Sero-epidemiological Investigation of the Major Abortive Bacterial Agents in Ewes of M'Sila Governorate, Algeria

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Bulletin UASVM Veterinary Medicine 77(2)/2020
Print ISSN 1843-5270; Electronic ISSN 1843-5378
doi:10.15835/buasvmcn-vm:2020.0004

Abstract

The aim of this study was to evaluate the seroprevalence, risk factors and zoonotic threats of the major abortive bacterial agents in sheep of M'Sila Governorate. A total of 184 serum samples were collected from ewes among 16 sheep flocks and tested for *Coxiella burnetti, Chlamydia abortus, Brucella spp.* and *Salmonella abortusovis* via ELISA. Simultaneously, a questionnaire was used to collect breeding management data. Seropositive results were as follows : *Coxiella burnetti* (27.9%), *Salmonella abortusovis* (15.9%), *Chlamydia abortus* (10.9%) and *Brucella spp.* (3.8%).The use of univariate analysis and multivariate logistic regression showed a highly significant correlation between *Coxiella burnetti* seropositivity and presence of cats in farms (OR = 5.75; 95% CI = 1.86-19.9; p= 0.001), while promiscuity with newly introduced animals was associated to *Chlamydia abortus* seropositivity (OR=3.37; 95%CI=1.01-14.9; p= 0.04). Additionally to the economic losses, the dissemination of *Coxiella burnetti* and *Brucella spp.* presents uncontrollable zoonotic hazards.

Keywords: abortive bacterial agents, seroprevalence, ELISA, risk factors, ewes.

Introduction

According to the FAO (FAOSTAT, 2019), the recorded ovine population in Algeria stands is 28.393.602 heads. As a main source of meat production, sheep farming is the most practiced compared to other domestic species in Algeria. According to Algerian Ministry of Agriculture and Rural Development (MARD, 2018), sheep represents 78% of the total livestock in Algeria.

However, abortions are considered as a major scourge in small ruminants' flocks. When installed, they are difficult to eradicate because of the lack of accurate diagnosis tools. They result in very significant economic losses due to abortion and stillbirth occurrences (Rekiki *et al.*, 2005, Borel *et al.*, 2014). The economic loss linked to the loss of 160 animals and 4146 L of milk due to abortions in Mali, a country neighbor of Algeria, has been estimated at 7,887,880 FCFA (11,989 Euros according to the conversion rate of June 22, 2020) (Sidibe *et al.*, 2013). This constitutes a considerable shortfall for farmers, particularly in countries where incomes are quite low. To these losses, we could add the indirect losses relating to research, prophylaxis and vaccination programs for each abortive agent.

Sheep farming in Algeria, is mainly conducted according to extensive and semi intensive systems, also breeding and lambing seasons are random and chronogically indefinite (Kardjadj *et al.,* 2015). The observation and notification of abortions which have multiple causes, often lack precision, consequently farmers habitually consider their ewes as infertile especially when these abortions occur at an early stage of gestation.

Algeria is known for its varied climate; Mediterranean in the North and Saharan in the South. This would explain the disparity in abortion rates across the different regions of the country according to Kardjadj *et al*. (2015) whom reported high prevalence of abortive agents in small ruminants' flocks ranging from 40 to 88.2%. Furthermore, Hamza and Bouyoucef (2013) reported abortion rates up to 90% in small ruminants' flocks of the North and the East of Algeria.

Infectious agents are the most plausible causes of abortions in sheep as compared to non-infectious agents. Nevertheless, their differential clinical diagnosis is often difficult and laboratory tests are essential to reveal presence of microbial organisms. The main infectious agents of abortions in sheep are ; *Brucella melitensis, Chlamydia abortus (C. abortus), Coxiella burnetii (C. burnetii), Toxoplasma gondii (T.gondii)*, *Salmonella abortusovis (S. abortusovis)* and Border disease virus (BDV) (Edmondson *et al.,* 2002; Fthenakis *et al.,* 2012; Borel *et al.,* 2014). Moreover, these agents are mostly zoonotic (Acha and Szyfres, 2003 ; Toma *et al.,* 2004 ; Borel *et al.,* 2014).

