Research Article

Critical Field Appraisal for the Use of Bovine Tuberculosis' Antibody Detecting-Serodiagnostics

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Abstract | Bovine tuberculosis is a worldwide disease that causes great economic losses to the dairy industry and constitutes a serious human public health hazard. Development of a wide range of serodiagnostic assays for mycobacterium has been an area of interest through the past two decades as a rapid, accurate, and easy-to-be-used and interpreted diagnostic kit as well as to be a competitive alternative to the conventionally established expert-dependent techniques. The current work's main objective is to evaluate the reliability of two widely distributed rapid kits for as a suitable substitution for the gold standard bovine tuberculosis diagnostic techniques. IQRT Anigen Rapid Bovine TB Ab kit and Ubio quick VET Bovine Tuberculosis Antibody kit were used in testing 3750 dairy cattle. The results obtained were compared with the ELISA, bacteriological examination, and tuberculin skin testing. By the tuberculin test, 69 (1.8%) animals reacted positively, from which 51 animals (73.9%) showed visible lesions on postmortem examination while the remaining 18 animals (26.1%) showed no visible lesions. Using bacteriological examination of tissue specimens, 48 out of the 69 positive tuberculin reactors (69.6%) revealed successful *M. bovis* isolation. Using ELISA on serum samples collected prior to skin testing from the positive tuberculin reactors, only 26 cases (37.7%) were positive. The IQRT Anigen lateral flow kit showed only 21 positive cases, 43.8% of 48 bacteriologically identified cases, and 30.44% out of the 69 tuberculin-positive cases. The Ubio quick VET kit has detected zero% of bovine tuberculosis-positive cattle. Using bovine tuberculosis rapid kits alone may be unreliable for the detection of tuberculosis-infected cattle.

Keywords | Bovine tuberculosis, tuberculin skin test, lateral flow kits for antibody detection, ELISA, Rapid diagnostics, dairy industry, Human health hazards, Granulomatous diseases

Received | November 21, 2022; **Accepted** | December 25, 2022; **Published** | January 27, 2023
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Citation | Soliman R, Waheeb N, Nasr E, El-Hariri M, Abo-Elyazeed H, Aboul-Ella H (2023). Critical field appraisal for the use of bovine tuberculosis' antibody detecting-serodiagnostics. Adv. Anim. Vet. Sci. 11(2):278-287.
DOI | https://dx.doi.org/10.17582/journal.aavs/2023/11.2.278.287
ISSN (Online) | 2307-8316

INTRODUCTION

Bovine tuberculosis (bTB) is a chronic granulomatous condition that mostly affects cattle's lymph nodes and lungs. It is caused by *M. bovis*, a member of the *M. tuberculosis* complex (Taylor et al., 2007). *M. bovis* zoonotically transferred to people; causing disease through ingestion; inhalation and less usually by contact with mucous membranes and torn skin (De la Rua-Domenech, 2006). Bovine tuberculosis can affect a wide range of species and is defined by the progressive development of granulomatous lesions (tubercles/granulomas) in any body organ (Borham et al., 2022).

The intradermal tuberculin skin test has been in use for almost a century in the diagnosis of bovine tuberculosis

and despite the technological advances of the last two decades, it is still the only reliable test for the diagnosis of tuberculosis in cattle (Cousins and Florisson, 2005). The purified protein derivative (PPD), the antigen used in the tuberculin test, contains many antigenic determinants of broad specificity, which leads to the appearance of non-specific reactors indicating false positive possibilities (Radostits et al., 2000).

Although the tuberculin test is still considered the golden test for the diagnosis of bovine tuberculosis, it is laborious and time-consuming. In addition, negative or doubtful tuberculin-tested cases cannot be retested 6 months after tuberculin testing. Therefore, the recently developed serodiagnostic methods may be suitable and reasonable alternative tools for the diagnosis of bovine tuberculosis. Therefore, the suggestion of (El-Sify et al., 2013) to use enzyme-linked immunosorbent assay (ELISA) with the tuberculin skin test to overcome the problems of the tuberculin test was very convincing. Aurtenetxe et al. (2008) developed the first ELISA kit for the serodiagnosis of bovine tuberculosis, and it was intended for use in wildlife applications with bovine-purified protein derivatives (b-PPD) as the target antigen. For the identification of TB in wild boar, a previously created ELISA kit was reported to have a sensitivity and specificity of 73% and 96%, respectively (Chambers, 2013).

