

Seroprevalence of *Brucella* species among women with miscarriage in Jordan

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معدل الانتشار السيرولوجي لأنواع البروسيلات بين النساء اللاتي أجهضن في الأردن محمود أبو شحادة، مروان أبو حلاوة

الخلاصة: تتباين النتائج حول تأثير عدوى البروسيلات أثناء الحمل على زيادة خطر الإجهاض. وقد قاس الباحثان معدل الانتشار السيرولوجي لأنواع البروسيلات في عينات مأخوذة من نساء أجهضن ونساء ليس لديهن سوابق إجهاض في الأردن خلال المدة من كانون الأول/يناير حتى تموز/يوليو عام 2003. وجمع الباحثان العينات المصلية من 445 امرأة لديهن سوابق إجهاض، ومن عدد مماثل من النساء ليس لديهن سوابق إجهاض، ولكنهن كنّ متوافقات مع المجموعة الأولى من حيث العمر، والمستوى الاجتماعي والاقتصادي، والإقامة. واختبر الباحثان الأمصال باستخدام اختبار طبق وردية البنغال واختبار تثبيت المتممة. وقد بلغ معدل الانتشار السيرولوجي الحقيقي بين النساء اللاتي أجهضن 1.8% (95% CI = 0.6-3.0)، في حين بلغ معدل الانتشار السيرولوجي الحقيقي في النساء اللاتي ليس هن سوابق إجهاض 1.0% (95% CI = 0.08-1.9). ولم يُشاهد أي اختلاف يُعتدُّ به إحصائياً بين الانتشار المصلي للبروسيلات بين النساء اللاتي أجهضن وبين اللواتي ليس هن سوابق إجهاض ($P=0.6$).

ABSTRACT Results differ as to whether *Brucella* infection during pregnancy increases a woman's risk of miscarriage. We determined the seroprevalence of *Brucella* spp. among a sample of women with miscarriage and women with no history of miscarriage in Jordan during January–July 2003. Serum samples were collected from 445 women with miscarriage and a similar number of women with no history of miscarriage, matched on age, socioeconomic status and residence. Sera were tested using the Rose Bengal plate test and complement fixation test. The true seroprevalence among women with miscarriage was 1.8% (95% CI: 0.6–3.0), while the true seroprevalence among women with no history of miscarriage was 1.0% (95% CI: 0.08–1.9). There was no significant difference between seroprevalences of *Brucella* spp. among women with miscarriage and those with no history of miscarriage ($P = 0.6$).

Séroprévalence de l'espèce *Brucella* chez des femmes ayant des antécédents de fausse couche en Jordanie

RÉSUMÉ Les résultats de la recherche sur l'augmentation du risque de fausse couche chez les femmes atteintes de brucellose pendant leur grossesse sont discordants. Nous avons déterminé la séroprévalence de *Brucella* spp. dans un échantillon de femmes ayant des antécédents de fausse couche et dans un groupe de femmes sans antécédent de fausse couche en Jordanie, entre janvier et juillet 2003. Des échantillons de sérum ont été prélevés auprès de 445 femmes ayant des antécédents de fausse couche. Un nombre équivalent d'échantillons a été prélevé dans un groupe de femmes sans antécédent, appariées pour l'âge, le niveau socioéconomique et le lieu de résidence. Les échantillons de sérum ont été analysés à l'aide de l'épreuve au rose Bengale et du test de fixation du complément. La séroprévalence réelle chez les femmes ayant des antécédents de fausse couche était de 1,8 % (IC à 95 % : 0,6–3,0), alors que chez les femmes sans antécédent, elle était de 1,0 % (IC à 95 % : 0,08–1,9). Aucune différence significative n'a été observée entre les séroprévalences de *Brucella* spp. chez les femmes ayant des antécédents de fausse couche et les autres ($P = 0,6$).

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Introduction

Brucellosis, which is caused by organisms belonging to the genus *Brucella* is one of the five common bacterial zoonoses worldwide [1]. It has a global distribution, and is endemic in the Mediterranean basin, the Middle East, India, and South America [2,3]. Human brucellosis is caused by different species and includes those that cause infection in cattle (*B. abortus*), goats and sheep (*B. melitensis*), pigs (*B. suis*), and dogs (*B. canis*), with both *B. melitensis* and *B. suis* being more virulent in humans [4,5].

