One Health 5 (2018) 21–26

Contents lists available at ScienceDirect

One Health

journal homepage: www.elsevier.com/locate/onehlt

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

Médiha Khamassi Khbou[a,*], Samaher Htira[a], Kaouther Harabech[b], M'hammed Benzarti[a]

[a] Laboratoire des Maladies Contagieuses, Univ. Manouba, Institut de la Recherche et de l'Enseignement Supérieur Agricoles, Ecole Nationale de Médecine Vétérinaire de Sidi Thabet, 2020 Sidi Thabet, Tunisia

[b] Direction des Soins de Santé de Base, Ministère de la Santé, 32 rue du Khartoum, Tunis, Belvédère. Tunisia

ARTICLE INFO

Keywords:
Clinical human brucellosis
Ruminants
Risk factors
Abortion
Tunisia

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

* Corresponding author.
E-mail address: mkhbou@hotmail.fr (M. Khamassi Khbou).

https://doi.org/10.1016/j.onehlt.2017.12.001
Received 23 October 2016; Received in revised form 21 September 2017; Accepted 18 December 2017
Available online 19 December 2017

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

Table 2
Clinical human brucellosis incidence in Gafsa district.

Incidence per 100,000 inhabitants	Formula
Overall	Total number of CHB cases/total human population in Gafsa
Per gender (male)	Number of male's CHB /total number of male's population in Gafsa
Per gender (female)	Number of female's CHB/total number of female's population in Gafsa
Per age	Number of CHB in each age group/total number of population in corresponding age group in Gafsa
Per locality	Number of CHB in each locality/total number of population in corresponding locality in Gafsa

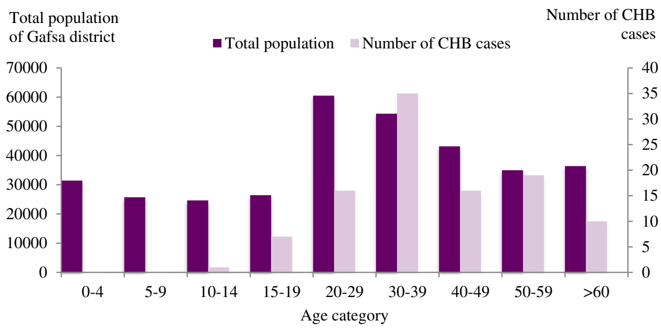

Fig. 2. Age-incidence of clinical human brucellosis (CHB) during 2015 in Gafsa district, Southwest Tunisia.

ruminants was reported during 2015 in 31 farms owned by CHB patients.

3.2. Case control study and risk factors of zoonotic brucellosis

The seroprevalence of *Brucella* spp. infection in both cases and controls was about 20.57% (93/452) and 1.9% (4/210), respectively ($p < 0.0001$)(Table 3). Overall *Brucella* seropositivity was associated with CHB in both flock (OR = 65.2; 95% CI: 13.3–318.7) and animal (OR = 13.3; 95%CI: 4.8–36.8) levels, respectively. The CHB risk's dramatically increases by the presence of seropositive goats (OR = 18.3; 95%CI: 2.4–137.6) and sheep (OR = 9.66; 95%CI: 2.9–31.5). Seropositivity among aborted female ruminants represents also a high risk factor for human brucellosis development (OR = 67.6; 95%CI: 21.6–211.5) (Table 4).

In addition to the impact of seropositivity of animals, handling aborted female ruminants and the presence of male ruminants in herds,

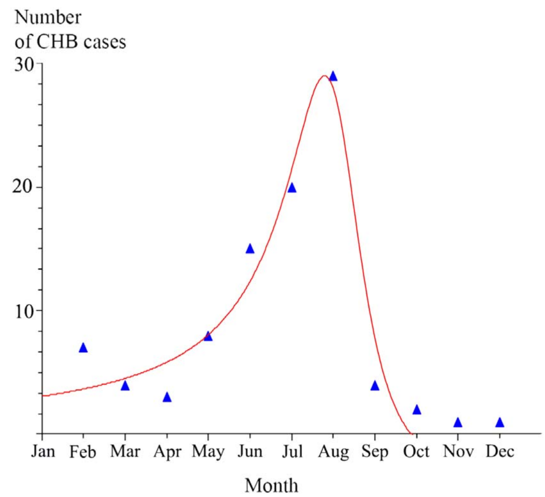

Fig. 4. Monthly number of clinical human brucellosis (CHB) cases in Gafsa district during 2015 (dots indicate the observed values, the line is the fitted curve).