As an alternative to tuberculin skin testing for antemortem diagnosis, quick and simple immune-chromatographic lateral flow assays (LFAs) for the serodiagnosis of bTB have been developed and presented (Pollock et al., 2005; Ameni et al., 2010). As both qualitative captures and detectors of specific antibodies against *M. bovis* in plasma, serum, and whole blood, these chromatographic immunoassays use special blends of chosen *M. bovis* antigens (Lyashchenko et al., 2004; Wernery et al., 2007). The common seroreactive antigens in bovine tubercle bacilli (TB) have been identified as the MPB83, 14-kDa protein, ESAT-6, MPB70, CFP-10, MPT51, MPT32, MPB59, MPB64, MPT63, Acr1, PstS-1, 16-kDa alpha-crystalline/MPB83 fusion protein, ESAT-6/CFP10 fusion protein, *M. bovis* purified protein derivatives, and *M. bovis* culture filtrate (Lyashchenko et al., 2004; Waters et al., 2006). The use of a lateral flow kit (LFK) in the serodiagnosis of infectious diseases has been widely applied and its first application in the diagnosis of bovine tuberculosis was first recorded by (Greenwald et al., 2013). Since then, these tests have become an extremely popular tool for the rapid diagnosis of bovine tuberculosis (Chambers, 2013). However, the specificity and sensitivity of several commercially available LFA kits are questionable.

In the present work, two LFKs were evaluated in the rapid detection of tuberculosis-infected cattle. The sensitivity and specificity of these kits were determined using the tuberculin skin test and bacterial isolation as a golden standard technique. The first kit was the IQRT Antigen Rapid Bovine TB antibody kit that employed recombinant *M. bovis* MPB70 antigen (specific for *M. bovis*) as capture and detector. The second kit was the Ubio quick VET Bovine TB Antibody Rapid Test, this kit uses a unique cocktail of recombinant antigens of *M. bovis* to detect specific antibodies of three immunoglobulin classes, IgG, IgM, and IgA developed during infection in cattle.

MATERIALS AND METHODS

Study design and animal population

Through a national study involving most of the Egyptian governorates, a total of 3750 crossbreed dairy cattle from different Egyptian governorates, all over Egypt were involved in the current study. The study design can be clearly described through the flowchart illustrated in Figure 1.

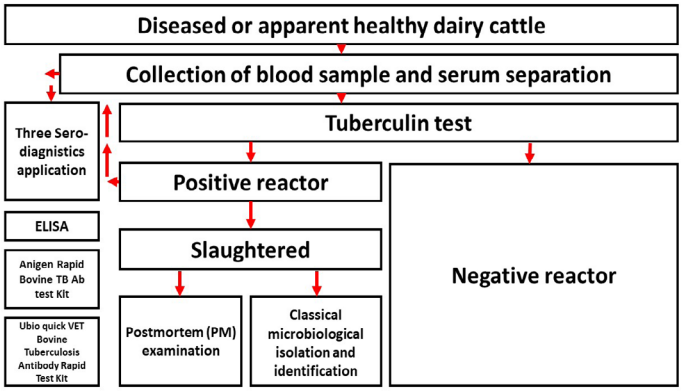

Figure 1: A flowchart illustrating the overall testing flow of each animal involved in the study.

General signs appeared on diseased/bovine tuberculosis suspected cases

Bovine tuberculosis is mainly represented clinically as a chronic debilitating disease in dairy cows. However, in the current study, most infected cattle were identified early before obvious clinical signs' development. Progressive emaciation, a low-grade fluctuating temperature, weakness, and decreased appetite are typical late-stage signs. Animals typically have a moist cough that gets worse in the morning, in cold weather, or after activity. They may also experience dyspnea or tachypnea, as well as occasional constipation and diarrhea. Symptoms of bovine tuberculosis in cattle generally take months to manifest. Additionally, infections may remain dormant for a very long time before resurfacing in tense situations or in the elderly. Therefore, the bTB can be difficult to diagnose based only on clinical symptoms, especially in cases when the prevalence of severe cases in animals with clinical evidence may be low or missing and the majority are discovered at the slaughterhouse or identified by routine testing.

