Prevalence and factors associated with a higher or lower risk of exposure to *Coxiella burnetii*, *Chlamydia abortus* and *Toxoplasma gondii* in dairy cows that have aborted in Algeria

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Summary

In Algeria, the prevalence of causes of abortion on dairy cattle farms (whether infectious causes or not) has been little studied. The current study involved a serological analysis conducted between October 2014 and June 2016 in northern Algeria using an enzyme-linked immunosorbent assay test on blood samples taken from 368 cows that had aborted on 124 farms. It was complemented by a survey to identify the factors associated with a higher or lower risk of exposure to *Coxiella burnetii*, *Chlamydia abortus* and *Toxoplasma gondii*, using univariate logistic regression and then multivariate logistic regression. The individual serological prevalences obtained were 8.4% (31/368) for *C. burnetii* and 12.2% (45/368) for *C. abortus*. For *T. gondii,* the individual seroprevalence was 13.8% (51/368); the factors associated with a higher risk of individual exposure were the fourth month of gestation (odds ratio [OR] = 22.68; 95% confidence interval [CI]: 1.38–392.97) and the fifth month of gestation (OR = 25.51; 95% CI: 1.47–442.11). All the other factors identified by the multivariate logistic regression were associated with a lower risk of exposure. They are the inspection visits in 2015 (OR = 0.0006; 95% CI: 0.000004–0.12) and in 2016 (OR = 0.0005; 95% CI: 0.000002–0.13) and artificial insemination (OR = 0.15; 95% CI: 0.05–0.44) for *C. burnetii*; winter (OR = 0.39; 95% CI: 0.15–1.00), spring (OR = 0.45; 95% CI: 0.20–0.97), and artificial insemination (OR = 0.27; 95% CI: 0.13–0.56) for *C. abortus*; and the number of gestations (OR = 0.38; 95% CI: 0.16–0.92) for *T. gondii.* The seroprevalence at herd level was 16.1% (20/124) for *C. burnetii* and 29.8% (37/124) for both *C. abortus* and *T. gondii.* At herd level, the risk factors associated with a higher risk of exposure to *C. abortus* and *T. gondii* were the practice of deworming (OR = 3.89; 95% CI: 1.53–9.89) and drilling individual wells as a source of drinking water (OR = 7.50; 95% CI: 2.11–26.69). For *C. burnetii,* the inspection visit in 2015 (OR = 0.02; 95% CI: 0.0008-0.65) and in 2016 (OR = 0.01; 95% CI: 0.0003-0.36), artificial insemination (OR = 0.21; 95% CI: 0.06-0.69) and rodent eradication (OR = 0.19; 95% CI: 0.06-0.57) were factors that reduced the risk of exposure.

Keywords

Abortion – Algeria – Cattle – *Chlamydia abortus* – *Coxiella burnetii* – Dairy cow – Enzyme-linked immunosorbent assay (ELISA) – Prevalence – Protective factor – Risk factor – *Toxoplasma gondii*.

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Introduction

Abortions are considered to be one of the main causes of economic losses suffered by cattle farmers, due to the longer calving interval, loss of calves, reduction in milk output, treatment costs and the purchase of replacement animals (1). The majority of abortions are caused by infections (bacterial, viral, parasitic or fungal) (2, 3). Their aetiological diagnosis requires blood samples for laboratory analysis in order to determine the serological concentration of specific antibodies and/or the presence of genomic elements and pathogenic agents. For this, many common techniques are used to identify *Coxiella burnetii*, *Chlamydia abortus* and *Toxoplasma gondii*, such as polymerase chain reaction (PCR), real-time PCR, enzyme-linked immunosorbent assay (ELISA) or the indirect immunofluorescence test (4, 5, 6).

