جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة ديالى
كلية الطب البيطري
فرع الطب الباطني والوقائي

مرض البروسيلا كأحد مشاكل الأمراض المشتركة في محافظة ديالى: انتشار *B.melitensis* في الأغنام والماعز والإنسان في مدينة بعقوبة

رسالة

مقدمة الى مجلس كلية الطب البيطري في جامعة ديالى وهي جزء من متطلبات نيل درجة الماجستير في علوم الطب البيطري/ الامراض المشتركة

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15 ربيع الثاني 1439هـ

2018/ كانون الثاني

Republic of Iraq
Ministry of Higher Education and Scientific Research
University of Diyala
College of Veterinary Medicine
Dep. Internal and preventive Medicine

Prevalence of *Brucella melitensis* in Sheep, Goats and Man in Diyala Governorate

A Thesis

Submitted to the Council of College of Veterinary of Medicine

University of Diyala

In Partial Fulfillment of the Requirements for the Degree of Master of Science in Veterinary Medicine-Zoonosis

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January/2018

ربيع الثاني-1439

1-1.Introduction

Brucellosis is a general term used for animal and human infections caused by several species of the genus *Brucella*, mainly *B. abortus*, *B. melitensis* and *B. suis* (OIE, 2016). It is one of the most important and widespread zoonosis in the world according to reports of FAO, WHO and the OIE organizations (Ariza et al., 2007; Lopez et al., 2010). Reports of the FAO, considered it second to rabies in the list of most important zoonotic disease in the world (FAO, 2003), and Brucellosis is still a major problem in the field of public health worldwide (Lopez et al., 2010, AL-Dahouk et al., 2013).

Brucellosis is highly infectious, re-emerging bacterial disease (Hadush and Pal, 2013); readily transmissible from animals to humans, by consuming unpasteurized milk, raw dairy products or undercooked meat, or inhalation of aerosols harboring the bacteria and through skin wounds or mucous membranes. In man, it causes acute febrile illness which may progress to a more chronic form (OIE, 2016). Human Brucellosis is a severely debilitating and disabling illness, with fever, sweating, fatigue, weight loss, headache, and joint pain persisting for weeks to months. Neurological complications, endocarditis and testicular or bone abscess formation can also occur (Avdikou et al., 2005).

In addition to the public health importance; Brucellosis causes significant economic losses for animal industry, resulting from abortion, stillbirths, loss in milk production, low fertility rates, and cost of replacement animals. Ingestion is the most frequent route of infection in animals, in addition to inhalation, through skin and mucosa of genital tract and conjunctiva. Infection via natural breeding in caw does not occur very often, unlike artificial insemination with contaminated semen (Radostits et al., 2007).

An accurate diagnosis of Brucellosis is important for the control of the disease in animals and consequently in man. The 'gold standard' for the diagnosis of Brucellosis is isolation and identification of the causative bacterium. Isolation of *Brucella spp.* requires high security laboratory facilities and it takes at least a week to be completed (Nielsen, 1997; Nielsen and Yu, 2010)

Serology is the most useful and widely used tool for the diagnosis of Brucellosis in man and animals. These tests are most commonly used because they are fast and safe. Serological methods recommended for large-scale eradication purposes includes mainly Rose Bengal plate test (RBPT), complement fixation test (CFT) and enzyme-linked immunosorbent assays (ELISAs) (OIE, 2009).

Anyhow, infected animals may not always produce all types of antibodies in measurable quantities; that is why results of different serological tests should be used as a monitor of infection (FAO, 2005).

Serological tests have drawbacks of giving false-positive results; this may be associated with infection by other cross-reacting bacteria like *Yersinia enterocolitica* O: 9, *Salmonella* spp. and *Escherichia coli* (Kaltungo et al., 2014; Ahmed, et al., 2016); or found in animals vaccinated with alive attenuated vaccines as *B. melitensis* Rev.1 strain, as they will stay serologically reactive after vaccination. Consequently, differentiation between infected and vaccinated animals by the standard serological tests is difficult. The competitive enzyme immunoassays were developed in order to eliminate some of the problems arising from residual vaccinal antibody, and from cross-reacting antibodies (Poester et al. 2010, Mustafa et al., 2012). The outer membrane proteins (OMP) of *Brucella* spp. were found to be alternative antigens rather than S-LPS

for serological diagnosis to minimize the post vaccinal false positive serological results (Debbarh et al., 1996; Ahmed, et al., 2016).

