

ORIGINAL ARTICLE

Seroprevalence of brucellosis among farmers in Morocco

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SUMMARY

From 17 to 31 December 2017, a cross-sectional study was conducted by selecting a study population in numerous farms spread over 30 douars covering the entire province of Sidi Kacem. A total of 430 people were interviewed, using a structured questionnaire in Arabic languages including clinical symptoms.

A 3-5 ml venous blood sample was collected from each patient. Of all patients, 189 (44%) were male and 241 (56%) were female. Sample sera were tested for *Brucella* antibodies using rose Bengal plate test and immunoglobulin (Ig) M/IgG enzyme-linked immunosorbent assay (ELISA) for confirmation.

Of all people surveyed, 212 were confirmed positive for brucellosis, giving an overall seroprevalence of 49.30% (212/430). Of these, 128 (60.4%) were males and most of whom were married. However, the marital status and educational level appears not to affect the seroprevalence of brucellosis for both sexes. Among patients who claim to have brucellosis related symptoms (n = 211), 101 subjects were IgG positive / IgM negative and 9 were IgM positive / IgG negative. However, 1 and 117 cases among those who profess not to have any symptoms of brucellosis were IgM positive / IgG negative and IgG positive / IgM negative, respectively.

It is concluded from the current study that brucellosis is highly prevalent in humans in Sidi Kacem province, where animal breeding is common. Preventive

and control measures should immediately and strictly be implemented to protect animals and humans from brucellosis.

Introduction

Bacteria of the genus *Brucella* cause brucellosis, one of the world's neglected zoonotic diseases. It is a disease of poverty; infections of livestock have a huge socioeconomic cost while human brucellosis starts as a debilitating acute infection that can become chronic with many complications. The figure of 500 000 new human cases each year is regularly cited in reviews and research papers, however this is a vast under estimation; many of the most affected countries do not have the infrastructure for diagnosis and the broad spectrum of symptoms are shared with other febrile infections (David O'Callaghan, 2020 ; Etemadi *et al.*, 2020).

The highest prevalence was observed in the Mediterranean basin (North Africa, Portugal, Spain, southern France, Italy, Greece and Turkey), Mexico, South and Central America, Eastern Europe, Asia, Africa, the Caribbean, and the Middle East (CDC 24/7). In the Maghreb region (Morocco/Algeria/Tunisia), brucellosis still remains a major public health problem, despite the various program strategies that have been implemented to control the disease. The presence of human brucellosis in the Maghreb still seems to be underestimated and its epidemiological situation remains largely unknown (Lounes *et al.*, 2014).

There are six species of *Brucella* and four of which are known to infect humans. They are *B. melitensis*, *B. abortus*, *B. suis* and *B. canis*. *B. abortus* is found principally in cattle, *B. melitensis* in goats and sheep, *B. suis* in swine and *B. canis* in kennel-raised dogs (Kang *et al.*, 2011).

In humans, the incubation period of brucellosis is usually 1-4 weeks; but in some cases, it may be several months.

This infection can cause a range of signs and symptoms, some of which may present for prolonged periods of time. Initial symptoms can include: fever, sweats, malaise, anorexia, headache, pain in muscles, joint, and/or back fatigue. Some signs and symptoms may persist for longer periods of time. Others may never go away or reoccur. These can include: recurrent fevers, arthritis, swelling of the testicle and scrotum area, swelling of the heart (endocarditis), neurologic symptoms (in up to 5% of all cases), chronic fatigue, depression, swelling of the liver and/or spleen (CDC 24/7; Unuvar *et al.*, 2019). On the other hand, the non-specific symptoms of brucellosis pose a challenge to the diagnosis of brucellosis (Shi *et al.*, 2018).

To our knowledge, in Morocco, no study has been conducted on human brucellosis during the last decade. Epidemiological surveillance of animal brucellosis by veterinary authorities has revealed that this disease is enzootic in different regions of the country with different prevalences in different categories of livestock. According to the survey on animal brucellosis conducted by the "Office National de Sécurité Sanitaire des Produits Alimentaires" (ONSSA) in 2010, estimated seroprevalences were 4.92% in livestock and 2.15% in cattle (ONSSA).

