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Full Length Research Paper

BACTEC MGIT 960 ™ system for screening of *Mycobacterium tuberculosis* complex among cattle

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Bovine tuberculosis remains a disease of economic and public health importance in developing countries. The largest number of new cases of tuberculosis usually occurs in South-East Asia region and Africa. This study was aimed to evaluate the recent technique (BACTEC MGIT 960 ™ system) for screening of *Mycobacterium tuberculosis* complex among cattle in Egypt. From the 1180 cattle examined in three different Governorates (El-Sharkia, El-Gharbia and El-Monefeia) by single intradermal tuberculin test, 29 animals (2.46%) were positive reactors. The post mortem examination of the positive reactors showed that 22 animals (75.9%) had visible lesions [respiratory form (31.0%), digestive form (13.8%), mixed form (20.7%) and generalized form (10.3%)], while seven (24.1%) did not show visible lesions. The results of isolation and identification using conventional culture method (Lowenstein-Jensen medium) were 22 mycobacterial isolates (75.9%), 20 (68.97%) *Mycobacterium bovis* and 2 (6.9%) unidentified slow growth. The BACTEC MGIT 960 ™ system was used for recovery of *Mycobacteria* and compared with conventional culture method (Lowenstein-Jensen medium). The recovery rate of BACTEC MGIT 960 ™ system was 82.8%, while that of Lowenstein-Jensen medium was 75.9%. The mean time for detection of *Mycobacteria* was 17.8 ± 0.9 days and 46.5 ± 0.4 days for BACTEC MGIT 960 ™ system and Lowenstein-Jensen medium, respectively while the contamination rate with BACTEC MGIT 960 ™ system was 6.9% and 10.3% in Lowenstein-Jensen medium.

Key words: Bovine tuberculosis, tuberculin test, Lowenstein-Jensen medium, BACTEC system.

INTRODUCTION

Bovine tuberculosis (TB) remains a disease of economic and public health importance even in developed countries. The cause of bovine tuberculosis in cattle mainly is *Mycobacterium bovis* (*Mycobacterium tuberculosis* complex), which is also pathogenic for a large number of other animals, and its transmission to human constitutes a public health problems (Ameni et al*.,* 2007). The economic losses due to the disease are represented by reduction of 10 to 20% in milk production and weight in cattle, in addition to infertility and condemnation of meat. Without considering the death, there is 10 to 25% loss of the productive efficiency of cattle (Lilenbaum et al., 1999).

The diagnosis of bovine tuberculosis in live animals mainly depends on clinical manifestations of the disease, skin testing, staining with Ziehl – Neelsen (Z.N.) stain and more recently by molecular methods. Subsequent identification of the pathogen has been made by culturing and biochemical tests (Mishra et al*.,* 2005). The Ziehl-Neelsen stain is a very rapid method, but lacks specificity and cannot be used to distinguish between the various members of the family *Mycobacteriaceae*, while culturing

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usually requires four to eight weeks to obtain good growth (Vitale et al., 1998). Although tuberculin skin testing has been a hallmark of bovine tuberculosis eradication campaigns and has been effective in reducing the prevalence of bovine tuberculosis in most developed countries, problems do exist with such tests as it lacks sufficient sensitivity and specificity (Palmer et al., 2006).

The use of new techniques for the isolation and identification of *Mycobacteria* has substantially reduced the time required by the clinical laboratory to report the results, with the advantage of improved sensitivity. This achievement is mainly due to the introduction and routine use of non-radiometric techniques as BACTEC MGIT 960 ™ system, which is a fully automatic non- radiometric system based on the early detection of positive cultures in broth media (Abe et al., 1999).

Rodriguez et al. (2009) evaluated the performance of an automated BACTEC MGIT 960 ™ system, a non radioactive, non-invasive liquid culture system for cultivation of *M. tuberculosis* complex and compared it with conventional Lowenstein-Jensen (L.J.) medium. They standardized p- nitro benzoic acid (PNBA) assay on BACTEC MGIT 960 ™ system for identification of *M. tuberculosis* complex and evaluated the usefulness by comparing the results with an in-house molecular assay and sequencing. They concluded that the BACTEC MGIT 960 ™ system with PNBA for identification of *M. tuberculosis* complex is a rapid and useful method in laboratories processing a large number of specimens. The objective of this study was to therefore isolate and detect *Mycobacteria* using conventional culture method (L.J.) and comparing it with BACTEC MGIT 960 ™ system.