Collective description of the most remarkable postmortem lesions revealed by the tuberculin test-positive reactors

This syndrome leads to the formation of granulomas that contain mycobacteria. These granulomas are normally yellowish, frequently encapsulated, caseous, caseo-calcareous, or calcified. Some tubercles are so small that they cannot be detected with the naked eye unless the tissue is dissected into parts. Cattle lymph nodes typically feature tubercles, especially those in the head and thorax. They frequently appear on the exteriors of internal organs such as the liver, spleen, and lung.

Direct microscopic examination using Ziehl Neelsen (ZN) differential stain

A potential secondary microscopic presentation of acid-fast bacteria serving as a diagnostic aid for post-mortem lesions. Ziehl-Neelsen staining, which is based on tinctorial properties shared by mycobacteria and other non-mycobacterial acid-fast opportunistic as well as pathogenic bacteria like *Nocardia, Rhodococcus*, and *Corynebacterium*, was used to detect the presence of mycobacteria in a given sample. This staining technique was followed by a light microscopy examination.

Using the Ziehl-Neelsen acid-fast technique, direct smears were made from tissues exhibiting tuberculous lesions. The gathered samples were homogenized with a homogenizer after being smashed with a pestle in a clean, sterile mortar. The homogenized tissues were then smeared with a sterile loop to create a smear (1cm x 2cm) on a fresh, clean, and labeled grease-free glass slide. The smeared slides were heated to a temperature of 50°C in a hot air oven (HAO) for 7–10 minutes to completely dry and fix them. As stated by (Saidu et al., 2015), the hot Ziehl Neelsen procedure was exactly carried out. A positive microscopic slide was defined as a red, acid-fast, straight, or slightly curved rod that was present alone or in groups.

Application of the single intradermal Tuberculin skin test (TST), performed according to the OIE manual (2009) as follows

Testing of each animal started with measuring skin thickness followed by intradermal (I/D) injection of 0.1mL of bovine PPD. Seventy-two hours post-injection, the skin thickness was measured and the differences between the 1st and 2nd readings in (mm) were recorded. According to the Egyptian general organization of veterinary services (Amin, 2019), an increase in skin thickness of 4mm or more was considered positive, less than 2mm is considered negative and from 3-4 mm was considered doubtful. All positive reactants were slaughtered due to the test and slaughter rule in case of TB-positive cases.

Isolation of acid-fast bacilli from collected tissue samples of slaughtered tuberculin-positive reactants

The tested tuberculin-positive cattle were slaughtered. Postmortem examination was conducted on them, and 69 tissue samples were collected. These samples were used for bacteriological isolation and identification of *M. bovis* according to (Corner et al., 2012). For the isolation of this organism, tissues, lymph nodes, and/or other organs were processed. All specimens were broken into small pieces, combined with fine sterile sand, diluted with 2mL of sterile distilled water, and then ground in a sterile mortar. The samples were then incubated for half an hour at 37°C with two mL of 4% H2SO4. After being further diluted with 16mL of sterile, distilled water, the liquid was centrifuged for 20 minutes at 3000rpm. The acquired sediment was re-suspended in 0.5mL sterile distilled water, inoculated into Lowenstein-Jensen slants, and cultured at 37°C in an inclined posture for at least 6-8 weeks with weekly examinations beginning three days after inoculation. The obtained data were recorded (Riello et al., 2016).

Serological diagnosis of bovine tuberculosis using locally developed ELISA and 2 types of commercially available lateral flow kits

All serodiagnostic tests were performed on the serum samples that were collected from the examined dairy cattle before being tested with the single intradermal tuberculin skin test (Kennedy et al., 2003). These samples were examined for diagnosis of bovine tuberculosis through the detection of *Mycobacterium bovis*-specific antibodies. The serum samples were examined using a locally prepared ELISA and two commercially available lateral flow kits, namely; the Antigen Rapid Bovine TB Ab test Kit (IQRT test kits) and the Ubio quick VET Bovine Tuberculosis Antibody Rapid Test Kit. The obtained results were comparably analyzed.

