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Application of Tuberculin screening tests for determination the prevalence of bovine tuberculosis in Basra governorate /Iraq

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Abstract
This study was intended to identify the prevalence of bovine Tuberculosis (TB) in Basra governorate/ Iraq. About 694 random samples were collected from cows and buffaloes that reared in Basra city center, Abu Alkhasib, Shat Alarab, Al Fao, Safwan, Al Qurna, Al Mdainah, Imam Sadiq, Imam Qaim and Al Zubair / Basra governorate. The study was extended between 14th of August to 11th of September 2014. Comparative intradermal tuberculin test (CITT) was used to determine the morbidity rates of bovine tuberculosis in all animals. Only four animals, from city center district showed suspected results and revealed increased in the thickness of the skin for CITT, while, all other animals were negative. Sixty days later blood samples were collected from elected animals by non-bias methods and tested by ELISA. The results of ELISA were negative for all samples as well for the suspected samples in the skin test. The efficiency of the CITT and ELISA tests was evaluated for the diagnosis of bovine TB. This study approved that TB screening tests are important in the eradication and control procedures of the bovine TB in Basra governorate because its ability to recognize the infected and suspected animals. The author recommend to apply the field CITT and ELISA laboratory test in the diagnosis and elimination of the infected animals in the bovine herds.

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Introduction

Mycobacterium bovis is causative agent of bovine Tuberculosis. It infects approximately 50 million animals all over the world causing economic losses of approximately 3 billion dollars per year (Steele, 1995). In 1890, Robert Koch demonstrated that intradermal instillation of live or killed tubercle bacilli or their extracts could elicit a delayed-type hypersensitivity response in experimentally infected guinea pigs with tubercle bacilli. The tuberculin skin test has remained the primary diagnostic test for tuberculosis in both cattle and humans since that time (Pritchard, 1988). *M. bovis*

is a member of the *M. tuberculosis complex*, a group that includes also *M. tuberculosis*, *M. africanum* and *M. microti* (Grange *et al.*, 1996). Tuberculosis remains a major public health issue in 2004 and according to the WHO, the mortality and morbidity statistics were 14.6 million chronic active cases, 8.9 million new cases, and 1.6 million deaths. These data were mostly found in developing countries with an expected 1% increase annually (WHO, 2006). The transmission of *M. bovis* to humans via milk and its products is eliminated by the pasteurization of milk (O'reilly and Daborn, 1995). Iraq considers as one of the highest TB burden country in the Eastern Mediterranean region. The estimated incidence of all TB forms accounted for 56/100,000 population according to WHO report (WHO/IUATLD, 2008). Cows are most susceptible to become infected in the first half year of their lives (Mitchell *et al.,* 2012). Bovine infection is characterized by a long subclinical phase with no or low intermittent shedding, ending in progressive infection with clinical signs in a small proportion of infected animals (Sweeney, 2011). The diagnosis of bovine TB in the live animal depend mainly on the basis of delayed hypersensitivity reactions. Bovine TB Infection is often subclinical. However, in case of the appearance of clinical signs, they are not specifically distinctive. The most prominent clinical signs of TB are weakness, anorexia, emaciation, dyspnea, enlargement of lymph nodes, and cough, particularly with advanced tuberculosis. After death, TB infection is diagnosed by necropsy and histopathological and bacteriological techniques. Rapid nucleic acid methodologies, such as the polymerase chain reaction (PCR), may also be used, although these are demanding techniques and should only be used when appropriately validated. Traditional mycobacterial culture remains the gold standard method for routine confirmation of infection (OIE, 2009). Although tuberculin skin test has been a hallmark of bovine tuberculosis eradication campaigns, it lacks sensitivity, and can be confounded by exposure to nontuberculous mycobacteria, and cannot be repeated for 60 days due to desensitization. To overcome these difficulties, an effective whole-blood cellular immunoassay for bovine gamma interferon has been developed (Palmer *et al.,* 2006). Al-Rubiaii *et al.,* (2013), reported that *M. bovis* infection was spreading in dairy cow within the mentioned areas and PCR was more sensitive, rapid, and accurate technique for *M. bovis* infection diagnosis. Kalaf *et al.,* (2014) recorded that twenty one cows (75%) were positive in the tuberculin test, while 7 cows (25%) were negative. Twenty two cows (78.57%) gave positive results to the antigen rapid bovine TB Ab test, and 6 cows (21.43%) gave negative results. It was noticed that most of the positive tuberculin animals were also positive to the antigen rapid bovine TB Ab test. There are a paucity in the research regarding bovine TB in Basra governorate, so this study intended to identify the prevalence of bovine Tuberculosis (TB) in different areas in Basra governorate/ Iraq.

