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REGULAR ARTICLES

Brucellosis in nomadic pastoralists and their goats in two provinces of the eastern Algerian high plateaus

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Abstract A 31-months study was conducted to elucidate the prevalence of brucellosis in nomadic pastoralists and their goats in two provinces of the eastern Algerian high plateaus. Five hundred eight human and 4955 animal sera were screened with the Rose Bengal plate test and the complement fixation test for confirmation. Uterine fluids from aborting goats were subjected to microbiological analyses to determine the biovars responsible for abortions. The overall seroprevalence was 0.98 % among animals and 15.84 % among herds. A significant correlation was recorded between occurrence of brucellosis and herd size (r=0.4046, P<0.0001) as well as age (χ^2=5.809, P=0.0159) and sex of animals (χ^2=20.09, P<0.0001); 89.65 % of human cases were related to positive herds and the infection rate was higher in men (7.6 %) than in women (6 %) and children (0.92 %). *Brucella melitensis biovar 3* was the only aetiology of brucellosis-associated abortion in goats of the studied region.

Keywords Algeria · Brucellosis · *B. melitensis* biovar 3 · Goats · Nomadic pastoralists · Seroprevalence

Introduction

Brucellosis is a worldwide zoonosis caused by several species of the *Brucella* genus mostly transmitted through direct or indirect contact with infected animals or their products (Pappas et al. 2006). This disease has heavy economic and social impacts. In humans, it is a systemic acute or chronic infection affecting the entire body and resulting in many complications (Buzgan et al. 2010), while in ruminants, it causes severe economic losses in relation with increased number of abortions, reduced fertility and productivity, decreased milk production and loss of trade opportunities (FAO 2010).

Goats are known to be a potential reservoir for the most pathogenic *Brucella* species all over the world, and in Algeria, they are considered as primary hosts and important source for human brucellosis (Boudilmi and Benhabylles 1991). In this country, there are about 3.8 million goats, mainly located in the steppe and pre-Sahara regions where they play an important role in the livelihood of many families by providing them with various products (Moustaria 2008).

Intensive efforts have been made by the Algerian authorities to eradicate brucellosis or at least to keep it at a controllable level (Benkirane 2001). Nevertheless, scarce information is available on its prevalence in the national livestock due to lack of research into it, deficiency in cooperation between the public health and veterinary sectors and absence of information exchange with surrounding countries (Aggad and Boukraa 2006).

The present study attempts to elucidate the prevalence of brucellosis in both nomadic pastoralists and their goats in Setif and Batna provinces (eastern Algerian high plateaus). This can complete the existing data about this infection and may lead to more efficient preventive and control strategies.

Materials and methods

Samples

From March 2011 to October 2013, 105 nomadic pastoralists were contacted and informed about the purpose and the

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methods of the study to obtain their consent. Only 101 accepted to participate to this survey. Each of their herds presented on average 48 goats of local breeds commingling with sheep in a traditional extensive production system.

Four thousand nine hundred fifty-five goats with no history of vaccination against brucellosis and 508 pastoralists and their family members were sampled. Blood was collected in plain Vacutainer® tubes labelled and conveyed quickly on ice to the laboratory. Sera were then separated from the clot by centrifugation and stored under refrigeration until analyses. Each herd was assigned an identification number (ID n°) and its characteristics (age and sex composition) (Table 1) as well as those of the corresponding family were recorded (Table 2).

Vaginal swabs were taken from 38 goats that have recently aborted in order to search and identify *Brucella* species in their uterine fluids (Table 3). They were transferred on ice to the laboratory for further processing. The parity number and the history of abortion of each female were noted.

Serological tests

Animal and human sera were subjected at first to the Rose Bengal plate test (RBT) as described by Alton et al. (1998) using standard Rose Bengal antigen (Bengatest, Synbiotics Europe, Lyon, France). Then, all RBT-positive sera were tested further for confirmation by the complement fixation test (CFT) according to the recommendations of the OIE (2009). The test was performed using micro-titration plates and *Brucella abortus biovar 1 strain S99* as antigen (Veterinary Laboratories Agency Weybridge, Addlestone, UK). The complement and the haemolytic system used as indicator were purchased from Institute Pourquier (Montpellier, France). A titre equivalent to 20 international complement fixation test units (ICFTU)/ml or greater was considered to be positive.

