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Correlation Between Microscopic Examination and Culture for Detection and Differentiation of Mycobacterial Isolates from Cattle in the Sudan

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Abstract: One hundred and sixty seven caseated lymph nodes and tuberculous lungs were collected from cattle slaughtered in various locations in Khartoum State (Sudan) and examined bacteriologically. Microscopic examination using Zeilh-Neelsen stain and culture on Lowenstein-Jensen (L-J) medium were carried out to detect and differentiate between the mycobacterial species in the specimens. Thirty-five, 35 (20.96%) samples were found to harbor acid-fast bacteria when examined microscopically. Out of the 35 acid-fast bacteria, 22 (62.86%) showed branching filaments and were identified as *Mycobacterium farcinogenes*. The remaining 13 (37.14%) were bacilli and identified as *Mycobacterium bovis*. The 35 specimens that proved to harbor acid fast bacteria were cultured on L-J medium. None of the 22 specimens with branching filaments (*Mycobacterium farcinogenes*) grew on L-J medium when incubated aerobically at 37°C between four and eight weeks. 12 (92.31%) of the 13 bacilli (*Mycobacterium bovis*) showed visible growth using the above growth conditions. In conclusion, microscopic examination can only detect acid fast organisms in the clinical samples whereas culture on L-J medium can differentiate between acid-fast mycobacterial species.

Key words: Mycobacteria, microscopy, culture

INTRODUCTION

Mycobacterial organisms are responsible for chronic infectious diseases of man and animals. These diseases are characterized by the progressive development of tubercles in many organs in most species[1]. Tuberculosis in human caused by *Mycobacterium tuberculosis* complex, an actinomycetes those are aerobic, non-motile, non-spore forming bacteria and characteristically acid fast[2]. *Mycobacterium bovis*, the cause of tuberculosis in cattle, is also pathogenic for a number of other animal species and its transmission to humans constitutes a public health problem[3,4].

Identification of the mycobacteria routinely based on methods such as the Ziehl-Neelsen stain, pigmentation, growth rate and gross and microscopic colonial morphology of cultures of the isolated organisms. Biochemical methods such as tests for niacin, catalase, nitrate reduction and urease are also used to identify different mycobacterial species[4]. The objective of this study was to correlate between microscopic examination and culture to detect and differentiate among mycobacterial organisms from bovine origin.

MATERIALS AND METHODS

Samples processing: One hundred and seventy six caseated lymph nodes and tuberculous lungs were collected from cattle slaughtered at some slaughterhouses in Khartoum State (Sudan). With each sample, 5 mL of 2% NaOH was mixed and incubated at room temperature for 20 min. The mixture was centrifuged at 4.000 rpm for 15 min and the sediment was re-suspended in 5 mL sterile distilled water and centrifuged again as above. Then the sediment was used for microscopy and culture.

Microscopic examination: All the above-prepared samples were examined microscopically by Ziehl-Neelsen staining essentially as described by Cowan and Steel[5].

Culture of samples: Only specimens that proved to harbor acid-fast bacilli when examined microscopically were cultured using Lowenstein-Jensen medium. Primarily, two slants of L-J medium (one with Na-puruvate and the other without Na-pyruvate) were prepared and inoculated with the previous materials and incubated aerobically at 37°C for 12 weeks. The slants were examined daily for

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growth. Subculturing was performed when growth appears. The growing organism was identified as slow or rapid growing acid fast if colonies seen before or after 7 days, respectively according to Nolte and Mitchok[6] recommendations.

RESULTS AND DISCUSSION

Microscopic examination of the smears made from prepared samples stained by Ziehl-Neelsen, revealed acid-fast organism in 35 (20.96%) of the 167 samples examined (Table 1). 22 (62.86%) samples showed branching filaments and were identified as *Mycobacterium farcinogenes*. 13 (37.14%) were bacilli and identified as *Mycobacterium bovis*. Only twelve (7.19%) out of the 35 samples examined showed visible colonies on Lowenstein-Jensen (LJ) medium incubated aerobically at 37°C for up to 12 weeks.

Tuberculosis occurs in many countries of the world affecting many species including man with major importance in dairy cattle. This is of particular importance for public health and animal production concern. Definite and rapid detection tools for the disease agent are therefore important. Present finding in this study confirmed the fact that Zeilh Neelsen stain can only differentiate between bacilli and filamentous acid-fast organisms. This substantiates previous reports of Quinn *et al.*[7] who affirmed the limited potential of the test to differentiate between the various members of the family mycobacteriaceae. In the present study, it was also noted that 12 (92.30%) out of 13 specimens, which showed acid-fast bacilli in smears were isolated using Lowenstein-Jensen (L-J) medium. This proved that decontamination method employed using 2% sodium hydroxide was effective in suppressing contaminants in the specimens, which confirm findings of Tageldin[8], Roberts *et al.*[9], Nolte and Mitchock[6]. However, Sulieman[10] confirmed that although this method was effective in suppressing contaminants, it might decrease cell viability and inhibit cell growth. In previous reports, L-J medium with pyruvate is regarded to be the most reliable medium for the isolation of the bovine type of mycobacterium[11] where the growth of *M. bovis* is enhanced by pyruvate. However, in the present study, L-J medium with and without pyruvate was used and was found to be equally reliable and hence concluded pyruvate is not a critical factor for the growth of the organism.

Atypical mycobacteria observed in 2 (0.1%) of the caseous lesion tested is of great interest to strengthen our believe that *M. bovis* is not the only pathogen responsible for bovine tuberculosis. This believe came to our mind when considering the typical gross lesions in the specimens, microscopical and cultural results of these 2 isolates. Similar conclusion had drawn previously by Sulieman and Hamid[12] who reported the isolation of some Nocardial species from caseated lymph node as well as Mycobacterium Other than Tuberculosis (MOTT). Corner[13] and Jorgensen[14] reported that certain atypical mycobacteria can produce in cattle localized lesions which macroscopically and histologically resemble lesions caused by *M. bovis*. Infection with these mycobacteria was confirmed to be originated from the environment and neither lateral nor vertical transmission has been recorded[15].

Table 1: Correlation between microscopic examination and culture of treated suspected samples

Specimen	Number Examined (%)	Microscopic examination: No. of branching Filaments (%)	Microscopic examination: No. of rod bacilli (%)	Culture on L-J medium
Tuberculous lung	5 (2.99)	-	-	-
Reteropharyngeal LN	23 (13.77)	3 (1.80)	3 (1.80)	3 (1.80)
Prescapular LN	27 (16.17)	3 (1.80)	-	-
Submandibular LN	40 (23.95)	1 (0.60)	-	-
Inguinal LN	10 (5.99)	-	-	-
Mediastinal LN	36 (21.56)	1 (0.60)	7 (4.19)	6 (3.59)
Unspecified LN	26 (15.57)	14 (8.38)	3 (1.80)	3 (1.80)

* All branching filamentous acid-fast bacteria fail to grow on L-J medium and identified tentatively as *Mycobacterium farcinogenes*. All rod bacilli acid-fast bacteria have grown on L.J medium and identified tentatively as *Mycobacterium bovis*. LN= Lymph node

In conclusion, it is not possible to differentiate between the different species of mycobacteria when employing microscopic examination for suspected specimens but culture in L-J medium will enhance the growth of acid fast rod bacilli. From the present findings and in view of the previous studies, we can also be able to conclude that the distribution of lesions indicated that lymphatic spread is the most common mean of dissemination of tuberculosis infection.

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