showed high odds ratios (43.12; 95%CI: 8.34–222.7 and 18.5; 95%CI: 5.18–66, respectively). The vaccination of animals against brucellosis was as protective factor (OR = 0.03; 95%CI: 0.004–0.25). Surprisingly, the odds ratio of raw milk or derivatives consumption was not statistically significant (Table 4).

The final model of the logistic regression analysis included three significant variables: handling aborted females, presence of ruminant males in the herds and vaccination of ruminants against brucellosis (Table 5). The risk of CHB was divided by 4, when ruminants were vaccinated against brucellosis. However, this risk increases of 2.9 and of 4, when CHB owned males ruminants in their herds and handled

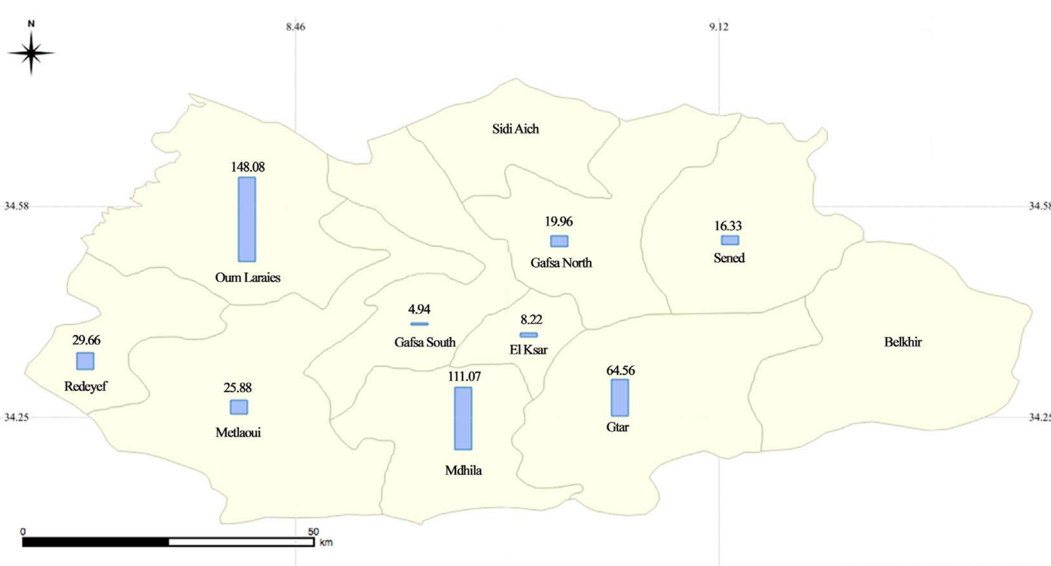

Fig. 3. Incidence per 100,000 inhabitants of clinical human brucellosis in Gafsa localities during 2015.

Table 3
Seroprevalence of *Brucella* spp. infection in ruminants owned by clinical human brucellosis cases and controls in Gafsa district.

		Age		Positive/tested		
Animal species	Gender	Mean	Range	Cases	Controls	Total
Cattle	Males	NA	18	0	0/1	0/1
	Females	58.2	18–120	9/42	0/22	9/64
	Subtotal	57.6	18–120	9/42	0/23	9/65
Sheep	Males	36.8	6–108	7/51	0/4	7/55
	Females	37.7	6–180	47/221	3/116	50/337
	Subtotal	40.1	6–180	54/272	3/120	57/392
Goats	Males	35.16	18–60	0/12	0/2	0/14
	Females	39.23	6–132	30/126	1/65	31/191
	Subtotal	39.85	6–132	30/138	1/67	31/205
Subtotals	Males	39.34	6–108	7/63	0/7	7/70
	Females	40.15	6–180	86/389	4/203	90/592
Total				93/452	4/210	97/662

aborted ruminants, respectively.