It has been estimated that more than 500 000 human *Brucella* spp. infections occur per year with most reported cases occurring in the Syrian Arab Republic, followed by Mongolia, Kyrgyzstan and Iraq [6]. *Brucella* spp. are transmitted to humans by direct contact with infected animals or their excreta or the consumption of unpasteurized milk or dairy products [7].

In animals, *Brucella* spp. is an established major cause of miscarriage and serious economic loss to dairy and small ruminant farms [8]. Conversely, many reports have excluded *Brucella* from being a cause of miscarriage in women. The reports are based on the lack of carbohydrate erythritol in women's placentas considered to be a growth factor for *Brucella* spp. in the placentas of animals, the presence of anti-*Brucella* activity in human amniotic fluid [9,10], the absence of a significant relationship between *Brucella* antibody and spontaneous miscarriage [11], and the unsuccessful isolation of *Brucella* spp. [12].

However, some of those same reports showed a significant relationship between raw milk consumption and spontaneous miscarriage [11] and a correlation between *Brucella* infection and preterm and intrauterine fetal death [12].

Miscarriage cases have been associated with *B. abortus* type 2 [13] and *B. melitensis* [14]. It has been suggested

that brucellosis can cause fetal death at any stage of pregnancy, whether maternal infection is mild or severe [9,15]. Associated pathological changes were preterm labour chorioamnionitis, placental abruption [13], intrauterine fetal death and Gram-negative septic shock with diffuse intravascular coagulation and septicaemia [14].

In a study in northern Jordan, the seroprevalence of *Brucella* was reported to be 8% among high-risk individuals (veterinarians, sheep and cattle farmers, and meat, milk and milk product handlers) compared to only 0.5% in the control sample [16], and an even higher seroprevalence of 55% was reported among veterinarians [17]. These findings emphasize the importance of contact infection and the occupational nature of the disease in Jordan.

A better understanding of the reported association between brucellosis and miscarriage in women is needed. Few community-based studies have been carried out in Jordan and little is known about human brucellosis and miscarriage in the Middle East, including Jordan. This study therefore aimed to determine and compare the seroprevalence of *Brucella* spp. among Jordanian women with miscarriage and women with no history of miscarriage.

Methods

Determination of sample size

The seroprevalence of *Brucella* spp. was reported as 8% among high risk people in Jordan [16]. According to Thrusfield [18], the appropriate sample size was 441, with a 99% confidence level and 1% absolute value precision.

Study setting and subjects

From January to July 2003, women with miscarriage ($n = 445$) visiting 3 main hospitals (2 public and 1 private) were surveyed for *Brucella* spp. The 2 public hospitals were Al-Bashir, the largest public hospital in Jordan, located

in Amman, which serves patients from central Jordan especially those of poor socioeconomic standard, and Al Hussein Hospital, the fifth largest public hospital, located in the city of Salt [19]. Hiba is a private maternity hospital in Amman serving mainly patients of the Christian community. The women were recruited from the outpatient clinics of the hospitals. A qualified nurse approached them and explained the objectives of the study. Of the women approached, 48 did not want to have their blood drawn; in that case the next eligible woman was approached and recruited.

The definition of a case of miscarriage was the spontaneous end of a pregnancy after the first 6 weeks and prior to 20 weeks of gestation.

A group of 445 control women with no history of miscarriage were also recruited from women who accompanied patients to the hospitals, nurses and other hospital workers and trainees. They were actively selected and matched with the women with miscarriage for age, socioeconomic status and residence (the same village of the case). There were 121 refusals and the next woman who matched was approached and recruited.

Serology

Blood samples were collected from all the women; for the study group, the sample was taken 7–10 days after miscarriage.

Sera were separated and stored at $-20\text{ }^{\circ}\text{C}$ until testing. Serum samples were tested for *Brucella* spp. using the Rose Bengal plate test (RBPT) (JO-VAC, Jordan). Positive samples were confirmed using the complement fixation test (CFT) (JOVAC, Jordan). In the case of CFT, serum samples with 17 IU/mL antibody or higher were regarded as positive. As suggested by the manufacturer, the sensitivity and specificity of RBPT are 89% and 97% respectively. CFT has a sensitivity of 88.1% and a specificity of 100% [20].