Among other factors, lack of hygiene and poor management which increase infection rate by abortive agents, the risk of abortions and stillbirths (Dabaja *et al.,* 2019). Thus, identification of the risk factors associated with abortions and infection occurrence with abortive agents can serve in optimizing flocks' reproductive efficiency. The objective of the present study is to evaluate the seroprevalence and risk factors for abortive bacterial agents in ewes of M'Sila Governorate, focusing on the most incriminated; *C. burnetti,*

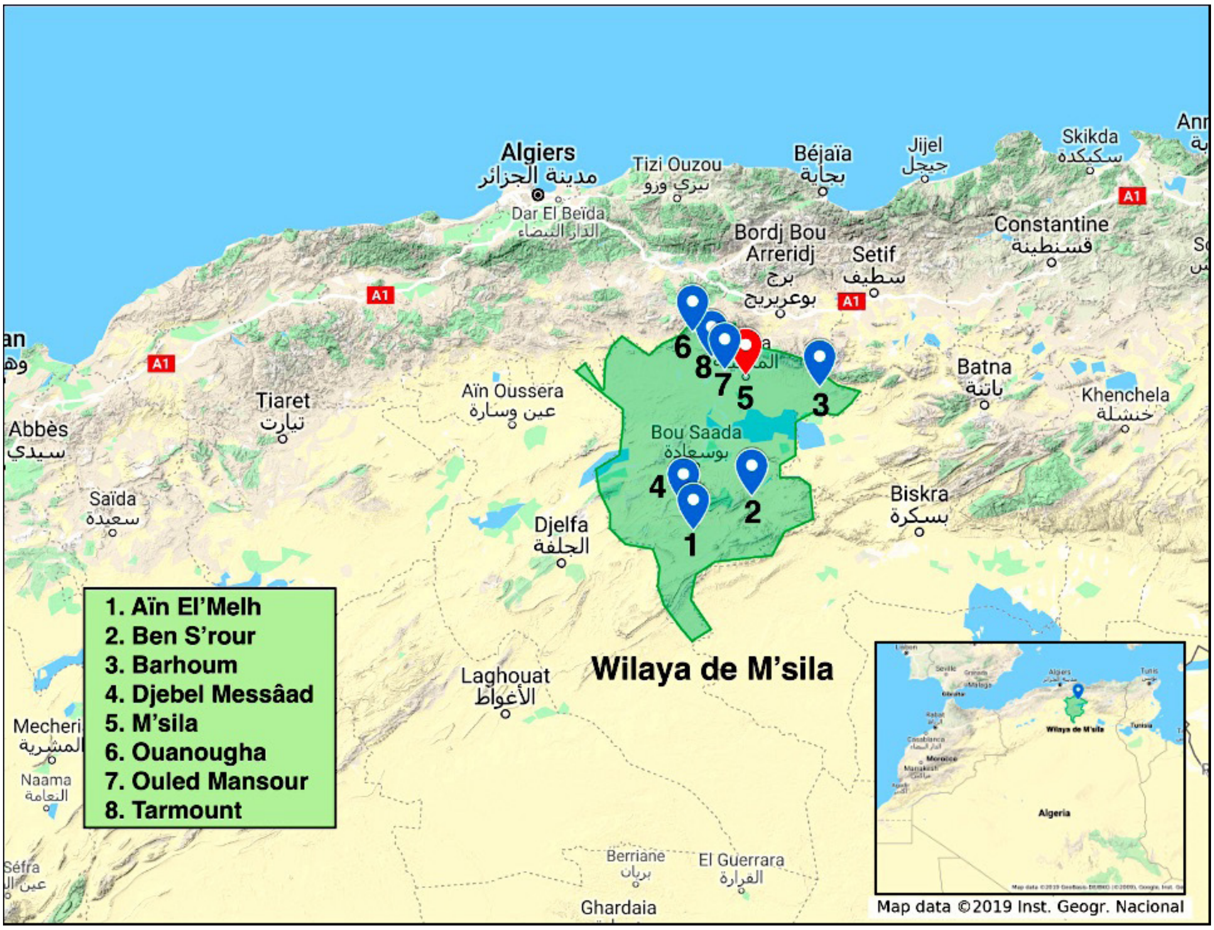

Figure1: Distribution of surveyed municipalities in study area

C. abortus, Brucella spp. and *S. abortusovis,* also highlight the eventual zoonotic risks.

Material and methods

Description of the study area

The study was carried out in eight municipalities of M'Sila Governorate (Figure 1). This region occupies the central highlands of Northern Algeria and covers an area of 18175 Km^2. Its population is estimated at 1.210.952 inhabitants, with an average density of 66 inhabitants/km^2.It is characterized by a continental climate; semi-arid to arid, very reduced maritime influences, a climatic gradient strongly influenced by the topography, since the mountainous zones are culminating at the altitude of 1800 m while the pre-saharan zones record an altitude of below 300m. Thus, the climate of M'Sila Governorate is contrasted with a long hot and dry summer season and cold and rainy winter season (ANDI, 2013). It depends on a low and irregular pluviometry not exceeding 250 mm per year. Regarding animal production, study region is a steppe land with an agro-pastoral vocation (Senoussi *et al.,* 2014). It includes an important sheep herd of over 1.630.000 heads (M'Sila DAS, 2018).

Study design and sampling

The flocks were selected based on the occurrence of at least one abortion. An epidemiological investigation using an interview via structured questionnaire was conducted focusing on the assessment of risk factors associated with abortion incidence and ELISA test seropositivity. This cross-sectional survey took place from January 2016 to June 2018. Questions asked concerned sampled ewes, with dominant items as age, breed, place of born (autochthonic or purchased from livestock markets). While other questions focused on herd management items; flock size, vaccination programs and close promiscuity with other animals.

Blood sample collection and serum separation

Blood samples were collected from December 2017 to June 2018, using a reasoned sampling according to recommendations of Toma *et al*. (2001); they were taken from ewes in which parturition or abortion occurred since lesser than one week and 2 weeks, respectively, according to owners' statements. Wholly, 184 sampled ewes reared in 16 flocks counting a total of 4359 sheep and a total of 2562 ewes. Blood had been aseptically collected from the jugular vein into 5 ml sterile vacutainer tubes. After identification, blood samples were transported into low temperature cooler to the university of M'Sila laboratory and then centrifuged at 3000 round per min for 10 min. The sera were stored at – 20° C until tested.