MATERIALS AND METHODS

A total of 1180 cows were examined in three different governorates (El-Sharkia, El-Gharbia and El – Monefeia) by the single intradermal (SID) cervical tuberculin skin test. The positive reactor animals (29 cows) were slaughtered and examined for post mortem findings.

Tissue samples

The internal organs (livers, spleens, lungs) and lymph nodes showing tuberculous-like lesions were subjected to bacteriological examination for isolation and identification of acid fast bacilli. Samples were stored in an ice box and sent as quickly as possible to the laboratory where they were processed for isolation of the organism.

Processing of samples for isolation of organism

Conventional culture method [Lowenstein-Jensen medium (L.J.)] (Marks, 1972)

Organs, lymph nodes and/or tissues showing gross lesions were cut into small pieces, ground with fine sterile sand, and mixed with 2 ml sterile distilled water in a mortar. 2 ml of 4% H_2SO_4 were added to the sample then incubated for ½ h at 37°C. The mixture was diluted with 16 ml sterile distilled water and centrifuged at 3000 rpm/20 min. The obtained sediment was re-suspended in 0.5 ml sterile distilled water, then inoculated onto Lowenstein-Jensen slants and incubated at 37°C in inclined position overnight and then vertically for at least six to eight weeks with weekly examination starting from three days post inoculation.

BACTEC MGIT 960™ system (Hines et al., 2006)

Only 15 ml of BACTEC MGIT growth supplement were reconstituted and lyophilized vial BBL MGIT PANTA antibiotic mixture. MGIT tubes were labeled with the specimen number and only 0.8 ml of the growth supplement / MGIT PANTA mixture were added to the MGIT 960™ liquid culture tubes to reduce the growth of contaminant bacteria and supplement the growth of bacteria. After then, 0.5 ml of processed specimens was added to MGIT 960™ liquid culture tubes. All MGIT 960 tubes were incubated at 37°C into BACTEC MGIT 960™ instrument for six weeks. This instrument automatically monitored the fluorescence that was emitted within each tube every hour. MGIT 960 culture tubes were interpreted as positive if the instrument signaled positive within six weeks and smear was made to detect acid fast bacilli by the Ziehl – Neelsen's method. However, MGIT 960 culture tubes were interpreted as negative if the instrument did not signal positive by the end of the sixth week of incubation.

RESULTS

Isolation of *Mycobacteria* from tuberculin positive cows by BACTEC MGIT 960 ™ system and conventional culture method

The results in Table 1 and Figure 1 show that out of 29 processed tissue samples from tuberculin positive cows, 24 (82.8%) were recovered by BACTEC MGIT 960 ™ system and 22 (75.9%) by conventional culture method (L.J.). It was also observed that 19 (86.4%) mycobacterial isolates were recovered from 22 cows showing visible lesions, while 5 (71.4%) of *Mycobacteria* were isolated from seven cows without any visible lesions (NVL) by using BACTEC MGIT 960 ™ system. However, when conventional culture method was used, 18 (81.8%) were isolated from 22 cows showing visible lesions and 4 (57.1%) *Mycobacteria* were isolated from seven cows without any visible lesions.

The mean time for detection of mycobacteria using the BACTEC MGIT 960 ™ system and conventional culture method (L.J.)

Table 2 shows the mean time to detection of mycobacterial isolates for the BACTEC MGIT 960 ™ system and conventional culture method. For the BACTEC MGIT 960 ™ system, the recovery of the organism required only 17.8 days (S.E. 0.9), while the conventional culture method required 46.5 days (S.E. 0.4).

Table 1. Results of the recovery rates of *Mycobacteria* from slaughtered tuberculin positive cows by BACTEC MGIT 960 ™ system and conventional culture method.

Type of lesion (number)	BACTEC MGIT 960 system		Conventional culture system (L.J.)	
	N	*%	N	%*
1. Visible lesions (22)	19	86.4	18	81.8
A. Localized:				
(i) Respiratory (pulmonary L.N + lung tissue) (9)	8	36.4	8	36.4
(ii) Digestive (liver + mesenteric L.N) (4)	3	13.6	3	13.6
(iii) Mixed (liver + lung tissue + L.N) (6)	5	22.7	4	18.2
B. Generalized (3)	3	13.6	3	13.6
2. Non visible lesions (7)	5	71.4	4	57.1
Total (29)	24	82.8	22	75.9

*% Calculated according to the type of lesions; L.N, lymph node.

