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SURVEY ON BOVINE TUBERCULOSIS IN LACTATING COWS IN KUWAIT; COMPARISON BETWEEN TUBERCULIN SKIN TESTS, ELISA AND INTERFERON-GAMMA ASSAY IN DIAGNOSIS

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ABSTRACT

A survey to screen bovine tuberculosis (BTB) in dairy cows in some cattle farms in state of Kuwait was carried out, For this purpose, a total of 3086 apparently healthy lactating cows was investigated. These included 2692 from Al-Sulaibya Area (700 from El-shawaaf, 276 from Al-Anizy, 203 from PAAF, 604 Al-Wataiya and 909 from Naif farms), 104 from El-Abdally Area (104 from El-mealy farm) and 290 from Al-Wafra Area (290 from Al-Yasimeen farm) in state of Kuwait were tested by single intradermal tuberculin test (SID) and single intradermal comparative tuberculin test (SIDC). The results revealed that the overall prevalence of bovine tuberculosis were 69 (2.23%) and 23 (0.74%) as determined by SID, and SIDC, respectively. It showed that in Al-Sulaibya Area 69 (2.56%) out of 2692 (50 (7.14%) from El-shawaaf, 3(1.08%) from Al-Anizy, 4(1.97%) from PAAF, 0(0%) from Al-Wataiya and12(1.32%) from Naif farms) were positive by SID, however, all 104 cattle from El-mealy farm (El-Abdally Area 104) and 290 from Al-Yasimeen farm (Al-Wafra Area) were negative by SID. While only 23 (3.23%) out of 700 cattle from El-shawaaf (Al-Sulaibya Area) were positive by SIDC. In the this study, ELISA was carried out for diagnosis of bovine tuberculosis on 90 blood samples from lactating cows, and revealed that the overall prevalence was 13 (14.44) as determined by ELISA. When the ELISA results compared with the results of same 90 lactating cattle by SID and SIDC. It found that overall prevalence as determined by ELISA versus SID and SIDC were 14.4% versus 5% and 1.1%. Furthermore, interferon gamma assay was applied on 161 blood samples taken from cattle of endemic farm and compared its results with the results of SID and SIDC tests. It revealed that 16 (9.9%) out of all 161 samples were positive by interferon gamma assay, whereas, 50 (31%) and 23 (14.3%) out of all 161 cattle were reactive by of SID and SIDC tests, respectively.

It could be concluded that BTB was prevalent among some examined lactating cow farms in Kuwait. This illustrated that the important role of these lactating cattle in disseminating and transmit and disseminate of such utmost zoonotic disease in the examined areas, In the present study, SIDC showed lower results than SID. This indicated that SIDC more specific in diagnosis of bovine tuberculosis than SID. ELISA showed higher sensitivities and specificities in diagnosis of BTB than SID and SIDC tests, and interferon gamma assay showed higher sensitivity than skin tests but less specific. The public health importance as well as the preventive measures which would be strictly applied for combating bovine tuberculosis were fully discussed.

INTRODUCTION

Bovine tuberculosis (BTB) is a chronic mycobacterial disease caused by the Mycobacterium bovis bacterium which mainly infect cattle and buffalo but can affects other species of mammals. BTB is a important zoonotic disease that can transmit to humans, by the inhalation of aerosols or the drinking of unheat treat milk which are typically route of transmission. TB is found ubiquitous the world

and is more prevalent in most of parts of Asia, Africa countries and of the Americas. In developed countries, eradication programs have been established to reduce or eliminate tuberculosis in cattle, therefore, human infection is now rare; however, complete eradication of the disease is difficult reservoirs due to its wildlife reservoir. BTB is still common in less developed and developing countries, and is the source of severe economic losses due to livestock deaths, chronic disease and trade restrictions, moreover, a serious health threat to humans **(The center for Food Security & public Health, 2006)**. Mycobacterium bovis is a zoonotic agent and should be treated as a risk/hazard group III microorganism with appropriate and strict precautions to prevent occurring of infection in humans must be carried out. Cattle shed M. bovis bacterium in respiratory secretions, milk and feces, and sometimes in the urine, vaginal secretions or semen. Large numbers of bacteria may be shed in the late stages of infection. Asymptomatic and carriers occur. In most cases, transmission of M. bovis between cattle through aerosols during close contact. Some animals become infected when they ingest the organism; this route may be particularly important in calves which suckle milk from infected cows **(The center for Food Security & public Health, 2009).** Dairy cows usually spend more time in crowded conditions or in enclosed areas, so, there is a higher risk of exposure. In addition unpasteurized colostrums and raw milk, which are two main sources of nourishment for calves, can also spread the disease infection among herd. BTB is difficult to diagnose with clinically only signs. In the first stages of the disease, clinical symptoms are not visible. In later stages, emaciation, weakness, lethargy, anorexia, low-grade fever, and pneumonia are the most clinical signs noticed however, may include a chronic stage, there was moist cough and lymph nodes may also be enlarged **(APHIS, 2015**).

