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Molecular prevalence of Chlamydia and Chlamydia-like bacteria in Tunisian domestic ruminant farms and their influencing risk factors

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Summary

Chlamydia and *Chlamydia*-like bacteria are well known to infect several organisms and may cause a wide range of diseases, particularly in ruminants. To gain insight into the prevalence and diversity of these intracellular bacteria, we applied a pan-*Chlamydiales* real-time PCR to 1,134 veterinary samples taken from 130 Tunisian ruminant herds. The true adjusted animal population-level prevalence was 12.9% in cattle, against 8.7% in sheep. In addition, the true adjusted herd-level prevalence of *Chlamydiae* was 80% in cattle and 25.5% in sheep. *Chlamydiales* from three family-level lineages were detected indicating a high biodiversity of *Chlamydiales* in ruminant herds. Our results showed that *Parachlamydia acanthamoebae* could be responsible for bovine and ovine chlamydiosis in central-eastern Tunisia. Multivariable logistic regression analysis at the animal population level indicated that strata and digestive disorders variables were the important risk factors of bovine and ovine chlamydiosis. However, origin and age variables were found to be associated with bovine and ovine chlamydiosis, respectively. At the herd level, risk factors for *Chlamydia* positivity were as follows: abortion and herd size for cattle against breeding system, cleaning frequency, quarantine, use of disinfectant and floor type for sheep. Paying attention to these risk factors will help improvement of control programs against this harmful zoonotic disease.

KEYWORDS
Abortion, *Chlamydiae*, Coculture, sequencing, TaqMan PCR, veterinary samples

1 | INTRODUCTION

Tunisia is one of the leading countries in livestock production in Africa, with an estimated livestock population of more than 9.2 million head of ruminants (Zaibet, Hammami, & Jabbar, 2009). The livestock sector is the second largest source of income, accounting for 35% of the agricultural gross domestic product. Beyond its macroeconomic importance, livestock farming occupies 8.8% of the working population. It contributes at 97% to the collective satisfaction of the population food needs. Despite its importance, food and sanitary problems hamper the development of this sector (Zaibet et al., 2009). Similarly, the poor diagnostic and epidemiological surveillance capacities of the veterinary services have resulted in a low rate of livestock health coverage (Zaibet et al., 2009). In addition, ruminants are exposed to several health problems, such as gastrointestinal parasitic and respiratory diseases (Akkari et al., 2014; Gharbi et al., 2013). Numerous pathogenic agents, including viruses, protozoa, fungi and bacteria, are already known to directly affect animal health and reproductive function (Yoo, 2010). Among these infectious agents, we note *Chlamydia* which is one of the

most important bacterial agents, causing chlamydiosis in ruminant species.

Chlamydiosis is a zoonotic disease with a global occurrence (Krawiec, Piasecki, & Wieliczko, 2015). It is usually caused in cattle by *Chlamydia abortus*, belonging to the *Chlamydiaceae* family. This bacterium is mainly involved in abortions and hypofertility in cattle. The abortion occurs during the third trimester of gestation, particularly in heifers during their first pregnancy. After the abortion, several factors cause the transmission of *Chlamydiae* to susceptible animals such as contact with infected foetuses, foetal membranes, vaginal discharge, uterine secretions and milk (Rodolakis, 2006). *Chlamydia pecorum* was also isolated from the digestive tract of cows clinically affected by reproductive disorders, conjunctivitis, mastitis and pulmonary inflammation (Ruhl et al., 2009). However, its zoonotic potential is still unknown (Berri, Rekiki, Sidi Boumedine, & Rodolakis, 2009). Moreover, there are increasing evidences supporting the role of *Chlamydia*-related bacteria such as *Waddlia chondrophila* and *Parachlamydia acanthamoebae* in bovine abortion (Baud, Thomas, Arafa, Regan, & Greub, 2007; Borel et al., 2007). Ovine chlamydiosis, also known as enzootic abortion of ewes (EAE), is principally caused by *C. abortus*. This infection is characterized by reduction in milk production and abortion or birth of weak lamb and can be transmitted by the same ways given in cattle (Barkallah et al., 2014). *C. pecorum* strains have also been associated with a variety of diseases in sheep including arthritis, conjunctivitis and enteric infections. Nevertheless, the involvement of *C. pecorum* in ovine abortion cases was also reported, almost 26 years ago, in southern France (Berri et al., 2009). Previous collaborative studies between veterinary institutes in Morocco, Algeria and Tunisia have confirmed the isolation of *C. pecorum* strains from abortion cases (Berri et al., 2009; Rekiki et al., 2004), suggesting the involvement of this bacterium in the spontaneous abortion of small ruminants in North African countries. To date, *W. chondrophila* has never been detected in cases of abortions in sheep. In contrast, authors showed that *P. acanthamoebae* was detected by molecular techniques and immunohistochemistry in aborted ewe placentas and in the lung of an aborted small lamb (Ruhl et al., 2009).