4. Discussion

The aims of this study were to describe the epidemiological feature of CHB in Gafsa district in Tunisia in 2015 and to determine the main risk factors for this disease. The incidence of CHB in Gafsa in 2015 was 30.8 per 100,000 inhabitants. During the same year, this incidence was significantly higher than the national incidence (4.35/100,000 inhabitants) [9]. This difference was reported since 1991, when the biggest human outbreak had occurred in Gafsa [16]. After this period, the prevalences in sheep and goats in Gafsa were 30 and 61%, respectively [17], confirming that brucellosis became endemic in the region. There was no analytical study attempting to explain this trend at the time. In 2001, in 2007 and 2011, three annual incidence peaks were registered in Gafsa (55.29; 63.63 and 48.87 per 100,000 inhabitants, respectively) [9]. These peaks were associated to a high brucellosis incidence in animals [18]. In the same period, in Algeria (West of Tunisia), from 2006 to 2014, the incidence ranged from 14.8 to 28.1 per 100,000 [19]. World Health Organisation (WHO) argued that human brucellosis incidence in Maghreb countries was 10 to 25 times underestimated [20].

The age category 30–39 years was the most at risk age category (60.8 per 100,000). Similar trend was reported in Tunisia for human brucellosis cases notified between 1989 and 1998 [10]. Farming and animal rearing are considering as hard activity and is usually better supported by younger people. In addition, people in this age category have finished their schooling and usually are seeking for work, especially in rural regions, where poverty is high. Moreover, males were the most at risk category (35.49%) and higher proportion of brucellosis in men were reported in Tunisia (65%) by Chakroun and Bouzouaia, [21] and in Bangladesh (75%) by Rahman et al., [22]. This trend is confirming that CHB is an occupational disease affecting mainly young farmers, butchers and herdsmen, which are masculine activities [23].

In 2015, in Gafsa, the incidence was higher during summer season (Mai-August)($p < 0.0001$). Ben Hamza, [24] reported that between 1986 and 2008, 75% of CHB occurred during summer and fall. This seasonal trend is likely to be associated to milk yield increase of female small ruminants during spring, when grass becomes abundant. Even if in Tunisia, small ruminant's parturition occurs in fall and winter [25], milk yield increases significantly in spring and people especially in southern Tunisia appreciate it so much. Moreover, milk consumption increases of > 70% during the Muslim fasting month (Ramadan) [26]. During last years, Ramadan was occurring during the summer season and even almost all the CHB cases (95.6%) reported consumption of raw milk products at least once, further investigations should be done to confirm this correlation.

Table 4
Univariate analysis of human zoonotic brucellosis occurrence in Gafsa during 2015.

Risk factor	Case	Control	OR [95% CI]	p
Herd composition				
Presence of small ruminants ($n = 63$)				
Present	29	22	3.95 [0.95–16.34]	0.04*
Absent	3	9		
Presence of cattle ($n = 63$)				
Present	9	9	0.95 [0.32–2.85]	0.9
Absent	23	22		
Presence of dogs in the farm ($n = 63$)				
Present	25	29	0.24 [0.04–1.29]	0.08
Absent	7	2		
Herd management				
Handling aborted females ($n = 63$)				
Yes	30	8	43.12 [8.34–222.74]	< 0.0001*
No	2	23		
Presence of males in herd ($n = 63$)				
Yes	27	7	18.51 [5.18–66.09]	< 0.0001*
No	5	24		
Livestock management ($n = 63$)				
Extensive	31	27	4.59 [0.48–43.62]	*0.15*
Semi-intensive	1	4		
Herd renewal ($n = 63$)				
Self-repopulation	3	3	0.96 [0.18–5.19]	0.9
Uncontrolled purchase	29	28		
Seropositivity to *Brucella*				
Seropositivity of aborted females ($n = 130$)				
Positive	73	4	67.63 [21.6–211.5]	< 0.0001*
Negative	17	36		
Seropositivity of flocks ($n = 63$)				
Positive	29	4	65.2 [13.3–318.7]	< 0.0001*
Negative	3	27		
Seropositivity of goats ($n = 205$)				
Positive	30	1	18.33 [2.4–137.6]	< 0.0001*
Negative	108	66		
Seropositivity of all ruminants ($n = 662$)				
Positive	93	4	13.3 [4.8–36.8]	< 0.0001*
Negative	359	206		
Seropositivity of sheep ($n = 392$)				
Positive	54	3	9.66 [2.9–31.5]	< 0.0001*
Negative	218	117		
Seropositivity of cattle ($n = 65$)				
Positive	9	0	N.A.	N.A.
Negative	33	23		
History of abortion in ruminants (n = 63)				
Yes	26	16	4.06 [1.30–12.61]	0.01*
No	6	15		
Raw milk consumption ($n = 63$)				
Yes	30	26	2.88 [0.51–16.13]	0.2
No	2	5		
Brucellosis prevention				
Vaccination against brucellosis ($n = 63$)				
Yes	1	16	0.03 [0.004–0.25]	< 0.0001*
No	31	15		