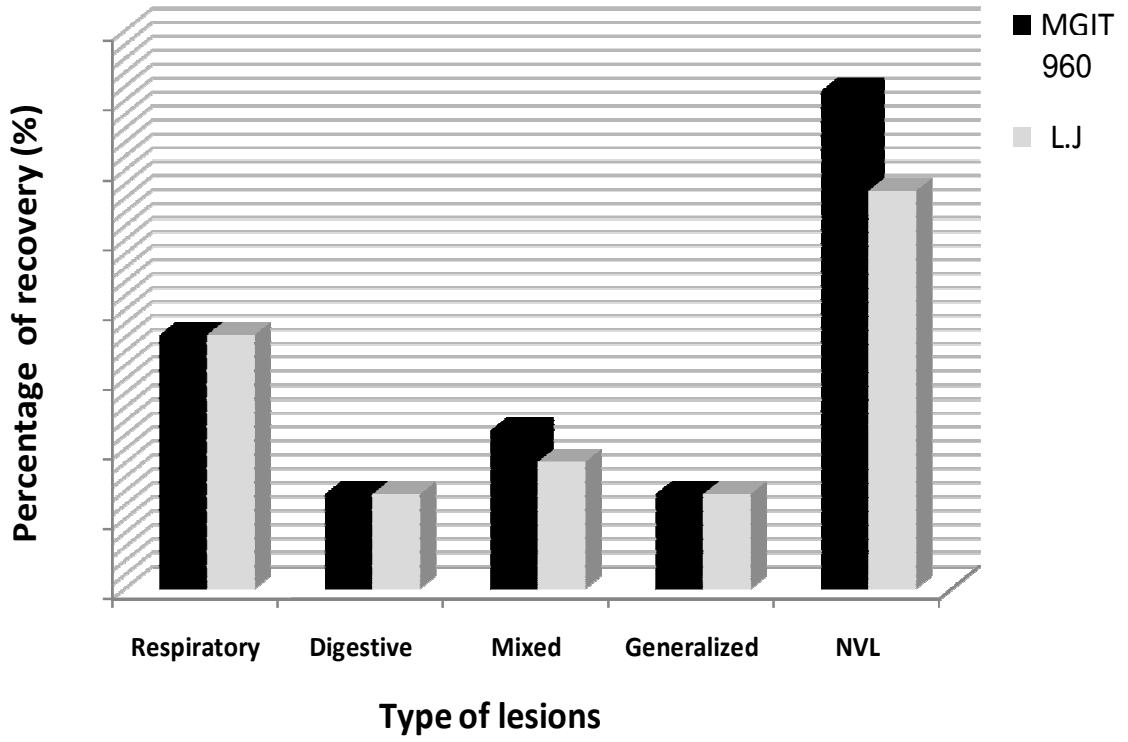

Figure 1. Comparison of the recovery rates of mycobacteria from slaughtered tuberculin positive cows by BACTEC MGIT 960 TM system and conventional culture method.

Table 2. Comparison between the mean time for detection of *Mycobacteria* using the BACTEC MGIT 960 ™ system and conventional culture method.

Culture system	Days of detection
BACTEC MGIT 960 ™	17.8 ± 0.9
Conventional culture method (L.J.)	46.5 ± 0.4

±, Standard error (S. E.)

Table 3. Comparison between the contamination rates of the BACTEC MGIT 960 ™ and conventional culture method.

Media system	Number of contamination / total number of sample	Percentage of contamination (%)
BACTEC MGIT 960 ™	2/29	6.9
Conventional culture method (L.J.)	3/29	10.3

The contamination rate of the BACTEC MGIT 960 ™ system and conventional culture method

The contamination rate of the each method is displayed in Table 3. The contamination rate of the MGIT 960 ™ system was 6.9%, while that of the conventional culture method was 10.3%.

DISCUSSION

The BACTEC MGIT 960™ system is a fully automated, high capacity and non-invasive instrument, which requires neither needles nor other sharp instruments (Rishi et al., 2007). This study was carried out to compare one such automated system (BACTEC MGIT 960™ system) with conventional culture method (L.J.) for the isolation of *Mycobacteria*. It was clear from Table 1 and Figure 1 that out of the 29 processed samples from tuberculin positive cows, 24 (82.8%) were positive culture for *Mycobacteria* by BACTEC MGIT 960™ system and 22 (75.9%) by conventional culture method (L.J.).