Many previous studies were conducted in different geographical regions of Tunisia and demonstrated the presence of antibodies against chlamydial species (Rekiki et al., 2004). However, there is still a gap of information on the existence of this infection in many parts of the country. This study aimed at (i) determining the true prevalence of *Chlamydiae* among ruminants in central-eastern Tunisia and (ii) identifying potential risk factors associated with chlamydial infection at the individual animal and herd levels.

2 | MATERIALS AND METHODS

2.1 | Ethical considerations

The study protocol was assessed and approved by the institutional review board (IRB) of the Regional Office of Agricultural Development of Sfax (CRDA) in collaboration with the Veterinary Research Center of Sfax in Tunisia. All breeders declared their verbal and written consent before animal sampling as well as for the related survey questions. Samples were collected by authorized veterinarians of the Veterinary Research Center of Sfax during annual vaccination campaigns following standard procedures of the usual screening scheme on farms and Tunisian ethical guidelines.

2.2 | Study area, animals and samples

This study was conducted in Sfax town (average altitude of 13 m), which is located in the East Centre of Tunisia, at 270 km from the capital Tunis. During this study, many geographical regions of Sfax have been visited as described by Barkallah et al. (2017). In each region, households owning cattle and/or sheep were identified through discussion with responsible veterinarians during the annual vaccination campaigns. The number of herds to be studied was calculated using the following formula $n = [t^2 \text{ Pexp (1-Pesp)}]/d^2$ as previously described by Barkallah et al. (2017). In total, 20 Holsteins bovine herds and 110 ovine herds were visited. Blood, vaginal swabs and milk samples were collected from 378 animals and sent to the laboratory. These samples were collected as described by Barkallah et al. (2016).

2.3 | Epidemiological data collection

Two detailed questionnaires were used separately to collect information on potential management and environmental risks factors related to bovine and ovine chlamydiosis at the individual animal and herd levels. The owners of ruminants were questioned by the principal researcher of this study.

2.4 | Cocultivation of *Waddlia chondrophila* in amoebae

Fresh swab samples positive by real-time PCR were used to isolate *W. chondrophila* in the coculture system. In fact, 500 μl of culture was taken from each swab and filtered through 0.22-μm pore size membrane. Each membrane was then shaken in 500 μl of Page's amoeba saline (PAS). Amoebae (strain *Acanthamoeba castellanii* ATCC 30010) were grown and prepared for culture as described by Lienard and Greub (2011). For inoculation, homogenized samples were inoculated into the wells by spinoculation at 2,000 *g* for 10 min. Then, cocultures were examined to exclude or confirm the growth of *W. chondrophila* (Goy, Croxatto, Posfay-Barbe, Gervaix, & Greub, 2009).

2.5 | DNA extraction

DNA from all blood, milk and vaginal swab samples was extracted by ZR Fungal/Bacterial DNA MiniPrep™ D6005 Kit (Zymo Research) as described by Barkallah et al. (2016).