Therefore, BTB is difficult to diagnose antimortem by clinical symptoms. The majority of bovine cases are diagnosed by routine testing or found at the slaughterhouse. In live cattle, tuberculosis is usually diagnosed in the field with the tuberculin skin test. In this test, tuberculin is injected in the skin a positive test result is a delayed hypersensitivity reaction (swelling). Intradermal tuberculin test is the definitive screening test that is currently most practically common used test which and also prescribed by OIE for international trade. Protein material extracted from the cell wall of the Mycobacterium was used as tuberculin in solution. When this protein solution is injected into the skin of an infected animal, the body's sensitised immune response will cause a localized inflammatory reaction that leads to the typical signs of a positive tuberculin test **(Department of Agriculture Forestry & Fisheries, Republic of South Africa, 2016).**

Although laboratory serological tests must be not regarded first choice of diagnostic techniques for BTB, however, The indirect ELISA technique measures the binding of specific antibodies to an antigen is a simple and specific test, but sensitivity is mostly limited because of the late and irregular development of response by humoral immune reaction in cattle during the course of the disease **(de la Rua-Domenech *et al.*, 2006)**.In order to diagnose cattle infected by *M. bovis*, antigens usually employed are the PPD and single or associated purified antigens from *M. bovis* which have achieved a sensitivity and specificity of the assay around 90% **(McNair *et al.*, 2001; Faye *et al.*, 2011).**

Since 2006, The lymphocytes taken from diseased cattle produced IFNγ after activation with PPD. Afterthat, IFNγ produced by T lymphocytes can be measured by using monoclonal anti-IFNγ. Cattle were negative for infection to *M. bovis*, by the lack of detection of IFNγ, whereas, the lymphocytes from

uninfected cattle do not produce of IFNγ. The interferon gamma had higher in the sensitivity **(Neill *et al*., 1994; Wood and Jones, 2001; Schiller *et al*., 2010; Faye *et al.*, 2011).**

Incidence of tuberculosis (per 100,000 people) in Kuwait was recorded at 22 in 2015, according to the World Bank collection of development indicators, compiled from officially recognized sources **(Trading Economics. 2017)**. There was little information and research about bovine tuberculosis in Kuwait, From the veterinary economic and zoonotic and public health significance of bovine tuberculosis, this work was performed to study the prevalence of bovine tuberculosis in dairy cows in Kuwait. Also, use of single intradermal tuberculin test (SID) and single intradermal comparative tuberculin test (SIDC) compared to ELISA and interferon gamma assay was assessed.

MATERIAL AND METHODS

Animals:A total of 3086 apparently healthy dairy cows. These included 2692 from Al-Sulaibya Area (700 from El-shawaaf, 276 from Al-Anizy, 203 from PAAF, 604 Al-Wataiya and 909 from Naif farms), 104 from El-Abdally Area (104 from El-mealy farm) and 290 from Al-Wafra Area (290 from Al-Yasimeen farm) in state of Kuwait were investigated in this study. All animals were assessed by single intradermal tuberculin test (SID) and single intradermal comparative tuberculin test (SIDC). The animals were rearing under different managemental system. The breeds of cattle are Friesian and Australian. Ages of cows were ranged from 2-8 old years.

Blood samples: Ninety blood samples were taken from examined animals. Of which, 70 from Al-Sulaibya Area (20 from El-shawaaf, 10 from Al-Anizy, 10 from PAAF, 10 Al-Wataiya and 20 from Naif farms), 10 from El-Abdally Area (10 from El-mealy farm) and 10 from Al-Wafra Area (10 from Al-Yasimeen farm) were testing by ELISA. Samples were kept overnight at 4° C to allow for separation of serum, then centrifuged at 1000 g. for 10 minutes to obtain amber clear serum. Sera were kept at -20°C each in 2 aliquots in sterile bijou bottles till examined. Meantime a total of 161 whole blood samples were taken from 161 animals from endemic farm to be tested by interferon gamma assay for compared its results tosingle intradermal tuberculin test and single intradermal comparative tuberculin test results.