* Statistically significant. N.A.: not applicable.

Table 5
Logistic regression results (the dependant variable is the occurrence of clinical human brucellosis in Gafsa during 2015).

Risk factor	A	*p* value	OR	95%CI OR
Handling aborted females	4.4	0.001	84.05	6.8–1030.4
Presence of males in the herds	2.96	0.01	19.43	1.8–203.3
Presence of vaccinated animals against brucellosis	− 4.4	0.004	0.012	0.001–0.2

A: regression coefficient; OR: Odds ratio; CI: Confidence interval.

Among the 11 Gafsa localities, Oum Laraies, showed the highest incidence (148.08 per 100,000 inhabitants). This locality has the highest small ruminants population, moreover, this is the only locality sharing borders with Algeria, where brucellosis occurs. Indeed, a recent study conducted in eastern high plateaus in Algeria showed that 89.6% of human brucellosis in pastoralists was correlated to *Brucella* infection in herds' goats [27]. So it's more likely that unregulated animal movement between Algeria and Tunisia participates to infection spread among animals and humans [28].

The overall seroprevalence in animals' cases (20.57%) was by far higher than in control animals (1.9%) (OR = 65; 95%CI: 13.35–318.71; $p < 0.001$) confirming that rearing infected animals is an important risk factor. In addition, the presence of *Brucella* seropositive goats increased the CHB risk (OR = 18.33; 95%CI: 2.4–137.6) more than it did in sheep (OR = 9.66; 95%CI: 2.9–31.5) and cattle. Similar findings were reported by Musallam et al., [29] in Jordan where the same OR was estimated to 6.9. These results appear to be true, since goats are considered as more susceptible to brucellosis infection than sheep [30].

Dogs are also involved in brucellosis transmission to ruminants when they consume aborted foetuses and placenta [31,32]. In our study, since the dogs were present in both case and control farms, their role in brucellosis epidemiological cycle could not be investigated and further studies targeting this species could be carried out in Tunisia.

CHB cases were more at risk when they were handling small ruminants (OR = 3.95, 95%CI: 0.95–16.34) than cattle (OR = 0.95, 95%CI: 0.32–2.85). In Bangladesh, Rahman et al., [22] (2012) and Islam et al., [32] reported that human brucellosis incidence was higher in farmers handling goats than those handling cattle. This could be explained by two facts: the number of cattle sampled in our study was lower than those of small ruminants, because in Gafsa district the biggest animal population is sheep and goats. In Tunisia, brucellosis infection is mainly occurring in small ruminants, contrarily Barkallah et al., [33] reported higher flock prevalence in cattle (55.6%) than in sheep (21.8%) in central-eastern Tunisia, but *Brucella abortus* DNA was detected in almost all samples including small ruminants.

In the present study, small ruminants with abortion history in the previous years increased significantly the odds of CHB. That's why handling aborted females was a high risk factor (OR = 43; $p < 0.0001$). This finding was also documented by Schelling [34]. Two factors contribute to human contamination: the relative long lasting survive of *Brucella* in the environment after abortion: between 21 and 81 days [35] and transmission through skin contact or inhalation [1]. As hazardous practices in Tunisia: at the moment of parturition and delivery, the whole family participates by pulling the offspring out, usually without gloves. The other practice is helping males during mating large tailed ewes, by orienting the penis to vulva. This needs further investigation to show if contact with penis of infected rams is risk factors.