A number of studies have characterised individual and herd seroprevalence and the factors associated with a higher or lower risk of exposure to *C. burnetii*, *C. abortus* and/or *T. gondii*, generally using an ELISA test. These studies show that individual seroprevalence varies from 0.8% to 89.5% for *C. burnetii* (7, 8), from 0.73% to 51.3% for *C. abortus* (9, 10) and from 2.7% to 83.4% for *T. gondii* (11, 12). Herd seroprevalence varies from 10% to 81.6% for *C. burnetii* (7, 13), from 57% to 66.3% for *C. abortus* (14, 15) and from 44.8% to 100% for *T. gondii* (12, 16).

The factors associated with a higher risk of exposure to *C. burnetii* reported in the literature are: older animals (17); autumn (18); the Holstein breed (17); an increase in the number of animals on the farm; the presence of ticks (13); cohabitation of cattle and sheep (13); the introduction of new cattle onto the farm (19); the absence of quarantine (20); increased rotation of cattle between farms (13, 21); and failure to comply with biosecurity rules for equipment or for artificial insemination (22). Older animals (23) or the size of herds (24), as well as previous cases of abortion (23), have been identified as factors associated with an increased risk of exposure to *C. abortus*. Finally, according to various studies, animal age, drinking water contamination and the presence of cats on the farm (23) are factors associated with a higher risk of exposure to *T. gondii*.

The above data illustrate the importance of these pathologies in cattle. Nevertheless, the role of these zoonotic agents in abortions has been very little studied in the Algerian context. The authors therefore deemed it worthwhile to assess the level of exposure to these three zoonotic agents, using ELISA tests, in cases of cattle abortion at individual and herd level, and to identify the various factors associated with a higher or lower risk of exposure to the three pathogens of interest. The ELISA test was selected because it is sensitive, specific, reliable and suitable for large-scale screening. It allows for contact between the animal and the infectious agent (exposure) to be suspected; however, this does not necessarily mean that the agent is responsible for the abortion. Only identification of the infectious agent and the associated products of the abortion, using the PCR method, for example, can determine whether the agent was responsible for the abortion.

Materials and methods

Scope of the study and sampling

The study was conducted between the months of October 2014 and June 2016. It relates to 368 cases of clinical abortion in 52 heifers and 316 cows on 124 farms declared free from brucellosis and tuberculosis in the north of Algeria (on the Mitidja plain). This sample represents around 1% of heifers and cows in the region. The number of females of reproductive age on each farm was between 4 and 180, and the number of abortions recorded during the study period ranged between 1 and 21, depending on the herd (Fig. 1).

The Mitidja region, which is composed of the *wilayas* (provinces) of Blida, Alger, Tipaza and Boumerdès, is situated on a large agricultural plain well known for citrus fruit production and vines. It extends over a surface area of 1,400 km^2 (100 km long and 5 km to 20 km wide), with an average altitude of 50 metres. The climate is Mediterranean with a continental influence (the Sirocco wind in summer), with mild rainy winters and hot dry summers. The animal population comprises approximately 67,000 cattle (including 34,000 cows), 155,000 sheep (including 61,500 ewes), 26,000 goats (including 12,000 nannies) and 700 horses (including 120 mares).

Because clinical abortion cannot be detected by farmers before the third month of gestation, only reports of abortions observed after this period were considered in this study. The farms concerned were inspected following such reports. The necessary data were collected to evaluate the factors associated with a higher or lower risk of exposure to the three causes of abortion under study. These data were: the year of the farm inspection visit (2014, 2015 and 2016); the number of cattle; the number of females; the type of housing (loose, stabled, or semi-stabled, i.e. confined indoors in the farm building during milking and during the night but kept outside the building during the daytime for grazing or for resting); the grazing practices; the method of insemination (artificial insemination or natural mating); the season of the abortion (spring, summer, autumn, winter); the feed storage conditions (good, average, poor); the watering source (communal water supply, tank, deep drilling, shallow well, stream); whether or not animals had been dewormed; whether or not there was a rodent control