Endemicity of Brucellosis is more pronounced in the underdeveloped countries; due to inadequacy of diagnostic and control services (Gwida et al., 2010). Nevertheless, Studies from developed countries indicate that elimination of Brucellosis from the animal reservoir has resulted in a pronounced decline in the incidence of disease in human (Pappas et al., 2006), and currently, laboratory workers are among those most frequently infected in such countries. In addition to that, the increase in business and travel to areas endemic with Brucellosis has led to importation of the disease into non-endemic areas (Corbel, 2006).

Human Brucellosis, is still endemic in the Mediterranean basin, Middle East, Western Asia, Africa, and South America (Maurine, 2005). Small ruminant populations in the region show seroprevalence values that are among the highest worldwide (Musallam et al., 2016). There are about 500,000 new human cases of Brucellosis reported annually worldwide making it the most common zoonosis (Saleem, 2010).

Problems of wars in the Middle East; led to insufficient preventive measures with inadequate control programs as well as uncontrolled animal movement through “open” borders increased the risk that Brucellosis will spread in some regions. New seroprevalence studies are needed urgently to evaluate the current situation and for continuous monitoring of necessary control programs (Gwida et al., 2010).

Brucellosis is enzootic and endemic in Iraq; it has a history dating back to 1937 when it was first isolated by an Iraqi physician (Al-Zahawi, 1938; Beattle et al., 1939, Saleem, 2010). Several studies on the seroprevalence of Brucellosis in Iraq have been conducted on man and

animals in the recent decades (Al-Rodhan, 2005; Al – Zayadi and Al-Yassaree, 2006; Gwida et al., 2010; Al- Saaidi et al., 2010; Al-Abdaly et al., 2013; Najum, 2014; Mohamed et al., 2015; Jaff, 2016; Suadad,2016). Because of the cultural habits and close proximity of animals to humans which favor spread of the disease; Brucellosis is of particular concern in Iraq (John et al., 2008).

In Diyala Governorate/Iraq, there are few studies concerning human and animal Brucellosis. (Qasim, et al., 1995; AL-Dileamy, 2010, Fadihl and Khalil.2016).

Aims of the study: This study aimed at detecting the incidence of infection with *B. melitensis* in sheep, goats and man in some areas of Diyala Governorate, in order to evaluate the importance of this infectious disease as a zoonotic problem in the area of the study. This is going to be carried through, targeting the diagnosis of Brucellosis in flocks with cases of abortion and still birth in ewes and does resident in some districts of Diyala Governorate during the study which done by:-

1. Bacteriological examination of aborted fetuses, placentas and vaginal discharge of recently aborted ewes and does for Brucellosis.
2. Serological study on Brucellosis in randomly collected serum samples from flocks of sheep and goats with previous history of reproductive problems as abortion, stillbirth and infertility.
3. Serological examination for Brucellosis in humans with clinical signs suggestive for Brucellosis in private and general hospitals in Baqubah city in addition in women with history of miscarriage.

Abstract

This work aimed at detecting the prevalence of *Brucella* infection in sheep, goats and humans in certain districts of Diyala Governorate/Iraq through a cross sectional bacteriological and serological study conducted from September/2016 to June / 2017.

Tissue samples were collected from does and ewes with history of abortion, still birth and infertility. Using Stamp stain; acid fast, bright red coco-bacilli were detected in primary smears prepared from 27 (39.70%) out of 68 tissue samples (placentas, fetuses and vaginal swabs from sheep and goats). Meanwhile, *Brucella* spp. was isolated from 2 (4.54%), 1 (33.3%) from vaginal discharge and placenta of ewes and didn't isolated from the vaginal discharge of goats, and fetal stomach contents. Results of biochemical activities and biotyping assays indicated that the two isolated strains of *Brucella* were *B. melitensis* biotype 1.

