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Comparison of LCD array and IS6110-PCR with conventional techniques for detection of *Mycobacterium bovis* isolated from Egyptian cattle and Buffaloes

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ABSTRACT

Bovine tuberculosis is a chronic bacterial and major infectious disease of cattle and buffaloes caused by *Mycobacterium bovis*. Rapid diagnosis of bovine tuberculosis is considered one of the cornerstones for worldwide control as it permits early epidemiological and therapeutic interventions. Therefore, this study was designed to evaluate conventional techniques (tuberculin test, Ziehl Neelsen staining and culturing) in comparison with proven molecular laboratory techniques (LCD array and IS6110 PCR) for identification of Bovine tuberculosis. A total of 902 Egyptian animals (480 buffaloes and 422 cattle) were examined by tuberculin test, and the positive reactors were slaughtered. Tissue samples were collected for staining as well as culturing. Moreover, LCD array and PCR using IS6110 on DNA extracted from tissue and culture samples were carried out for molecular identification of *M. bovis*. According to the results, the tuberculin positive cases for cattle and buffaloes were 2.14% (9 cases) and 5.62% (27 cases), respectively. After post-mortem examination, the prevalence of tuberculin positive cases with visible lesions was 88.9% for cattle and 14.8% for buffaloes. Alternatively, these percentages were 11.1% and 85.2% for cattle and buffalo carcasses with non-visible lesions. The percentage of cattle and buffaloes showing positive culture was 88.9% and 62.9%, respectively. This percentage was 69.5% after staining with Ziehl Neelsen. In contrast, LCD array and IS6110 were 100%, confirming the isolation results. In conclusion, LCD array depending on 16S RNA and DNA hybridization with specific probes for detection of *M. bovis* are rapid, sensitive and labor-saving when combined with IS6110-PCR.

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Introduction

Bovine tuberculosis (BTB) is a primitive infectious disease caused by *Mycobacterium bovis* (*M. bovis*) that typically affects the lungs (pulmonary TB), but can affect other sites as well (extra-pulmonary TB) [1]. Tuberculosis caused by *M. bovis* represents the most important zoonotic disease and in turn a significant cause of economic losses. Shitaye et al. [2] indicated that Egypt is considered one of the developing countries that suffer from chronic infectious diseases such as BTB. Although the fact that BTB is a public health danger and in addition leads to economic losses [3], in Egypt fast diagnosis and control of livestock tuberculosis has not received as much attention as human tuberculosis. In the past, diagnosis of tubercular infection was detected mainly by tuberculin reaction test (Mantoux intradermal reaction). The tuberculin test was developed over 100 years ago by Robert Koch and consists of a mixture of approximately 200 purified protein derivatives (PPD) present in *Mycobacterium tuberculosis* (MTB), but established as well in *M. bovis* Bacilli Calmette-Guerin (BCG) strain and in many environmental mycobacteria [4]. Regrettably, the tuberculin test has numerous restrictions: it has a low sensitivity, and the antigenic cross-reactivity resulting from the widespread of several antigens in BCG and in environmental mycobacteria is responsible for its low specificity. Therefore, a positive reaction can be indicative not only of contact with *M. bovis*, but also of an earlier vaccination with BCG or contact with environmental mycobacteria. In addition, false-positive reactions may be caused by frequent tests as a result of the enhancing effect of continuous sensitization with PPD. These different limits of the tuberculin test have encouraged researchers to develop another method that could solve the PPD faults [4]. Therefore, a fast and accurate diagnostic technique is needed for the detection and identification of the pathogenic mycobacteria in various clinical samples. This is very necessary for the promotion of diagnosis, treatment and control of BTB [5].

Culturing of microorganisms has a specificity that moves toward 100% and permits sensitivity testing of the strains, but the most important difficulty is that growth of the organism may take a long time (6–8 weeks); culture methods as well necessitate viable pathogens and this represents a big problem when tissues are improperly handled [6]. Furthermore, mycobacterial strains are identified traditionally by phenotypic and biochemical methods, but these methods are difficult and time consuming. Therefore, an alternative method such as the molecular amplification of DNA using polymerase chain reaction (PCR) is considered a fast and accurate technique for identification of mycobacterial species [7] and appears to have more sensitivity than that obtained by the culture technique [8,9]. From all the above-mentioned data, it was observed that the quick molecular detection and identification of various *Mycobacterium* spp. of clinical importance is necessary for proper management of animals with BTB. Watt et al. [10] indicated that the insertion sequence (IS) 6110 is considered specific for the Mycobacterium tuberculosis complex (MTBC). In addition, Waddell [11] noticed that mycobacterial strains typically contain several copies of IS6110 (about 25 per genome). In contrast, Casas et al. [12] identified several strains of *M. bovis*

with low copy number. The high changeability in copy number and location of IS6110, in addition to its stability over time, gives IS6110 a useful epidemiological and diagnostic tool.

