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EVALUATION OF THE 48 HOUR, 72 HOUR AND 96 HOUR READINGS OF TUBERCULIN TEST FOR THE SCREENING OF TUBERCULOSIS IN CATTLE

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In this study, a cattle farm with a history of tuberculosis was examined over a period of three years to determine the usefulness of reading tuberculin tests (single intradermal cervical tuberculin test (SICTT) and single intradermal comparative cervical tuberculin test (SICCTT)) at 48 hrs, 72 hrs and 96 hrs intervals in the diagnosis of tuberculosis. On the onset, SICTT was conducted on a total of 145 cattle, 52 (35.9%) of these were positive at 48 hours, 56 (38.6%) at 72 hours and 65 (44.8%) at 96 hours (X^2 = 1.54, p = 0.46). After one year, 171 cattle were screened using SICCTT, 10 (5.8%) animals were positive at 48 hours, 12 (7.0%) at 72 hours and 14 (8.2%) at 96 hours (X^2 = 0.67, p = 0.72). During the third test conducted almost one year after the second test, 136 cattle were screened using SICCTT, 13 (9.6%) were positive at 48 hours, 17 (12.5%) at 72 hours and 17 (12.5%) at 96 hours (X^2 = 0.68, p = 0.71). With the pattern of this result, there may be need to review the policy which gave the 72 hr reading a preference over the 96 hr reading of tuberculin test.

INTRODUCTION

According to Francis *et al.*, (1) various forms of the tuberculin test provided the essential means for the diagnosis and control of tuberculosis but these had been modified over time. The tuberculin test in cattle has proven to be the most widely accepted *in vivo* diagnostic test when compared to other sero-diagnostic tests that have been employed in the diagnosis of bovine tuberculosis.

Serological tests for screening tuberculosis are ELISA technique, complement fixation, fluorescent antibody, direct bacterial agglutination, precipitin and haemagglutination tests (2); but these have little potential value for the routine diagnosis of tuberculosis. However, a diagnostic technique exploring the *in vitro* assay of cell mediated reactivity known as the interferon-gamma assay has been developed (2).

The more recent adaptation of the IFN-gamma assay is ELISPOT assay (enzyme linked immunospot) using synthetic peptides (ESAT-6 and CFP-10), which have been reported by Vordermeier *et al* (3) to be promising in the detection of *M. bovis* in cattle. Despite these elegant detection procedures for tuberculosis, the single intradermal test using the purified protein derivative of *Mycobacterium tuberculosis* (PPD) is still the routine screening tool for detecting carriers of tuberculosis (3).

PPD tuberculin is universally used in medical practice and has been the official tuberculin for testing cattle in Britain, Europe, South Africa and some of the developing countries including Nigeria (4, 5, 6). Reactivity to tuberculin made from either human or bovine bacilli (the mammalian tuberculins) is similar and usually greatest in sensitized hosts.

However, sensitization to *M. avium, M. paratuberculosis* and many tuberculoid bacilli may produce a state of greater sensitivity to tuberculin made from *M avium* (avian tuberculin) than to the mammalian tuberculins. This difference according to Francis *et al.*, (1) provided the basis for the comparative tuberculin test, which has been used universally.

The accuracy of this tuberculin test lies greatly on the time when readings are done post-inoculation of the PPD. Several authors, (1, 5, 6, 7) supported reading on the 72hours post-inoculation; while others (4), suggested taking the average of readings at 72hours and 96hours post inoculation. Radostits *et al,* (2) however supported readings between 48 hours and 96hours after injection with a preference for 48-72hours for maximum sensitivity and at 96hours for maximum specificity.

This study evaluated the 48hour, 72hour and 96hour readings of tuberculin test in making accurate diagnosis of tuberculosis. The results of tuberculin tests were also compared with other diagnostic tools of tuberculosis.

MATERIALS AND METHOD

This work was conducted in a resident cattle herd in Southwestern Nigeria over a period of three years. Before this study was carried out, postmortem findings revealed five cases of tuberculosis. All the cattle were screened using either the single intradermal tuberculin test (SICTT) or the single intradermal comparative cervical tuberculin test (SICCTT) with bovine purified protein derivative (B-PPD) and avian purified protein derivative (A-PPD) obtained from the Central Veterinary Laboratory Weybridge, UK. Measurements of skin thickness were taken before and after the inoculation as described by OIE (6).

Further confirmation of the disease was based on clinical symptoms, gross pathology lesions, histopathology, Ziehl Neelsen staining technique (acid fast microscopy), culture and microscopy using the Lowenstein-Jensen medium (5, 6).

1st year: This involved the screening of 145 cattle using SICTT followed by carrying out postmortem on two cattle that were positive following this test.

2nd year: This test was conducted nine months after test 1 was completed and 171cattle were screened using the SICCT. This was followed by postmortem examination of 11 of the positive animals. 3rd year: This was carried out one year after test 2. In all, 136 cattle remaining in the herd were screened using SICCTT.

RESULTS

Figure 1 shows the results of this study as explained below;

1st year: Altogether, 85 (58.6%) of the cattle were classified as reactors when individual hours are taken into consideration without counting the same animal twice between 48 to 96 hours. Fifty-two (35.9%) animals were positive at 48hours, 56(38.6%) at 72hours and 65(44.8%) at 96hours (X^2 =1.54, p=0.46). Of the two animals culled, postmortem findings in one of them showed no visible lesion in all the organs and lymph nodes, while the other animal revealed generalized tuberculosis, which affected all the lymph nodes, major organs and the reproductive tract.