Single intradermal tuberculin test (SID): Single intradermal (SID) tuberculin test was performed according to **Ovdienkop *et al.* (1987).** Briefly, A narrow zone at the middle third of the neck of the tested animals was marked after clipping the hair using curved scissors with rounded end points and disinfected withunirritant antiseptic. The skin thickness was measured using the caliper. Thereafter, 0.1 ml of mammalian PPD tuberculin prepared by Bacterial Diagnostic Products Research Department, Veterinary Serum and Vaccine ResearchInstitute, Abbassia, Cairo, Egypt) was injected intradermal by using an automatic syringe, The skin thickness was measured 72 hours post injection and the results were interpreted as follows: A reaction is usually considered to be positive if the increase in the skin thickness of 4 mm or more, whereas, less than 3 mm was considered negative result, and from 3-4 mm was considered doubtful.

Single intradermal comparative tuberculin test (SIDC): SIDC was carried out according to **OIE (2009)** using bovine or avian PPD tuberculin. 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. 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 **(OIE, 2009)**.

Enzyme Linked Immuno Sorbant Assay (ELISA): It carried on 90 bovine serum samples according to **Hall and Thoen (1985)**: In brief, Bovine PPD antigens kindly provided from Bacterial Diagnostic Products Department of Vaccine and Serum Veterinary Research Institute, Cairo, Egypt, were firstly diluted to 15 µg /ml in 0.1 M sodium carbonate (pH 9.6). Each well of round-bottomed, poly vinyl plates were coated with 50 µl of Bovine PPD antigens with the exception of 4 wells which served as blank wells. Each well then received 50 µl of carbodiimide in 0.1 M sodium carbonate buffer. The plates were placed in plastic bags and incubated at 4^0C over night. After incubation, the plates were decanted, washed 3 times with phosphate buffered saline solution and air dried for 10 minutes, then incubated for 30 minutes at 22^0C with 0.01 M ammonium chloride 100 µl/ well except the blank wells, and washed 3 times with ELISA wash solution. Sera were diluted 1:40 in ELISA diluent. One hundred microliters was added to the first row of wells of the micro titer plates. Serial dilutions of serum were made by transferring 50 µl of serum to separate wells that contained 50 µl of diluent (two fold dilutions were made down the plates). Each plate received negative sera run in the same dilutions to calculate the cut-off value, and then incubated at room temperature for 30 minutes on a horizontal shaker. After incubation, the plates were decanted, washed 8 times with ELISA wash solution and allowed to stand inverted for 30 minutes. thereafter, to each well, 50 µl of antibovine IgG alkaline phosphates diluted 1.5000 in diluting buffer was added then the plates were incubated for 30 minutes at room temperature on a horizontal shaker. The plates were washed 8 times with ELISA wash again and allowed to stand inverted for 30 minutes. The optical density was measured at 405 nm using spectra III. The result was calculated as a serum dilution was considered positive if it yielded a mean OD of each group equal to / or greater than the cut off value.**(Dimitri *et al.*,1990**). Which equal to the mean OD of negative serum plus 2 standard deviation according to **(Nassau *et al.* 1976)**.

Interferon Gamma Assay (IFNγ assay): It performed on 161 bovine whole blood samples according to **OIE (2009)** and Manufacturer's manual of BOVIGAM. The assay is based on the release of IFN-γ from sensitised lymphocytes during a 16–24-hourincubation period with specific antigen (PPD-tuberculin) **(Wood, *et al.*, 1990 and Coad *et al.*, 2007)**. The detection of bovine IFN-γ is carried out witha sandwich ELISA that uses two monoclonal antibodies to bovine gamma-interferon **(Ryan *et al.*, 2000)**. The *Mycobacterium bovis* Gamma Interferon test kit for cattle, BOVIGAM, Product Number: 63329/63320/63326, USA, which is In Vitro diagnostic test kit for detection of bovine tuberculosis infection in cattle were used according to Manufacturer's manual.Antigens: BOVIGAM bovine and BOVIGAM avian PPD tuberculin (0.3mg/ml). The dilution and handling of all BOVIGAM kits were according to according to guideline outlines in procedures notes of Manufacturer's manual.

