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Epidemiological Studies, Seroprevalance and Some Risk Factors of Brucellosis in Sheep and Goats in the South Province of West Bank

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ABSTRACT

Between 2010 and 2011, a study of seroprevalence and some risk factors for *Brucella* seropositively in sheep and goats has been carried out in the south province of West Bank. A total of six hundred twenty sheep sera from 15 flocks and 145 goats sera from 5 flocks were randomly collected and analyzed. Rose Bengal test was used to screen all serum samples. The positive samples were subjected to conformation by complement fixation test. Moreover, a complete history was compiled from each flock. The true prevalence of *Brucella* seropositive in sheep was 21.1% and in goats was 24.6%. *Brucella melitensis* biotype 3 was isolated from 11 aborted fetuses and from 24 milk samples. It was concluded that brucellosis is still common in sheep and goats in West Bank. It is recommended for prevention of the disease in animals by vaccinating young female animals aged from three to six months with standared full dose of 1 to 2×10^9 CFU also recommendations were given to control the disease in animals and to avoid brucellosis in humans.

Key words: Brucellosis, sheep, goat, epidemiology, serology prevalence, west bank

INTRODUCTION

Brucellosis is a disease of highly economic and public health importance and has a worldwide distribution. It is considered as the most wide-spread zoonosis in the world (Nicolleti, 1989). Brucellosis is also known as Mediterranean fever caused by *Brucella* species. The causative agent, a gram-negative, facultative, intracellular bacterial pathogen can cause serious infections in people and animals. Each *Brucella* spp. has a preferred natural host that serves as a reservoir (Quinn *et al*., 1994). Ovine and caprine brucellosis are still endemic in countries of the Mediterranean basin, the middle east, central Asia and Latin America (Awad *et al*., 1975). The disease has been reported in Jordan, Syria, Saudi Arabia and Iraq, with varying incidences and the main *Brucella* spp. in these countries was *Brucella melitensis* (Nicolleti, 1989; Aldomy *et al*., 1992; Mustafa *et al*., 1985; Polydorou, 1992; Karim *et al*., 1979; Al-Talafhah *et al*., 2003).

This zoonosis is easily transmitted from one animal to another and from animals to human. The epidemiological evidence indicates that human infection due to *Brucella* from sheep and goats is known to occur mostly through the consumption of unheated milk or unpasteurized milk and milk products and to direct contact with infected animals (Baron and Finegold, 1990). In West Bank there was some evidence that brucellosis does occur in sheep, goats, cattle and humans. However, the exact incidence and distribution of brucellosis infection within the West Bank is unknown. For control and eradicate this disease, a vaccination program had been begun since more than ten years using *Brucella melitensis* Rev. 1 vaccine. This vaccine is administered by conjuctival route, at a reduced dose of 1×10^5 Colony Forming Units (CFU) of *Brucella* melitensis.

The aim of this study was to investigate the seroprevalence of brucellosis and to evaluate some risk factors to be associated with the occurrence of *Brucella* infection in Awassi sheep and goats in South of West Bank. Also the study estimated the incidence of abortion due to brucellosis and biotyped the isolated *Brucella* organisms from aborted fetuses and milk samples.

MATERIALS AND METHODS

Sheep and goats: Breeding season usually start in West Bank in July. All participating flocks grazed during the spring season until the end of harvest season (March to end of August) with little feed supplementation. Lambing season usually is from November until March. To determine the seroprevalence of brucellosis in some sheep and goat flocks, a total of 620 sheep form 15 flocks and 145 goats from 5 flocks were examined during the years 2010 and 2011. A pre-tested questionnaire was developed together information about factors influence the spread of *Brucella* infection, also incidence of abortion, vaccination programme, time of lambing and other diseases causing abortions within or between flocks. Other information of each animal sampled was also obtained including herd size, age, sex, health status, history of abortion and management practices. A history of each flock was taken and blood samples from the adult animals were collected. These samples were 20% from all sheep and goats flocks. Blood samples were collected from adult females and males of each flock. The blood was allowed to clot and the sera were separated by centrifugation and stored at -20°C until testing. The collected sera were screened for antibodies against *Brucella* using Rose Bengal Plate Test "RBPT". Positive and inconclusive serum samples were further tested using Complement Fixation Test "CFT" as described by Alton *et al.* (1988). Both tests were manufactured by the Jordan Bio-Industries center (JoVac, Amman, Jordan).