Serological tests

All serological tests were done in the laboratory of serology of Batna Univesity Hospital Center (UHC). *Salmonella* serology was performed using the recently developed and validated ELISA kit (Diatheva S.r.l, ITALY). The test intended for the detection of IgG anti-*Salmonella abortusovis*. The assay had sheep-level characteristics of 98 % for specificity and 96% for sensitivity. Antibodies to *C. burnetti, C. abortus and Brucella spp.*, were detected by a commercial indirect ELISA kit (ID-VET, Grablels-FRANCE) according to the manufacturer's instructions. The presence or absence of antibodies against bacterial abortive agents is determined by calculating the positive to sample ratio for each serum.

Data analysis

Data was analyzed using R studio version (version 3.6.2; R Foundation for statistical Computing, Vienna, Austria). Using generalized linear mixed effects models (glmer function of the lme 4 package).We used farm identity as a random variable to account for variation between farms and unequal sample sizes for each farm. We analyzed binary data using logistic regression. Tables representing serology results to the various abortive agents and their percentages were made. A flock is considered positive for an abortive agent if at least one ewe was seropositive for the reliable ELISA test. The disparity of seropositivity between the eight municipalities of study area, were analyzed using Chi-square test. Univariable then multivariable logistic regression analyses were performed for quantifying the associations between seropositivity and specific risk factors. Variables with a p-value of ≤ 0.2 in univariable analysis were used in the multivariable logistic regression model. The explanatory variable was assessed with p-value < 0.05 which was considered to be statistically significant.

Results and discussions

Herd management and global overview on abortion occurrences in study area

In whole surveyed flocks of M'sila Governorate, sheep farming is the main activity with obvious dominance of *Ouled Djellal* ovine breed. Many authors agree to recognize several advantages to this breed: maternal skills, reproductive performance, resistance to difficult conditions, walking skills (Dekhili and Benkhlif, 2005, Dekhili, 2014). Hence, it constitutes a socioeconomic pillar for steppic areas' development. Nevertheless, in several regions of Algeria, including M'Sila Governorate, sheep production is even hurdled by reproductive disorders, primordially; abortions, stillbirth and fertility troubles, engendering thus great losses for herders and serious uncontrolled zoonotic risks (Hireche *et al.,* 2014, Feknous *et al.,* 2018 ; Mohamed-Cherif *et al.,* 2019 ; Rouatbi *et al.,* 2019).