Materials and Methods

The tuberculin tests were used to determine the prevalence of bovine tuberculosis in the selected animals (bovine and buffalo), in addition, to the laboratory test. Totally, 694 including 379 and 315, cows and buffalos respectively, were selected randomly from different regions of Basra city according to the density of animals (City center, Abu Alkhasib, Shat Alarab, Al Fao, Safwan, Al Qurna, Al Mdainah, Imam Sadiq, Imam Qaim and Al Zubair).

Principles of Comparative intradermal tuberculin test

The standard method for detection of bovine tuberculosis is the tuberculin test, which involves the intradermal injection of bovine tuberculin purified protein derivative (PPD) and the subsequent detection of swelling (delayed hypersensitivity) at the site of injection. This may be performed using bovine tuberculin alone or as a comparative test using avian and bovine tuberculin (OIE, 2009). According to Anonymous, (2006) the comparative tests are performed by intradermal injection of the PPD, bovine and avian at adjacent sites, on the neck of the animal. The interpretation of the test is based on observing, measuring and recording 72 hours after inoculation the nature and extent of any increase in skin thickness at the bovine PPD injection site. In the comparative test this response is compared to that observed 72 hours after injection of avian PPD. The reaction is considered to be inconclusive if the increase in skin thickness at the bovine site of injection is from 1 to 4 mm greater than the avian reaction. The reaction is considered to be negative if the increase in skin thickness at the bovine site of injection is less than or equal to the increase in the skin reaction at the avian site of injection.

Test procedure

According to OIE, (2009) the accuracy of field technique is important. The injection sites were clipped and cleaned in the neck of the animal and about 4 square centimeters for each with 5 cm distance between them. A prior to injection the folds of skin within each clipped area is measured using skin calipers. The site of injection was marked prior the test. Both avian and bovine tuberculin antigens supplied by (Prionics, Healthy animals Inc., UK). A short needle, bevel edge outwards and graduated syringe charged with tuberculin attached, is inserted obliquely into the deeper layers of the skin. The dose of 0.1 ml of avian antigen injected in the upper site and the dose of 0.1 ml of bovine antigen injected in the lower site of the middle third of the neck. The accuracy of injection is confirmed by palpating a small pea-like swelling at each site of injection. After 72 hours of injection, the skin-fold thickness of each injection site is re-measured. Skin thickness measurements to the nearest millimeter were reported. The change in skin thickness due to swelling or induration was calculated by subtracting the pre injection skin thickness from the post injection skin thickness (Good *et al.*, 2011).

Blood laboratory test (ELISA)

ELISA was used to detect the presence of antibody against the bovine TB infection and also to compare its results with CITT for the diagnosis of the morbidity rates of bovine tuberculosis. After 6o days, blood samples were collected from the elected animals including the animals which reveal positive results with CITT. Whole blood were collected in the test tube (without anticoagulant) from each cow and buffalo to obtain serum for the antigen rapid bovine TB Ab test. ELISA test were done in the central laboratory in Baghdad.