2.6 | *Chlamydiales*-specific real-time PCR

A pan-*Chlamydiales* real-time PCR that may amplify all members of the *Chlamydiales* order was performed as described previously by

Lienard, Croxatto, Aeby, Jaton, and Posfay-Barbe (2011). The detection limit of this assay was five DNA copies per reaction of the positive control (with an efficiency of 75%). The amplified products were sent to GATC Biotech SARL (Germany) for sequencing. Sequences were compared, with the BLAST server, to existing sequences on GenBank which classifies them based on the percentage identity of the best known species. Positive samples for *W. chondrophila* and *P. acanthamoebae* were quantified using previously described real-time PCRs (Casson, Posfay-Barbe, Gervaix, & Greub, 2008; Goy et al., 2009). Both PCR assays were able to detect bacterial DNA over a linear range of 10–10^7 copies per reaction mixture. However, *C. abortus* and *C. pecorum* were quantified in veterinary samples using two commercial kits "PrimerDesign™ genesig Kits for *C. abortus* and *C. pecorum* genomes quantification" (Genesig). Both assays could detect DNA to levels less than 10 copies/μl (Pantchev, Sting, Bauerfeind, Tyczka, & Sachse, 2010).

2.7 | Statistical analysis

The true prevalence (TP) of chlamydial infection at the individual animal level was estimated using Epitools epidemiological calculators (http://epitools.ausvet.com.au/content.php?page=home) and the following formula: TP = (AP + Sp-1)/(Se + Sp-1) (Rogan & Gladen, 1978). Herd-level prevalence was calculated as the number of herds with at least one positive animal divided by the total number of herds tested. The true herd prevalence (THP) was estimated from distributions of herd sensitivity (HSe) and specificity (HSp) as described by Christensen and Gardner (2000).

The risk factor analysis was separately performed at the animal and herd levels for cattle and sheep as described by Barkallah et al. (2016). The overall goodness of fit of final models was assessed using Pearson's chi-square and Hosmer–Lemeshow tests (Hosmer & Lemeshow, 1980).

3 | RESULTS

3.1 | Detection of *Chlamydiae*

For bovine samples, chlamydial DNA was detected in 27/214 (12.61%) vaginal swab samples. To know the responsible species of infection, PCR products were sequenced and analysed using the BLAST web interface (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Among the 16S rRNA sequences of these 27 test-positive samples, 12, 8 and 7 sequences with hits in the BLAST analysis belonged to *Waddliaceae*, *Parachlamydiaceae* and *Chlamydiaceae* families, respectively (Barkallah et al., 2014). No chlamydial DNA was detected in milk and blood samples. All amoebal cocultures (n = 12) remained negative for *W. chondrophila*.

For ovine samples, chlamydial DNA was detected only in 14/164 (8.53%) vaginal swab samples. The amplicon sequences of four test-positive samples were found to be 99% identical to the *P. acanthamoebae 16S rDNA* gene in the database. All of them had already been confirmed by the *P. acanthamoebae*-specific PCR-TR-TaqMan as previously described (Casson et al., 2008). Five among the 10 remaining sequences belonging to the *Chlamydiacaeae* family demonstrated ≥99% sequence similarity with *C. abortus*, whereas the remaining sequences exhibited ≥99% sequence similarity with *C. pecorum*. These results were confirmed by two commercial kits specific for the quantification of *C. abortus* and *C. pecorum*. As for cattle, a very high bacterial load was found in the ovine vaginal samples (35–1434 copies/μl). All milk and blood samples were shown to be negative.

3.2 | Prevalence of chlamydial infection

The apparent individual animal prevalence (AP) of chlamydial infection in cattle (12.61%; 95% CI: 8.82%–17.73%) was found higher than in sheep (8.54%; 95% CI: 5.15%–13.82%) (Z test: p = .101). Sixteen of 20 (80%; 95% CI [65.2%–94.7%]) bovine herds had at least one positive animal for *Chlamydia*. Compared with sheep, an overall 12.7% (14/110) (95% CI [7.48%–17.92%]) herd-level prevalence of chlamydial infection was found (Z test: $p < .0001$). The AP of chlamydial infection on both individual animal and herd levels was adjusted to the sensitivity (97.7%) and specificity (100%) of the pan-*Chlamydiales* PCR (Lienard et al., 2011). In cattle, the true prevalence (TP) at the animal level was 12.9% (95% CI: [8.4%–17.5%]), against 8.7% (95% CI: [4.4%–13.1%]) in sheep. The average number of animals tested in each herd of cattle was 10, while for sheep, the number was shown to be between 1 and 5 animals, according to the herd/flock sizes. Therefore, the herd specificity (HSp) was equal to 1 for the two species of ruminants. For cattle, the herd sensitivity (HSe) was $1 - (1 - 0.8)^{10} = 0.9999$, while for sheep, it was $1 - (1 - 0.127)^5 = 0.5$. According to these calculated parameters, the THP of chlamydial infection was equal to 80% for cattle and 25.5% for sheep.