Therefore, a good knowledge of abortive agents' epidemiology in M'Sila Governorate, is the key to diminish abortion incidence in its small ruminant flocks. Subsequently, strict measures are required to eradicate abortive enzootic agents via continuous control of specific risk factors associated with abortion occurrence. The dilemma is that diagnostic tests for these abortive agents, excepting for *Brucella*, are not routinely applied in Algerian veterinary diagnostic laboratories. Moreover, differential clinical diagnosis seems to be eventually confused by non bacterial organisms, as it was remarquably shown by Feknous *et al.* (2018) the record of apparent seroprevalence of 71.9 % for BDV in M'Sila Governorate.

Seroprevalence of the bacterial abortive agents

The ELISA serological tests of the four abortive agents indicated that the highest seroprevalence was recorded, respectively, for; *C. burnetti, S. abortusovis, C. abortus and Brucella spp.* (Table 1). Among the 50 positive sera with *C. burnetti*, 33 were strongly positive. All flocks revealed at least one positive test for *C. burnetti* (Figure 2).

Table 1. Seroprevalence results of tested abortive agents in M'Sila Governorate

Abortive agents	Seropositive ewes	Seronegative ewes	Seropositivity (%)
***C.**burnetii*	50/184	134	27.2
***C.**abortus*	20/184	164	10.9
***S.**abortusovis*	14/88	74	15.9
Brucella spp.	7/184	177	3.80

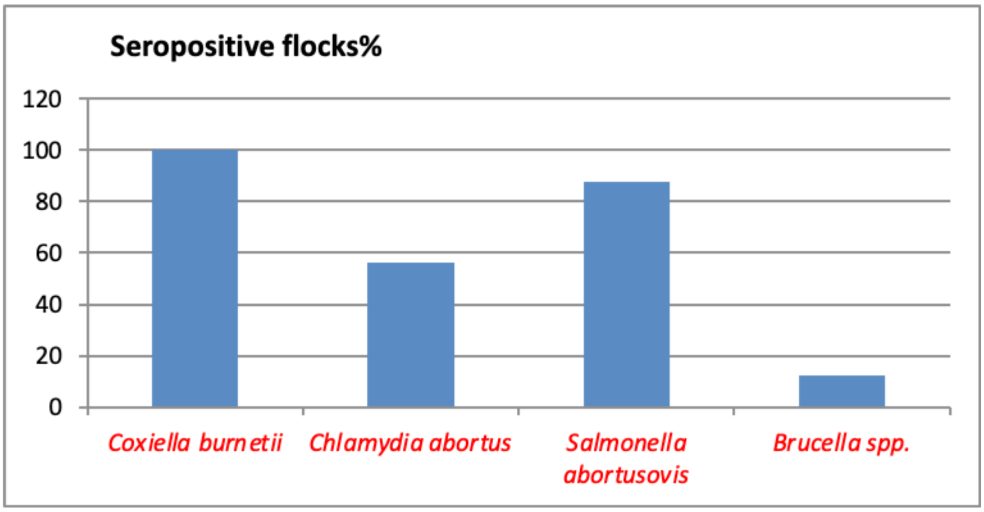

Figure 2: Seroprevalence percentages of abortive agents in whole surveyed flocks

The highest seroprevalence was recorded for *C. burnetti* within flock level, for ewes (27.2%). Comparatively, this value is higher than that reported by Khaled *et al.* (2016) for Algeria;14.1%, and worldwide, higher than those reported by Dabaja *et al.* (2019) for Lebanon; 24.2%, Kennerman *et al.* (2010) for Turkey; 20%, Asadi *et al.* (2013) for Iran;19.5%, Benkirane *et al.* (2015) for Morocco ; 15.3%, , Ruiz-Fons *et al.* (2010) for the South East of Iran ; 11.8% and Van Den Brom et Vellema (2009) for Netherlands; 2.4%.However, *C. burnetti* seroprevalence for this present study is lower than results reported by Bisias *et al.* (2009) for Greece; 48,8% and Gebretensay *et al*. (2019) for Ethiopia; 38%.