Examination of the collected serum samples using the Anigen Rapid Bovine TB Ab test Kit (IQRT test kits) produced by Anigen Animal Genetics Inc., South Korea

The test was performed per the manufacturer's instructions as follows; three drops (about 60-90µL) of tested serum samples were dispensed into the sample application well. One drop of phosphate buffered saline was added into the sample well if the flow migration is not observed within 30 seconds in the result window, Results were read in 20 minutes with instructional invalid readings and interpretation after 20 minutes. Positive results appeared as two purple color bands (T band and C band) within the result window, positive results can be visible in as short as 1 minute. If the intensity of the purple band color is faint, it is interpreted as positive as it appears within 20 minutes.

Examination of the collected serum samples using the Ubio quick VET bovine tuberculosis antibody rapid test kit produced by Ubio, India

The test was performed per the manufacturer's instructions as follows; three drops (about 60-90µl) of the tested serum were added to the sample hole on the test card using a dropper. The result was recorded in 5-10 minutes. The control line, marked C, should always develop color if the test procedure is performed properly and the test reagents are working. A red test line marked T will be visible if there are enough bovine TB-specific antibodies in the serum sample.

Examination of the collected serum samples using locally prepared ELISA

The antigen used for coating the microtiter plate wells was b-PPD which was obtained from the bacteriological diagnostic products department, veterinary serum and vaccine research institute (VSVRI), Abbasia, Cairo, Egypt. It was prepared according to the protocol of the central veterinary laboratories, in Weighbridge, United Kingdom (UK). Briefly, the test was developed as follows; polystyrene 96-well microtiter plates were coated with the b-PPD antigen using 15µg/mL in 0.1M carbonate-bicarbonate buffer (pH 9.6). The antigen solution was dispensed in 50uL/well. In each microtiter plate, 4 wells were left uncoated with b-PPD antigen and served as blank control wells. Each of these blank wells received 50µL of carbodiimide in 0.1M carbonate bicarbonate coating buffer. The plates were incubated overnight at 4°C then decanted, washed 3 times with phosphate-buffered saline (PBS), and air dried for 10 minutes. Then all wells except the blank ones were filled with 100µL/well of 0.01M ammonium chloride as a blocking buffer and further incubated for 30 minutes at 22°C. Finally, the plates were washed 3 times with PBS washing buffer (Waters et al., 2011). In each microtiter plate, all wells except the wells of the first column were filled with 50ul/well PBS buffer. For each serum sample, one row is used. The tested serum samples were diluted 1:40 in PBS and 100µL of each tested serum sample was added to the first well of the corresponding row. This was followed by two-fold serial dilutions of each sample. Each plate received negative serum samples run in the same dilutions to calculate the cut-off value. The plates were incubated at room temperature for 30 minutes on a horizontal shaker. The microtiter plates were decanted, washed 4 times with ELISA washing buffer, and allowed to stand inverted for 30 minutes. To each well, 50µL of anti-bovine IgG alkaline phosphatase diluted 1:5000 in diluting buffer was added and the plates were then incubated for 30 minutes at room temperature on a horizontal shaker. The plates were again washed 4 times with ELISA washing buffer and allowed to stand inverted for 30 minutes. The substrate solution composed of P-Nitrate phenyl phosphate (one tablet 5mg/5mL substrate buffer) was prepared and 50µL/well was dispensed in all wells of the plates and were further incubated for 20 minutes at room temperature. The color development was stopped by the addition of 100µl/well of 0.4 M NaOH-stopping solution. The results were recorded by optical density measurement at 405nm using spectra III ELISA reader. A titer of 1:80 or higher was considered positive for bovine tuberculosis (El-Seedy et al., 2007). The results obtained by the applied serological tests were compared with the tuberculin testing and the results of the culture isolation, which is considered the gold standard test. The diagnostic value of the tested serological methods was determined in the terms of accuracy index, sensitivity, specificity, positive predictive value, and negative predictive value.

RESULTS AND DISCUSSION

Mycobacteria can be detected using a microscope; however, the sensitivity is typically low (19%). Additionally, the genus *Mycobacterium* and *Nocardia* have many characteristics, such as the unique acid fastness staining properties, making it often challenging to distinguish between the two. This is primarily because effective microscopic investigation requires a large bacterial burden Figure 2.