3.3 | Univariable analysis of related risk factors

At the individual animal level, four variables (age range, strata, abortion and digestive disorders) were found to be significantly associated with chlamydial infection in cattle ($p < .05$) (Table 1). However, the variables (age range, abortion, conjunctivitis and digestive disorders) were significantly associated with chlamydial infection in sheep (Table 1).

At the herd level, all risk factors assumed to be associated with chlamydial infection in cattle and sheep are presented in Table 2. All significant and non-significant variables ($p < .2$) were then entered to the multivariable logistic analysis (Table 2).

3.4 | Multivariable logistic regression analysis

The Hosmer–Lemeshow goodness of fit test indicated adequate fit for both the cattle (p = .639) and the sheep (p = .772) models (Table 3). From the final model for cattle, it can be seen that the odds of *Chlamydia* positivity were significantly higher in homebred cows compared to purchased cows with an OR of 3 (p = .032). We

TABLE 1 Potential risk factors associated with individual animal level *Chlamydia* positivity among 214 cows and 164 ewes

	Cattle				Sheep		
Variable and level	**No. of animals**	**No. of positive (%)**	***p*-Value**	**Variable and level**	**No. of animals**	**No. of positive (%)**	***p*-Value**
Age range				Age range			
<3	120	11 (9.1)	.006	1-2	83	2 (2.4)	.004
3–5	74	9 (12.16)		2-3	81	12 (14.8)	
>5	20	7 (35)					
Strata				Strata			
Rural	9	1 (11.11)	.049	Rural	40	6 (15)	.236
Peri-urban	76	4 (5.26)		Peri-urban	83	5 (6)	
Urban	129	22 (17)		Urban	41	3 (7.3)	
Origin				Origin			
Home bred	85	12 (14.11)	.591	Home bred	130	9 (6.9)	.148
Purchased	129	15 (11.62)		Purchased	34	5 (14.7)	
History of abortion				History of abortion			
Yes	150	27 (18)	<.001	Yes	100	14 (14)	.002
No	64	0 (0)		No	64	0 (0)	
Conjunctivitis				Conjunctivitis			
Yes	7	1 (14.2)	.892	Yes	8	3 (37.5)	.003
No	207	26 (12.5)		No	156	11 (7.1)	
Digestive disorders				Digestive disorders			
Yes	43	12 (27.9)	<.001	Yes	29	10 (34.48)	<.001
No	171	15 (8.77)		No	135	4 (2.96)	
Respiratory disorders				Breed			
Yes	6	2 (33.3)	.297	Barbarine	144	11 (9.7)	.27
No	193	23 (11.91)		Others	20	3 (5)	
Not mentioned	15	2 (13.3)					

noted also that the risk of *Chlamydia* positivity was 6.18 times higher in affected cows by digestive disorders than in healthy cows ($p < .001$). For sheep between 2 and 3 years old, the odds of *Chlamydia* positivity were 166 times higher compared with those that are 1 year of age. We also noted that the risk of *Chlamydia* positivity was 65 and 143 times higher in rural than in urban and peri-urban areas (Table 3). In addition, the risk of *Chlamydia* test positivity was significantly higher in ewes affected by digestive disorders compared to the healthy ones (OR = 35.71; $p < .001$).

At the herd level, risk factors associated with *Chlamydia* positivity in the multivariable logistic regression analysis were as follows: abortion and herd size for cattle against breeding system, handling, cleaning frequency, floor type and use of disinfectant for sheep. The Hosmer–Lemeshow test showed that the two final models fit well the data for the cattle herds ($p = .8$) and sheep flocks ($p = .963$) (Table 4).