Recently, the technology of DNA Biochip is similar to that of the hybridization techniques, in that PCR is carried out and the products are hybridized to a solid phase containing the oligonucleotide primers designed to target regions of interest [13]. LCD array has been developed by Chipron GmbH (Berlin, Germany) for rapid identification of various microorganisms. LCD arrays do not require particular tools, and the working procedures are similar to those used with line probe assays. Furthermore, the LCD array offers increased throughput (8 samples per chip) and permits the direct recognition of up to 14 animal species.

Materials and methods

Sample collection

A total of 902 animals (480 buffaloes and 422 cattle) from Gharbia and Minufiya Governorates, Egypt, were examined by tuberculin test and the positive reactor animals were slaughtered. The samples were collected at the time of slaughter from tuberculin-positive cases under aseptic technique. Blood samples were collected from all animals at the time of tuberculin testing and from each slaughtered animal. 10 ml of blood was collected in sterile labeled McCartney tubes from the jugular vein. The tubes were put in slope position for two hours in an ice box and transported to the laboratory for centrifugation at 3000 rpm for 15 min. The Egyptian Laws of General Organisation for Veterinary Services (GOVS) for routine work of meat inspection in slaughterhouses were applied. The case was considered as localized or generalized infection. After collection of the samples, they were divided into two parts (the first part for cultivation and the second part for PCR). Samples intended for PCR were stored at 20 °C and subjected later for PCR testing. Suspected tissue samples such as lung, liver, spleen, kidney, heart, mammary glands and lymph nodes showing tubercle-like lesions were also collected from cattle and buffalo carcasses.

Tuberculin test

Two sites on one side of the neck (12 cm apart) were shaved and the initial skin thickness was recorded; 0.1 ml of avian tuberculin was injected intradermally into the upper site and an equivalent dose of bovine tuberculin was injected into the lower site of the neck. Three days after injection, the skin fold thickness at each site was measured again and recorded. The results were interpreted based on standard interpretation (i.e., after 72 h). Bovine positive reactors and avian positive reactors were obtained using the following formulae: (Bov72-Bov0)-(Av72-Av0) and (Av72-Av0)-(Bov72-Bov0) respectively; Bov0 and Av0 indicated skin-fold thickness before injection of tuberculin, Bov72 and Av72 indicated skin-fold thickness after injection of tuberculin with 72hrs. The differences of the increase of thicknesses between bovine and avian antigens were interpreted as follows: (a) Skin-fold thickness < 3 mm negative; (b) Skin-fold thickness = 3 mm

regarded doubtful inconclusive; (c) Skin-fold thickness > 4 mm positive reactor.

Bacteriological examination of the collected samples

Three grams of tissue samples collected from tuberculin positive cases were sectioned using sterile blades, minced with scissors and homogenized with a sterile mortar and pestle under a biological safety cabinet. The homogenates were decontaminated by adding an equal volume of 4% NaOH on the sample in order to remove contaminants and incubated for 30 min at 37 °C, thereafter, centrifuged at 3000 rpm for 15 min to concentrate the mycobacteria. The supernatant was discarded, and the sediment was neutralized by 1% (0.1 N) HCl acid using phenol red as an indicator. Neutralization was carried out when the color of the solution changed from yellow to brackish. Next, 0.1 ml of suspension from each sample was spread onto a slant of Lowenstein Jensen (LJ) medium. Cultures were incubated aerobically at 37 °C for 8–12 weeks with weekly observation for growth of colonies. Positive cultures were confirmed with Ziehl Neelsen (ZN) staining.