Total of 163 serum sample of adult sheep and goats (107 from sheep and 56 from goats) of different sexes has been collected from the different flocks. Animal sera were subjected to the Rose Bengal plate test (RBT) and Competitive enzyme- linked immunosorbent assay (c-ELISA) for the serological diagnosis of Brucellosis.

Human serum samples were collected from 84 patient referring to different health institutes in Baqubah city/Iraq with clinical signs suggestive for Brucellosis (23 men and 61 women "27 none aborted and 34 aborted"); they were tested for Brucellosis using Rose Bengat test (RBT), Rapid serum agglutination test (RSAT) and Indirect enzyme-linked immunosorbent assay (i- ELISA).

From a total of 163 animal serum samples tested (107 from sheep and 56 from goats); positive results were detected in 19 (11.655) and

97(59.5%) of samples using RBT and c-ELISA, respectively. This was represented by 62 (57.49%) sheep and 35 (62.5%) goats. Statistically significant difference was not detected in seroprevalence of Brucellosis in ovine and caprine included in this study.

Using the RBT, from the 163 serum sample, 19 (17.75%) of sheep sera were positive, they were represented by 7 (9.72%), 11 (47.82%) and 1 (8.33%) of none aborted ewes, aborted ewes and rams, respectively, while all goats were negative.

Using c-ELISA, from a total of 56 goat serum samples 35 (62.5%) gave positive reaction represented by 26 (65%), 2 (40%) and 7 (63.63%) of none aborted, aborted does and bucks, respectively. For different groups of sheep, c-ELISA yielded 36 (50%), 21 (91.30%) and, 5 (41.66%) in none aborted, aborted ewes and rams, respectively.

Results for serology in different groups of sheep declared that aborted ewes gave a statistically significant ($p<0.05$) higher positive reaction (47.82% and 91.30%) when compared to none aborted ones (9.72% and 50%) using RBT and c-ELISA, respectively. While in goats, none aborted dose gave significantly ($P <0.05$) higher seropositivity than the aborted one.

Seropositivity in different groups of sheep is significantly ($P<0.05$) higher using c-ELISA than with RBT.

According to sex, 28 (62.2%) of does and 7 (63.63%) of bucks reacted positively to the c-ELISA. While ewes showed significantly ($p<0.05$) higher positivity 18 (18.94%) than rams 1 (8.33%) using RBT, but using c- ELISA ewes gave statistically none significant more seropositivity than rams. Using c-ELISA significantly ($p<0.05$) higher positivity than RBT in both sexes were observed.

Statistically significant ($p<0.05$) differences were detected in seroprevalence of animal Brucellosis in different areas of Diyala Governorate/Iraq included in this study. The highest percentage of seropositivity was detected in Bardiya and Qarra-Tabbah followed by Agriculture college animal farm, Door Mandli and Khan Bani Saad, the least prevalence was detected in AL-Anbakiya district.

Concerning humans; from a total of 84 human serum samples tested by RBT, RSAT and i-ELISA; positive results were detected in 11 (13.09%), 13 (15.47%) and 9 (10.71%) of total samples, respectively. When using RBT; this result is represented by 5 (13.04%), 4 (11.76%) and 2 (8.69%) of none aborted women, aborted women and men, respectively. For RSAT; 9 (33.33%) and 4 (17.39%) of samples were positive for none aborted women and men respectively, while all aborted women reacted negatively to this test.

according to the i-ELISA, 5 (18.51%), 3 (8.82%) and 1 (4.34%) of none aborted women, aborted women and men reacted positively, respectively.

Sex wise result in human samples showed that using i-ELISA; women revealed significantly ($p<0.05$) higher seropositivity than men, while using RBT and RSAT women gave a statistically none significant higher seropositivity.

Comparing different serological tests; statistically significant difference was detected in men, as significantly higher ($p<0.05$) seropositivity was obtained using RSAT than RBT, in addition to that, the RSAT failed to detect infection in aborted women.