Table (1): Prevalence rate of tuberculosis in examined farms as determined by single intradermal tuberculin test in relation to locality.

Farm Name	location	No. of animals	SID +ve No	Prevalence rate
El-shawaaf	Al-Sulaibya	700	50	7.14
Al-Anizy	Al-Sulaibya	276	3	1.08
PAAF	Al-Sulaibya	203	4	1.97
Al-Wataiya	Al-Sulaibya	604	0	0
Naif	location	909	12	1.32
Al-Sulaibya Area		**2692**	**69**	**2.56**
El-mealy	El-Abdally	104	0	0
El-Abdally Area		**104**	**0**	**0**
Al-Yasimeen	Al-wafra	290	0	0
Al-wafra Area		290	0	0
Total		**3086**	**69**	**2.23**

Table (2): Prevalence rate of tuberculosis in examined farms as determined by single intradermal comparative tuberculin test in relation to locality.

Farm Name	location	No. of animals	SIDC suspected		SIDC Positive	
			No	%	No	Prevalence rate
El-shawaaf	Al-Sulaibya	700	27	3.85	23	3.28
Al-Anizy	Al-Sulaibya	276	1	0.36	0	0
PAAF	Al-Sulaibya	203	3	1.47	0	0
Al-Wataiya	Al-Sulaibya	604	0	0	0	0
Naif	Al-Sulaibya	909	9	0.99	0	0
Al-Sulaibya Area		**2692**	**40**	**1.48**	**23**	**0.77**
El-mealy	El-Abdally	104	0	0	0	0
El-Abdally Area		**104**	**0**	**0**	**0**	**0**
Al-Yasimeen	Al-wafra	290	0	0	0	0
Al-wafra Area		**290**	**0**	**0**	**0**	**0**
Total		**3086**	**40**	**1.29**	**23**	**0.74**

Table (3): Prevalence rate of tuberculosis in examined farms as assessed by ELISA test in relation to locality.

Farm Name	location	No. of Serum samples	No. of ELISA+ve	Prevalence rate
El-shawaaf	Al-Sulaibya	20	3	15
Al-Anizy	Al-Sulaibya	10	1	10
PAAF	Al-Sulaibya	10	0	0
Al-Wataiya	Al-Sulaibya	10	2	20
Naif	Al-Sulaibya	20	5	20
Al-Sulaibya Area		**70**	**11**	**14.28**
El-mealy	El-Abdally	10	1	10
El-Abdally Area		**10**	**1**	**10**
Al-Yasimeen	Al-wafra	10	2	20
Al-wafra Area		10	2	20
Total		**90**	**13**	**14.44**

Table (4): Results of single and single intradermal comparative tuberculin test compared to ELISA results in diagnosis of bovine tuberculosis with respect to locality.

Farm Name/ Location	**No. of tested**	**SID test**		**SIDC test**		**ELISA**	
		No. of +ve	**%**	**No. of +ve**	**%**	**No. of +ve**	**%**
El-shawaaf	20	2	10	1	5	3	15
Al-Anizy	10	1	10	0	0	1	10
PAAF	10	1	10	0	0	0	0
Al-Wataiya	10	0	0	0	0	2	20
Naif	20	1	5	0	0	5	20
Al-Sulaibya Area 70		5	7.4	1	1.42	10	14.3
El-mealy	10	0	0	0	0	1	10
El-Abdally Area 10		**0**	**0**	**0**	**0**	**1**	**10**
Al-Yasimeen	10	0	0	0	0	2	20
Al-wafra Area 10		**0**	**0**	**0**	**0**	**2**	**20**
Total	**90**	**5**	**5.5**	**1**	**101**	**13**	**14.4**

Table (5): Relationship between results of tuberculin tests and Gamma interferon assay in diagnosis of bovine tuberculosis in endemic farms.