4 | DISCUSSION

In the diagnosis of chlamydiosis, which it is connected with surveillance investigations of the disease in ruminants, different types of methods are used. The choice of a diagnostic method largely depends on the type and number of samples to be tested and the stage of infection in animals. Since serological and traditional culture techniques are ineffective, the pan-*Chlamydiales* real-time PCR was chosen for mass screening because is robust, rapid, sensitive and highly specific (Lienard et al., 2011). In addition, this real-time PCR could detect low concentrations of chlamydial DNA from various types of samples (Lienard et al., 2011).

Based on the present study results, *Chlamydia* and *Chlamydia*-like microorganisms do not seem to be excreted in the milk of ruminants. This is in accordance with the results of other studies showing that an infected ruminant does not necessarily excrete *Chlamydia* in milk and, if so, it may be irregular (Thomas, Davison, & Wilsmore, 1990). Thus, our results confirm that milk is not the best sample to detect chlamydial DNA, a statistically significant and clinically important finding. The low incidence of *Chlamydia* in blood samples can be explained by the low chlamydial load particularly in cases of chronic chlamydiosis.

The *Chlamydiaceae* family is frequently found in domestic ruminant herds and generally associated with reproductive problems (Merdja et al., 2015). Thus, it is not surprising that 17 of the 41 positive PCRs performed on vaginal swab samples were positive for

TABLE 2 Analysis of potential risk factors associated with herd-level prevalence of chlamydial infection in ruminants from different geographical regions of Sfax in Tunisia

	Cattle				Sheep		
Variable and level	No. of herds	No. of positive (%)	*p*-Value	Variable and level	No. of herds	No. of positive (%)	*p*-Value
Study regions				Study regions			
El hajeb	3	3 (100)	.603	El hajeb	12	2 (16.7)	.73
El amra	6	5 (83.3)		El hincha	10	2 (20)	
Skhira	7	5 (71.4)		Sakiet ezzit	3	0 (0)	
Sakiet eddaier	2	2 (100)		Sakiet eddaier	27	2 (7.4)	
Sidi mansour	2	1 (50)		Sidi mansour	25	3 (12)	
				Bir ali ben khelifa	21	2 (9.5)	
				Agareb	12	3 (25)	
Herd size				Herd size			
<50	5	2 (40)	.01	<30	46	2 (4.3)	.025
>50	15	14 (93.3)		>30	64	11 (18.8)	
Herd composition				Herd composition			
Cattle	13	13 (100)	.008	Sheep	4	0 (0)	<.001
Cattle+ sheep or Goat	3	1 (33.3)		Sheep + cattle or Goat	91	7 (7.7)	
Cattle +sheep+ goat	4	2 (50)		Sheep + cattle + Goat	15	7 (26.6)	
Type of breeding				Management system			
Artificial insemination	5	2 (40)	.032	Extensive	96	9 (9.37)	.006
Use natural service	5	5 (100)		Intensive	14	5 (35.71)	
Both	10	9 (90)					
Abortion in the herd				Abortion in the herd			
Yes	15	14 (93.33)	.01	Yes	80	14 (17.5)	.014
No	5	2 (40)		No	30	0 (0)	
Mortality				Mortality			
Yes	4	3 (75)	.78	Yes	42	11 (26,2)	.004
No	16	13 (81.3)		No	53	2 (3.8)	
				No information	15	1 (6.7)	
Housing				Housing			
Close house	9	7 (77.7)	.822	Solid enclosure with roof	47	6 (12.8)	.992
Open house	11	9 (81.81)			63	8 (12.7)	
Hygiene/floor type				Hygiene/floor type			
Solid floor	5	2 (40)	.01	Solid floor	46	2 (4.3)	.025
Soil	15	14 (93.3)		Soil	64	12 (18.8)	
Origin of silage				Origin of silage			
Home made	3	2 (66.66)	.531	Home made	39	2 (5.1)	.144
Purchased	17	14 (82.35)		Purchased	18	2 (11.1)	
				Both	53	0 (0)	
Mineral supplementation				Mineral supplementation			
Yes	8	4 (50)	.006	Yes	15	2 (13.3)	.94
No	12	12 (100)		No	95	12 (12.6)	
Water source				Water source			
Communal water source	19	15 (78.94)	.608	Communal water source	53	8 (15.1)	.734
Own supply	1	1 (100)		Own supply	50	5 (10)	
				Not indicated	7	1 (14.3)	