The results of tuberculin skin testing are presented in Table 1 were out of 3750 tuberculin-tested cross-bred dairy cattle, 69 were found to be positive reactors with a prevalence rate of 1.8%.

Table 1: Tuberculin skin test result in dairy farm cattle from some Egyptian Governorate

Farm	Total no. of examined animals	Positive tuberculin	
		Number	%
1	170	2	1.2
2	220	5	2.3
3	290	7	2.4
4	140	3	2.1
5	320	8	2.5
6	500	12	2.4
7	410	11	2.7
8	250	3	1.2
9	180	2	1.1
10	290	4	1.4
11	370	5	1.4
12	155	2	1.3
13	225	2	0.9
14	110	2	1.8
15	120	1	0.83
Total	3750	69	1.8

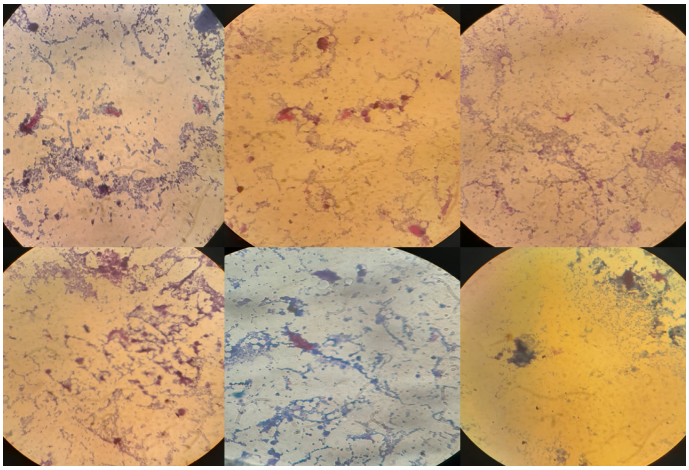

Figure 2: A representative sample of the direct Ziehl Neelsen technique stained slides.

This is comparatively lower than that recorded by other investigators in Egypt (Dimitri et al., 1990), (6.9%), (Lotfy et al., 1960), (4.6%), (Elbattawy, 2008), (2.2%) and in some other African countries as in Ethiopia (Nasr et al. 2008), (11.6%), while in Chad (Ameni and Erkihun, 2007), (8%). This noticeably reduced incidence might be attributed to the fact that the farms included in this research perform the tuberculin test regularly and applied the test and slaughter strategy (Borna et al., 2009). On the other hand, the prevalence rate recorded in the present study is comparatively higher than that reported by other investigators in other countries, 0.9% to 1.3% in Tanzania (Gonzalez-Liamazares et al., 1999; Shirma et al., 2003), respectively, 0.54 % in Venezuela (Cleaveland et al., 2007), 0.4 %, in New Zealand (Delgado and Trujillo, 1975), 0.05 to 0.15 % in Australia (Johns, 1969), and 0.02 % in Japan (Clay, 1971).

The postmortem findings of the slaughtered TB reactors revealed that 73.9% of these animals showed visible lesions. At the same time, the percentage of tuberculin reactors with non-visible lesions was 26.1%, Table 2.

Table 2: Result of Postmortem examination of tuberculin-positive slaughtered cattle.

Total no. of animals	Visible lesions										Non-visible lésions	
	Generalized		Head		Pulmonary		Digestive		Mixed			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
69	8	11.6	5	7.2	22	31.9	6	8.7	10	14.5	18	26.1

These results agreed with those reported by (Nemoto, 1972; Olivera et al., 1983). As well as, a much higher percentage was recorded by (Zivkovic et al., 1984) who reported (86.9%) in Queensland. A lower percentage was also reported in Egypt (Guindi et al., 1980) by (Kuczyuski, 1970), (46%), (Yoon et al., 1979), (52.7%), and (Rodriguez et al., 1983), (40.8%). Sensitization by atypical *Mycobacteria* or even closely related microbes, particularly members of the genus *Nocardia*, or a combination of liver fluke infestation and saprophytic *Mycobacteria* was blamed for the proportion of tuberculin-positive animals with non-visible lesions (Waddington, 1965; Byrne et al. 2017). In addition, (O'Reilly, 1992; Huitema, 1994) attributed the non-specific reaction to the possibility that those animals were slaughtered at an early stage of the illness when the tuberculous lesions were still invisible, or lesions may have been found in body parts not usually examined in the carcass, such as bone or brain. The relation between the postmortem findings in different ages of tuberculin reactor cattle is presented in Table 3.

