected by development of a red colour which indicated a negative test i.e nitrate was not reduced. On the other hand, lack of colour change after the addition of zinc dust indicate a false negative nitrate reduction test (Lampe, 1981).

RESULTS

Of the 60 organ specimens culture, 38 (63.3%) produced growth. Biochemical characterization revealed that 26 (68.4%) of the isolates were *Mycobcterium bovis* 2 (5.3%) *Mycobacterium tuberculosis* and 10 (26.3%) uncertain species of acid fast bacilli. *Mycobacterium bovis* growth was slower, colonies began to appear after 6-7 weeks with no pigmentation. They were negative to both niacin production and nitrate reduction tests. *M. tuberculosis* growth was also slow, colonies began to appear 6-7 weeks with pigmentation. Unlike M. bovis they were positive to both niacin production and nitrate reduction tests. Other acid-fast bacilli showed rapid growth (1-3 weeks), produced pigmentation and were negative to both niacin production and nitrate reduction test.

Table 1. Summary of isolation and biochemical identification of *Mycobacteria* from bovine granulomatus organs.

Number of Isolates	Growth	Pigment production	Niacin production	Nitrate reduction test	Identification
26	S	-	-	-	*M. bovis*
02	S	+	+	+	*M. tuberculosis*
10	R	+	-	-	Other acid-fast bacilli

S = slow; R = rapid

Table 2. Bacteriological identification of *Mycobacteria* species from bovine granulomatous organs

Specimen sources	No. of specimens selected	No. of specimens yielding isolates	*M. bovis*	*M. tuberculosis*	Other acid-fast bacilli
Zone A (Ogoja)	30	20	13	02	05
Zone B (Ikom)	20	15	11	00	03
Zone C (Bakoko Calabar)	10	03	02	00	02
Total	60	38(63.3%)	26(68.4%)	2(5.3%)	10(26.3%)

DISCUSSION

The study has shown that Mycobacterium bovis is the major causative organism causing tuberculosis in slaughtered cattle examined in Cross River State of Nigeria. However, other tubercle bacilli such as *M. tuberculosis* and other uncertain acid-fat bacilli were also involved in bovine tuberculosis.

Gross appearance of tuberculosis, nocardiosis and mycobacteriosis lesions are similar, and often, meat inspectors do lump then together as Tuberculosis and carcasses are disposed of (Alhaji, 1976). The public health problems posed by the occurrence of nocardiosis and mycobacteriosis in cattle are difficult to assess. This is because the causative agents are opportunistic saprophytes whose epidemiology is poorly understood. The presence of acid-fast rods alone is not diagnostic of Tuberculosis because Nocardia and other Runyon group of organisms

are also acid-fast bacilli and mixed infections could occur between *M. bovis* and *N. asteriodes* (Alhaji, 1976). Therefore, there is need for both acid-fast staining techniques and biochemical tests to be used in the confirmatory diagnosis of tuberculosis.

The involvement of species of tuberculosis other than *M. bovis* in cattle infection has been reported world wide and also the involvement of *M. bovis* in human tuberculosis has also been reported (Idigbe et al., 1986, Kolo, 1991 and Garba, 2002).

Zoonotic bovine tuberculosis caused by *M. bovis* and *M. tuberculosis* could be present in the study area. This therefore calls for a comprehensive field study of tuberculosis in the state so that effective planning of the disease control programme could be made and to highlight the mechanism of transmission between man and animals. Efforts should be made by the veterinarians in the state for disease surveillance and meticulous meat inspection to safe guard public health by preventing affected animals and carcasses from being sold to the public for human consumption.

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