Results and discussion

The accurate diagnosis of bovine tuberculosis remains an elusive goal because no method has yet been developed to detect Bovine TB precisely and the presence of the microorganism in live animals in all cases **(**Solmaz *et al.,* 2009). The results of this study, showed negative results in the field CITT for the examined animals from different area in Basra governorate. Moreover, the results of ELISA test were also negative in all examined samples as well in the animals which were suspected in the CITT. These results probably due to the fact that the main factor in the infection and spread of tuberculosis is related with management of the animal such as the closed hygiene and crowded of animals in the farms. The open farm management system in Basra governorate support the results of this study, which recorded absence of the positive reaction in the examined animals. The results of this study approved the successful control and prevention program used by the veterinary directorate and the veterinary hospital for TB eradication in Basra city. This prevention program help in the elimination of infected animals and so the control of bovine tuberculosis. The mean ages of the cows and buffalos included in this study was 3.68 and 4.17 years respectively, however, the high mean of animal ages 5.10 and 7.01 years respectively in Al Zubair region was recorded. The sex of selected animals was also reported and the number of male and female cows was 90 and 289 respectively and for buffalos 43 and 272 respectively. In this study, the comparison between the results of tuberculin test and ELISA test was similar. Many researcher referred to the high percentage of cattle infected with *M. bovis*, these results might be due to the lack of efficiently control program, false result of routine skin test, and wild reservoir. In addition, to the poor nutritional condition of the animals, and the infected animals that shaded the microorganism to the environment, all these factors facilitated spreading the disease among animal herds and human population. In Iraq, the low infection rates in dairy stations maybe due to improving the routine control programs of bovine TB which include efficient diagnosis using the CITT and slaughtering of the infected animals. Solmaz *et al.,* (2009), performed the tuberculin test on 210 cattle In addition, nasal swab and milk samples were obtained from the tested animals. Three cattle that had a positive tuberculin test also revealed positive for the DNA target, a 580-bp fragment of IS6110 specific for members of the *Mycobacterium tuberculosis complex*. This fragment was also recovered from milk taken from the third animal revealed positive results for tuberculin test. All the other cattle were negative for bovine tuberculosis on both tuberculin test and PCR assay. The results of the tuberculin test and the PCR were in close correlation with each other. The Van is a border province in eastern Anatolia. In the city center and its villages, the prevalence of bovine tuberculosis was estimated as 1.42%. The negative results of rapid bovine TB. Ab. test may occur due to the fact that the low titer of Ab. to the mycobacteria Ag *M bovis* infection where the Ag is secreted to the blood circulation in a large amount causing temporary suppression for Ab formation (Krambovitis, 1986 ; Amadori *et al.,* 1998). Barak, (2012), tested 850 cows with the Comparative tuberculin test, a positive results were appeared in 373 cows with 43.88% while, 112 (13.17%) and 365 (42.94%) cows were showed suspected and negative results respectively. The immunologic test was performed on 260 serum samples taken from tuberculin tested cattle, where 135 serums gave positive results with a percentage of 51.92% , while 125 serum samples were negative with a percentage of 48.08%. It was observed that the incidence of bovine TB is rising in many parts of the world specially in Asia and Africa , whereas the standards of living is poor and this may be attributed to

lack of organized and practicable test for mass screening (Amini *et al.*, 2003). According to Al-Graibawi *et al.*, (2014), all animals in the first cow station showed negative results for the tuberculin test, whereas the percentage of positive tuberculin cows in the second and third stations were 0.4% and 16.64%, respectively. Nine months after the first investigation the rest of cows (59, 3934 and 968) in the three stations were subjected for tuberculin test and bacterial isolation as previously. All cows in the first station remained negative for tuberculin test. On the other hand, tuberculin positive cows decreased to 0.31% and 8.26% in the second and third stations, respectively. Al-Saqur *et al.*, (2009), studied sixty eight milk samples taken from AL-Fthelia- Baghdad. Conventional and molecular methods were used for the diagnosis of the disease. The results showed that three positive cases in the direct smear, seven positive by culturing on Lowenstein Jansen media and the result of conventional methods were confirmed by PCR.

The results of the present study disagreed with other studies performed in Iraq 9.23% (Kustandi, 1984), and with other studies in many countries, in Niger that was 56.2% (Boukary *et al.*, 2011) and in 58.7% in Egypt (Hassanain *et al.*, 2009). Asiak *et al.*, (2007), reported the increase in the titer of antibodies in the serum of *M bovis* infected cows, in addition, the testing cows with tuberculin test will lead to increase in production of antibodies but, it agreed with other studies that reported the decrease in infection percentage and the sensitivity of ELISA (Kazwala *et al.*, 1997; Otero *et al.*, 2003). The differences between these studies may be due to the fact that the production of antibodies in TB infected animals is variable according to the stage of the disease, where the level of antibodies is low in the early stages of the disease and rises in the late stages (Plackett *et al.*, 2008).

Table 1: The random samples of cows and buffaloes (sex and ages) from different regions in Basra city

No.	Regions	Cows		Mean of ages (year)	Buffalos		Mean of ages (year)
		male	female		male	female	
1.	City center	10	9	2.46	3	16	5.88
2.	Abu Alkhasib	8	1	1.98	8	10	2.07
3.	Shat Alarab	9	25	4.89	9	79	2.07
4.	Al Fao	1	35	4.18	-	1	6.00
5.	Safwan	-	11	4.64	-	-	-
6.	Al Qurna	-	33	2.33	-	94	2.71
7.	Al Mdainah	49	135	2.58	21	40	1.54
8.	Imam Sadiq	-	10	3.82	2	11	6.10
9.	Imam Qaim	2	9	4.86	-	-	-
10.	Al Zubair	11	21	5.10	-	21	7.01
Total		90	289	3.68	43	272	4.17

In conclusion, this study approved that CITT and determination of the antibody responses in cows and buffalos using ELISA are useful methods in identifying *M. bovis* infection. In addition, these tests can be used efficiently in the eradication and control program of the Bovine TB in Iraq. The author also suggests the collaboration between the veterinary and medical sectors in order to diagnosis, monitoring, prevention and control of bovine TB.

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