Contrarily to several findings, the consumption of raw milk and derivatives was not a risk factor for human brucellosis in Gafsa, may be because small ruminants' milk is reserved to feeding young animals.

All farms were extensively managed; this factor was not significant in the present study, contrarily to others [36,37]. In extensively managed herds, rams are either co-grazing with ewes or borrowed [29]. In this study, the presence of males was an important risk factor (OR = 18; 95%CI: 5.18–66.09). Indeed, rams could be sub-clinically infected and transmit sexually *Brucella* to ewes [38]. For this reason, rams could be included in the brucellosis vaccination programme. Despite vaccination being a protective factor for CHB (OR = 0.03; $p < 0.0001$), a small animal population was vaccinated (27%).

5. Conclusion

This study showed that human brucellosis is still a prevalent disease in Tunisia, especially seen in the Gafsa district. Young males in contact with ruminants are the most exposed category indicating that it is an occupational disease, which could be relatively easy to prevent. The analysis of risk factors revealed that handling female ruminants during abortion or parturition increased significantly the odds of CHB. Contrarily to our expectations, consumption of raw milk and derivatives was not considered as a risk factor in Gafsa. Since handling infected animals is a high risk, awareness and educational programme targeting farmers should be carried out. Increase animal vaccination rate especially in remote regions have to be targeted in each control programme. Moreover, vaccinating ruminant males in the control scheme might prevent sexually transmitted brucellosis.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors would like to address thanks to people working in the regional health services of Gafsa district for providing data. The authors are also grateful to private veterinarians of Gafsa district, for helping in animal sampling.

Special thanks to Pr. Gharbi Mohamed from the National Veterinary School of Sidi Thabet for revising and correcting the manuscript.

Funding

This work was supported by *Laboratoire d'épidémiologie d'infections enzootiques des herbivores en Tunisie* (Ministère de l'Enseignement Supérieur, de la Recherche Scientifique; Grant number: LR02AGR03).

References

[1] World Health Organization, Brucellosis in Humans and Animals, WHO/CDS/EPR/2006.7 http://www.who.int/csr/resources/publications/Brucellosis.pdf, (2006) , Accessed date: 20 September 2017.
[2] M.J. Ducrotoy, K. Ammary, H. Ait Lbacha, Z. Zouagui, V. Mick, L. Prevost, W. Bryssinckx, S.C. Welburn, A. Benkirane, Narrative overview of animal and human brucellosis in Morocco: intensification of livestock production as a driver for emergence? Infect. Dis. Poverty 4 (2015).
[3] V.P. Meador, B.L. Deyoe, N.F. Cheville, Pathogenesis of Brucella abortus infection of the mammary gland and Supramammary lymph node of the goat, Vet. Pathol. Online. 26 (1989) 357–368.
[4] E. Diaz Aparicio, Epidemiology of brucellosis in domestic animals caused by Brucella melitensis, Brucella suis and Brucella abortus, Rev. Sci. Tech. 32 (1) (2013) 43–51 OIE.
[5] S. Mazeri, F. Scolamacchia, I.G. Handel, K.L. Morgan, V.N. Tanya, B.M. deC Bronsvoort, Risk factor analysis for antibodies to Brucella, Leptospira and C. burnetii among cattle in the Adamawa region of Cameroon: a cross-sectional study, Trop. Anim. Health Prod. 45 (2013) 617–623.
[6] M.N. Seleem, S.M. Boyle, N. Sriranganathan, Brucellosis: a re-emerging zoonosis, Vet. Microbiol. 140 (2010) 392–398.
[7] J. Karadzinska-Bislimovska, J. Minov, D. Mijakoski, S. Stoleski, S. Todorov, Brucellosis as an occupational disease in the republic of Macedonia, Maced J. Med. Sci. 3 (2010) 251–256.
[8] Center for Disease Control and Prevention, Signs and Symptoms | Brucellosis | CDC, https://www.cdc.gov/vhf/ebola/symptoms/index.html, (2012) , Accessed date: 20 September 2017.
[9] DSSB (Direction des soins et de santé de base), Ministère de la santé, Bulletin épidémiologique, *Annual report* (2016).
[10] M. Gharbi, A. Rejeb, M. Bejaoui, A. Jemli, Coût de la brucellose humaine en