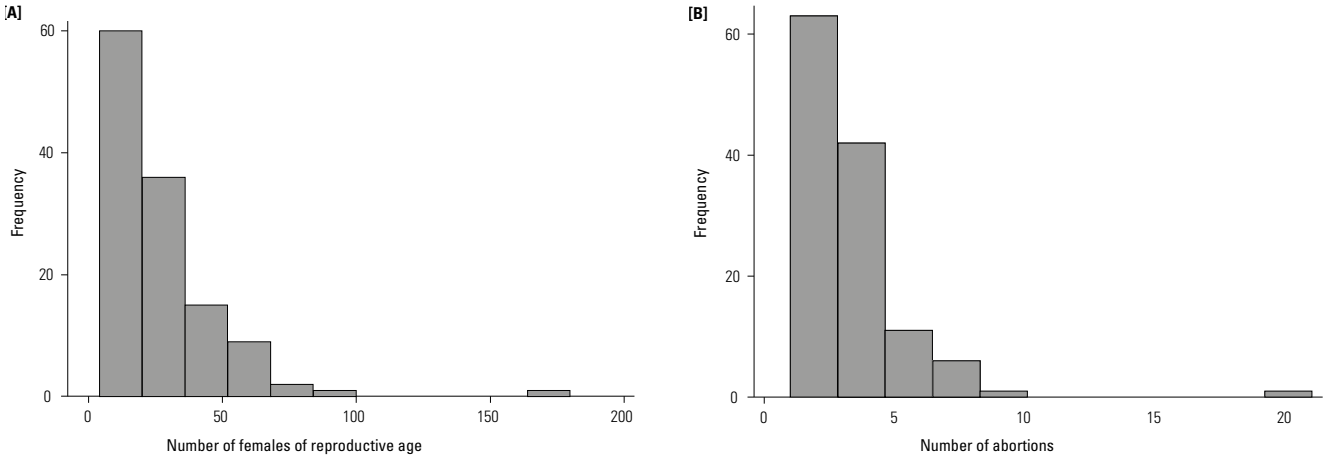

Fig. 1
Frequency histograms of the number of females of reproductive age on each farm [A] and the number of clinical abortions observed per herd [B] during the study period

plan; the estimated frequency of abortions; whether or not new animals had been purchased during the previous 12 months; and the number of cows that aborted, based on the identification of abortion (clinical abortion) or return to heat of the animal after confirmation of gestation (sub-clinical abortion).

A 5-ml blood sample was taken by a veterinarian from the caudal vein of every cow that had aborted, using a Vacutainer tube. The samples were taken within a period of no more than two months after the abortion was reported, reducing the probability of finding a very low level of antibodies. The samples were then dispatched to the laboratory in a coolbox at a temperature of +4°C and then centrifuged for 5 minutes at 3,000 rpm. The sera were stored at a temperature of –20°C until the serological tests were performed.

Serological analyses

The presence of anti-*C. burnetii*, anti-*C. abortus* and anti-*T. gondii* antibodies was detected using ELISA kits (IDVET, Montpellier, France). The kit used to search for anti-*C. burnetii* antibodies was the ID Screen® Q Fever Indirect Multi-species Kit, which uses a *C. burnetii* strain phases I and II isolated in France from the placenta of an aborted cow. The diagnostic specificity and sensitivity of this test according to the manufacturer are, respectively, 100% (95% confidence interval [CI]: 98.49–100) and 100% (95% CI: 88.65–100). The kit used to screen for anti-*C. abortus* antibodies was the ID Screen® *C. abortus* Indirect Multi-species Kit, which uses a synthetic antigen from the major outer membrane protein (MOMP) with specificity for *C. abortus*. The manufacturer claims diagnostic specificity and sensitivity for this test of, respectively, 100% (95% CI: 93.38–100) and 70% (95% CI: 53.5–83.4). Screening for the anti-*T. gondii* antibody in cattle serum was carried out using the ID Screen® Toxoplasmosis Indirect Multi-species Kit, which uses the specific antigen P30 of *T. gondii*. According to the manufacturer, the diagnostic specificity and sensitivity of this test are, respectively, 99.42% (95% CI: 98.5–99.7) and 98.36% (95% CI: 95.29–99.44). The tests used were validated by an optical density positive control (ODPC) greater than 0.35 for *C. burnetii*, *C. abortus* and *T. gondii*, and a ratio between the average of positive controls (ODPCs) and the average of optical density negative controls (ODNCs) greater than 3 for *C. burnetii* and *C. abortus* and 3.5 for *T. gondii*. With these two conditions satisfied, the optical densities of the samples tested were measured at a wavelength of 450 nm. The S/P (Sample/Positive) percentages were calculated using equation 1 and interpreted according to the instructions of the manufacturers of the ELISA tests (Table I).