The results of this study therefore demonstrated that the number of *Mycobacteria* recovered in BACTEC MGIT 960™ system was greater than those recovered using conventional culture method (L.J.). The obtained results are in agreement with that reported by Hanna et al. (1999), which showed that the BACTEC MGIT 960™ system had recovery rate greater than those in BACTEC 460 TB ™ system and with solid media. Tortoli et al. (1999) showed that the overall rates of recovery obtained with the BACTEC MGIT 960™ and BACTEC 460 TB ™ systems were clearly higher than those achieved with solid media. Moreover, Dongsi and Dunnc (2002) found that the BACTEC MGIT 960™ system consistently provided better recovery of all *Mycobacterium* species from a variety of clinical specimens than did traditional L.J. slants. In addition, Hines et al. (2006) showed that the BACTEC MGIT 960™ system had higher recovery rate of *M. bovis* (122/129) than did the solid media system (96/129), and finally Rishi et al.(2007) found that the BACTEC MGIT 960™ system was the most rapid and efficient system to isolate *Mycobacteria*. However, for maximum recovery of mycobacteria, it is important to use both types of media.

The higher recovery rate of the BACTEC MGIT 960™ system than those of L.J. medium may be due to the fact that the BACTEC MGIT 960™ system is a liquid medium system, hence more isolates were recovered than with the solid medium. Bacteria are able to grow and spread more easily through liquid media than through solid media. Solid media have low recovery rates because the bacteria can use the nutrients only in the vicinity of the colony. The difference in the nutrients contained in the solid media tubes and liquid culture media may be more conducive to recovery of *M. bovis*. It is also possible that recovery was reduced in the solid media system because smaller amount of samples were inoculated on the solid media versus the liquid media (Hines et al*.,* 2006). In addition, the low positivity rate shown by conventional culture method (L.J.) in these studies could be because of the fact that sample slants were grossly contaminated and considered negative, whereas in BACTEC MGIT 960™ system, since the smear were made from all instrument positive MGIT 960 tubes, it was found that there were samples which had both contamination, as well as mycobacterial growth in them. Such tubes were considered positive by BACTEC MGIT 960™ system (Rishi et al., 2007).

Beside higher isolation rate, even the time to detect *Mycobacteria* was shorter on the BACTEC MGIT 960™ (17.8 ± 0.9) days than the conventional culture method (46.5 ± 0.4) days as shown in Table 2. This study is in agreement with that recorded by Tortoli et al. (1999) who showed that the mean detection time where significantly shorter for methods that used a liquid medium than for L.J. medium. Moreover, Hines et al. (2006) recorded that the BACTEC MGIT 960™ had a significantly lower mean time to detection (15.8 ± 0.8) days than BACTEC 460 TB (28.2 ± 1.0) days and solid media (43.4 ± 1.0) days, and finally Rishi et al. (2007) found that the time to detect *Mycobacteria* was shorter on the BACTEC MGIT 960™ than on the L.J. medium; average been 9.66 days with BACTEC MGIT 960™ and 28.81 days with L.J. medium.

It is clear from Table 3 that the high contamination rate was found with conventional culture method (L.J.) (10.3%) than with the BACTEC MGIT 960™ system (6.9%). The results in this study were lower than that reported by Hanna et al. (1999) who showed that the rate of contamination was higher with solid media (21.1%) than with the BACTEC MGIT 960™ system (8.1%). Moreover, Hines et al. (2006) found that the solid media had higher contamination rate (21.7%) than did the BACTEC MGIT 960™ (6.9%). In addition, Rishi et al. (2007) also found that the break through contamination rates were 13.4% for the BACTEC MGIT 960™ system

and 27.2% for L.J. medium. However, these results disagree with that reported by Somoskovi et al. (2000) who found that the rates of contamination were 3.7 and 1.2% for the BACTEC MGIT 960™ and L.J. medium, respectively. The high contamination rate seen with solid medium in this study may be because of the lack of inclusion of antibiotics. Antibiotic cocktail was added to the liquid media, but no antibiotics were added to the solid media (Hines et al., 2006).

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