Fast molecular identification of M. bovis by LCD array technique DNA extraction from tissues and bacterial culture

The DNA extraction was mainly carried out by Qiagen spin column genomic DNA isolation kit according to the manufacturer's instructions. In brief, 25 mg of tissue (up to 10 mg of spleen) was cut up into small pieces, and then placed in a 1.5 ml microcentrifuge tube; 20 μl proteinase K was added, mixed thoroughly by vortexing, and incubated at 56 °C until the tissue was completely lysed. 200 μl of Buffer AL was then added to the sample, and mixed thoroughly by vortexing. Then 200 μl of ethanol (96%–100%) was added and mixed again thoroughly by vortexing. The mixture from the previous step was put (including any precipitate) into the DNeasy Mini spin column placed in a 2 ml collection tube (provided) and centrifugation was carried out at 6000×g (8000 rpm) for 1 min. Then the DNeasy Mini spin column was placed in a new 2 ml collection tube (provided) and 500 μl Buffer AW1 was added and centrifuged for 1 min at 6000×g (8000 rpm). After that the DNeasy Mini spin column was placed in a new 2 ml collection tube (provided), then 500 μl Buffer AW2 was added and centrifugation was done for 3 min at 20,000×g (14,000 rpm) to dry the DNeasy membrane. Finally, the DNeasy Mini spin column was then placed in a clean 2 ml microcentrifuge tube (not provided), and put 200 μl Buffer AE directly onto the DNeasy membrane. Incubation was done at room temperature for 1 min, and then centrifugation for 1 min at 6000×g (8000 rpm) to elute was carried out. For maximum DNA yield, elution was repeated once as described in the prior step. In addition, from the obtained cultures, three to five colonies were collected and re-suspended in 180 μl Buffer ATL and the manufacturer's protocol for extraction was followed.

DNA hybridization MYCO Direct 1.7 LCD-Array

DNA hybridization is chiefly used for the preliminary identification of the presence of M. bovis; this process is carried out by using MYCO Direct1.7 LCD-Array Kit DNA-based identification of Mycobacterium tuberculosis complex (MTUB) and other Mycobacteria (MOT); these kits are mostly dependent upon the use of two primer sets. Primer Mix A: Genus Amplification of rRNA gene region from the most members of the genus Mycobacteria with fragment size: 225–265 bp dependent on the species. Primer Mix B: TUB Amplification of a fragment from the repetitive element IS6110 from members of the MTUB-Complex with fragment size: 126 bp, then using the specific probes, and the practice was done and the obtained results were interpreted according to the manufacturer's recommendations (www.chipron.com).

Confirmation of M. bovis infection by PCR

Four cattle cases positive for tuberculin test and LCD array system were confirmed by PCR using six designated oligonucleotide primers as shown in Table 1. The letters A-E indicates the PCR reaction and the group F was performed as haphazard repeat control for the results and to evaluate the various lengths beside the marker. The utilized program by the thermal cycler was modified as follows: 95x10x1, 94x30sx40, 60x60sx40, 72x90sx40, 72x7mx1, 4C. The reaction components were also modified (added DMSO as an enhancer). Per 25 μl reaction: H2O (10.5 μl), Dimethyl Sulfoxide (DMSO) (1.5 μl), Master Mix (Qiagen, Germany) (5 μl), Primers (2x 3 μl, 10 ng) and DNA (2 μl). After the end of the PCR, the next step was to detect the presence of specific products. Agarose gel electrophoresis was used for detecting PCR products. In brief, the procedure was carried out as follows: 1.0 g of agarose was dissolved in 100 ml of 1X Tris–acetate-EDTA (TAE) buffer in a boiling water bath. The melted agarose was cooled to 60 °C. The comb was put at 0.5–1.0 mm above the plastic tray so that complete wells are formed when agarose was added. The melted agarose was poured into the mold. The thickness of the gel should be between 3–5 mm. After solidification of the gel, the comb was carefully removed. The gel with plastic support was put in the electrophoresis tank. Sufficient amount of 1XTAE was added to the electrophoresis tank to cover the gel by about 1-mm. 5 μl of the PCR product were mixed with 1 μl of 6X gel loading buffer .The mixture was slowly loaded into the wells of submerged gel using disposable micropipette. One well of the gel was loaded with 2 μl of 100 bp DNA ladder. The lid of the electrophoresis tank was closed and the electrical leads were connected. A constant voltage of 100 V was applied and the gel was run until the bromophenol blue migrated half way down the gel. The electrical current was switched off. The leads and lid were removed from the gel tank. The gel was stained with 5 μg of ethidium bromide in TAE buffer for 30–45 min at room temperature. The stained gel was visualized by UV-transillumination, (UVP ultraviolet products, TLW-20 transillumination, Amersham Pharmacia Biotech, Inc.) and examined for the presence of predictable bands.