Positive and negative control sera were used.

Data collection

The women were interviewed using a questionnaire in Arabic which had been pilot tested. They were interviewed by a qualified nurse during the outpatient visit and at the time of blood collection. Information collected included name, age, place of residence, number of children, medication or health history.

Ethical considerations

The study protocol was approved by the concerned committees at Jordan University of Science and Technology. Verbal consent was obtained from all participants. All identifying information was kept confidential.

Data analysis

Data were analysed using SPSS, version 10.0. $P < 0.05$ was considered statistically significant. The true seroprevalence and 95% confidence interval (CI) was calculated according to Rogan and Gladen [21]. The true seroprevalence is derived from the apparent seroprevalence by

adjusting for the test sensitivity and specificity. The chi-squared and Fisher exact tests were used to test the significance of differences between proportions.

Results

Women with miscarriage were aged between 15 and 45 years with quartiles of, Q1 = 24 years, Q2 = 27 years and Q3 = 32 years. Only 9 and 7 aborted women were positive by RBPT and CFT respectively, while 5 and 4 control women were positive by RBPT and CFT respectively. The true seroprevalence among aborted women was 1.8% (95% CI: 0.6–3.0), while the true seroprevalence among women with no history of abortion was 1.0% (95% CI: 0.08–1.9). There was no significant difference between seroprevalence of *Brucella* among women with miscarriage and women with no history of miscarriage ($\chi^2 = 0.37$, $df = 1$, $P = 0.6$). The Fisher exact test also showed no significant difference ($P = 0.27$). Table 1 summarizes the results of positive sera.

All women with high titres were treated by the attending physician and relevant education was provided by a nurse who discussed the disease with the patients and provided a leaflet on the disease transmission.

Discussion

There was no significant difference between the true seroprevalences of women with miscarriage and women with no history of miscarriage, indicating equal exposure for both groups. However, 3 women with miscarriage had high titres of anti-*Brucella* antibodies (over 1:1000). The true seroprevalence among women with no history of miscarriage found in our study was similar to the reported seroprevalence of the lower risk group (0.5%, 5 of 910) [16].

As far as we know, this is the first *Brucella* seroprevalence study among aborted women. The same sample was tested for *Toxoplasma gondii* and *Neospora caninum* (M.N. Abo-Shehada, unpublished observations). High

Table 1 Characteristics of the women with and without miscarriage testing positive for *Brucella* spp.

Case No.	Age (years)	No. of miscarriages	Rose Bengal test intensity	CFT titre ^a
Women with miscarriage				
1	25	2	4	1:2560
2	37	1	1	0
3	32	1	2	1:160
4	42	6	1	1:40
5	30	3	2	1:40
6	34	2	1	1:80
7	37	1	1	0
8	30	3	3	1:1280
9	22	1	3	1:1280
Women with no history of miscarriage				
1	35	0	3	1:2560
2	28	0	2	1:160
3	31	0	1	0
4	32	0	4	1:2560
5	26	0	2	1:1280

^aTitres = 0 were under the cut-off value of 17 IU/mL and hence negative. Titres $\geq 1:10$ were above the cut-off value and hence positive. CFT = complement fixation test.

seroprevalences of both *T. gondii* and *N. caninum* were found, but none of the *Brucella*-seropositive samples were found positive for *T. gondii*, and *N. caninum*.

Because of the low prevalence of brucellosis in both groups of women and hence the low number of seropositive women (cases) we were not able to conduct risk analysis (case-control study).

We did not examine other causes of miscarriage such as malaria, typhoid fever, syphilis and antiphospholipid-antibody syndrome. Jordan is free from

malaria, and malaria cases encountered are found only in Jordanian travellers to endemic countries [19].

Even though positivity for *Brucella* does not appear to increase the risk of miscarriage in women, in northern Jordan, more than half of the sheep and goat flocks are seropositive for *B. melitensis* [22,23], and this represents a major source of infection to humans directly through contact and indirectly through contact and consumption of animal products [1]. *B. melitensis* is the

only species isolated in Jordan [24] and infects humans causing severe disease.

We conclude that *Brucella* seropositivity among Jordanian women with miscarriage and women with no history of miscarriage was similar.

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