Culture and identification of *Brucella spp*

Each vaginal swab was streaked on two Farrel's medium plates. Half of the plates were incubated in aerobiosis and the other half in microaerobiosis (with 5 to 10 % CO_2) at 37 °C. Plates were observed for bacterial growth on the 3rd, 5th and 7th day. Suspected colonies were subjected to biotyping tests: staining, oxidase, catalase, H_2S production, CO_2 requirement, growth on media supplemented with thionin and fuchsin dyes and finally agglutination with monospecific A and M antisera (Alton et al. 1998).

Data analysis

An animal was considered seropositive if it tested positive to both RBT and CFT, and a herd was defined as infected if it included at least one seropositive animal. As there was no history of vaccination, seropositivity was considered to be due to natural infection.

Person *r* correlation coefficient and χ^2 statistical test (or *Fisher's exact test* when necessary) were performed using the statistical software GraphPad Instat prism 5 (2007) to assess the influence of the recorded variables on *Brucella* seroprevalence rates. Statistical significance was set at *95 % confidence interval* (*CI*) and a *p* value <5 %.

Table 1 Prevalence of brucellosis in herds and individual goats

Province	Herds/animals (age in months)	No. tested	RBT+CFT	
			No. positive (ID n° of the corresponding herds)	% positive
Setif	Herds	45	5 (10, 14, 26, 37, 39)	11.11
	♀ (>6)	1450	14 (10, 14, 26, 37, 39)	0.97
	♂ (>6)	130	5 (14, 26, 37, 39)	3.84
	♂+♀ (2–6)	465	1 (37)	0.21
	Sub-total	2045	20	0.98
Batna	Herds	56	11 (48, 55, 62, 65, 74, 79, 82, 85, 89, 93, 98)	19.64
	♀ (>6)	1820	19 (48, 55, 62, 65, 74, 79, 82, 85, 89, 93, 98)	1.04
	♂ (>6)	181	7 (55, 62, 85, 89, 98)	3.86
	♂+♀ (2–6)	909	3 (62, 89)	0.33
	Sub-total	2910	29	0.99
Overall	Herds	101	16	15.84
	♀ (>6)	3270	33	1.01
	♂ (>6)	311	12	3.85
	♂+♀ (2–6)	1374	4	0.29
	Total	4955	49	0.98

Table 2 Prevalence of brucellosis in nomads

Province		Age (years old)	RBT+CFT			ID n° of the corresponding herds	
			No. tested	No. of positive	Positive (%)	Infected	Non-infected
Setif	Man	15–45	70	2	2.85	10, 14, 26, 37, 39	2, 6
		46–82	30	6	20		
		Sub-total	100	8	8		
	Woman	15–40	52	0	0		
		41–80	18	2	11.11		
		Sub-total	70	2	2.85		
	Child	5–9	15	0	0		
		10–15	30	0	0		
		Sub-total	45	0	0		
	Total		215	10	4.65		
Batna	Man	15–45	102	3	2.94	48, 55, 62, 65, 74, 79, 82, 85, 89, 93, 98	68
		46–82	48	8	16.66		
		Sub-total	150	11	7.33		
	Woman	15–40	62	2	3.22		
		41–80	18	5	27.77		
		Sub-total	80	7	8.75		
	Child	5–9	16	0	0		
		10–15	47	1	2.12		
		Sub-total	63	1	1.58		
	Total		293	19	6.48		
Overall	Man	15–45	172	5	2.9		
		46–82	78	14	17.94		
		Sub-total	250	19	7.6		
	Woman	15–40	114	2	1.75		
		41–80	36	7	19.44		
		Sub-total	150	9	6		
	Child	5–9	31	0	0		
		10–15	77	1	1.29		
		Sub-total	108	1	0.92		
	Total		508	29	5.7		

Table 3 Results of bacterial identifications

Province	No. of swabbed goats		No. of goats infected with *B. melitensis*	Goats infected with *B. melitensis* (%)
Setif	Primiparous	14	10	71.42
	Multiparous	2	1	50
	Sub-total	16	11	68.75
Batna	Primiparous	19	13	68.42
	Multiparous	3	2	66.66
	Sub-total	22	15	68.18
Overall	Primiparous	33	23	69.69
	Multiparous	5	3	60
	Total	38	26	68.42