(Continues)

TABLE 2 (Continued)

	Cattle				Sheep		
Variable and level	No. of herds	No. of positive (%)	*p*-Value	Variable and level	No. of herds	No. of positive (%)	*p*-Value
Herd prophylactic measures[a]				Herd prophylactic measures[a]			
Yes	14	13 (92.9)	.028	Yes	43	2 (4.7)	.042
No	6	3 (50)		No	67	12 (17.9)	
Cleaning frequency				Cleaning frequency			
Weekly	15	11 (73.3)	.435	Weekly	85	7 (8.2)	.025
Monthly	4	4 (100)		Monthly	13	3 (23.1)	
No cleaning	1	1 (100)		No cleaning	12	4 (33.3)	
Use of disinfectant				Use of disinfectant			
Yes	12	8 (66.7)	.068	Yes	16	4 (25)	.111
No	8	8 (100)		No	94	10 (10.6)	
Purchase of animal in the past 3 years				Purchase of animal in the past 3 years			
Yes	16	15 (93.75)	.002	Yes	99	13 (13.1)	.703
No	4	1 (25)		No	11	1 (9.1)	
Handling				Handling			
Quarantined	11	7 (63.6)	.043	Quarantined	54	3 (5.6)	.027
Mixed	9	9 (100)		Mixed	56	11 (19.6)	
Breeder Knowledge of Chlamydiosis				Breeder Knowledge of Chlamydiosis			
Yes	2	1 (50)	.264	Yes	11	1 (9.1)	.703
No	18	15 (83.3)		No	99	13 (13.1)	
Socio-economic status of farmer				Socio-economic status of farmer			
Full-time	5	4 (80)	.392	Full-time	98	11 (11.2)	.401
Part-time	10	9 (90)		Part-time	8	2 (25)	
Not indicated	5	3 (60)		Not indicated	4	1 (25)	
Specialist attending to animals[b]				Specialist attending to animals[b]			
Yes	16	12 (75)	.264	Yes	22	1 (4.5)	.198
No	4	4 (100)		No	88	13 (14.8)	
Gynaecological exam[c]							
Yes	1	1 (100)	.608				
No	19	15 (78.94)					

[a]Regular deworming, tick control, haemoparasite control or vaccination.
[b]Veterinarian or livestock assistant attending to the ruminants.
[c]Regular or occasional rectal palpation or gynaecological/obstetrical examination.

Chlamydiaceae. The high prevalence of positivity (60%) for *Parachlamydiaceae* is important to investigate in our case as *P. acanthamoebae*, a species of this chlamydial family, is well known to cause reproductive disorders, especially in cattle (Barkallah et al., 2014; Borel et al., 2007). In our study, 12 samples from five ruminant herds were positive for *P. acanthamoebae*. This is the first description of this *Chlamydia*-like bacterium in ovine abortion in African farms. *W. chondrophila* was absent in all ovine samples tested in our study. This result is in agreement with those of a previous study showing the non-susceptibility of ewes to be infected with *W. chondrophila*. Recently, an in vitro study has shown that *W. chondrophila* is able to infect and multiply in ovine trophoblastic cells while inducing an immune and inflammatory response in a dose-dependent way similar to that observed with *C. abortus* (Wheelhouse et al., 2014). More samples should be collected to survey the reproductive problems caused by this chlamydia-like bacterium in Tunisian sheep flocks.