A serum sample with an antibody titer equal or more than 1:4 was classified as a CFT-positive. *Brucella* seropostive animals are animals with both positive RBPT and CFT results. According to the manufacturing company, the sensitivity and specificity of RBPT are 89 and 97%, respectively. CFT has a sensitivity of 88.1% and a specificity of 100% (Blasco, 2006).

Bacteriological examination: Sixty milk samples were collected with sterile universal bottles from adult lactating sheep and goats. In the field Swabs of lungs, livers, spleen and stomach contents were collected by aspiration using a sterile syringe. All the samples were cultured on duplicated plates of sheep-blood agar and *Brucella* agar selective media (oxoid). Also fetal stomach contents and the vaginal swabs were used to prepare slide smears, stained by the modified Zeih-Neelsen stain and examined microscopically for *Brucella*-like organisms. The inoculated plates were incubated at 37°C in the presence of 10% Co_2 for up to 2 weeks. *Brucella* initially were identified by colony morphology, gram stain and modified Ziel-Neelsen stain as described by Quinn *et al.* (1994). The colonies that appeared (after 3-4 days of inoculation) pinpoint, glistening, smooth and translucent and the bacterial cells that appeared as gram-negative coccobacilli and showed red color by modified Ziel-Neelsen stain were subjected to confirmatory tests. Also some tests were performed as catalase, oxidase, motility, urease, indole, nitrate reduction and growth on MacConkey agar. For identifying and typing, the tests used according to Alton *et al.* (1988).

Statistical analysis: The collected sera were screened for the presence of antibodies against *Brucella* antigens by using "RBPT" and "CFT". The *Brucella*-seroprevalence was estimated by adjusting the apparent prevalence to the sensitivities and specificities of the serological tests (inseries) and typed using the following formula:

$$TP = \frac{AP-(1-Sp_1)(1-Sp_2)}{Se_1Se_2-(1-Sp_1)(1-Sp_2)}$$

where, TP is the true prevalence; AP is the apparent prevalence, Sp_1 and Sp_2 are RBT and CFT tests specificities, respectively, Se_1 and Se_2 are RBT and CFT sensitivities, respectively (Noordhuizen, 1997).

RESULTS AND DISCUSSION

Out of 620 sheep sera tested, 115 (18.5%) were positive by RBT. When tested by CFT, 103 (16.6%) out of the 115 RBT positive sera were positive by CFT. Also out of 145 goat sera tested, 31 (21.4%) were positive by RBT. When tested by CFT, 28 (19.3%) out of 31 RBT positive sera were positive by CFT. Therefore, the true seroprevalence of sheep and goats brucellosis in South province of West Bank as adjusted to RBT and CFT sensitivities and specificities are 21.1 and 24.6%, respectively. The design of this study is summarized in Table 1. The study included sheep and goat flocks from different areas in South of West Bank. 15 flocks of sheep (620 animals) and 5 herds of goats (145 animals) were studied. All these flocks had known histories of abortion. In general this table shows 545 female sheep and 75 male sheep were serologically tested. The 94 (17.2%) and 9 (12%) were found positive, respectively. Also Table 1 shows 122 female goats and 23 male goats were serologically tested. 24 (19.7%) and 4 (17.4%) were found positive, respectively for the disease. From 60 sheep milk samples 16 isolates 26.7% were identified as *Brucella* micro-organism. From 22 goats milk samples 8 isolates 36.4% were identified as *Brucella* micro-organism. Of 18 aborted fetuses (12 from sheep and 6 from goats) that were bacteriologically examined, *Brucella* melitensis was isolated from 11 fetuses (7 from sheep and 4 from goats). The abortion rate among sheep and goats varies from flock to flock ranging from 16 to 45%. The main sign of brucellosis in sheep and goats during this study was abortion during the last 40 days of pregnancy or premature expulsion of the fetus. Metritis and retention of placenta were also observed. The fetuses were somewhat oedematous with blood-tinged fluid in body cavities. From abomasal contents, a pure culture of *Brucella meletenis* was isolated. All the isolates from milk and fetuses were characterized as *Brucella melitensis* biotype 3.