Thus, the objective of this study was to determine the prevalence of human brucellosis among farmers in the province of Sidi Kacem, Morocco. As well as associated clinical symptoms.

Material and methods

Study period and location

From 17 to 31 December 2017, a cross-sectional study was

conducted by selecting a study population in numerous farms spread over 30 douars (in Morocco, the term “douar” refers to a “small village [or] a group of rural dwellings” which consists of 50 to 400 households, with a habitat more dispersed in the plains than in mountains) covering the entire province of Sidi Kacem, located in northwestern Morocco. According to the Kingdom of Morocco's High Commission for Planning (KMHCP) (web), the population of Sidi Kacem province in 2017 was approximately 476,600, the majority of whom lived in rural areas with a population density of 150.6/km².

Study design and sample size determination

In the absence of data on human brucellosis seroprevalence estimates in Morocco, we first considered an overall convenience sample of approximately 450 individuals in the farms based on a power of 80%, a type I error of 5%, a nonresponse rate of 10%, and an expected seroprevalence around 12% according to published international studies (Dreyfus *et al.*, 2014; Onyemelukwe, 1993).

Study participants and data collection

A total of 430 people were interviewed, using a structured questionnaire in Arabic languages. For the collection of data, all authors' elaborated close-ended questionnaires consisted of two sections. The first section included sociodemographic data question to collect information on age, sex, marital status, education level. The second section for respondent's clinical manifestation: symptoms such as fever (including low-grade fever) lasting for days or weeks, hyperhidrosis, fatigue, arthralgia and myalgia, etc.

Inclusion criteria: (1) Person was a resident of the selected douar for more than six months. (2) Subjects have a history of animal contact or consuming animal products. (3) Subject willing to participate in a research study and sign

for written consent. (4) 18 years and above individual presented in the douar at the time of screening and interview.

Exclusion criteria: (1) Subject was the resident of selected douars for less than six months, (2) Age of the subject less than 18 years, (3) Subject not willing to participate in the research study.

Ethical approval

Ethical approval was obtained from the "Ethics Committee for Biomedical Research, Rabat, Morocco" prior to the study (CERB reference number N/R 34/16). From each eligible subject, informed consent was obtained before the questionnaire administration. The study was conducted in accordance with the principles of Helsinki Declaration. Participation to our study was voluntary, anonymous, confidential, and only for research purposes to protect privacy and ensure data integrity.

Blood examination for brucellosis

According to CDC and WHO guidelines, two tests, agglutinating and non-agglutinating, should be used to confirm the disease (Corbel 2006; Dean *et al.*, 2012). Sera were screened using the Rose Bengal Plate Test (RBPT) and positive samples were then subjected to indirect enzyme-linked immunosorbent assay (IgM ELISA and IgG ELISA).

A 3-5 ml venous blood sample was collected from each patient using a standard vacutainer tube. The sample was kept at room temperature for 30 minutes to facilitate coagulation and centrifuged at 1509 g for 5 minutes to obtain the clear serum. All sera were separated into labeled 1.8 ml cryotubes and transported to the Pasteur Institute of Morocco in a cold box and stored at 4°C until use.

Sera, RBPT, ELISA reagents, and controls were thawed and brought to room temperature before screening for anti-*Brucella* antibodies in the microbiology laboratory of the Pasteur Institute of Morocco. Rose Bengal plate test was performed with a commercial *Brucella* antigen (*B. abortus*, Crescent Diagnostics, KSA) according to the manufacturer's instructions. Briefly, 30 μ L of test serum was mixed with 30 μ L of Rose Bengal antigen on a clean glass slide and stirred with a disposable stick. The slide was rotated manually for 5–6 min. The presence of agglutinating clusters indicates a positive reaction, while their absence indicates a negative test. A known positive and negative sera (confirmed as positive or negative in our laboratory and stored at -80°C) were used as controls. The RBPT-positive sera were then subjected to an ELISA as a confirmatory test, while the negative sera were stored at -20°C . *Brucella*-specific IgG and IgM ELISA antibody titers in serum samples were determined as previously described (Vircell *Brucella* IgM ELISA and Vircell *Brucella* IgG ELISA, Spain). Briefly, 96-well microtiter plates were coated with LPS of *B. abortus* strain S-99 diluent and serum samples were added to the wells. The plates were incubated at 37°C for 45 min and washed before the addition of peroxidase-conjugated anti-human IgG or IgM was added to the wells. Plates were incubated at 37°C for 30 min, washed again and tetramethylbenzidine was added. The reaction was stopped by the addition of sulfuric acid after 20 min of incubation in the dark and the plates were read at 450 nm in a spectrophotometer (Institut Pasteur du Maroc, Morocco).