Table 3: Results of the correlation between the age of the animal and PM finding of the slaughtered cases.

Age (years)	No. of tested animals	Positive tuberculin reactors		PM findings in slaughtered tuberculin reactor cattle											
				Visible lesions (51)										Non visible lesions (18)	
				Generalized		Head		Respiratory		Digestive		Mixed			
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1-3 years	850	16	1.9	1	6.3	3	18.8	3	18.8	2	12.5	1	6.3	6	0.7
3-5 years	1850	30	1.6	3	10	2	6.7	9	30	3	10	6	20	7	0.38
Over 5 years	1050	23	2.2	4	17.4	0	0	10	43.5	1	4.3	3	13	5	0.48
Total	3750	69	1.8	8	11.6	5	7.2	22	31.9	6	8.7	10	14.5	18	26.1

The percentage of reaction-positive animals increased with age, reaching a maximum in animals over 60 months old. Similar studies in Great Britain recorded an increase in the incidence of bTB with increased age (Brooks-Pollock et al., 2013). It was suggested earlier by (Mackay and Hein, 1989) that, the high positivity of old age might be due to the reduced number γ and δ T cells responsible for anti-mycobacterial immunity. [These cells are predominantly found in the circulation of young calves. It has also been suggested that the increased incidence of TB in older animals can be due to a waning protective capability in aged animals (O'Reilly and Daborn, 1995; Parlas and Rossi, 1964). The correlation between the site of the lesion and the isolated mycobacteria species is shown in Table 4.

Table 4: Result of bacteriological isolation and identification of mycobacteria isolated from tuberculin positive cattle.

No. of processed tissue samples	Bacteriological isolation (52 isolates)					
	M. bovis		Unidentified mycobacteria			
	No.	%	Slow growers		Rapid growers	
			No.	%	No.	%
69	48	69.6	3	4.3	1	1.4

The total acid-fast bacilli isolated from the 69 slaughtered cattle were 52 isolates (75.4%). According to the morphological characters, growth rate, pigmentation, growth at different temperatures and biochemical tests these isolates were identified into 48 (69.6%) *M. bovis*,

1 unidentified rapid grower strain (1.44%) and 3 (4.3%) unidentified pigmented slow grower strains. The recovery rate of *M. bovis* figured up to 69.6% but other authors reported a lower *M. bovis* recovery rates, 14.8% by (Lesslie and Birn, 1970), 20.2% by (Beck and Bibrack, 1971), 41% by (Osman, 1974), 35.4% by (Gallo et al., 1983), 29.1% by (Payeur and Marquardt, 1988), 5.6% by (Abuo-Eisha et al., 1995), and 42.9% by (Chul, 1981). On the contrary, (Rogers et al., 1980), a much higher isolation rate (92.1%), has been reported in the Republic of Korea. These findings are mostly influenced by the actual disease state existing in the tested herd, and to a lesser extent by the investigators' experience and the method employed to decontaminate tissue samples. Data presented in Figure 3, shows the relation between the post-slaughter findings of positive tuberculin reactors and the type of mycobacteria recovered from the 69 carcasses, *M. bovis isolates*, rapid grower isolates and slow growers pigmented isolates were recovered at rates of 69.6%, 4.3%, and 1.44%, respectively. *M. bovis* was isolated at a rate of 100% from generalized TB, 91% from pulmonary TB, 81.8% from extra-pulmonary TB, 90% from mixed TB, and 11.1% from non-visible lesion (NVL) reactors. These results coincide with those reported by (Good et al., 2018) who isolated *M. bovis* from NVL reactors. On the other hand, the recovery rate of typical mycobacteria rapid growers and slow growers pigmented was 16.7% of the extra-pulmonary for both of them and 33.3% of the mixed for the rapid growers. None of the cases of generalized tuberculosis yielded atypical mycobacteria, since *M. bovis* has a tendency to induce progressive lesions and generalization (Huitema, 1994; Palmer et al., 2006). All cases with generalized TB yielded *M. bovis, a* finding indicating the high susceptibility of cattle to *M. bovis* infection. Comparison between bacteriological examination and serological testing by lateral flow kits of tuberculin-positive cattle are shown in Figure 4.