Zoonosis continues to present an important health hazard in most parts of the world, particularly in developing countries. Brucellosis is now considered to be the most important disease transmitted from animals to human beings in West Bank. This disease can have a considerable impact on human and animal health as well as a socioeconomic impact. Due to its economic importance and the threat it poses to human health, it is necessary to take steps to fight brucellosis in animal population. The epidemiological evidence in West Bank indicates that ingestion of raw milk and milk products, such as cottage of cheese as well as direct contact with infected animals,

Table 1: Results of serological diagnosis of brucellosis by RBT and confirmed by CFT and isolation of Brucella in aborted fetuses and milk samples in the South province of West Bank. Values in bracket are percentages

	No. of samples collected		Females		Males		No. of aborted fetuses examined		No. of milk samples collected		
No. of flocks examined	Total	No. of Pos.	Total	No. of Pos.	Total	No. of Pos.	Total	No. of Pos.	Total	No. of Pos.	No. of human infected
15 Sheep	620	103 (16.6)	545	94 (17.2)	75	9 (12.0)	12	7 (58.3)	60	16 (26.7)	12
5 Goats	145	28 (19.3)	122	24 (19.7)	23	4 (17.4)	6	4 (66.7)	22	08 (36.4)	5

Values in brackets are percentage

are the main sources of human infection. There is very important risk factor for brucellosis at the flock level observed in this study was grazing at communal pasture. This will allow contact between flocks and will increase the chance for disease transmission to susceptible animals. In addition to that, this is not surprising in abscence of restricted quarantine practices where sheep and goats owners move their animals freely from one area to another.

This is the first study to report some epidemiological aspects related to small ruminant brucellosis in West Bank. The true seroprevalence of brucellosis in sheep and goats in South West Bank was found 14.9 and 22%, respectively. The crude seroprevalence in this study were within the ranges reported in surrounding and other neighbouring countries (Al-Talafhah *et al*., 2003; Al-Majali, 2005; Lafi *et al*., 2001; Dawood, 2008; Al-Magali *et al*., 2009; Al-Ani *et al*., 2004). The *Brucella* agglutination tests such as (RBT) are known to have high analytical sensitivity (OIE, 2000). Complement fixation test have slightly lower diagnostic sensitivity than that of the buffered of any of other convential tests. So due to this fact, CFT has been recognized as a confirmatory serological test for brucellosis.

In this study *Brucella melitensis* was isolated from 24 milk samples (16 from sheep and 8 from goats) and from 11 aborted fetuses (7 from sheep and 4 from goats). All the isolates from milk and fetuses were characterized as *Brucella melitensis* biotype 3. These results are in agreement with those previous studies in some neighbouring countries (Nicolleti, 1989; Polydorou, 1992; Karim *et al*., 1979; Al-Talafhah *et al*., 2003). It is clear from this study that *Brucella melitensis* biotype 3 is the principal field strain which causes brucellosis in animals. In this study two *Brucella melitensis* biotype 3 was identified in two milk samples from sheep flock vaccinated with reduced dose of Rev. 1 before more than 30 months. In this respect, brucellosis is still diagnosed in vaccinated as well as non-vaccinated flocks which may indicate the low efficiency of the vaccine in protecting animals which means that the efficacy of the reduced dose of the Rev. 1 vaccine is questionable.

Many researchers recommended vaccinating female lambs and kids with a single subcutaneous standard dose of Rev. 1 vaccine (Al-Ani *et al*., 2004), so it is recommended for prevention of the disease in animals by vaccinating young female sheep and goats aged from three to six months with standard dose $1\text{-}2\times10^{9}$ CFU of the Rev. 1 vaccine. Also the control of human brucellosis can be achieved by continuing the vaccination of sheep and goats and by education the public to consume only boiled or pasteurized milk and milk products.

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