Test		SID		Total	SIDC test			Total
		-Ve	+Ve		-Ve	±Ve	+Ve	
Gamma IFN=(161)	+Ve	5 (4.5%)	11 (22%)	16 (9.9%)	5 (4.5%)	4 (14.8%)	7 (30.4%)	16 (9.9%)
	-Ve	106 (96.4%)	39 (78%)	145 (90%)	106 (95.5%)	23 (85.2%)	16 (69.6%)	145 (90%)
Total		110 (68.3%)	50 (31%)	161	111 (68.9%)	27 (16.8%)	23 (14.3%)	161

RESULTS & DISCUSSION

Bovine tuberculosis is a chronic mycobacterial disease of ubiquitous worldwide occurrence that causes economic losses and public health problems. *Mycobacterium bovis* (*M. bovis*) is the cause of BTB which is also pathogenic for a large number of other animals, and its transmission to human constitute a public health hazard (**Ameni *et al.*, 2007**). In a large numbers of developing countries BTB is a major infectious disease among cattle, other domesticated animals and cretin wildlife population **(OIE 2009).**Although human tuberculosis is mainly caused by *M.tuberculosis* but in areas and countries where BTB is prevalent in animals, human Tuberculosis cases due to *M. bovis* may occur (**Thoen *et al.*, 2006**). BTB is the disease of cattle in intensive rearing resulted in a serious effect on animal production, has a significant public health importance (**O'Reilly and Daborn, 1995**). Diagnosis of the BTB in the herd based on tuberculin skin testing which is of utmost importance as a good herd test and in eradication program for BTB in most developed countries. However this test lacks sufficient sensitivity and specificity (**Palmer *et al.*, 2006, Costello *et al.*, 1997**).

In the present study, single intradermal tuberculin test (SID) and single intradermal comparative tuberculin test (SIDC) were used for diagnosis of bovine tuberculosis and detection of naturally infected cases in a total of 3086 apparently healthy lactating cows. Which included 2692 from Al-Sulaibya Area (700 from El-shawaaf, 276 from Al-Anizy, 203 from PAAF, 604 Al-Wataiya and 909 from Naif farms), 104 from El-Abdally Area (104 from El-mealy farm) and 290 from Al-Wafra Area (290 from Al-Yasimeen farm) in state of Kuwait. In the present study the overall prevalence of bovine tuberculosis were 69 (2.23%) and 23 (0.74%) as determined by SID, and SIDC, respectively (Table 2, 3). The suspected overall prevalence of bovine tuberculosis result by SIDC was 40 (1.29%). The results revealed that in Al-Sulaibya Area was 69 (2.56%) out of 2692 (50 (7.14%) from El-shawaaf, 3(1.08%) from Al-Anizy, 4(1.97%) from PAAF, 0(0%) from Al-Wataiya and12(1.32%) from Naif farms) were positive by SID, while, all 104 cattle from El-mealy farm (El-Abdally Area 104) and 290 from Al-Yasimeen farm (Al-Wafra Area) were negative by SID. While only 23 (3.23%) out of 700 cattle from El-shawaaf (Al-Sulaibya Area) were positive by SIDC. The suspected prevalence of bovine tuberculosis results by SIDC were in Al-Sulaibya Area was 40 (1.48) out of 2692 (27(3.85%) from El-shawaaf, 1(0.36%) from Al-Anizy, 3(1.47%) from PAAF, 0(0%) from Al-Wataiya and 9(0.99%) from Naif farms (Table1,2). Nearly similar results were previously reported in Egypt by

El-Sawah (2008) and Hazem (2012) as they recorded the overall occurrence bovine TB was 0.96 % in Egypt as determined by SID tuberculin test**.** However, higher occurrence has been previously recorded in Egypt by many investigators as, **ELSabban *et al.* (1992)**; **Hassan (2008)**, **Naglaa (2008)**; **Mossad *et al.* (2009)**;**Marwa (2010)** and **Moussa *et al.* (2011)**. Their respective percentages were(24%), (12.4%), (21.41%), (4.6%), (2.46%) and (2.46%). Also the present results were lower the Annual report of the General Organization of Veterinary Services in Egypt **(GOVS, 1992)** which announced that the prevalence of bovine tuberculosis in cattle during 2009 was high in certain governorates such as Dakahlia and Behera and Alexandria which were 9.6% 14.06 and % 6%, respectively. Also it was lower than that reported in other countries by **Waddington(1965)** (6.6 %) in Kenya; **Eid, 1975** (25 %) in Northern Nigeria, **Oliveira *et al.*, 1983** (3.2 %) in Brazil, 4.3% in Argentinaby **Pan America Zoonoses Center (1988)**, **Ameni *et al.* (2007)** (48%) and (13.5%) in Ethiopia by **Dinka and Duressa (2011).** However the present results was higher than recorded by **Johns, 1969** (0.4 %) in New Zealand and **Clay, 1971** (0.05 % to 0.15 %) in Australia. **Nemoto (1972)** (0.02%) in Japan; **Delgado and Trujllo (1975)** (0.54%) in Venezuela and **Mohamed (2003) (**0.18%) in Egypt. Tuberculin skin testing is recognized as a universally test for the diagnosis of TB in live cattle, and forms and it is the basis for national test and slaughter programmes (**Ayele *et al.*, 2004**). In the present study, SIDC tuberculin test showed lower results than SID in diagnosis of bovine tuberculosis (0.74% versus 2.23). This might be SIDC more specific in diagnosis of bovine tuberculosis as previously mentioned by **OIE (2009).** Moreover, **Rhodes *et al.*, (2015).** reported that the use of avian and bovine tuberculin allows the differentiation between bovine TB and other mycobacterial species, principally avian TB, thereby significantly improving the specificity. The advantage of this test lies in its excellent specificity (generally considered around 99.5%) which makes it very suitable for herd level testing, Furthermore, Single Intradermal Comparative Cervical Tuberculin (SICCT) test for bovine tuberculosis showed specificity of 99.98% equating to one false positive result per 5000 uninfected animals tested in Great Britain **(Goodchild, *et al.*,2015).**