The presence or absence of *Brucella* anti-LPS antibodies was determined by comparing optical densities (OD) to cut-off values obtained from the positive control. Therefore, samples considered positive are those with OD

values \geq positive cut-off values, samples considered negative are those with OD values $<$ positive cut-off values. Moreover, a person was considered seropositive when he/she tested positive for both RBPT and ELISA (IgM and/or IgG).

Results

Over a ten consecutive day's period, 430 volunteers participated in this study. Forty-eight of these cases were from Douar Chaouia, the others from Lahrarta (30 cases), Zwayed (30 cases), Oulad Mbarek (24 cases), Coop Lkhaoula (23 cases), Rhamna (22 cases), Oulad Sass (21 cases), Mhichra (19 cases), Majd Zitoune (18 cases), Coop Nouirate (16 cases), Zmamra (16 cases), Oulad Mrabet (16 cases), Louamra (15 cases), Allague (14 cases), Oulad Douar (14 cases), Beni Ouarzguen (12 cases), Ait Youssa (10 cases), Azib Nih (10 cases), Mhaya (10 cases), Oulad Massoud (10 cases), Oulad Sghir (10 cases), Zyayna (10 cases), Legdadra (8 cases), Ait Baha (7 cases), Oulad Larbi (7 cases), Trabna (5 cases), Ramra (4 cases), Oulad Ayad (3 cases), Hricha (1 case) and Rmila (1 case). All recruited farmers live in contact with domestic animals such as cattle, sheep and goats and consume their products. Of the 430 patients, 189 (44%) were male and 241 (56%) were female, with ages ranging from 18 to 83 years old (median age: 45.3 years). Based on educational qualifications, two hundred and sixteen (50.20%) had no education, 153 (35.60%) had primary education, 53 (12.30%) had secondary education while 8 (1.90%) had tertiary education. On the other hand, a total of 211 (49.1%) had one or more symptoms related to brucellosis while 219 (50.9%) have not. The most common symptoms were fever (46.3%), followed by chills (29.8%), arthralgia (29.8%), headache (29.1%), sweating (27.3%), fatigue (22.6%), Anorexia (10.5%) and weight loss (10.3%) (**Table 1**).

Table 1. Characteristics of recruited patients in Sidi Kacem province (n = 430)

Characteristics	No patients tested (%)
Gender	
Male	189 (44.0)
Female	241 (66.0)
Marital status	
Married	287 (66.7)
Unmarried	143 (33.3)
Education level	
None	216 (50.2)
Primary	153 (35.6)
Secondary	53 (12.3)
Tertiary	8 (1.9)
Clinical symptoms	
Presence of:	211 (49.1)
Fever	199 (46.3)
Chills	128 (29.8)
Arthralgia	128 (29.8)
Headache	125 (29.1)
Sweating	117 (27.3)
Fatigue	97 (22.6)
Anorexia	45 (10.5)
Weight loss	44 (10.3)
Absence	219 (50.9)

Of the total samples tested, brucellosis seroprevalence was found to be 53.0 % (228/430) by RBPT, of which 202 (88.6%) were positive for brucellosis with human IgG ELISA test, 10 (4.4%) were positive with IgM ELISA test and the remaining 16 (7.0%) were false positive by ELISA, giving an overall seroprevalence of 49.30% (212/430). Of these 212 subjects tested positive to RBPT and ELISA, 128 (60.4%) were male and more of them were married. The marital status and educational level appears not to affect the seroprevalence of brucellosis (**Table 2**).

