Comparative Identification of *Mycobacterium bovis* By Using A Serological ELISA (IDEXX) and Tuberculin Test in Cattle in Wasit Province /Iraq

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**Abstract**

In Iraq, due to semi-full-scale dependence on tuberculin test, and presence, only, limited serological and molecular studies, the practical *Mycobacterium bovis* infection rate still dispersed especially in Wasit province that consider as an endemic area for bovine tuberculosis. In this study, two diagnostic techniques were used, IDEXX ELISA and tuberculin test; 186 cattle (155 females and 31 males), selected randomly and divided into two age groups, the first with less than 5 years and the second with more than 5 years. All cattle submitted to both tests and the results showed that (9.13%) were positives for tuberculin test and (17.2%) for IDEXX ELISA. Also, in age groups, the result of <5 years group by tuberculin test was 5.04%, and 11.76% by IDEXX ELISA test; while in >5 years group, was 16.41% and 26.86%, by tuberculin test and IDEXX ELISA test, respectively. Significant, the statistical different, P ≤ 0.05, was reported through the positive results of both tests as well as the two age groups, while in sex groups, the variance had not shown through both sexes.

**Keywords:** *Mycobacterium bovis*, IDEXX ELISA, Tuberculin test, Cattle, Wasit province
تشخيص المقارن للابقار المصابة ببعضات السل البقرية باستخدام اختبار الاليزا 
واختبار السمين في ابقار محافظة واسط/ العراق

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الخلاصة

نتيجة للاعتماد شبه الكامل في العراق على اختبار السمين ووجود عدد محدود من الدراسات السيرولوجية والجزئية، فإن المعدل الواقعي للابقار المصابة بال Mycobacterium bovis لا يزال مبهمًا خصوصًا في محافظة واسط التي تعتبر كمنطقة مسوبوءة لداء السل البقرية. في هذه الدراسة، تم استخدام اثنين من التقنيات التشخيصية، اختبار السمين واختبار الاليزا IDEXX ELIZA، واستخدام 681 من الابقار، من الإناث 611 وذكور 31، تم اختيارها عشوائياً وتم تقسيمها إلى مجموعتين من الفئات العمرية، الأولى بعمر أقل من 1 سنة والثانية بعمر أكثر من 5 سنوات. خضعت جميع الابقار لكلا الاختبارين وكانت النتائج كالتالي: (3.69)٪ في اختبار السمين و (6.1)٪ في اختبار الاليزا IDEXX. اما بالنسبة للمجاميع العمرية فقد كانت النتائج في الابقار التي تقل اعمارها عن خمس سنوات باختبار السمين 5.04 ٪ و 11.76 ٪ باختبار الاليزا IDEXX. اما بالنسبة للابقار التي تزيد اعمارها عن خمس سنوات، فقد كانت النتائج (61.61٪) باختبار السبين والاليزا IDEXX على التوالي. احصائيًا تم ملاحظة وجود اختلافات معنوية 0.05 بين نتائج اختبار السمين والاليزا IDEXX وبين نتائج المجموعات العمرية. بالاعتماد على جنس الابقار، لم يتم ملاحظة وجود فرق معنوي 0.05 بين الذكور والإناث في معدل الاصابة.
Introduction

*Mycobacterium bovis,* a classical pathological causer of tuberculosis in cattle, is an aerobic, non-motile, acid fast, slowly growing, non-photochromogen bacterium that affined to the family of Mycobacteriaceae (1, 2). Bovine tuberculosis (BTB) considers as serious and chronic infection which afflicted a large scale of animals such as cattle, camel, goat, deer, pigs, bison, domestic cats, foxes and rarely, sheep, horses and elephants (3, 4, 5). Its worldwide distribution in most countries involving Iraq, although, several epidemiological and public health features of disease were stay, extremely, obscure (6, 7). Steele (1995) reported that *M. bovis* could result nearly 50 million infections worldwide resulting economically in large losses of nearly 3 billion dollars yearly (8). In Latin-America, BTB was accountable for nearly 2% and 8% of recently human pulmonary and extra-pulmonary tuberculosis focuses (9). In Asia, 94% of total cows and less than 99% of total buffaloes were be existence in regions had BTB with partial control or without it exposing a humanly human populations at risk in areas where these animals without or with only limited BTB control (10). WHO (2013) and Abdul sahib et al (2015) reported that Iraq was represented an elevated BTB rises region in Eastern Mediterranean region (11, 12). Recently, the increasing in attentions for BTB is focused to founding new methods for identifying the actual herd schemes that could be impressed with hazards of infection transition (3). “Although tuberculin skin test has been a hallmark of BTB eradication programs but it lacks to the required sensitivity, not efficient in detecting the disease at its different stages particularly at early and advanced ages, and can be confounded by exposure to non-tuberculous mycobacteria as well as *Fasciola hepatica,* and cannot be repeated for 60 days due to desensitization “(13, 14). For controlling on those obstacles, several diagnostic methods are obtained to improve a predictive value of tuberculin skin such as the cellular immunoassay of gamma interferon, ELISA and PCR besides to bacteriological, histopathological and necropsy techniques (15, 16). The antibodies that responses to *M. bovis* have been shown to be positives in correlation with *mycobacterium* emerged as pathogenesis and antigens overburden (17). However, the continuous development of serological tests provided a high degree in diagnosis of BTB and became as an integral to other tuberculin diagnosing methods (18). In addition, the application of multiple tests will gain more diagnostic accuracy through detecting of subsidiary infections that negative by tuberculin skin test (19). IDEXX *M. bovis* antibody kit (USA) is a new commercial ELISA test that manufactured by IDEXX Laboratories for the diagnosis of BTB, and could
To improve BTB detection because it’s easy, cost effective for surveillance, need a few of time (15, 20). It was validated and certified by the World Organization of Animal Health (OIE) in 2012 at approval number of 20120107 (21). In Iraq, all surveillance or control programs as well as the academic studies were depended on tuberculin test as the only method in detection of BTB. Therefore, the goals were to:

1. Detect the most actual and practical incidence rate of BTB in cattle of Wasit province by using two methods (skin test and IDEXX ELISA test).

2. Assessment the efficacy of IDEXX ELISA test that used for first in Iraq in detection of the specific antibodies against Mycobacterium bovis.

3. Comparison the positive results between the two age groups and between both sexes through depending on the results of both selected tests.

Materials and method

1. Study’s areas, animals and specimens collection

During the first three months / 2016, the study was performed in rural areas of Wasit province / Iraq, involved (186) apparently healthy cattle that selected randomly from both sexes (155 females and 31 males), and divided into two age groups (less than 5 years and more than 5 years). From each one, 5 ml of blood was collected under aseptic condition with using a disposable syringe that installed in the numbered without anti-coagulant tubes for serological diagnosis (22).

2. Skin test or the Single Intradermal Comparative Cervical Test (SICCT)

OIE prescribed the skin tuberculin test for BTB diagnosis throughout the intradermal utilization of purified protein derivatives (PPD) in field diagnostics. An increasing of skin fold thickness (>4 mm) was mean positive, (1-4 mm) doubtful and negative if (<1mm) (23).
IDEXX ELISA

Bovine serums were examined through using an indirect IDEXX ELISA kit (USA) for bovine tuberculosis (Mycobacterium bovis) according to the manufacturer’s instructions (IDEXX laboratories) that recommended the cut-off with S/P ratio of 0.3. The S/P ratio of ≥ 0.3 was considered as the test had been positive.

3. Statistical Analysis

All data submitted to IBM SPSS v.23. program for statistical analysis. The efficiency of SICCT was validated against an IDEXX ELISA test that used as a gold standard. The positive associations were verified by Chi-square test with a level of P ≤ 0.05 (24).

Results and Discussion

The incidence of BTB had been observed to be increasing in several regions of the world whereas the living’s measures is weak particularly in Asia and Africa as a result of absence the regular practical method for effective screening (25). Although, the skin test and culture are available for diagnosis of tuberculosis in animals or humans, but the serological techniques promised and provided a high sensitivity and specificity results in detection of infection (26). The results of this study revealed that the positive BTB with SICCT and IDEXX ELISA test were 17/186 (9.14%) and 32/186 (17.2%), respectively.

Table 1/ Total results of SICCT and IDEXX ELISA

<table>
<thead>
<tr>
<th>Test</th>
<th>No.</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>SICCT</td>
<td>186</td>
<td>17</td>
<td>169</td>
</tr>
<tr>
<td>ELISA</td>
<td>186</td>
<td>32</td>
<td>154</td>
</tr>
</tbody>
</table>

The different small letters had differences at level P < 0.05

Although, several studies were done in Iraq by using different methods but, comparatively, the results were less than those recorded by this study and this variation could be attributed to several agents like the “herd size, density of animals, breeding and management system, uncontrolled animal movement, unhygienic local habits and stress factors due to other diseases and mass
vaccination against various diseases “(27). The high positives results of IDEXX ELISA in compared with SICCT may refer to the high sensitivity rate of first test and inability of SICCT to detect the antibodies at all stages of disease’s development or could be due the cross reaction of *Mycobacterium* spp. with SICCT (28, 29). In herds, where the control on infection is based on an identification and removal of reactors by using the tuberculin method, some diseased cattle with chronic phase or with unblock lesions, fail to reveal the reaction for PPD and, thus, could still in a farm as a latent provenance for disease in oversensitive cows (30). As well as, although the SICCT is valid method in cattle’s group, but it’s admitted that is restricted for using in detection the individualist diseased cattle. Also, it’s a slowly method for gaining results because of the period that required for showing the interaction (31). In addition, many factors can lead to false-positive or false-negative results such as the PPD antigen quality and manipulation, skin induration interpretation, and injection dose protocols (32, 33). Whilst, the major disadvantages of SICCT are an absence of specificity and increasing the numbers of no-visible-lesion reactors which occurs (34).