At the individual animal level, our results concerning both bovine and ovine chlamydiosis are highly consistent with those observed in other studies realized in Tunisia (Zaibet et al., 2009) and Zambia (Ghirotti et al., 1991) and in other continents (Blumer, Greub, Waldvogel, Hassig, & Thoma, 2011; Szymańska-Czerwińska, Niemczuk, & Galińska, 2013). At the herd level, our AP data are proportionally similar to those described in other studies realized in Switzerland (Borel et al., 2004), Germany (Lenzko et al., 2011), Ireland (Wilson et al., 2012) and in Algeria (Merdja et al., 2015). The adjustment of prevalence according to the performance of the used molecular test gave TP values of 12.9% in cattle and 8.7% in sheep. The TP value of chlamydial infection in sheep was higher than those obtained in other studies carried out in other governorates of Tunisia

TABLE 3 Final model of animal population-level risk factors associated with chlamydial infection among cattle and sheep

	Multivariable logistic regression							
							95% CI of O.R	
Chlamydiae[a]	Variable	Level	*b*	S.E(*b*)	*p* Value	O.R	Lower	Upper
Positive cows	Digestive disorders	Yes	1.822	.497	<.001	6.183	2.334	16.38
		No	0	–	–	1.000	–	–
	Strata	Rural	1.59	1.207	.188	4.902	0.462	52.26
		Urban	1.78	0.618	.004	5.932	1.766	19.93
		Peri-urban	0	–	–	1.000	–	–
	Origin	Purchased	−1.065	0.497	.032	0.345	0.13	0.914
		Home bred	0	–	–	1.000	–	–
		Intercept	−2.933	1.181	.013	–	–	–
Positive ewes	Age	1-2	−5.181	1.513	.001	0.006	0.000	0.109
		2-3	0	–	–	1.000	–	–
	Digestive disorders	No	-3.581	0.87	<.001	0.028	0.005	0.153
		Yes	0	–	–	1.000	–	–
	Strata	Rural	4.967	1.5	.001	143.59	7.6	2714.38
		Urban	0.788	0.925	.394	2.2	0.359	13.485
		Peri-urban	0	–	–	1.000	–	–
		Intercept	−4.365	1.033	<.001	–	–	–

[a]The reference category is negative.

TABLE 4 Risk factors for herd-level chlamydial infection in 130 ruminant herds in different regions of Sfax, Tunisia: results of a multiple logistic regression model

	Multivariable logistic regression							
							95% CI of O.R	
Chlamydiae[a]	Variable	Level	*b*	S.E(*b*)	*p* Value	O.R	Lower	Upper
Positive bovine herds	Abortion	No	0	–	–	1.000	–	–
		Yes	2.986	1.420	.04	19.85	0.655	420.12
	Herd size	<50	−3.202	1.406	.042	0.047	0.004	0.943
		>50	0	–	–	1.000	–	–
		Intercept	−4.203	1.47	.035			
Positive ovine herds	Breeding system	Extensive	−1.739	0.823	.035	0.176	0.035	0.882
		Intensive	0	–	–	1.000	–	–
	Cleaning frequency	Weekly	−2.194	0.966	.023	0.111	0.017	0.74
		Monthly	−1.19	1.039	.252	0.304	0.04	2.329
		No cleaning	0	–	–	1.000	–	–
	Handling	Quarantined	−2.037	0.975	.037	0.13	0.019	0.882
		Mixed	0	–	–	1.000	–	–
	Floor type	Solid floor	−2.354	1.073	.028	0.095	0.012	0.778
		Soil	0	–	–	1.000	–	–
	Use of disinfectant	Yes	−3.542	1.192	.003	0.029	0.003	0.3
		No	0	–	–	1.000	–	–
		Intercept	5.111	1.748	.003			

[a]The reference category is negative.

(Elandalousi, Ghram, Maaroufi, & Mnif, 2015; Zaibet et al., 2009). The signalled high prevalence of chlamydial infection of 37.16% in cattle of Kalaat El Andalous (northern Tunisia) could be due to the specificity lack of some serological techniques based on chlamydial antigen preparations and purified chlamydial LPS (Elandalousi et al., 2015). At the herd level, the TP of chlamydial infection in sheep

(80%) was higher than those reported in Tunisia (6.25%) (Zaibet et al., 2009), in Zaer and Morocco (21.5%) (Hamzy-El Idrissi, Manyari, & Benkirane, 1995) and in Mauritania (20%) (Chartier & Chartier, 1988). In fact, all these investigations were based only on *Chlamydiaceae*-specific serological tests, and therefore, infected animals with *Chlamydia*-like bacteria were not considered. In addition, the changing of diagnostic techniques and the choice of sampling methods are important factors that contribute to the variability of results among investigations. On the other hand, Rekiki et al. (2004) reported a relatively similar prevalence rate of 26% in Tunisian sheep flocks.

