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# First case-control study of zoonotic brucellosis in Gafsa district, Southwest Tunisia

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ABSTRACT

A case-control study was conducted, aimed to describe the clinical human brucellosis (CHB) pattern during 2015 in the Gafsa region (Southwest Tunisia) and to investigate the main risk factors involved in the disease occurrence. One hundred and four CHB cases were notified in 2015 in Gafsa district. All CHB cases that own ruminants were contacted, but only 32 accepted to participate in a matched case-control study. Thirty-two and thirty-one CHB cases and controls, respectively, were included in the study. The subjects were interviewed using a structured questionnaire. A total of 662 domestic ruminants (cattle, sheep and goats) belonging to cases and controls, were screened using the Rose Bengal Test, as recommended by the World Organisation of Animal Health. During 2015, the incidence of CHB was estimated to 30.8 per 100,000 inhabitants affecting mainly males aged between 30 and 39 years. The overall animal seropositivity to *Brucella*, was 21 and 1.9% in case and control farms, respectively ($p < 0.0001$). Only five risk factors were found to be significant: overall animal seropositivity (OR = 65.2; 95%CI: 13.3–318.7); handling aborted females (OR = 43.1; 95%CI: 8.3–222.7); presence of male ruminants in the herds (OR = 18.5; 95%CI: 5.18–66); owning seropositive goats (OR = 18.3; 95%CI: 2.4–137.6), owning seropositive sheep (OR = 9.66; 95%CI: 2.9–31.5) and history of abortion during the previous year in the herd (OR = 4.6; 95%CI: 1.3–12.6). Vaccination of animals against brucellosis was associated with lower odds of human brucellosis (OR = 0.03; 95%CI: 0.004–0.2). Raw milk and derivatives consumption was not a risk factor of human brucellosis. Based on this study, ruminants' vaccination coverage should be increased by enhancing the number of vaccinated animals and systematically including male ruminants in Tunisia. Comprehensive education programmes targeting both farmers and general population should be implemented.

## 1. Introduction

Brucellosis is a zoonotic disease transmitted from ruminants to humans, caused by a gram-negative bacteria belonging to the *Brucella* genus. *Brucella melitensis* infects mainly sheep and goats and is the most pathogen species for humans, widespread throughout the Mediterranean basin [1]. *Brucella* colonizes preferably in female mammals' reproductive tract leading to infertility, placental retention, abortion and stillbirth [2]. It has also a predilection for mammary glands and is occasionally excreted in milk [3]. The transmission between animals occurs mainly through direct contact with infected placenta, genital discharges and through sexual route [4]. The main risk factors include introduction in herds of infected animals or borrowing rams, and co-grazing and contact in watering points [5]. Animal mass vaccination is the only effective control option leading to prevent humans' and animals contamination.

The infection of humans occurs through either consumption of infected milk and derivatives or contact with infected animals (close contact with female ruminants during parturition or abortion, separating the placenta with necked hands and milking) [6]. Brucellosis is considered as an occupational disease, with animal workers' herd managers, slaughterhouse workers and veterinarians as the most exposed categories [7]. In humans, *Brucella* infection leads to several clinical forms ranging from mild fever to neurobrucellosis, found in 5% of the cases [8].

In Tunisia, the overall incidence of human brucellosis ranged between 2.9 and 3.9 per 100,000 inhabitants in 2008 and 2015, respectively [9]. These findings confirm that human brucellosis is still an important disease in Tunisia. Indeed, the annual mean cost of CHB per patient was estimated to 2200 Tunisian dinars (995.5 $US) [10]. During

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**Fig. 1.** Map of Tunisia with Gafsa district location.

this period, all 24 Tunisian districts notified CHB cases, among them 23 to 46% were from Gafsa district (Southwest of Tunisia) [9].

In Tunisia, both human and animal brucellosis are notifiable diseases [11,12]. Despite the implemented control programmes, animal brucellosis is still occurring in Tunisia with variable annual incidence. The aim of this study was to describe the epidemiological pattern of clinical human brucellosis (CHB) in Gafsa during 2015 and to identify the main risk factors associated to animals' infection.

## 2. Materials and methods

### 2.1. Study region

The present survey was carried out from September 2015 to January 2016, in Gafsa district (34°25' North; 8°47′ East). It consists of 11 localities and is situated in Southwest Tunisia; it shares 50 km of borderlands with Algeria (Fig. 1). The climate is arid, with a maximal annual rainfall of 200 mm and a mean temperature in winter and summer of 0 and 43 °C, respectively. The sheep and goats populations per locality ranged between 17,000 and 34,000 heads and from 4.5 to 8 thousand heads, respectively [13] (Table 1).

### 2.2. Descriptive human brucellosis in Gafsa

Epidemiological indicators were calculated according to demographic data provided by the National Institute of Statistics [14].