In the present study, ELISA was performed for diagnosis of bovine tuberculosis on 90 blood samples from lactating cows. Which included 70 from Al-Sulaibya Area (20 from El-shawaaf, 10 from Al-Anizy, 10 from PAAF, 10 Al-Wataiya and 20 from Naif farms), 10 from El-Abdally Area (10 from El-mealy farm) and 10 from Al-Wafra Area (10 from Al-Yasimeen farm) in state of Kuwait.In the present study, the overall prevalence of bovine tuberculosis as determined by ELISA was 13 (14.44) (Table 3). The results revealed that in Al-Sulaibya Area was 11 (15.71) out of 70 (3(15%) from El-shawaaf, 1(10%) from Al-Anizy, 0(0%) from PAAF, 2(20%) from Al-Wataiya and 5(20%) from Naif farms) were seroreactors, while, 10% in El-Abdally Area (El-mealy farm) and 20% of Al-Wafra Area (Al-Yasimeen farm) were seropositive by ELISA (Table 3). ELISA technique was applied as a sensitive method for measurement of antibodies in sera of tuberculous animals as one of important in serodiagnosis of bovine tuberculosis **(Engvall and Perlmann, 1972).Dimitri (1987)** recorded that the sensitivity and specificity of ELISA used for diagnosis of tuberculosis reach to 100% and 81.8% in cattle and buffalo respectively. **Soad *et al.* (1994)** reported that the accuracy parameters of measurement of IgG were index, sensitivity, specificity, positive and negative predicative values on using PPD antigen in ELISA were 84.8%, 73.3%, 94.4%, 91.4%, 91.6%, and 80.95%, respectively. **Liu *et al.***

(2007) demonstrated that the sensitivity of the ELISA was 69.4 % (59/85) and specificity was 96 % (96/100). Moreover, **Marwa (2010)** declared that 20 out of 29 (68.96%) cattle were positive and 9 out of 29 (31.03 %) cattle were negative in ELISA using Mycobacterium bovine sonic extract antigen, while 22 out of 29 (75.86 %) cattle were positive and 7 out of 29 (24.13 %) cattle were negative in ELISA using Bovine purified protein derivatives antigen. **Anita *et al.*(2015)** reported that of the 2661 cattle tested, 1.4 % (95 % CI) were ELISA positive whereas, of the 154 herds tested, 18.1 (95 % CI) contained at least one ELISA positive animal**.**