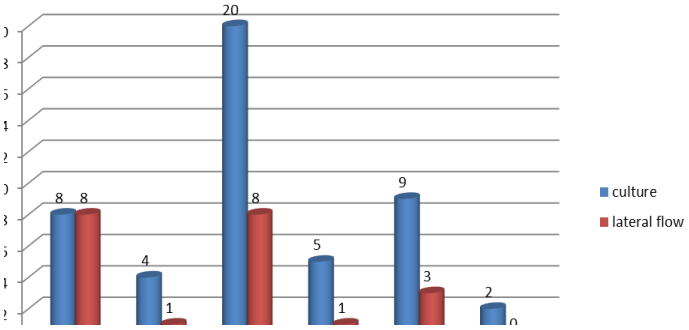

Figure 3: Correlation between site of lesion and the isolated *mycobacteria* spp.

The results of the application of lateral flow kits on sera from tuberculin-positive cattle

The One-step Anigen Rapid Bovine Antibody Test (IQRT) employed recombinant *M. bovis* MPB70 antigen specific for *M. bovis* as capture and detector antigen. It is crucial to note that TST was performed after serological testing because the latter can enhance antibody responses in infected cattle and stresses the significance of the timing of blood sample collection on the interpretation of the test (Harrington et al., 2008). In contrast to the 69.6% of calves whose tuberculin positivity was verified by bacterial isolation of *M. bovis*, this Anigen TB antibody test kit only detected 43.8% of them. While the single-directional lateral-flow serological test used in the other lateral flow assay Ubio quick VET test was developed to quickly determine the presence of M. Bovis antibody has not identified any cattle that are tuberculin positive (Danbrini et al., 2010, 2013).

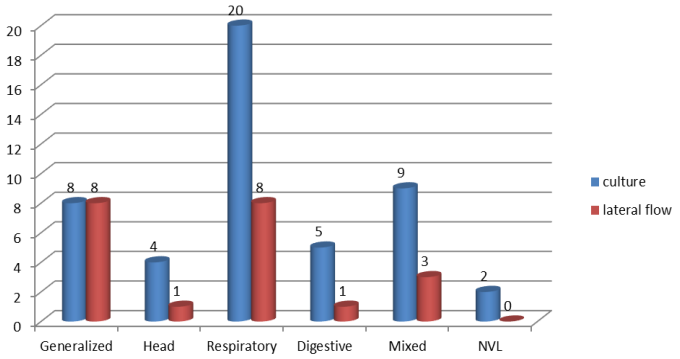

Figure 4: Comparison between bacteriological examination and serological testing (lateral flow kits) of tuberculin positive cattle.

Comparison between the result of the ELISA, rapid lateral flow test, and bacteriological isolation of tuberculin-positive cattle showed that 8 tuberculin positive reactors with generalized tuberculous lesions were bacteriologically positive *(M. bovis* isolation) and ELISA positive using b-PPD as coating antigen (100%).

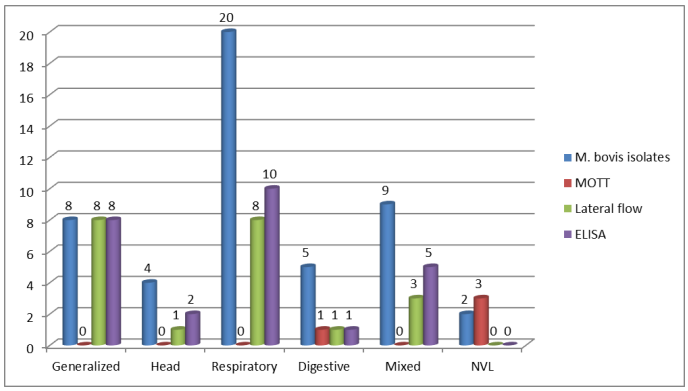

Figure 5: Comparison between the result of the ELISA test, rapid lateral flow test, and bacteriological isolation of tuberculin-positive cattle. MOTT; Mycobacteria Other Than Tuberculosis.