Table 2. Demographic characteristic of seropositive and seronegative among the farmers in Sidi Kacem Province, Morocco (n=430)

Characteristics	Sero positive individuals	Seronegative individuals
	N = 212 (49.3) n (%)	N = 218 (50.7) n (%)
Gender		
Male	128 (60.0%)	61 (28.0)
Female	84 (39.6%)	157 (72.0)
Marital status		
Married	139 (65.6)	148 (67.9)
Unmarried	73 (34.4)	70 (32.1)
Education level		
None	106 (50.0)	110 (50.5)
Primary	74 (34.9)	79 (36.2)
Secondary	28 (13.2)	25 (11.5)
Tertiary	4 (1.9)	4 (1.8)

Among patients who claim to have brucellosis related symptoms (n = 211), 101 subjects were IgG positive and IgM negative and 9 were IgM positive and IgG negative. However, 1 and 117 cases among those who profess not to have any symptoms of brucellosis were IgM positive-IgG negative and IgG positive-IgM negative, respectively (**Table 3**)

The table 4 summarises the clinical symptoms found in the 10 anti-*Brucella* IgM positive cases.

Discussion

Brucellosis is an occupationally-associated disease and reports from different parts of the world have shown that one of the high risk groups for brucellosis are livestock farmers (Ahmad *et al.*, 1999; Bamaiyi, 2016; El Sherbini *et al.*, 2007; Jama'ayah *et al.*, 2011)

All samples (430) were first screened for anti-*Brucella* antibodies using RBPT, and samples that tested positive were confirmed as having *Brucella* antibodies using IgM

and IgG ELISA tests. The overall seroprevalence was 49.3% (212/430), of which the prevalence of IgG antibodies was 95.3% (202/212) and IgM was 4.7% (10/212), suggesting that a very large proportion of farmers in Sidi Kacem province was already infected with *Brucella*.

Our result (49.3%) is higher than the prevalence of 9.7% reported in India on subjects living in direct contact with large ruminants, either by their profession or by the family herd (Holt *et al.*, 2021) and higher than rates ranging from 2.6% to 27.1% in Saudi Arabia (Alkahtani *et al.*, 2020). Another study in India screened veterinarians, veterinary pharmacists and animal handlers working for the Department of Animal Husbandry for *Brucella* spp. and found 21.9% had a positive RBPT result, 19.7% had a positive IgM ELISA result, and 53.8% had a positive IgG

ELISA result (Proch *et al.*, 2018). However, our finding remains lower than 63.6% reported among butcher workers in Nigeria (Cadmus *et al.*, 2006). The high prevalence of brucellosis in farmers determined in the current study can be explained by the fact that people involved in the study consume dairy products such as butter, white cheese and cream traditionally made of raw and/or insufficiently heated milk and are in a direct contact with the infected animals and their aborted fetuses and discharges.

Table 2. Demographic characteristic of seropositive and seronegative among the farmers in Sidi Kacem Province, Morocco (n=430)

Characteristics	Seropositive individuals N = 212 (49.3)	Seronegative individuals N = 218 (50.7)
	n (%)	n (%)
Gender		
Male	128 (60.0%)	61 (28.0)
Female	84 (39.6%)	157 (72.0)
Marital status		
Married	139 (65.6)	148 (67.9)
Unmarried	73 (34.4)	70 (32.1)
Education level		
None	106 (50.0)	110 (50.5)
Primary	74 (34.9)	79 (36.2)
Secondary	28 (13.2)	25 (11.5)
Tertiary	4 (1.9)	4 (1.8)

Table 3. Clinical characteristics of patients by diagnosis of brucellosis.

Symptoms	Overall N = 430	ELISA IgM ^{+ve} N = 10	ELISA IgG ^{+ve} N = 202	RBPT ^{-ve} or ELISA IgM-IgG ^{-ve} N = 218
	n (%)	n (%)	n (%)	n (%)
Presence	211 (49.1)	9 (90.0)	101 (50.0)	101 (46.3)
- Fever	199 (46.3)	9 (90.0)	90 (44.6)	100 (45.9)
- Chills	128 (29.8)	9 (90.0)	64 (31.7)	55 (25.2)
- Arthralgia	128 (29.8)	3 (30.0)	65 (32.2)	60 (27.5)
- Headache	125 (29.1)	9 (90.0)	59 (29.2)	57 (26.1)
- Sweating	117 (27.3)	8 (80.0)	55 (27.2)	54 (24.8)
- Fatigue	97 (22.6)	7 (70.0)	48 (23.8)	42 (19.3)
- Anorexia	45 (10.5)	9 (90.0)	19 (9.4)	17 (7.8)
- Weight loss	44 (10.3)	0 (0)	25 (12.4)	19 (8.7)
Absence	219 (50.9)	1 (10.0)	101 (50.0)	117 (53.7)

Table 4. Clinical symptoms of 10 patients positive for *Brucella* IgM antibodies.