$$\frac{S}{P}\% = \frac{OD\ sample}{OD\ positive\ control} \times 100 \qquad \text{(Equation 1)}$$

A farm was considered to be seropositive if at least one cow on the farm was seropositive. The seroprevalence was calculated by dividing the number of serologically positive and doubtful sera by the total number of sera analysed.

Statistical analysis

The individual true prevalence (TP) of *C. burnetii*, *C. abortus* and *T. gondii* was estimated from the apparent prevalence (AP) calculated in this study (i.e. the seroprevalence) and the individual diagnostic specificity (Sp) and sensitivity (Se) claimed by the manufacturer of the kits, using the Rogan-Gladen formula (25):

$$TP = \frac{PA + Sp - 1}{Se + Sp - 1} \qquad \text{(Equation 2)}$$

Table I
Interpretation thresholds of the enzyme-linked immunosorbent assay kits used for detection of antibodies against *Coxiella burnetii*, *Chlamydia abortus* and *Toxoplasma gondii*

Interpretation	*Coxiella burnetii*	*Chlamydia abortus*	*Toxoplasma gondii*
Acute infection	–	–	S/P ≥ 200%
Strongly positive result	S/P > 80%	–	–
Positive result	50% < S/P ≤ 80%	S/P ≥ 60%	50% ≤ S/P < 200%
Doubtful result	40% < S/P ≤ 50%	50% < S/P < 60%	40% < S/P < 50%
Negative result	S/P ≤ 40%	S/P ≤ 50%	S/P ≤ 40%

S/P: sample (sample tested) / positive (positive control sample)

For the AP, Sp and Se values of the different ELISA tests, a uniform variable was used, taking into account the extreme values of the confidence interval of 95%, and a stochastic simulation (1,000 Monte Carlo simulations) was performed, using @Risk 7.5.2 software (Palisade Corporation, Ithaca, New York, USA) to estimate the TP with a CI of 95% using equation 2.

The statistical identification of factors associated with a higher or lower risk of exposure to the three pathogens of interest was made using STATA/SE 14.2 (StataCorp., College Station, Texas, USA). First, a mixed-effects model at different levels was used to take into account any 'herd' level as a random effect. Because no random effect was observed, a logistic regression was then used to model the probabilities of an animal being seropositive or doubtful depending on factors associated with a higher or lower risk of exposure to the pathogens under study. The initial identification of these potential factors was made using a univariate regression. Following this, a multivariate logistic regression was performed using those variables that had a value of $P < 0.20$ in the univariate analysis. Finally, in the initial multivariate model, the non-significant variables ($P > 0.05$) were eliminated in a step-by-step approach (starting with the least significant variable, i.e. the variable with the highest *P* value). At each stage, a likelihood ratio test was used to compare the complex and simplified models. Where there was no significant difference between them, the simplified model was used. The correlations between variables that underwent the univariate analysis were not tested because they were of no biological relevance.

Results

Individual and herd prevalence rates

The individual apparent seroprevalence (positive and doubtful results) of *C. burnetii*, *C. abortus* and *T. gondii* were, respectively, 8.42% (31/368), 12.23% (45/368) and 13.86% (51/368) (Table II and Appendix 1). No cow had antibodies against more than one of these pathogens. The percentages of doubtful sera for *C. burnetii*, *C. abortus* and *T. gondii* were, respectively, 1.6%, 3% and 3.5%.