As shown in Figure 5, out of 22 tuberculin-positive reactors 10 (45.5%) showed pulmonary lesions, out of 11 tuberculin-positive reactors 3 (27.3%) showed extra-

pulmonary lesions (head and digestive), and out of 10 tuberculin reactors showing mixed TB lesions (pulmonary and extra-pulmonary), 5 (50%) isolates of *M. bovis* were positive for ELISA by using b-PPD as coating antigens. Out of 18 tuberculin reactors showing NVL, 2 (11.1%) isolates of *M. bovis* could be isolated from them and zero (0%) serum samples were positive by ELISA by using b-PPD as a coating antigen.

As reported by (Lepper and Corner, 1983; Waters et al., 2010), antibody response to *M. bovis*. The findings of the present study were consistent with those of other studies. Unevenness in bovis infection is unquestionable. This phenomenon was first mentioned in a serological examination by researchers looking at the humoral immune response to *M. bovis* (Hanna et al., 1992). They came to the conclusion that bovis infection in cattle was characterized by very diverse antigen recognition. It is determined that in order to establish the true prevalence of bTB in the herd, a single intradermal tuberculin test, culture, and isolation of the mycobacterium are advised.

The recent lateral flow rapid kits could be used for initial tuberculosis screening in combination with TST for improving the sensitivity of bovine tuberculosis screening, thereby leading to more successful control programs in developing countries. The rapid test is proposed as a potentially useful ancillary assay for bTB. In addition, it may be most suitable for surveillance, especially if an immediate result is needed. The differences in the sensitivity and specificity of the different LFA kit sources available in the market necessitate that, the importance of choosing the type of diagnostic kits used for the rapid detection of bovine tuberculosis. Also, these kits alone may be not enough for an accurate diagnosis of bovine tuberculosis.

CONCLUSIONS AND RECOMMENDATIONS

An important zoonotic disease for public health is bovine tuberculosis (bTB). To fully comprehend the epidemiology and zoonotic potentials of bovine TB, as well as to significantly reduce and manage the disease in livestock, the optimum identification of the disease in Egypt's cow herds is essential. The tuberculin skin tests (TST), which are based on delayed hypersensitivity reactions, are now the best strategies for worldwide field diagnosis of bTB in live animals. By cultivating the bacteria prior, one can gain a significantly higher sensitivity. Despite some drawbacks, such as the challenge of collecting representative samples from live animals, the requirement for pretreatment, slow growth, and additional time for identification by alternative methods, culture is still recognized as the gold standard for TB diagnosis.

ACKNOWLEDGMENTS

We thank all general practitioner veterinarians and dairy animal specialists all over Egypt due to their help during the sample collection stages.

NOVELTY STATEMENT

The outcome of the current work clearly describe the actual sensitivity and specificity of more than one antibody based serodiagnostics detectors in comparison with the gold standard diagnostic tests of tuberculosis cases.

AUTHOR'S CONTRIBUTION

All authors contributed equally during the whole stages of the study.

Abbreviations

bTB, bovine tuberculosis; PPD, purified protein derivatives; ELISA, enzyme linked immunosorbent assay; b-PPD, bovine purified protein derivatives; LFAs, lateral flow assays; TB, tubercle bacilli; LFKs, lateral flow kits; ZN, Ziehl Neelsen; HAO, hot air oven; TST, tuberculin skin test; I/D, intradermal; VSVRI, veterinary serum and vaccine research institute; UK, United Kingdom; NVL, nonvisible lesion; MOTT, Mycobacterium other than tuberculosis.

Ethics approval and consent to participate

This work is designed and performed with complete guidance by the (Animal Research: Reporting of In-Vivo Experiments-ARRIVE) and the (Institutional Animal Care and Use Committee-IACUC of the faculty of veterinary medicine, Cairo University) guidelines.
All sample collection procedures, which are (no) or (low) pain procedures, were done based on permission from the cattle farm owners and managers.

Consent for publication

Not applicable.

Availability of data and materials

The data collected, used, and/or analyzed related to the current work are available from the corresponding author upon request.

Funding

Not applicable.

Conflict of interest

The authors have declared no conflict of interest.

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