Results

Tuberculin test

The tuberculin test was carried out on 422 Egyptian cattle and 480 buffaloes showing signs of TB infection. As can be seen from the results (Fig. 1), the percentage of tuberculin positive

Table 1 – PCR primer sequences used for confirming the identification of M. bovis.

Group	Primer designation	Forward PCR primer sequence (5′–3′)	Reverse PCR primer sequence (5′–3′)	References
A	Miru 26	TAGGTCTACCGTCGAAATCTGTGAC	CATAGGCGACCAGGCGAATAG	[14]
B	VNTR 3232	TGCCGCCATGTTTCATCAGGATTAA	GCAGACGTCGTGCTCATCGATACA	[15]
C	Miru 24	CGACCAAGATGTGCAGGAATACAT	GGGCGAGTTGAGCTCACAGAA	[14]
D	QUB 4156c	CTGGTCGCTACGCATCGTG	TGGTGGTCGACTTGCCGTTGG	[16]
E	QUB 1451	GGTAGCCGTCGTCGAGAAGC	CGCCACCACCGCACTGGC	[16]
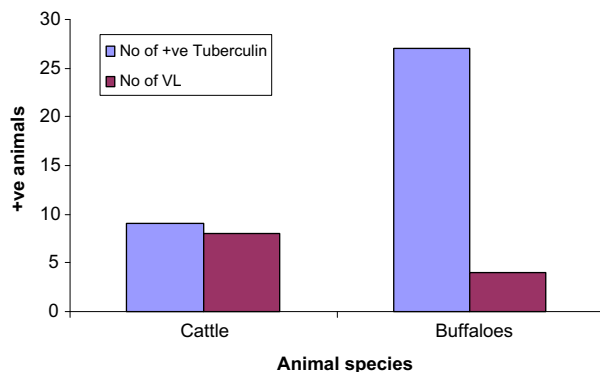
Fig. 1 – Postmortem findings in the positive tuberculin tested cattle and water buffaloes.

cases obtained after testing all clinical cattle and buffaloes was 2.14% (9 cases) and 5.62% (27 cases), respectively. After post-mortem (PM) examination, the prevalence of tuberculin positive cases with visible lesions (VL) was 88.9% for cattle and 14.8% for buffaloes. Alternatively, these percentages were 11.1% and 85.2% for cattle and buffalo carcasses with non-visible lesions (NVL).

Bacteriological examination and staining using ZN stain of tissue samples

A total of 36 positive tuberculin samples of cattle (9 cases) and buffaloes (27 cases) were examined bacteriologically by add-ing 0.1 ml of suspension from each sample onto a slant of Lowenstein Jensen (LJ) medium. Duplicate slants were used, one enriched with sodium pyruvate and the other enriched with glycerol. Positive cultures were confirmed with ZN stain-ing. The slopes after 48 h of incubation were examined for contamination by other organisms; this may be evident as visible growth or as a discoloration or softening of the med-ium. Consequently, the slopes were observed weekly for up to 8 weeks and any bacterial growth was examined micro-scopically for acid fastness by the ZN method. Visible growths of M. bovis were rare before 3 weeks and usually occurred after 4 or 5 weeks' incubation. Colonies of tubercle bacilli were noticed as off-white (buff) in color. According to the results, 9 samples (M. bovis) were grown on glycerol-contain-ing media while 16 samples were grown exclusively on sodium pyruvate-containing media. After staining the total samples with ZN stain, it was found that 25 samples (69.5%) gave positive results. In addition, Table 2 demonstrated that the percentage of cattle and buffaloes showing culture was 88.9% and 62.9%, respectively. Results are shown in Table 2.

Detection of M. bovis by PCR and LCD Hybridization array techniques

After comparing the effectiveness of PCR and LCD Hybrid-ization array techniques with bacteriological examination and PM finding of tuberculin reactors, it was found that 100% of samples (8/8) isolated from cattle with VL were

Table 2 – Relationship between bacteriological examination and ZN staining of slaughtered tuberculin reactors.