In depending on age groups (Table 2), cattle of less than five years group reported 6/119 (5.04%) positives by SICCT and 14/119 (11.76%) with IDEXX ELISA, whereas, in more than five years group, the SICCT and IDEXX ELISA tests were 11/67 (16.41%) and 18/67 (26.86%) positive cases, respectively.

<table>
<thead>
<tr>
<th>Test</th>
<th>Positive cases in 119 cattle &lt; 5 Years</th>
<th>Positive cases in 67 cattle &gt; 5 Years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>SICCT</td>
<td>6</td>
<td>5.04&lt;sup&gt;aA&lt;/sup&gt;</td>
</tr>
<tr>
<td>ELISA</td>
<td>14</td>
<td>11.76&lt;sup&gt;bA&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The small and large letters refers to vertical and horizontal significant differences, respectively, at level *P* < 0.05

Both, SICCT & IDEXX ELISA, tests reported that the increasing in BTB infection was directly proportional with age’s progression, and reaching to maximum at 5-9 years. For cows, the age reliance risk factor has still to be quantified, although all age groups can be affected, because of the little information or finiteness of diagnostic methods (34, 35). The increasing of BTB expansion with cattle’s age was reported in Ethiopia, Tanzania, Latvia, Ireland, United Kingdom, Canada and Mexico (36, 19, 37). Mackay and Hein (1989) concluded that the influences of γ-DT cells that found, mostly, in young calves’ blood flow might be play a role in
anti-mycobacterial immunity. Also, he proposed that the increasing in BTB incidence with aged cattle could interpreted as a result of reducing the defensive ability of these cows (38, 39, 40). Kazwala et al. (2001), Cleaveland et al. (2007) and Cadmus et al. (2010) believed that the age, alone, hasn’t an important sufficient value for disease’s sensibility and the existing belief, in endemic situation, is that due to increasing the exposure’s time for tuberculosis through advancing of age (41, 42, 43). Moreover, Phillips (2005) reported that the evidence of genetic variation in susceptibility of cattle to M. bovis infection, at least in families and genera, and susceptibility also increases with age (44). Barak (2012) recorded that the difference in results between age stages of cattle could be as a result of slow progression of the disease until reach to the detectable level (45). Francis et al. (1978) writes “The evidence suggests that even when young cattle are pastured with heavily infected old stock, the incidence in the former remains low until they enter the cow shed “(46).

Significantly, the results of (Table 3) didn’t reveal any differences between both sexes and this agreement with those reported by (47, 42). In opposite, (48, 43) reported that the females being at greater risk for infection with M. bovis than males, while (35) showed that the males had more readiness for infection than females. Katale et al. (2013) reported that BTB incidence appear to be gain with age’s advancing but only in females, particularly, in those cows over 4 years (37). McCrindle (2007) observed that, during controlling studies, there was no relationship between the sensitivity for acquiring the infection with sex of animal (49).

<table>
<thead>
<tr>
<th>Test</th>
<th>Positive cases in 155 Females</th>
<th>Positive cases in 31 Males</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>SICCT</td>
<td>14</td>
<td>9.03aB</td>
</tr>
<tr>
<td>ELISA</td>
<td>27</td>
<td>17.41BB</td>
</tr>
</tbody>
</table>

The small and large letters refers to vertical and horizontal significant differences, respectively, at level $P<0.05$

In conclusion, IDEXX ELISA that licensed by OIE in 2012 was used for first time in Iraq as a new diagnostics for BTB in cattle. Its efficiently, can be used in detection, eradication and control programs as a rapid screening test, because the disease is still represents a public health hazard, and the efforts should be made to
control on it. The lack of veterinary infrastructure that required for BTB surveillance may be the main cause for increasing the disease’s incidence.

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References


17. Waters, WR.; Whelan, AO.; Lyashchenko, KP.; Greenwald, R.; Palmer, MV.; Harris, BN.; Hewinson, RG. and Vordermeier, HM. (2010). Immune responses in cattle inoculated with Mycobacterium bovis, Mycobacterium tuberculosis, or Mycobacterium kansasii. Clinical and Vaccine Immunology, 17(2), 247-252.


23. Schiller, I.; Oesch, B.; Vordermeier, HM.; Palmer, MV.; Harris, BN.; Orloski, KA. and Waters, WR. (2010). Bovine tuberculosis: a review of current and emerging diagnostic techniques in view of their relevance for disease control and eradication. Trans boundary and emerging diseases, 57(4), 205 -220.


38. Mackay, CR. and Hein, WR. (1989). A large proportion of bovine T cells express the γ T cell receptor and show a distinct tissue distribution and surface phenotype. Int. Immun., 1(5), 540-545.


40. Barwinek, F. and Taylor, NM. (1996). Assessment of the socio-economic importance of Bovine Tuberculosis in Turkey and possible strategies for control or eradication: Turkish-German animal health information project General Direktorat of Protection and Control, Ankara. GTZ. p. 3-45.


483