

## The incidence of bovine tuberculosis and its public health hazards in a dairy cattle station in Iraq

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### Abstract

Bovine tuberculosis (TB) considered one of the important zoonotic diseases in the world specially in economic and public health field. The aim of this study is to determine the incidence of bovine TB in a dairy cattle station in Iraq, also the hazards on public health. The study was conducted on 850 cows and on 25 workers and veterinary doctors in the station. The comparative intradermal tuberculin test (CITT) was done on the cows and the single tuberculin test was done on the workers using the mammalian antigen. 260 Serum samples were taken from cows and 25 serum from workers and submitted to ELISA test, it was found that 43.88% of cows were positive to comparative tuberculin test and 51.92% were positive to ELISA, while in workers the positive results were 36% and 32% in tuberculin and ELISA respectively. 45 milk, 45 nasal swabs, 20 liver, 18 spleen, 30 lung and 30 lymph nodes from cattle and 25 sputum samples from workers were taken for bacterial isolation on Lowenstein- Jensen and stone brink media and the tubercle bacilli were isolated from 20 milk sample, 11 nasal swabs, 13 livers, 10 spleens, 23 lungs and 26 lymph nodes and 103 Mycobacterium isolates were diagnosed according to the biochemical tests results as 100 *M bovis* and 2 Runyon IV mycobacterium and one isolate as Runyon III. The presence of *M bovis* in milk samples represent an important factor in infection of humans and other animals.

نسبة حدوث مرض السل البقري وخطورته على الصحة العامة في إحدى محطات الأبقار في العراق

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### الخلاصة

يعتبر مرض السل من الأمراض المشتركة المهمة في العالم خصوصا من الناحية الاقتصادية والصحة العامة، إن الهدف من هذه الدراسة هو التعرف على نسبة الإصابة بمرض السل البقري في إحدى محطات الأبقار في العراق وخطورته على الصحة العامة، حيث أجريت الدراسة على 850 بقرة و 25 عامل وطبيب بيطري في المحطة حيث اجري فحص السلين المقارن على الأبقار وفحص السلين الأحادي على العاملين، وأخذت 260 عينة مصل من الأبقار و 25 عينة مصل من العاملين واجري عليها فحص الاليزا ووجد إن 43.88% من الأبقار أعطت نتائج موجبة لفحص السلين المقارن و 51.92% أعطت نتائج موجبة لفحص الاليزا بينما في العمال كانت النتائج الموجبة 36% بفحص السلين و 32% بفحص الاليزا. أخذت عينات من الأبقار والعمال لإجراء العزل الجرثومي لعصيات السل حيث اخذ 45 عينة حليب و 45 مسحة أنفية و 20 كبد و 18 طحال و 30 رئة و 30 عقدة لمفية من الأبقار وكذلك أخذت 25 عينة قشع من العمال وعزلت عصيات السل من 20 حليب و 11 مسحة أنفية و 13 كبد و 10 طحال و 23 رئة و 26 عقدة لمفية و 2 عينة قشع، وبعد إجراء الاختبارات الكيمياءحياتية على العزلات شخصت 100 عزلة على أنها *M bovis* وعزلتان من المايكوبكتريا الرمية Runyon IV وعزلة واحدة من المايكوبكتريا Runyon III. إن وجود *M bovis* في عينات الحليب تمثل عاملا مهما في إصابة الإنسان والحيوانات الأخرى.

## Introduction

Bovine tuberculosis is a chronic infectious disease caused by *Mycobacterium bovis*, Which remain an economic and public health problem in several countries, it can be transmitted from animal to human and vice versa and the most common route of infection is inhalation of aerosols containing mycobacterium which may be coughed out by infected animals, another source of infection is by ingestion, through breaks in the skin and congenital by infected semen (1). The disease is a significant zoonosis can spread to humans by inhalation of aerosols or ingestion of unpasteurized milk, cattle shed *M. bovis* in feaces, milk and some times in urine, vaginal secretions, so bovine tuberculosis still wide spread in Africa, parts of the middle east countries (2). T.B. is a major opportunistic infection of HIV-infected persons. The epidemic of HIV infection in developing countries particularly countries in which *M. bovis* is present in animals and the conditions favor zoonotic transmission, could make zoonotic TB a serous public health threat to persons at risk (3, 4). The successful control programs for bovine TB depend on the use of a vary efficient and reliable diagnostic tests to determine the infected animals (5). Many methods are available for diagnosis of bovine tuberculosis in animals and tuberculin test has been used to determine the prevalence of infection in animal and human. Also, the purified protein derivatives (PPD-tuberculin) is used to diagnose T. B. in human and animals (6). Serological tests used also in the diagnosis of T.B. as alternative to skin tests and culture. Many researches recorded high specificity of ELISA for diagnosis of T.B. which make it an important tool in the detection of tuberculosis in cattle in disease free areas (7). The main purpose of this study is to estimate the incidence and public health importance of bovine tuberculosis in dairy cattle maintained in a privet cattle station in Iraq using the comparative intradermal tuberculin test and ELISA for detecting the source of infection to farm workers through mycobacterial culture of samples.