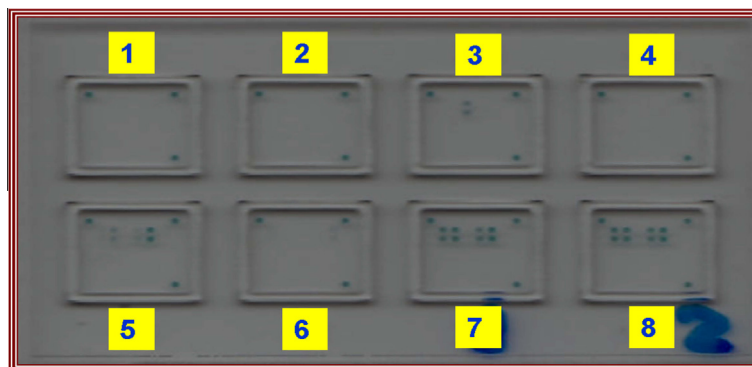| PM finding	No. of samples	Growth on isolation media			Examination With Ziehl Neelsen staining of obtained colonies	Total and percentage animals showing culture
		Glycerol containing media	Sodium pyruvate containing media	No. of animals	Cattle	Buffalos
VL 1-Generalized	1C	–	1C	1C/1C	8	4
2- Pulmonary	3C	–	3C	3C/3C		
3- Digestive	2C + 4B	4B	2C	2C + 4B/2C + 4B		
4- Mixed	2C	–	2C	2C/2C		
A – subtotal VL	8C + 4B	4B	8C	8C + 4B/8C + 4B		
NVL	1C + 23B	5B	8B	13B/23B	–	13
Total	36	9	16	25/36 69.5%	8/9 88.9%	17/27 62.9%

Where: C, cattle; B, buffalo.

Fig. 2 – A slide demonstrate LCD array pattern, wells 1, 2, 4 and 6 are negative; well 3 is Mycobacteria (Species not identified by Chip) one of buffalo cases; well 5 is one cattle case showing MTUB Complex (weak positive, ~10–50 genome equivalents/μl) and wells 7 and 8 are cattle cases showing MTUB Complex (strong positive > 50 genome equivalents/μl).
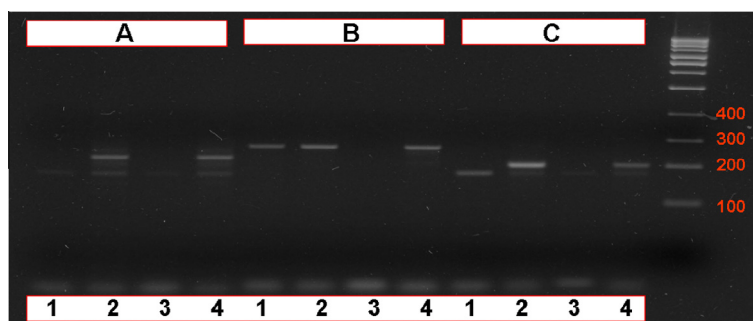

Fig. 3 – Verification of M. bovis by PCR of haphazard four isolates isolated from positive reactor cattle (Miru 26, VNTR 3232, and Miru 24). The numbers from 1 to 4 indicate the strain number and the letters from A to C indicate the PCR reaction where (A) Miru 26, (B) VNTR 3232, and (C) Miru 24. M means 100 base pair (bp) DNA marker.
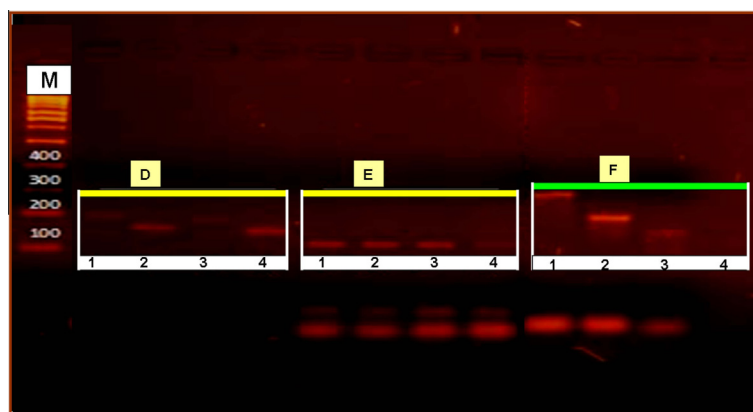

Fig. 4 – Verification of M. bovis by PCR of four isolates isolated from positive tuberculin reactor cattle (QUB4156c and QUB 1451). The numbers from 1 to 4 point to the strain numbers and the letters D–F point to the PCR reaction where (D) QUB 4156c, EQUB 1451, while F group was done as a random repeat control for the results and to compare the various lengths beside the 100 bp DNA marker.

positive after bacterial culture and staining with Ziehl Neelsen, while one sample with NVL was negative after culture and positive after staining with ZN stain. Moreover, LCD Hybridization array identified 100% of cattle samples (9/9) isolated from tissues and positive cultures with VL and NVL (Fig. 2). Four samples isolated from positive culture were confirmed by PCR (Figs. 3 and 4). Results are presented in Table 3.