Results

Animal seroprevalence

The prevalence of brucellosis in the study area was 0.98 % in individual animals and 15.84 % in herds. In Setif province, brucellosis was prevalent in 11.11 % of herds and 0.98 % of goats, while in Batna, 19.64 % of herds and 0.99 % of goats were tested positive. There was a difference in prevalence of brucellosis at herds' level among the two regions, but it was not significant (p=0.2839); however, no difference was observed at the individual animals' level (χ^2=0.0065, p= 0.9356). Sex distribution showed that 3.85 % of males and 1.01 % females were seropositive confirming the association between *Brucella* infection and sex of animals (χ^2=20.09,

$p<0.0001$). Similarly, there was an increasing tendency of *Brucella* infection with age ($\chi^2=5.809$, $p=0.0159$) since goats aged over 6 months had recorded higher seroprevalence (1.29 %) than those aged 2 to 6 months (0.29 %). It appears also that herd size is significantly associated to the seroprevalence of caprine brucellosis ($r=0.4046$, $p<0.0001$) (Table 1).

Human seroprevalence

In the overall, prevalence of brucellosis among pastoralists and their families was 5.7 % (7.6 % in men, 6 % in women and 0.92 % in children). No difference in prevalence in men was recorded between the two zones ($p=1.00$); however, the infection was more predominant in women and children in Batna than in Setif but not significantly ($p=0.1753$ and $p=1.00$, respectively).

Gender distribution in this study was 62.5 % males and 37.5 % females. The overall seroprevalence is higher in men than in women (7.6 against 6 %), but the difference was not significant ($p=0.6864$). Brucellosis infection rate was higher among men in Setif, while it was approximately the same in both sexes in Batna ($p=0.7976$). Men at age band 46–82 years old were more infected ($p<0.0001$) as well as women at the band 41–80 years old ($p=0.0007$) in both zones. The positive child case was associated to a high prevalence in women in Batna, and 89.65 % of positive human cases were related to positive herds; however, no correlation could be established between brucellosis frequency in nomadic families and their sizes ($r=0.1244$, $p=0.6118$) or its occurrence within their respective herds ($r=0.1671$, $p=0.5363$) (Table 2).

Bacterial isolation

Twenty-six bacterial isolations were characterized with success: 11 isolates from Setif and 15 from Batna with frequencies of 68.75 and 68.18 %, respectively. *Brucella melitensis biovar 3* was the only identified strain in both zones with no significant difference in isolation rates ($p=1.00$). No bacterial isolations were made from seronegative goats and brucellosis-associated abortion was six times more frequent in primiparous goats compared to multiparous ones (Table 3).

Discussion

Algerian high plateaus are grazing lands used for 3 or 4 months (springtime) to feed about 16.8 million sheep and 1.6 million goats. More than 7.5 million people live in this region, 10 % of them are nomads. Movements of the mobile population are of two types: nomadism (wherein the whole family moves with the herds) and transhumance (which only affects the shepherds and the livestock) (Nedjraoui 2012). This lifestyle characterized by persistent migration and lack of health service facilities, allows appearance, persistence and spread of zoonosis like brucellosis in both humans and their livestock.

The prevalence rate reported in goats of this study is lower than those reported by Aggad (2003) and Nehari et al. (2014) in Tiaret and El-Bayadh provinces (western Algerian high plateaus) (2.6 and 3 %, respectively). Bachir-Pacha et al. (2009) found an average prevalence of 11 % among goats in Msila province (central Algerian high plateaus). Our low prevalence may be explained by a certain success of the control programmes in association to a developing awareness in nomadic populations of the hazard that represents this disease. Furthermore, it is known that brucellosis is less frequent in extensive grazing conditions where both animal-to-animal contact and contamination of pastures are reduced under the dry climate (Crawford et al. 1990).

Prevalence of brucellosis in livestock is influenced by many factors such as age and sex of animals, herd size and composition, hygienic status, contact between infected and susceptible animals, biosecurity measures and contact with wildlife (McDermott and Arimi 2002; Radostitis et al. 2007). Nehari et al. (2014) observed high seroprevalence in sexually mature goats (in agreement with our findings) and especially in females (in contrast with our results) which are usually more physiologically stressed (Walker 1999; Mangen et al. 2002) and more susceptible to *Brucella* infection because of D-erythritol, a 4-carbon sugar alcohol found in higher concentrations in foetal and uterine tissues of ruminant females than in seminal vesicles and testes of males. This polyol stimulates the growth of *B. melitensis* and *B. abortus* (Keppie et al. 1965). The high prevalence of *Brucella* infection in males in our study may be due to their small number as compared to the number of females. Furthermore, a large number of males are kept for a shorter time thus the chance of their exposure to the infection is lower than females; however, the remaining males are more actively involved in the breeding process which makes them more susceptible to acquire and to spread the infection.