In the present study, the results of single and single intradermal comparative tuberculin test compared to ELISA results in diagnosis of bovine tuberculosis with respect to locality were evaluated on 90 blood samples from lactating cows. Which included 70 from Al-Sulaibya Area (20 from El-shawaaf, 10 from Al-Anizy, 10 from PAAF, 10 Al-Wataiya and 20 from Naif farms), 10 from El-Abdally Area (10 from El-mealy farm) and 10 from Al-Wafra Area (10 from Al-Yasimeen farm) in state of Kuwait. In the present study the overall prevalence of bovine tuberculosis as determined by ELISA versus SID and SIDC were 14.4% versus 5% and 1.1% (Table 4). The results revealed that in Al-Sulaibya Area was 14.3% versus 7.7% and 1.42% (from El-shawaaf was 15% versus 10% and, from Al-Anizy was 10% versus 10% and 0%, from PAAF was 0% versus 10% and 0%, from Al-Wataiya was 20% versus 0% and 0% and from Naif farm was 20% versus 5% and 0%) seroreactors, while, in El-Abdally Area (El-mealy farm) was 10% versus 0% and 0%, lastly from Al-Wafra Area (Al-Yasimeen farm) was 10% versus 0% and 0% seropositive (Table 4). The present results revealed that ELISA showed higher sensitivities and specificities in diagnosis of bovine tuberculosis than SID and SIDC tests. These was confirmed by studies of many authors **(Engvall and Perlmann, 1972; Ayanwale, 1987; Daniel and Debann, 1987; Dimitri, 1987** and **Soad *et al.*, 1994). Plackett *et al.*, (1989)** suggested the use of ELISA with the tuberculin test to overcome the problems of tuberculin test. Moreover, **Silva *et al.* (1997)** examined 1808 cattle from farms in areas notified free of tuberculosis. They performed tuberculin test and took serum samples to be tested by ELISA. Specificity of ELISA was 95.7%. The specificity and ease of use of ELISA make it an important tool in detection of tuberculosis antibodies in cattle in disease free areas. **FAO (2001)** mentioned that serological tests as ELISA can be useful when used in conjunction with intradermal tuberculin test or (IFN) test in diagnosis of tuberculosis. **Bonar *et al.* (2004)** who reported that the sensitivity of ELISA was 70.9 % and specificity was 84 %. **Anita *et al.* (2015)** concluded that the lack of agreement between the results of the two tests (skin and ELISA) may reflect different elements of the immune response (humoral and cell-mediated immunity) to be measured by both tests. They recommended, in future, cattle should be sensitized by the intradermal injection of tuberculin 14 days prior to the collection of blood samples, which would then be tested by the Mycobacterium bovis ELISA Test in order to determine more accurately the prevalence of infection.

In the present study we tested 161 blood samples from cattle of endemic farm by interferon gamma assay and compared its results with the results of SID and SIDC tests. The results revealed that 16 (9.9%) out of all 161 samples were positive by interferon gamma assay, whereas, 50 (31%) and 23 (14.3%) out of all 161 cattle were reactive by of SID and SIDC tests, respectively (Table 5). The results revealed that interferon gamma assay showed higher sensitivity than skin tests but less specific. These finding was supported by**(Rothel *et al.*, 1992)** who found that the

assay system has proven to be a sensitive, rapid, and inexpensive method for measuring antigen specific cell-mediated reactivity. Moreover, **Defra (2008)** stated that the g-IFN test has good sensitivity, appears to detect infected animals earlier than the skin test and can be repeated as often as necessary without the need to wait 60 days between tests. Furthermore, **Agriculture and Horticulture Development Board (2015)** announced the gamma test is more sensitive than the skin test (~90% compared to ~81%), and on average, the gamma test has a specificity of 96.6%1, while, the tuberculin skin test has a higher specificity of 99.98%. So, the gamma test is a supplementary blood test used alongside the skin test to increase the chances of identifying TB-infected cattle in specific TB breakdown situations.

It could be concluded that bovine tuberculosis was prevalent in some dairy cattle farms in Kuwait state, SIDC showed lower results than SID in diagnosis of bovine tuberculosis (0.74% versus 2.23). This might be SIDC more specific in diagnosis of bovine tuberculosis. ELISA showed higher sensitivities and specificities in diagnosis of bovine tuberculosis than SID and SIDC tests. Furthermore, interferon gamma assay showed higher sensitivity than skin tests but less specific. Effective implementation of control measures including test and slaughter strategy of the infected animals, rigidity of meat inspection, public health education and other strict hygienic measures which help in the control of tuberculosis in the dairy farms must be applied. Tuberculous carcasses should be incinerated immediately after aseptic removal of tissues for culture and histopathology. Further studies concerning isolation and biochemical, molecular typing and sequencing of the recovered mycobacterial strains in Kuwait are necessary required.