Table 3 – Detection of Mycobacterium by PCR & LCD Hybridization array tests compared with bacteriological examination and PM finding of tuberculin reactors.

No. of sample	Type of animal	PM findings	Bacteriological examination C + ZN+	PCR & LCD Hybridization array for detection of *Mycobacterium Tuberculosis* complex + ve		Primers for confirmation of *M. bovis*
				Tissue	Culture	Culture
1	Cattle	Generalized	C + ZN+	+ve	+ve	Not tested
2		Mixed (pulmonary + digestive)		+ve	+ve	Not tested
3		Mixed (pulmonary + digestive)		+ve	+ve	+ve with BCDE
4		Pulmonary		+ve	+ve	+ve with ABCDE
5		Pulmonary		+ve	+ve	+ve with DE
6		Pulmonary		+ve	+ve	+ve with ABCD
7		Digestive		+ve	+ve	Not tested
8		Digestive		+ve	+ve	Not tested
9		NVL	C − ZN+	+ve	No culture	Not tested
Total	9			9/9(100%)	8/8(100%)	
10	Buffaloes	NVL	C + ZN+	MOTT	MOTT	
11		NVL		MOTT	MOTT	
12		Digestive		MOTT	MOTT	
13		Digestive		MOTT	MOTT	
Total	4			0/4 (0%)	0/4 (0%)	

NVL: Non visible lesions, C+: culture positive, ZN+: Ziehl Neelsen positive (staining of the obtained culture), MOTT: Mycobacteria other than tuberculosis complex and not typed in the list of LCD array types.

Discussion

Recently, bovine tuberculosis (BTB) is considered one of the most dangerous diseases to cattle and buffalo health. It is caused by *M. bovis* and can affect a large number of species, including humans [17]. In addition, it is of huge economic and zoonotic significance in developing countries. The traditional laboratory diagnosis of tuberculosis is based mainly on the technique of staining with ZN and culturing of BTB [18]. The ZN stain is a fast and inexpensive technique, however it lacks sensitivity. Moreover, the culture method needs time and viable pathogens, which are not easy to obtain, particularly in treated animals. Numerous fast techniques for BTB diagnosis, such as DNA probes that need complicated apparatus have been developed [19]. Therefore, the polymerase chain reaction (PCR) is considered an alternative technique that can enlarge a small piece of DNA with high specificity for detection of infectious diseases [18,20]. However, the tuberculin test is still the widest diagnostic method used even though it has some sensitivity and specificity shortages and needs a subsequent examination of the animal for its interpretation together with the three established diagnostic methods used for detection of animal tuberculosis after slaughtering (post-mortem inspection, Ziehl Neelsen stain and bacteriological examination [1].

According to the results obtained from the current study, it was demonstrated that 9 cattle (2.14%) were positive to tuberculin test, 8 (88.9%) of them gave visible lesions (VL) and 1 (11.1%) gave no visible lesions (NVL). In buffaloes, the results of positive tuberculin test were 27 (5.62%) buffalos, 4 (14.8%) of them gave VL and 23 (85.2%) gave NVL. The current results are in agreement with those obtained by Essam [21] and El Shafei [22] who obtained 1.23%, 1.18% and 3% of positive tuberculin reactor in cattle but not in agreement with El-Mahrouk and El-Balawy [23] who found that this percentage was 30.11%. Unluckily, the tuberculin test has numerous restrictions such as low sensitivity, and the antigenic cross-reactivity as a result of the distribution of several antigens in *Bacillus Calmette-Guerin* (BCG). Therefore, a positive reaction for tuberculin test can be investigative not only of contact with BTB but also of prior vaccination with BCG. In addition, false positive results may be caused by repeated tests as a result of the improving effect of continual sensitization with purified protein derivative (PPD). These various restrictions of the tuberculin test have encouraged researchers to develop another technique that could overcome the PPD faults [1].