The seroprevalence of *C. abortus* in this present study was 10.9%. Comparatively, it is lower than results reported by Hireche *et al*. (2014); 24.5% for the North-Eastern of Algeria and by Merdja *et al*. (2015) ; 35% for the North-central of Algeria and worldwide, it is lower than results found by Gebretensay *et al*.(2019) in Ethiopia; 58.2%, Benkirane *et al*. (2015) in Morocco ; 27.2%, Bisias *et al*. (2009) in Greece; 14.9%. Although, our finding as regard to seroprevalence of *C. abortus,* is higher than that reported by ; Abd El-Razik *et al*. (2011) for Saoudi Arabia; 5,04%. Unfortunately, there is no current vaccination program against animal coxiellosis, chlamydiosis and salmonellosis in Algeria, this implies infection persistence and more spreading of these abortive agents.

Several authors indicate that *C. abortus* is a major cause of abortions in sheep (Rodolakis and Yousef, 2010 ; Hireche *et al.,* 2014). However, results of the present study showed that *C. burnetii* was most dominant in study area, so that all flocks had at least one seropositive test to *C. burnetti*. In non-pregnant females, infection is typically subclinical with reactivation occurring during pregnancy and excretion of *Coxiella* in large quantities during parturition in the placental, vaginal and uterine discharges, as well as in milk, urine and feces (Woldehiwet, 2004). This suggests that the disease is already spreading within the flocks, which would explain the high seroprevalence recorded for *C.burnetti*, respectively, in Barhoum, Ain El'Melh and Djebel Mesâad municipalities (Table 2).

There was significant difference in seropositivity of *C.burnetti* between municipalities (Chi-square=14.98, p=0.036) (Table 2). The highest seropositivity rate for *C.burnetti* was recorded in the municipality of Barhoum (Table 2). No disparity in *C. abortus* seropositivity between municipalities was registered Within this context, the existence of large markets for livestock in Barhoum and Ain El'Melh cities, also, the vast grasslands in Djebel Mesâad limits that could be considered as an im-

Table 2. Animal level seroprevalence of *C. burnetti C. abortus* and *Brucella spp.* in municipalities of M'sila Governorate

Municipalities	Surveyed flocks (n)	Total of flocks (n)	Tested sera (ewes) (n)	**C.burnetti* seropositivity		*C. abortus* seropositivity		*Brucella spp.* seropositivity	
				(n)	(%)	(n)	(%)	(n)	(%)
Djebel Messâad	2	385	23	5	21.7	3	13	0	0
Ain El'Melh	3	287	35	12	34.3	4	11.4	6	17.1
BenS'rour	2	310	25	10	40	3	12	0	0
Tarmount	2	132	24	6	25	0	0	0	0
Barhoum	1	332	12	7	58.3	0	0	0	0
Ouanougha	1	620	10	1	10	0	0	0	0
Ouled Mansour	1	560	15	1	6.66	4	26.7	0	0
M'Sila	4	640	40	8	20	6	15	1	2.5
Total	16	4359	184	50	-	20	-	7	-

(n) : number * : Chi-square : 14.98 ; p = 0.036

portant gathering areas for herds transhuming from neighboring regions, could be in relationship with high seroprevalence recorded for *C.burnetti,* by shedding bacteria via several pathways and sharing pasture. Commonly, transhumance has already been identified as a risk factor by previous studies in M'Sila Governorate for BDV in sheep by Feknous *et al*, (2018) and for *C.burnetii* spreading in Lebanon (Dabaja *et al.,* 2019).

The vaccination of small ruminants against brucellosis with *Rev 1* vaccine is practiced in Algeria since more than one decade which could be the cause of the relative low seropositivity for *Brucella spp.* (3.8%), compared to the other studied abortive agents. In the present study, there was no significant association between vaccination against brucellosis (p=0.46) and *Brucella spp.* seropositivity. Several studies confirmed that vaccination contributes to decrease the chances of brucellosis infections in flocks (Kardjadj and Ben Mahdi, 2014; Kardjadj *et al*., 2015). In neighbour countries, in Morocco, brucellosis was nearly absent when vaccination campaigns were done, however, the spread of the infection in the whole of the country was noted after stopping of vaccination, so that Benkirane *et al*. (2015) indicated a seroprevalence of 13.4% for brucellosis in small ruminants. Similarly, in Tunisia, brucellosis still has a high prevalence in humans and transmitted particularly by ruminants (Khamassi *et al*., 2018).