## Materials and Methods

- **Collection of samples:** This study was done in a dairy cattle station around Baghdad suffering from emaciation, chronic respiratory disorders and loss of milk production. 260 serum samples, 45 milk samples and 45 nasal swabs were collected from cattle, while 25 serum samples and 25 sputum samples were collected from farm workers and veterinarians that are in contact with the animals and the milk samples were collected in a sterile tubes 50-100 ml while the nasal swabs were collected by a sterile cotton swabs. 20 liver, 18 spleen, 30 lungs and 30 lymph nodes were collected after slaughtering the infected animals All samples were transported to the laboratory in a cold and sterile containers, the milk and nasal swabs were kept at 4°C in the laboratory until the bacterial isolation is done next day, while the organs were preserved at -20 °C.
- **Comparative intradermal tuberculin test:** The test was carried out on 850 cows present in the station according to (8) by injecting the avian and bovine antigens (Institute of animal science and health- Leyland, Holland) intradermally and reading the results after 72 hrs.
- **Tuberculin test of farm workers:** Human tuberculin antigen prepared from *M. tuberculosis* (biomerix- France) was injected in the skin of the dorsum of the hand. The skin was read after 48-72 hrs. It is considered positive if the injection was followed by in duration of 10 mm or more diameter (9).

- **Bacterial isolation:** The samples were processed for isolation of mycobacterium following standard procedures for homogenization, suspension, centrifugation and decontamination. The processed samples were inoculated on Lowenstein- Jensen and stone brink media with and without pyruvate and incubated at 37c for a period of 6-8 weeks. Then the colonies were identified by the following biochemical tests (Nitrate reduction test- Niacin test- Catalaze test- tween-80 hydrolysis test) (10).
- **Enzyme linked immunosorbant assay (ELISA):** It was performed on 260 serum samples from cattle and 25 serum sample from farm workers by using ELISA kit for tuberculosis (SVANOVIR- Sweden) (11) and the optical density was measured by micro-ELIZA reader and the animals conceded positive if the optical density was above 0.2 nm (12).

### Results

- **Comparative tuberculin test in cattle:** Out of 850 cows tested with the Comparative tuberculin test 373 cows with 43.88% gave positive results, where 112 cows with 13.17% gave suspected results, while the negative cows were 365 with 42.94%(Table 1).
- **Bacterial isolation:** The bacterial isolation was performed on the Comparative tuberculin test positive cows and *M. bovis* was isolated from 20 milk with percentage 44.44% and 11 nasal swabs 24.44% and 13 liver 65% and 10 spleen 55.5 and 23 lungs 76.66 and 26 lymph nodes 86.66 (Table 2).

It was noticed the growth of the colonies on culture media after 6-8 weeks of culturing and most of colonies were small, moist, smooth, shiny, white to gray in color, non-pigment forming and some of the colonies were large, rough, dry and attached to the media. Smears from colonies were stained with zehal-nelson stain showed the small red rods of the *M. bovis* and all the isolates were negative to the niacin test, catalaze test, nitrate reduction test and the tween-80 hydrolysis test which indicate that it is *M bovis* except (2) isolates that gave variable results for niacin test, nitrate reduction test and tween-80 hydrolysis test and positive result to catalaze test which indicate that it is mycobacterium Runyon IV, and one isolate was negative to nitrate reduction and niacin tests and positive to catalaze test and variable to tween-80 hydrolysis test which indicate it is mycobacterium Runyon III(Table3). The number of *M bovis* isolates was 100 and it was isolated from all samples while the two isolates of mycobacterium Runyoun IV were isolated from milk and nasal swab, while the mycobacterium Runyon III was isolated from milk. Out of 25 sputum samples from the workers only 2 (8%) gave positive culture for *M bovis*.

**Table (1) Results of the comparative tuberculin test in cattle**

Test	Total no. of animals	Positive	Suspected	Negative
Comparative tuberculin test	850	373	112	365
		43.88%	13.17%	42.94%

**Table (2) Results of the bacterial isolation from samples taken from tuberculin positive cows**

Sample	Total no.	Positive	Isolation percentage
Milk	45	20	44.44%
Nasal swabs	45	11	24.44%
Liver	20	13	65%
Spleen	18	10	55.5%
Lung	30	23	76.66%
Lymph nodes	30	26	86.66%

**Table (3) Results of the biochemical tests for the bacterial isolates**

Type of isolate	No. of isolates	Niacin test	Nitrate reduction	Catalyze	Tween-80 hydrolysis
<i>M. bovis</i>	100	-	-	-	-
Mycobacterium Runyon IV	2	Variable	Variable	+	Variable
Mycobacterium Runyon III	1	-	-	+	Variable

- **ELISA for cattle:** It was performed on 260 serum samples taken from tuberculin tested cattle and 135 serums gave positive results with a percentage of 51.92% while 125 serum samples were negative with a percentage of 48.08%, (Table 4).
- **Tuberculin test and ELISA in workers:** It was found that 9 36% of examined workers were positive to the tuberculin test, while the ELISA results showed that 8 (32%) persons were positive and all the positive ELISA samples were also positive tuberculin reactors (Table 5).