After culturing and staining of the positive reactive tuberculin samples, the total acid fast bacilli isolated from 9 cattle positive with tuberculin tests (8 with VL and 1 with NVL) were 8 (88.9%), all from the (8) cattle with visible lesions on Lowenstein-Jensen medium with sodium pyruvate and all 8 isolates gave positive with ZN stain. While in buffaloes, the total acid fast bacilli isolated from 27 buffaloes with positive tuberculin tests were 17 (62.9%), 4 with VL and 13 with NVL. They were 9 colonies on LJ with glycerol and 8 colonies on LJ with sodium pyruvate. With examination by ZN stain, all 17 isolates gave positive results. These results nearly approach that of El-Mahrouk and El-Balawy [23] who recorded that the total acid fast bacilli isolated from 137 slaughtered tuberculin cattle reactors were 97 (70.8%) isolates and El Shafei [22] which was 17 (77.3%) from 22 tuberculin-positive cattle; 16 were *M.bovis* and 1 was MOTT. In contrast, lower results were obtained by Gallo et al. [24] who recorded that out of 1287 reactors, mycobacteria were recovered from 374 (29.1%) and, in addition, Figueiredo et al. [25] recorded that *mycobacteria* colonies were isolated in Lowenstein-Jensen medium with sodium pyruvate from 17 (50%) out of 34 positive tuberculin tested cattle. Culturing of *M. bovis* from different tissues is

considered the gold standard of diagnostic tests for animal and human tuberculosis. Nevertheless, a low level of colonization in the tissues may prevent a precise diagnosis, principally in apparently healthy animals. Additionally, the long incubation period needed for the primary culture of the microorganism (two–three months) delays the management decisions to cull the infected animals and control the spread of the disease. Furthermore, acid-fast staining of suspected tissues is fast and needs diminutive optimization; nevertheless, ZN staining cannot differentiate among the different mycobacterial species.

The technology of DNA Biochip is similar to that of the hybridization techniques, in that PCR is carried out and the products are hybridized to a solid phase containing the oligonucleotide primers designed to target regions of interest [13]. Combination of the DNA with the primer creates a fluorescent sign which can be identified by confocal microscopy. At the same time, these Biochips are capable of the identification of *Mycobacterium tuberculosis* [13,26,27]. In the current study, identification of the extracted DNA from tissue samples and obtained isolates using LCD array was carried out and according to the results obtained, identification of *M. bovis* depending on 16S RNA and DNA hybridization with specific probes gave 100% for both clinical and apparently healthy cattle in contact with them. It was known that LCD microarrays are characterized by a low cost and density microarray (LCD) to detect different microorganisms which has been developed by Chipron GmbH (Berlin, Germany, http://chipron.com/index.html). Owing to high costs, complex protocols and the requirements for considerable supplementary laboratory tools, microarrays have to become part of routine molecular diagnostics. However, LCD arrays do not require particular tools and the working procedures are similar to those used with line probe assays. Furthermore, the LCD array offers increased throughput (8 samples per chip). In addition, the LCD Array permits the direct recognition of up to 14 animal species: cattle, buffalo, pig, sheep, goat, horse, donkey, rabbit, hare, chicken, turkey, goose, and two duck varieties. The assessment system here depends on the detection of specific sites within the 16S rRNA mitochondrial locus of all tested samples, and positive samples can be visually identified or by applying the scanner and software provided by the kit manufacturer [28]. Confirmation of BTB using PCR on DNA extracted from tissue samples and culture from both clinical and apparently healthy cattle also gave 100%. Similar results were obtained by Nahar et al. [29], who revealed that out of nine samples, seven (88%) gave amplified bands indicating positive and higher sensitivity of the technique. The rest of the samples failed to yield amplicon specific for *M. bovis* infection and this could be due to the fact that the cattle may be infected with Mycobacterium spp. other than *M. bovis*.

Conclusion

Punctual diagnosis of BTB particularly in countries where the disease is still endemic as in Egypt is of great significance to detect and identify infectious cases for accelerating the control measures. As the molecular method is much faster than culture and reduces the time for diagnosis from numerous weeks to 48 h, this study was concentrated on using LCD array which depends on 16S RNA and DNA hybridization with specific probes for the detection of BTB when combined with IS6110-PCR.

Authors' contributions

No authors other than Dr. Rasha Nabil Zahran and Dr. Ayman El-Behiry, Dr. Eman Marzouk and Dr. Tarek Askar, who carried out all the laboratory work, collected, analyzed the data, and drafted the manuscript.

Conflict of interest

We have no conflict of interest to declare.

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