Using the Rogan-Gladen formula (25) (Equation 2), the individual true prevalence rates of *C. burnetii*, *C. abortus* and *T. gondii* were estimated at 8.45% (95% CI: 5.33–11.78), 17.67% (95% CI: 11.17–25.97) and 13.70% (95% CI: 10.04–17.45), respectively.

The apparent seroprevalence rates at herd level for *C. burnetii*, *C. abortus* and *T. gondii* were, respectively, 16.13% (20/124), 29.84% (37/124) and 29.84% (37/124) (Table II). An analysis of the serological responses to the three agents screened showed the simultaneous presence of anti-*T. gondii*, anti-*C. abortus* and anti-*C. burnetii* antibodies on 0.8% of farms, anti-*T. gondii* and anti-*C. abortus* antibodies on 2.4% of farms, and anti-*T. gondii* and anti-*C. burnetii* antibodies on 0.8% of farms.

Table II
Individual and herd seroprevalences of *Coxiella burnetii, Chlamydia abortus* and *Toxoplasma gondii*

Zoonotic pathogen	Number (individuals)			Individual prevalence rate (95% CI)	Number (herds)			Herd prevalence rate (95% CI)
	P	D	N	= (P + D)/(P + D + N)	P	D	N	= (P + D)/(P + D + N)
Coxiella burnetii	25	6	337	8.42 (5.80–11.74)	17	3	104	16.13 (10.14–23.80)
Chlamydia abortus	34	11	323	12.23 (9.06–16.02)	31	6	87	29.84 (21.96–38.71)
Toxoplasma gondii	38	13	317	13.86 (10.50–17.82)	28	9	87	29.84 (21.96–38.71)

CI: confidence interval P: positive D: doubtful N: negative

Factors associated with a higher or lower risk of exposure to the three pathogens of interest

At an individual level, none of the factors analysed by the multivariate logistic regression model proved to be associated with an increased risk of exposure to *C. burnetii*. However, the 2015 visit (OR = 0.0006; 95% CI: 0.000004–0.12; *P* = 0.006) and the 2016 visit (OR = 0.0005; 95% CI: 0.000002–0.13; *P* = 0.007), as well as the practice of artificial insemination (OR = 0.15; 95% CI: 0.05–0.44; *P* = 0.001), were factors associated with a reduced risk of exposure to *C. burnetii*.

Similarly, winter (OR = 0.39; 95% CI: 0.15–1.00; *P* = 0.05), spring (OR = 0.45; 95% CI: 0.20–0.97; *P* = 0.04) and the practice of artificial insemination (OR = 0.27; 95% CI: 0.13–0.56; *P* < 0.001) were factors associated with a reduced risk of exposure to *C. abortus*.

For *T. gondii*, the fourth month of gestation (OR = 22.68; 95% CI: 1.38–392.97; *P* = 0.03) and the fifth month of gestation (OR = 25.51; 95% CI: 1.47–442.11; *P* = 0.03) were identified as factors associated with an increased risk of exposure. Conversely, the third lactation was associated with a reduced risk of exposure to *T. gondii* (OR = 0.38; 95% CI: 0.16–0.92; *P* = 0.03).

At herd level (Table III), deworming of animals and drilling individual wells for drinking water were factors associated, respectively, with an increased risk of exposure to *C. abortus* (OR = 3.89; 95% CI: 1.53–9.89; *P* = 0.005) and to *T. gondii* (OR = 7.50; 95% CI: 2.11–26.69; *P* < 0.001). For *C. burnetii*, no factor associated with an increased risk of exposure was identified by the multivariate logistic regression analysis. However, the following factors analysed were associated with a reduced risk of exposure to this pathogen: year of inspection 2015 (OR = 0.02; 95% CI: 0.0008–0.65; *P* = 0.027) and 2016 (OR = 0.01; 95% CI: 0.0003–0.36; *P* = 0.011); artificial insemination by a veterinarian or a trained inseminator (OR = 0.21; 95% CI: 0.06–0.69; *P* = 0.01); and a control plan for rodents and other vermin (OR = 0.19; 95% CI: 0.06–0.57; *P* = 0.003).