In Algeria, seroagglutination test was the habitual technique to identify infections by *Salmonella*. No abortions due to *S. abortusovis* had been reported since this serotype is not researched in routine laboratory diagnosis. In addition, *S. abortusovis* grows much slower than other *Salmonella* serotypes, and small colonies could easily be overgrown by other bacteria, especially *Escherichia coli* which inhibits the growth of *S. abortusovis* (Borel *et al*., 2014). The used ELISA test in the present study represents high specificity (98%) and sensibility (96%). For *S. abortusovis* seroprevalence, results showed 15.9% and 87.5% at ewes' and flocks' levels, respectively. These are higher than results found in Tunisia by (Rekiki *et al*., 2005); 7.44, and in Switzerland by (Wirz-Dittus *et al*., 2010); 5% at flock level. Abortion outbreaks caused by *S. abortusovis* with up to 70% stillbirth were described in sheep flocks in Switzerland (Belloy *et al*., 2009). *S. abortusovis* is specifically adapted to sheep, however, other *Salmonella* serotypes were found causing abortion in sheep. Indeed, in Spain, *Salmonella Indiana* was responsible for abortion in dairy ewes (Luque *et al*., 2009).

Table 3 summarizes the distribution of seropositivity results with regard to more than one abortive agent at the animal and flock levels. Respectively, 56.3 % and 5.43% of flocks and ewes were seropositive to more than one species of bacterial abortive agent. Mixed infections prevailed in 5.43% of tested ewes. The major surveyed flocks were infected by two or more abortive agents. This situation is common in many parts of the world (Rekiki *et al*., 2005; Gebretensay *et al*., 2019), which need careful investigations. Since they are difficult to interpret, it is essential to confirm strong serological suspicion by using direct diagnosis such as bacteriological isolation and Polymerase Chain Reaction. Concomitant seropositivity to both *C. burnetti*, *C. abortus* and *Brucella spp.*, was found in one ewe's serum belonging to one flock of Ain El'Melh municipality.

Risk factors associated with *s*eropositivity of the abortive agents

Univariable logistic analysis was used for possible associations between risk factors and seropositivity of the studied abortive agents. For multivariable analysis, linear mixed model logistic regression was used for variables with p≤ 0.2 in

Table 3. Distribution of flocks and ewes seropositive to more than one abortive agent

Abortive agents coexistence	Seropositive flocks (%)	Seropositive ewes (%)
C. burnetti + *C. abortus*	9/16 (56.3)	6/184 (3.26)
C. burnetti + *C. abortus* + *Brucella spp.*	2/16 (12.5)	1/184 (0.54)
C. burnetti + *Brucella spp.*	2/16 (12.5)	5/184 (2.72)
C. abortus + *Brucella spp.*	2/16 (12.5)	1/184 (0.54)
C. burnetti + *S.abortusovis*	1/8 (12.5)	2/88 (2.27)

univariable logistic regression. The variable (flock) was included in the logistic regression model as a fixed effect. It had been shown that *C. burnetti* seropositivity is significantly higher in farms having not stillbirth problem (OR= 3.36; 95% CI = 1.18- 10.2; p= 0.01) (Table 4). It is possible that the cause of stillbirth was infection by neither *C. burnetti* nor other abortive agents investigated in this study, but other eventual infectious agents, as reported by several studies revealing very high incidence of BDV (Feknous *et al*, 2018) and *Toxoplasma gondii* (Mohamed-Cherif *et al*., 2019 ; Rouatbi *et al*. 2019) in Algerian sheep. Indeed, vaccination of pregnant ewes protects them, as well as their lambs, against diseases which are a frequent cause of neonatal mortality like clostridial infections (Fthenakis *et al.,* 2012).

The presence of cats in farm increases 5.7 times infection by *C. burnetti* (95% CI = 1.86-19.9; p =0.001) (Table 5). Although, presence of dogs was not associated with seropositivity for the studied abortive agents, most of farmers keep guard dogs near of flocks. May be the dog can prevent the increase of the disease by eating aborted fetuses and preventing the spread of infectious organisms to the environment in which the sheep grazed. However, cats and dogs could play a principal role in the transmission of several abortive and zoonotic agents, especially when they are not treated and vaccinated, as in the case for cats in visited farms of the present study.