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الملخص العربي

مسح لمرض الدرن البقري في الأبقار الحلابة في الكويت: مقارنة بين اختبارات التيوبروكلين الجلدي والأليزا والانترفيرون جاما في التشخيص

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في هذه الدراسة تم إجراء مسح لاستبيان مدي انتشار مرض الدرن في الأبقار الحلابة في بعض مزارع الألبان بالكويت. لهذا الغرض قد تم فحص عدد كلي حلابة سليمة صحيا والتي شملت ٢٦٩٢ من منطقة الصلبية (٧٠٠ من مزرعة الشواف، ٢٧٦ من مزرعة العنزي، ٣٠٨٦ بقرة ٢٠٣ من مزرعة الهينه(PAAF) و ٦٠٤ من مزرعة الواطنية و ٩٠٩ من مزرعة نايف)، ١٠٤ من منطقة العبيدلي (١٠٤ من مزرعة الميلي) و٢٩٠ من منطقة الوافرة (٢٩٠ من مزرعة الياسمين) في دولة الكويت بواسطة اختبارات التيوبروكلين الجلدي الفردي (البقري) والمقارن(البقري والداجني)

by single intradermal tuberculin test (SID) and single intradermal comparative tuberculin test (SIDC).

أوضحت النتائج إن معدل الانتشار الكلي لمرض الدرن في الأبقار الحلابة كان ٦٩ (٢,٢٣%) و٢٣ (٠,٧٤%) بواسطة اختبارين التيوبروكلين الجلدي الفردي والمقارن علي التوالي. حيث ان ٦٩ (٢,٥٦%) من مجموع ٢٦٩٢ من منطقة الصلبية (٥٠ (٧,١٤%) من مزرعة الشواف، ٣(١,٠٨%) مزرعة العنزي، ٤(١,٩٧%) من مزرعة الهينه، (PAAF), (٠%) من مزرعة الواطنية و ١٢(١,٣٢%) من مزرعة نايف) أظهرت نتائج ايجابية بواسطة اختبار التيوبروكلين الجلدي الفردي، في حين إن كل ١٠٤ بقرة من منطقة العبيدلي (١٠٤ من مزرعة الميلي) و٢٩٠ من منطقة الوافرة (٢٩٠ من مزرعة الياسمين) كانت سالبيه لاختبار التيوبروكلين الجلدي الفردي. بينما ٢٣ بقرة (٣,٢٣%) من مجموع ٧٠٠ بقرة من مزرعة الشواف (منطقة الصلبية) كانت موجبة لاختبار التيوبروكلين الجلدي المقارن. في هذه الدراسة تم استخدام اختبار الأليزا لتشخيص مرض الدرن في٩٠ عينة دم من ٩٠ من الأبقار الحلابة وكان معدل الانتشار الكلي بها هو١٣ (١٤,٤٤%). وعندما تم مقارنة تلك النتائج بنتائج اختبارين التيوبروكلين الجلدي الفردي والمقارن وجد الانتشار الكلي بواسطة اختبار الأليزا مقارنتا بنتائج اختبارين التيوبروكلين الجلدي الفردي والمقارن هو١٤,٤% مضادا ٥% و ١,١% بواسطة اختبارين التيوبروكلين الجلدي الفردي والمقارن علي التوالي.

علاوة علي ذلك تم في هذه الدراسة استخدام اختبار الأنترفرون جاما لتشخيص مرض الدرن في ١٦٠ عينة دم من ١٦٠ من الأبقار الحلابة في المزرعة المتوطن بها المرض مقارنتا بنتائج اختبارين التيوبروكلين الجلدي الفردي والمقارن. أثبتت النتائج ان ١٦ (٩,٩%) موجبة لاختبار الأنترفرون جاما، في حين ان كان ٥٠(٣١%) و ٢٣(١٤,٣%) من ١٦١ بقرة كانت موجبة بواسطة اختبارين التيوبروكلين الجلدي الفردي والمقارن علي التوالي.

ويستخلص من الدراسة ان مرض الدرن البقري منتشر في الأبقار الحلابة في بعض مزارع الألبان بالكويت التي شملتها الدراسة مما يؤكد الدور المحوري الهام لتلك الحيوانات في انتشار تلك المرض المشترك ذات الأهمية الصحية. اختبار التيوبروكلين الجلدي المقارن اظهر نتائج ايجابية اقل من اختبار التيوبروكلين الجلدي الفردي، ويشير ذلك ان المقارن أكثر نوعية من الفردي في تشخيص مرض الدرن في الأبقار. وكذلك اختبار الأنترفرون جاما كان أكثر حساسية ولكنه اقل ونوعية من اختبارين التيوبروكلين الجلدي الفردي والمقارن في تشخيص مرض الدرن. وقد تم مناقشة الأهمية الصحية وكذلك الإجراءات الوقائية التي يجب تطبقها بصرامة لمكافحة المرض.