For 2nd year, 18 (10.5%) animals were considered to be positive between 48 to 96hours following the criterion used in 1st year above. On individual hours, 10 (5.8%) animals were positive at 48hours, 12 (7.0%) at 72hours and 14 (8.2%) at 96hours using the total of 171 animals as the common denominator (X^2=0.67, p=0.72). Eleven animals were culled for postmortem examination with the revelation of varying degrees of tuberculosis lesions. The organs affected included the lungs, kidney, spleen, heart, liver, lymph nodes (i.e. retropharyngeal, mandibular, bronchial, mesenteric, mediastinal, and hepatic).

Histopathology revealed varying

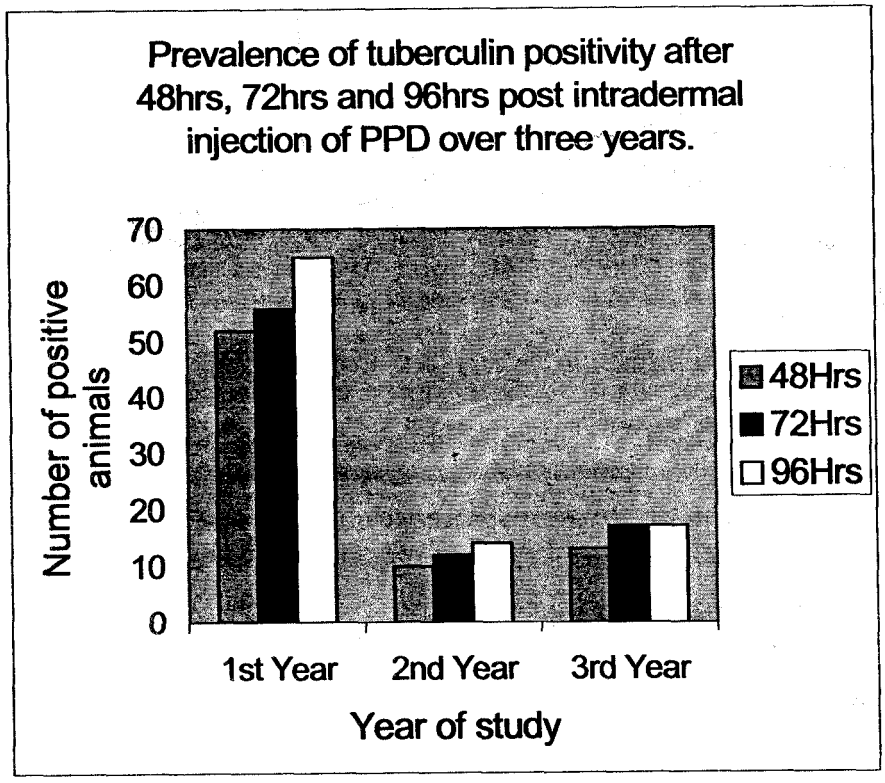
degrees of caseating granuloma, extensive areas of necrosis with parenchyma fibrosis and some areas of calcification. All the samples were acid fast positive. Only one of the 20 pairs of L-J media had growth on both L-J with glycerol and L-J with pyruvate indicating that there was a mixed infection in one of the animals. The other 19 pairs of LJ had growth only on the LJ with pyruvate. The confirmation of these positive growths was revealed by the numerous pink staining acid-fast bacilli on staining each culture smear with Ziehl Neelsen stain.

3rd year: Out of the 136 animals that were screened, 20 (14.7%) animals tested positive between 48 hours to 96hours following the criterion used in 1st year. 13 (9.6%) animals were positive at 48hours, 17 (12.5%) by 72hours and 17 (12.5%) at 96 hours ($X^2 = 0.68$, p=0.71). All the five animals examined for gross-pathology had gross lesions of TB with a very young calf of about six months also showing gross pathological lesions on the liver.

DISCUSSION

It was pointed out that paratuberculosis, an avian-tuberculosis, and nocardiosis gives positive

Figure 1:



reaction to SICTT (2, 8), therefore SICCTT, which is a more confirmatory test in confirming bovine-TB is recommended. This assertion is corroborated by Ayanwale (8). This explains why both SICTT and SICCTT were used in this study. Our finding shows that more animals were positive during the 96-hour readings than at the 48hours or 72 hours readings. This could be explained from the immunological point of view that at 96-hour, all the cells responsible for cell mediated immunity would have

been fully activated; hence, more reactors at this hour than at the 48 or 72-hour when fewer cells would be immunologically active. This is in line with the findings of Radostits *et al.*, (2) who reported that at 96 hour, maximum specificity is achieved for tuberculin test; while at 48-72 hour maximum sensitivity is obtained.

Histological study of this work also revealed that positive reactors were more in proportion in 72 hours and 96 hours post inoculation. This assertion is partially supported by Ayanwale (4) when he suggested the use of average reading at 72 hours and the 96 hours post inoculation for the diagnosis of TB.

CONCLUSION

The results obtained over the three years were consistent with the fact that the 96hour readings produced more positive reactors than the 48 and 72-hour readings; suggesting that more tuberculous animals would have been identified and destroyed in order to reduce the spread of TB within the herd.

This investigation strongly supports readings tuberculin test at 96-hour post inoculation, and therefore calls for the review of preference for 72 hour readings of tuberculin tests.

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