Table 4. Risk factors associated with *C. burnetti and C. abortus* seropositivity using univariable logistic regression analysis in flocks of M'Sila Governorate

Risk factors	**Categories**	**Tested n**	***C. burnetti***				***C. abortus***			
			Positive n (%)	**OR**	**95% CI**	***P***	**Positive n (%)**	**OR**	**95% CI**	P
Flock size	<100	20	4 (8)	1		0.92	2 (10)	1.51	0.15-20.9	0.91
	100-300	92	27 (54)	1.34	0.28-6.83		11 (55)	1.24	0.23-6.55	
	>300	72	19 (38)	1,2	0.22-6.88		7 (35)	1		
Age (month)	12-23	40	15 (30)	1		0.92	5 (25)	1.51	0.15-20.9	0.91
	24-35	81	15 (30)	1.34	0.28-6.82		4 (20)	1.24	0.23-6.55	
	>36	63	20 (40)	1.19	0.22-6.88		11 (55)	1		
Sheep pox vaccination	Yes	124	27 (54)	1		0.13	14 (70)	1.24	0.27-7.46	0.76
	No	60	23 (46)	2.23	0.74-6.73		6 (30)	1		
Presence of cats	No	89	14 (28)	1		**0.01**	9 (45)	1		0.83
	Yes	95	36 (72)	3.39	1.32-9.14		11 (55)	1.15	0.25-5.15	
Stillbirth occurence	Yes	141	31 (62)	1		**0.04**	12 (60)	1		0.16
	No	43	19 (38)	3.16	1.03-10.2		8 (40)	2.58	0.63-13.3	
Nervous disoders	Yes	83	16 (32)	1		0.13	5 (25)	1		0.12
	No	101	34 (68)	2.18	0.75-6.38		15 (75)	2,69	0.73-12.1	
Contact with newly purchased animals	No	91	22 (44)	1		0.51	5 (25)	1		0.04
	Yes	93	28 (56)	1.41	0.47-4.39		15 (75)	3.37	1.01-14.9	
Fertility disorders	++	40	7 (14)	1		0.54	1 (5)	1		0.13
	+	111	32 (64)	1.97	0.48-8.58		16 (80)	6.71	0.99-132	
	+++	33	11 (22)	2.36	0.41-14		3 (15)	3.78	0.3-87.3	

(n) : number

Table 5: Risk factors associated with *C. burnetti and C. abortus* seropositivity using multivariable mixed effect logistic regression analyses in flocks of M'Sila Governorate

Pathogenic agent	Risk factors	Categories	OR	95% CI	*P*
C. burnetti	Sheep pox vaccination	Yes	1		
		No	1.42	0.61-3.29	0.37
	Presence of cats	No	1		
		Yes	5.72	1.86-19.9	**0.001**
	Stillbirth occurrence	Yes	1		
		No	3.36	1.18-10.2	**0.01**
	Nervous disorders	Yes	1		
		No	0.34	0.08-1.19	0.07
C. abortus	Contact with newly purchased animals	No	1		
		Yes	8.25	1.25-167	0.06
	Stillbirth occurrence	Yes	1		
		No	0.81	0.16-3.56	0.76
	Nervous disorders	Yes	1		
		No	0.57	0.02-5.42	0.65
	Fertility disorders	++	1		
		+	15.8	1.95-478	**0.03**
		+++	5.81	0.45-228	0.23

Table 6. Association between risk factors and *S.abortusovis* seropositivity using univariable and multivariable logistic analyses in flocks of M'Sila Governorate