Symptoms	Fever	Chills	Arthralgia	Headache	Sweating	Fatigue	Anorexia	Weight loss
Patient N°								
Patient 1	+	+	+	+	+	+	+	-
Patient 2	+	+	+	+	+	+	+	-
Patient 3	+	+	-	+	+	+	+	-
Patient 4	+	+	-	+	+	+	+	-
Patient 5	+	+	-	+	-	+	+	-
Patient 6	+	+	+	+	+	-	+	-
Patient 7	+	+	-	+	+	-	+	-
Patient 8	+	+	-	+	+	+	+	-
Patient 9	+	+	-	+	-	+	+	-
Patients 10	-	-	-	-	-	-	-	-
Total symptoms	9	9	3	9	7	7	9	0

(+) : Presence, (-) : Absence

Our study identified gender as a potential risk factor for

Brucella seropositivity in men. Men residents who lived in Sidi Kacem province were more likely to be seropositive

for brucellosis compared with women. Studies in Saudi Arabia, Libya, and Tunisia found similar results (Alkahtani *et al.*, 2020; Ahmed *et al.*, 2010; Khbou *et al.*, 2018). The higher rate of brucellosis seroprevalence in men is probably due to their greater involvement in feeding animals, raising domestic animals and handling their products, managing vulnerable animals (calves, small ruminants, sick, injured, or pregnant animals), cleaning barns, transporting farm manure, and selling animals, milk, and its by-products (cheese, butter...), which puts them at greater risk of infection. In contrast, women are mostly occupied with household chores, child rearing and spend less time with animals.

However, educational level appears not to affect the seroprevalence of brucellosis. This is in agreement with finding of the study done in Yemen where the socioeconomic and educational factors were independent risk factors for brucellosis (Al-Shamahy *et al.*, 2000). In contrast, Alhoshani *et al.* in 2016 reported that the least educated individuals in Saudi Arabia had a higher prevalence rate than individuals with higher education (Alhoshani *et al.*, 2016). We believe that having higher level of education will not ensure protection against brucellosis but having the right information (regardless of education level) about brucellosis as well as taking heed to all precautionary measures against brucellosis is very important to guard against the infection. Those who had some knowledge of brucellosis and how to protect themselves from it have less seroprevalence of brucellosis because knowledge of the mode of transmission is protective as it helps individuals to take necessary precautionary measures to protect themselves from being infected by the bacteria (Kozukeev *et al.*, 2006).

Brucellosis may present with various symptoms (Franco *et al.*, 2007). The patients most frequently present with

complaints of high fever, weakness, sweating, joint pains, and lack of appetite (Pappas and Memish, 2007 ; Young, 2010). Among the patients included in this study who were positive for IgM antibodies, the most frequently observed complaints were high fever, chills, headache and anorexia, followed fatigue and sweating. Therefore, it is believed that brucellosis should be included in the differential diagnosis of patients with complaints of high fever, chills, headache and anorexia. As much as, Dean *et al.*, reported that documented fever was common, with an estimated 78% of patients affected and estimates of the proportions of patients with self-reported symptoms of sweats, chills, fatigue, headache, and malaise, were 33–81% for adults. Weight loss was lower than the 31% reported in adults (Dean *et al.*, 2012).

Conclusion

It is concluded from the current study that brucellosis is highly prevalent in humans in Sidi Kacem province, where animal breeding is common. Preventive and control measures should immediately and strictly be implemented to protect animals and humans from brucellosis. In order to achieve this, domestic animals such as cattle, sheep and goat should be tested for brucellosis and seropositive animals should be slaughtered and sero-negative animals vaccinated systematically to efficiently eradicate brucellosis. Farmers should be educated about infectious animal diseases and small-scale family farming should be encouraged to perform more industrialized farming.

Conflicts of Interest

Authors must declare all relevant interests that could be perceived as conflicting. If no conflicts exist, the authors should state this.

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