Several reports agree with the fact that the risk of transmission of *Chlamydiae* in cattle varied significantly depending on the strata and the involved systems of production (Igayara-Souza, Genovez, Ferreira, Paulin, & Scarcelli, 2004; Jaouad, 2004). Our results have shown that the probability of having a positive animal was higher in urban (17%) than in peri-urban (5.26%) and rural (11.1%) areas. This could be explained by the fact that most dairy farmers practicing the intensive farming are situated in urban areas where there is a high demand for milk (Barkallah et al., 2017; Jaouad, 2004). In this type of areas, reproductive infections associated with *Chlamydiae*, particularly abortions, appear mainly as enzootic diseases, resulting in severe economic losses (Igayara-Souza et al., 2004). On the other hand, the risk of chlamydial infection in sheep was equal in urban and rural areas, where keeping sheep is a method to save money. The observed association between *Chlamydia* status and the ruminant's age was consistent with what is mostly known about the biology of infection (Qin et al., 2014; Zhou et al., 2013). This association with aged animals may be due to (i) the cumulative probability of exposure to *Chlamydiae* (Qin et al., 2014) and (ii) the immune system failure (Zhou et al., 2013). Similarly, the observed relationships between chlamydial infection and abortive and digestive disorders were consistent with what has generally been observed (Longbottom et al., 2013; Merdja et al., 2015; Wheelhouse et al., 2014). Under these conditions, the infection finds favourable ground for transmission through placental and foetal membranes, faeces and urines (Barkallah et al., 2014). In addition, conjunctivitis was found to be a major risk factor significantly associated with chlamydial infection in sheep. These results are in agreement with those of other recent studies (Jelocnik et al., 2014; Polkinghorne et al., 2009).

As expected, different herd/flock properties were significantly associated with the probability of a herd/flock being positive. Our results showed that the risk of contracting *Chlamydia* increases significantly with the herd size. These results are in agreement with those of other studies, who observed that the prevalence of chlamydial infection was higher in herds with large numbers of animals (Al-Qudah, Sharif, Raouf, Hailat, & AL-Domy, 2004; Merdja et al., 2015). In general, larger herds might be expected to be associated with intensive management practices that are characteristically more difficult to control and let for closer contact between susceptible and infected animals. Similarly, our findings showed that herd composition was linked to *Chlamydiae* test positivity. This finding is in accordance with those of Zaibet et al. (2009), who reported that the risk of chlamydial infection in sheep is multiplied by 4 and 1.08 in the presence of cattle and goats in the same farm. We also found that flocks exposed to *Chlamydiae* showed not only risks of abortions, but also high sheep mortality rates. Indeed, chlamydial infection induces high animal mortality that finally reduces the financial capital of breeders and increases costs of production (Hireche et al., 2014). The reduction in mortality and abortion rates requires that chlamydial infection be combated through divers management and sanitation practices such as the use of artificial insemination, the addition of mineral supplements and the installation of suitable prophylactic measures and animal quarantine services (Longbottom et al., 2013; Müller, Sachse, Kemmerling, Rietz, & Sauerwein, 2013; Ostermann et al., 2013). All these applications play a crucial role in keeping the immune status of the herds healthy and may evenly give an indication of top herd management. Concerning the floor type, our results showed that the exposure risk to chlamydial infection was more important in ruminants in direct contact with soil than in those that are in contact with solid ground. These results corroborate those of several other authors, who showed that the soil could be a source of contaminated residues (Coulon et al., 2012; Kebbi-Beghdadi & Greub, 2014).

Chlamydiosis is endemic at great levels in domestic ruminants in central-eastern Tunisia. The infection is heterogeneously distributed, with some farms at high risk as a result of practices such as the introduction of new animals without quarantine, changing of rams for reproduction and the intensification of the production system. The adoption of hygiene and biosecurity practices is recommended as a control strategy of chlamydial infection in Tunisian farms.

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CONFLICT OF INTEREST

The authors declare that there are no conflict of interests.

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