Risk factors	Categories	Tested n	Positive n (%)	Univariable analysis			Multivariable analysis		
				OR	95% CI	*P*	OR	95% CI	*P*
Flock size	<300	19	1 (7.14)	1		0.11	1		0.33
	≥300	69	13 (92.8)	4.17	0.71-78.2		1.19	0.88-1.64	
Presence of poultry	yes	73	9(64.3)	1		0,06	1		0.96
	No	15	5 (35.7)	3.55	0.93-14.6		1.01	0.68-1.49	
Dystocia occurrence	Yes	31	3 (21.4)	1		0.2	1		0.1
	No	57	11 (78.6)	2.22	0.56-10.5		1.22	0.95-1.6	
Stillbirth occurrence	Yes	78	14 (100)	1					1
	No	10	0 (0)	NA			0.89	0.65-1.22	
Digestive disorders	Yes	64	8 (57.1)	1		0.17	1		0.51
	No	24	6 (42.9)	2.31	0.62-8.25		0.81	0.79-1.75	
Metabolic diseases	+	37	8 (57.1)	1		0.2	1		0.4
	++	51	6 (42.9)		0.58-7.46		0.87	0.65-1.15	
Infectious diseases	++	64	8 (57.1)	1		0.17	1		-
	+	24	6 (42.9)	2.31	0.62-8.25		NA	-	
Presence of cats	Yes	73	9 (64.3)	1		0.06	1		-
	No	15	5 (35.7)	3.55	0.93-14.6		NA	-	
Nervous disorders	No	64	8 (57.1)	1		0.17	1		-
	Yes	24	6 (42.9)	2.31	0.62-8.25		NA	-	

(n) : number

The univariable logistic regression revealed that the seropositivity of the four abortive agents was not significantly associated with flock size and ewes age (p>0.05) (Table 4). However, other studies found association between those risk factors and *C.burnetti* seropositivity (Gebretensay *et al*., 2019), and between age and *C. abortus* seropositivity (Hireche *et al*., 2014). Contact with newly purchased and introduced animals increases 3.3 times (Table 4) infection by *C. Abortus* (OR=3.37; 95% CI=1.01-14.9; p = 0.04).

Using univariable and multivarible regression analyses, all variables were not considered as significant risk factors for *S. abortusovis* seropositivity (Table 6).

Zoonotic risks reliable to abortive agents

Among socioprofessional categories at high risk of contamination; sheep owners, shepherds households and veterinarians, due to the close permanent promiscuity with anonymous infected animals. Several studies reported zoonotic risks due to abortive agents in Algerian sheep (Hireche *et al.,* 2014, Feknous *et al.,* 2018 ; Mohamed-Cherif *et al.,* 2019 ; Rouatbi *et al.,* 2019). Regarding zoonotic threat, *C. burnetti* was the subject of several studies worldwide. Animal coxiellosis is considered to be the primary source of human Q fever infections (Angelakis and Raoult, 2010). During an epidemic of Q fever in humans, which occurred between 2007 and 2009 in the Netherlands, *C. burnetii* was identified in small ruminants belonging to 30 farms. The proximity of these farms suggested that they were the main cause of 3523 human cases (Roest *et al.,* 2010). In a study carried out in Algeria by Lacheheb and Raoult (2009) between 1995 and 1996 on humans, it had been reported about 18.5% of seropositive cases for *Coxiella*, and that among the positive cases; 35% lived in a rural area versus 7.7% in an urban area.

Worldwide, brucellosis is considered as a redoubtable bacterial zoonosis with chronic or acute forms (Acha and Szyfres, 2003; Toma *et al.,* 2004). Brucellosis in Algeria is an enzootic disease in small ruminants' flocks and it has a high prevalence in humans, especially in rural areas, due to culinary attitudes and nomadic lifestyle. Similar observations has been reported for Tunisia by Khamassi *et al*., (2018) and for Morocco by Benkirane *et al.,* (2015). In the same context, companion and guard animals, especially dogs and cats, would play the role of reservoir responsible of shedding *Brucella* and eventually other zoonotic agents, and thus they should be considered as veritable contaminators for their environment.

Conclusion

Results of the present study revealed the presence of antibodies against four abortive bacterial agents, with predominance of *C. burnetti* in ewes' sera sampled from M'sila Governorate. Presence of cats in close promiscuity of flocks is considered as risk factor for animal and human coxiellosis. Uncontrolled newly introduced animals, public livestock markets and pasture areas would play a major role for diseases' spreading. The diversity of abortive agents within reproductive sheep may hurdle diagnostic accuracy and increase zoonotic risks. Small ruminants' preventive and therapeutic programs should be systematically and rigorously respected by breeders. It seems necessary to complement research of infectious agents by microbiological isolation in placentas and foetuses of aborted ewes, and after lambing. Control of newly introduced animals in flocks and quarantine should be strictly respected to avoid dissemination and transmission of pathogens.

Acknowledgments. Authors thank Diatheva (S.r.l, ITALY) for financing this study by ELISA kits, also the laboratory of serology staff in Batna Univesity Hospital Center (UHC) for assistance.

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