**Table 1**
Human and animal populations in the different localities of Gafsa district (in thousands) [[13,14].]

| Locality | Human population | Flocks | | Animals | | |
|---|---|---|---|---|---|---|
| | | Small ruminants | Cattle | Sheep | Goats | Cattle |
| Belkhir | 14 | 0.9 | 0.023 | 22 | 8 | 0.1 |
| Gtar | 20 | 0.65 | 0.077 | 20 | 7.5 | 0.55 |
| Gafsa North | 10 | 1.15 | 0.295 | 34 | 5 | 2.4 |
| Gafsa South | 101 | 1 | 0.376 | 25 | 6 | 1.7 |
| El Ksar | 36 | 0.65 | 0.174 | 20 | 4.5 | 0.7 |
| Mdhila | 15 | 0.85 | 0.02 | 30 | 6.7 | 0.1 |
| Metlaoui | 38 | 0.173 | 0.026 | 18 | 6.7 | 0.25 |
| Oum Laraies | 27 | 0.78 | 148 | 32 | 7.5 | 1.5 |
| Redeyef | 26 | 0.65 | 24 | 17 | 5.5 | 100 |
| Sned | 36 | 1.05 | 0.126 | 32 | 5.3 | 65 |
| Sidi Aich | 10 | 1.05 | 0.528 | 30 | 7.3 | 3.95 |
| Total | 331 | 9.103 | 1.817 | 280 | 70 | 12 |

### 2.3. Risk factors analysis

In order to identify the CHB risk factors, a case-control study, including 32 and 31 human cases and controls, respectively, living in 4 localities of Gafsa district was carried out. The CHB incidence in these four localities was the highest in Gafsa between 2008 and 2015 [9]. The inclusion criteria of cases was, expressing CHB during 2015 and being a ruminant owner. Controls were selected randomly and were matched to cases, according to their activities, region of origin and ages. They never expressed symptoms of brucellosis, nor their family members. All the notified CHB owning ruminants (n = 49) in Gafsa for the year 2015 were contacted and invited to participate to the survey, only 32 accepted. An informed consent was obtained from all participants.

Information's on risk factors were collected using a structured questionnaire.

### 2.4. Animal sampling and laboratory analysis

A total number of 662 ruminants (65 cattle, 205 goats and 392 sheep) handled by both cases and controls were included in the survey. All animals were sampled if their numbers in the herd was below 30; otherwise, 10% of the present animals were randomly sampled. The cattle were aged between 18 months and 10 years; sheep and goats were aged between 6 and 180 months (Table 2).

Ten millilitres of whole blood were collected from jugular vein of each animal. Sera were separated by high centrifugation at 1500*g* for 10 min and stored at − 20 °C until testing. The Rose Bengal Test (RBT) was performed as described in the World Animal Health Organisation terrestrial manual [15]. Briefly, 30 µl of each serum was mixed with 30 µl of antigen (inactivated *Brucella abortus*, S99) and checked for agglutination after 4 min of incubation at room temperature. Positive (seropositive sheep serum) and negative controls (seronegative sheep serum) were included in each RBT run.

### 2.5. Statistical analyses

The overall incidence and the incidence per gender, age and locality were estimated (Table 2):

The monthly incidence curve of CHB was fitted with Curve Expert, V 1.4 (Hyams, D. G., Curve Expert software, http://www.curveexpert.net, 2010). Odds ratio and chi square test were calculated for all risk factors. Fisher exact test was performed for small sized samples.

A logistic regression using forward stepwise procedure was performed with SPSS 21 for Windows software (IBM, USA). Only the statistically and biologically significant variables were kept in the final model. The threshold value for all statistical tests was 0.05.

## 3. Results

### 3.1. Demographic characteristics of clinical human brucellosis cases notified in Gafsa district in 2015

During 2015, a total number of 104 CHB cases were notified in Gafsa corresponding to a mean annual incidence of 30.8 per 100,000 inhabitants. The highest incidence was reported for persons aged between 30 and 39 years ($p < 0.001$) (Fig. 2). There was no statistically significant difference of CHB incidence according to gender (35.49% and 28.66% in men and women, respectively) ($p = 0.49$).

The highest incidences were observed in Oum Laraies (148.08), Mdhila (111.07) and Gtar (64.56) localities ($p < 0.001$) (Fig. 3). The number of CHB per month during 2015 is bell shaped with a peak during August (n = 29; 27.8%) (Fig. 4).

The majority of CHB cases (97/104; 93.26%) reported a consumption of raw milk or derivative products during the previous year and more than half of them (59/104; 56.7%) handled ruminants. From the latter, 49 were farmers and 1 butcher ($p < 0.001$). Abortion history in