In young animals, brucellosis is less frequent because of the lack of sex steroid hormones and erythritol, which stimulate the growth and multiplication of *Brucella* organisms (Radostitis et al. 2007). The presence of *Brucella* infection in goats less than 6 months of age may result from their contact with infected animals and/or contaminated environment at pastures and watering points where the mixture of animals from different herds and many species may occur and facilitate the spread of the disease.

Compared to surrounding countries, caprine brucellosis in our studied regions is less frequent than in Libya (31 %) (Ahmed et al. 2010), Tunisia (6.25 %) (Khammassi-Khabou et al. 2009), Morocco (4.1 %) (Benkirane 2006) and Mali (1.3 %) (Sow et al. 2011), but it is higher than in Niger (0.4 %). These differences between countries may be attributed to variations in husbandry practices, susceptibility of

animals, virulence of organisms, transhumance and absence of veterinary services in addition to lack of consciousness about the disease among the different populations (Mohammed et al. 2011).

The recorded seroprevalence in the nomads of the two provinces is higher than those reported in the normal population by the National Institute of Public Health (INSP) in 2009 (19.4 cases/100,000 inhabitants) and by Nehari et al. in 2014 (113 cases/100,000 inhabitants). The occurrence of brucellosis in humans depends upon several factors such as dietary habits, methods of processing milk and meat products, husbandry practices and environmental hygiene (Gwida et al. 2010). The high prevalence recorded in our nomad population could be explained by the culture and traditions of this population such as ingestion of raw milk or milk products, mostly during the springtime which coincide with the parturition and lactation period of goats and sheep. Consumption of uncooked meat (liver and spleen) is also incriminated as well as the participation of the whole family in the slaughtering process which exposes them (including children) to infected blood and raw products.

Men are more infected than women because of their direct involvement in slaughtering animals and doing most of the livestock obstetric work. This is supported by Nehari et al. (2014) who additionally found brucellosis more frequent in adults (20 to 44 years old). In contrast, brucellosis in children is uncommon, and raw milk ingestion is described as the primary source of infection in these cases (Shaalan et al. 2002). Mother-to-child transmission of brucellosis (through breast milk) has been reported but is exceptional (Palanduz et al. 2000), and some habits and traditions may also play a foremost role in children infection (Alexander et al. 2012).

It is worth noting that no *brucella* screening test is perfect, and all tests have specificity and sensitivity limitations, especially when testing individual animals or humans. This is why it is necessary to couple the clinical history with a combination of at least two tests in order to minimize diagnostic errors. Confirmatory diagnosis must be provided by isolation of aetiological agents (Bricker 2002).

Brucella melitensis infects mostly sheep and goats, and the *biovar 3* is known to be the major aetiology of animal and human brucellosis in the Mediterranean basin (Refai 2002). This biovar has been previously isolated in Tiaret province from human blood and cow milk samples (Aggad and Boukraa 2006).

Similarly to our results, Mellado et al. (2004) found Brucella-positive primiparous goats to be less likely to kid than multiparous ones. This may be attributed to the fact that after the first abortion, animals acquire natural immunity and subsequent gestations become generally normal. Abortions in the seronegative and *Brucella*-free goats may be caused by other infectious or non-infectious agents (Haskell and Anttila 2001).

In conclusion, the current investigation confirms that brucellosis is prevalent in nomadic pastoralists and their goats in the Algerian high plateaus where animal breeding activities are very important. It reveals also that *B. melitensis biovar 3* remains the main aetiology of brucellosis-associated abortion in this livestock. This indicates a serious situation with goats being one of the major sources of infection.

Additional ample and multi-sectoral investigations are needed to assess the risk factors and to determine the status and transmission routes of this zoonosis in and between sedentary and nomadic populations, traditionally and nomadically reared livestock and wildlife using efficient diagnostic and epidemiological procedures such as more sensitive screening tests, molecular tools and geographic information systems.

The control of brucellosis in animals and consequently its prevention in humans imposes more cooperation between the public health and veterinary institutions, in addition to information exchange with bordering countries where brucellosis is frequent.

Conflict of interest The authors declare that they have no conflict of interest.

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