Tunisie : étude sur 10 ans (de 1989 à 1998), J. Econ Med. 19 (2001) 230–239.
[11] Journal Officiel de la République Tunisienne, Décret n°2009–2200 du 14 juillet 2009 fixant la nomenclature des maladies animales réglementées et édictant les mesures générales applicables à ces maladies, http://www.cnudst.rnrt.tn/jortsrc/2009/2009f/jo0592009.pdf , Accessed date: 20 September 2017.
[12] Journal Officiel de la République Tunisienne, Arrêté du ministre de la santé du 1er décembre 2015, fixant la liste des maladies transmissibles à déclaration obligatoire, http://www.ordre-medecins.org.tn/pdf/liste-des-Maladies-transmissibles.pdf, (2015) , Accessed date: 20 September 2017.
[13] Commissariat Régional au Développement Agricole de Gafsa, Ministère de l'Agriculture, des Ressources Hydrauliques et de la Pêche, *Annual report* (2014).
[14] Institut National de la Statistique, Gafsa à travers le recensement général de la population et de l'habitat, http://www.ins.tn/fr/resultats, (2014).
[15] OIE (World Organisation for Animal Health), Terrestrial Manuel. Brucellosis (*Brucella abortus*, *B. melitensis* and *B. suis*) (Infection with *B. abortus*, *B. melitensis* and *B. suis*) Chapter 2.1.4, http://www.oie.int/fileadmin/Home/fr/Health_standards/tahm/2.01.04_BRUCELLOSIS.pdf , Accessed date: 20 September 2017.
[16] N. Bouzouaia, M. Chakroun, J. Rachdi, T. Rachdi, Epidemiological, clinical and therapeutic aspects of brucellosis in Tunisia. A propos of epidemics in Gafsa, Tunis. Med. 73 (1995) 443–448.
[17] S. Akremi, La brucellose des petits ruminants et de l'homme en Tunisie, étude épidémiologique dans le gouvernorat de Gafsa et la localité Borj El Akerma, Ecole Nationale de Médecine Vétérinaire, 1999, p. 78 DVM thesis.
[18] Direction Générale des Services Vétérinaires, Ministère de l'Agriculture, des Ressources Hydrauliques et de la Pêche, *Annual report* (2013) (265 pp).
[19] M. Kardjadj, B. Kouidri, D. Metref, P.D. Luka, M.H. Ben-Mahdi, Abortion and various associated risk factors in small ruminants in Algeria, Prev. Vet. Med. 123 (2016) 97–101.
[20] D.S. Berger, Brucellosis: Global Status, GIDEON Informatics Inc, 2016, www.gideononline.com , Accessed date: 20 September 2017.
[21] M. Chakroun, N. Bouzouaia, La Brucellose: une zoonose toujours d'actualité. Brucellosis: a topical zoonosis, Rev Tun. Infect. 1 (2007) 1–10.
[22] A.K.M.A. Rahman, B. Dirk, D. Fretin, C. Saegerman, M.U. Ahmed, N. Muhammad, A. Hossain, E. Abatih, Seroprevalence and risk factors for brucellosis in a high-risk group of individuals in Bangladesh, PLoS One 9 (2012) 190–197.
[23] M. Maurin, Brucellosis at the dawn of the 21st century, Méd. Mal. Infect. 35 (2005) 6–16.
[24] A. Ben Hamza, La brucellose: étude épidémiologique, clinique et thérapeutique à propos de 117 cas, Thèse de médecine de la Faculté de Médecine de Tunis (2009), p. 95.
[25] M. Rekik, R. Aloulou, M. Ben Hammouda, Small Ruminant breeds in Tunisia, in: L. Inguinez (Ed.), Characterisation Small Ruminants Breeds in West Asia and North Africa, International Centre for Agricultural Research in the Dry Areas (ICARDA), 2005, pp. 91–140.