**Table (4) Results of ELISA test in cattle comparing with the comparative tuberculin test**

Test	Result	
	Positive	Negative
ELISA	135	125
	51.92%	48.08
Comparative tuberculin test	206	54
	79.23%	20.77

**Table (5) Results of ELISA and tuberculin test in farm workers**

Test	Test	Tuberculin test		Total
		+	-	
			9	
		36%	64%	100%
ELISA	+	8	-	8
		32%	-	32%
	-	1	16	17
		4%	64%	68%

### Discussion

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 (13, 14). The comparative tuberculin test gives a high sensitivity and specificity in cattle herds and the disease had been controlled in many developed countries by following efficient diagnostic and control programs and slaughtering infected animals, but the disease still spread in developing countries that don't have control programs for bovine TB (15, 16). The incidence of bovine TB in cows present in the dairy station was high 43.88% and this due to leaving the routine testing with tuberculin test, also due to the overcrowding in the barns which play an important role in the direct transmittion of infection from animal to another by breathing and also the breed of animals, housing, grazing sites and gathering of animals during drinking have an influence on the prevalence of the disease(17, 18, 19). The results of this study agreed with other studies performed in Iraq 9.23% (20) and 85% (21) and with other studies in many countries, in Nigeria it was 56.2% (22) and in Libya was 4.3% (23) and 58.7% in Egypt (24). Mycobacterium was isolated from milk 44.44% and this high percentage agreed with many studies that indicated the possibility of isolation of *M. bovis* from cow milk and that cows with tuberculous mastitis excrete mycobacterium in quantities

enough to contaminate the milk collected from 100 cows together and this form a public health danger when the pasteurization procedures are not enough leading to the infection of humans and also increasing the possibility of infecting the newborn calves (20, 25). Mycobacterium also isolated from nasal swabs 24.44% and 100 isolates were *M bovis* and 2 isolates were mycobacterium Runyon IV and one isolate was mycobacterium Runyon III. This high percentage of isolation in the tuberculin positive cows indicates that the disease is an active form, this agree with studies reported that TB in cattle is mainly the pulmonary form and it is transmitted by the exogenous route which lead to contamination of the surrounding environment and transmittion of the disease to the other animals either by direct contact or by contamination of food and water with the nasal discharges, In addition to the hazard on the public health due to the infection of veterinary doctors and workers by the direct contact with infected animals or breathing the contaminated aerosols with the bacilli (14, 26, 27, 28). Mycobacterium was isolated from all the internal organs that have lesions and some organs without lesions and the isolation percentage was 65% in liver and 55.5% in spleen and 76.66% in lungs and 86.66 in lymph nodes and this indicate the failure of the gross examination sometimes in the diagnosis of TB and this agree with many researchers who isolated mycobacterium from grossly healthy organs specially in the early stages of the disease (29, 30). ELISA gave 51.92% positive results in cattle and this agreed with many studies that reported the increase in the titer of antibodies in the serum of *M bovis* infected cows, in addition to that testing cows with tuberculin test will lead to increase in production of antibodies (31, 32). but, it disagreed with other studies that reported the decrease in infection percentage and the sensivity of ELISA (33, 34). 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 degree of the developing of the disease, the level of antibodies is low in the early stages of the disease and rises in the late stages when the cellular immunity is weak and many studies had recommended the using ELISA as complementary test with the tuberculin test in field screenings on cattle herds (35, 36, 37). When examining the workers in the dairy station the positive results were 36% in tuberculin test and 32% in ELISA, that in addition to the isolation of *M bovis* from two cases and this agrees with many studies reported that *M bovis* considered one of the major causes of pulmonary TB in humans specially when drinking unpasteurized milk which considered a main source of transferring infection from infected cows to man and other animals, in addition to that the direct contact between workers and infected animals also lead to transferring the infection by inhalation of the aerosols from infected animals (14, 38, 39). Conclusions, This study confirm that bovine TB is endemic in cattle and the risk of transmission to human is potentially high. Testing and eradication of infected animals is the most efficient way in controlling the disease and collaboration between the veterinary and medical sectors in the diagnosis, monitoring, prevention and control of bovine TB is strongly encouraged.

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