[26] Bizerte Competitiviness Pole, Developing the typical dairy product of Bizerte and Beja: Diagnosis and local strategy, http://www.lactimed.eu/sites/default/files/kcfinder/files/Lac_DiagnosisReport_Tunisia_EN_Final.pdf, (2013) , Accessed date: 20 September 2017.
[27] A. Gabli, A. Agabou, Z. Gabli, Brucellosis in nomadic pastoralists and their goats in two provinces of the eastern Algerian high plateaus, Trop. Anim. Health Prod. 47 (2015) 1043–1048.
[28] E.M. Fèvre, B.M. Bronsvoort, A. Hamilton, S. Katie Cleaveland, Animal movements and the spread of infectious diseases, Trends Microbiol. 14 (2006) 125–131.
[29] I.I. Musallam, M. Abo-Shehada, M. Omar, J. Guitian, Cross-sectional study of brucellosis in Jordan: prevalence, risk factors and spatial distribution in small ruminants and cattle, Prev. Vet. Med. 118 (2015) 387–396.
[30] M. Sobhani-Moltagh, A.R. Bahonar, An evluation of Brucellosis among Sheep Goats Qom Province of Iran, https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&cad=rja&uact=8&ved=0ahUKEwjF2KzEzLHWAhVD6xQKHdFnCHAQFggqMAA&url=http%3A%2F%2Fwww.sciquest.org.nz%2Felibrary%2Fdownload%2F68140%2FT2-P40_-_An_evaluation_of_prevalence_of_brucellosi.pdf&usg=AFQjCNFQpI4_hn_JDxdnntGtMx16fw0K8w, (2005) , Accessed date: 20 September 2017.
[31] B.J. Bricker, S.M. Halling, Differentiation of Brucella abortus bv. 1, 2, and 4, Brucella melitensis, Brucella ovis, and Brucella suis bv. 1 by PCR, J. Clin. Microbiol. 32 (1994) 2660–2666.
[32] M.A. Islam, M.M. Khatun, S.R. Werre, N. Sriranganathan, S.M. Boyle, A review of Brucella seroprevalence among humans and animals in Bangladesh with special emphasis on epidemiology, risk factors and control opportunities, Vet. Microbiol. 166 (2013) 317–326.
[33] M. Barkallah, Y. Gharbi, S. Zormati, N. Karkouch, Z. Mallek, M. Gautier, R. Gdoura, I. Fendri, A mixed methods study of ruminant brucellosis in central-eastern Tunisia, Trop. Anim. Health Prod. (2016) 1–7.
[34] E. Schelling, C. Diguimbaye, S. Daoud, J. Nicolet, P. Boerlin, M. Tanner, J. Zinsstag, Brucellosis and Q-fever seroprevalences of nomadic pastoralists and their livestock in Chad, Prev. Vet. Med. 61 (2003) 279–293.
[35] K. Aune, J.C. Rhyan, R. Russell, T.J. Roffe, B. Corso, Environmental persistence of Brucella abortus in the greater Yellowstone area, J. Wildl. Manag. 76 (2012) 253–261.
[36] F.J. Reviriego, M.A. Moreno, L. Domínguez, Risk factors for brucellosis seroprevalence of sheep and goat flocks in Spain, Prev. Vet. Med. 44 (2000) 167–173.
[37] A.J. Ogugua, V.O. Akinseye, M.C. Ayoola, O.O. Oyesola, F.K. Shima, A.O. Tijjani, A.N.A. Musa, H.K. Adesokan, L. Perrett, A. Taylor, J.A. Stack, I. Moriyon, S.I.B. Cadmus, Seroprevalence and risk factors of brucellosis in goats in selected states in Nigeria and the public health implications, Afr. J. Med. Med. Sci. 43 (2014) 121–129.
[38] M.N. Xavier, T.A. Paixao, A.B. den Hartigh, R.M. Tsolis, R.L. Santos, Pathogenesis of Brucella spp. Open Vet. Sci. J. 4 (2010).