Discussion

Prevalence and factors associated with a higher or lower risk of exposure to *Coxiella burnetii*

Seroprevalence

Individual seroprevalence for *C. burnetii* was estimated at 8.4% (of the population of aborted dairy cows). This can be compared with the results of other studies conducted in the same context (cattle abortions) using the same serological diagnostic technique (ELISA). The seroprevalence observed in this study is lower than the 23.9% recorded in the Tiaret region of Algeria (26), 16.2% in Tunisia (27), 25% in Iran (28), 44.9% in Italy (18) and 11.6% in the People's Republic of China (29). In the present study, the 'herd' seroprevalence of *C. burnetii* was 16.1%, slightly lower than the 22% found in the Bejaïa region of Algeria in 2016 (30). It was, however, quite similar to the 13.4% found in Latvia in 2017 (31) and 15.6% in Bangladesh in 2016 (32).

Factors associated with a higher or lower risk of exposure to *Coxiella burnetii*

No significant statistical link was found between the seroprevalence of *C. burnetii* and the different factors associated with a higher risk of exposure, which were screened for at both individual and herd level. Only factors associated with a reduced risk of exposure were identified. At both individual and herd level, analyses for the years 2015 and 2016 presented fewer seropositive cases for *C. burnetii* than those for 2014. The most probable explanation is rainfall. In Algeria, the average monthly number of rainy days during the years 2014 (study covered the last three months of the year), 2015 (study covered the entire year) and 2016 (study covered the first six months of the year) were, respectively, 8 days, 4.42 days and 4.67 days. This reduced risk of exposure to *C. burnetii* linked to rainfall has already been observed in Sweden in dairy cow herds (21). Unfortunately, the lack of free access to data concerning the wind speed and direction during the study period in the geographical zone concerned prevented an assessment of the impact of these factors on *C. burnetii*.

The practice of artificial insemination by a veterinarian or a trained technician is associated with a lower risk of exposure to *C. burnetii* at both individual and herd level. This observation may be related to the fact that, in a previous study, the prevalence of exposure to *C. burnetii*, estimated in bulk tank milk, was higher in dairy herds where artificial insemination was carried out by a person other than an artificial insemination technician (OR = 7.7; 95% CI: 2.1–17.0) (22).

At herd level, the implementation of a plan to control rodents and other vermin was related to lower exposure to *C. burnetii*. The deoxyribonucleic acid of *C. burnetii* has already been found in brown rats (*Rattus norvegicus*) and black rats (*Rattus rattus*) on cattle farms in the Netherlands (33). As rats are considered to be a potential host for *C. burnetii*, excretion of bacteria by rats is likely to soil cattle feed.

Table III
Results of the multivariate logistic regression at individual and herd level for risk factors and protection factors associated with exposure to *Coxiella burnetii, Chlamydia abortus* and *Toxoplasma gondii*

Zoonotic pathogen	Multivariate logistic regression at individual level*				Multivariate logistic regression at herd level#			
	Variable	Modality	OR (95% CI)	Value of *P*	Variable	Modality	OR (95% CI)	Value of *P*
Coxiella burnetii	Year	2014	Reference	–	Year	2014	Reference	–
		2015	0.0006 (0.000004–0.12)	0.006		2015	0.02 (0.0008–0.65)	0.027
		2016	0.0005 (0.000002–0.13)	0.007		2016	0.01 (0.0003–0.36)	0.011
	Artificial insemination or natural mating	Natural mating	Reference	–	Artificial insemination or natural mating	Natural mating	Reference	–
						Artificial insemination	0.21 (0.06–0.69)	0.01
		Artificial insemination	0.15 (0.05–0.44)	0.001	Rodent eradication	No	Reference	–
						Yes	0.19 (0.06–0.57)	0.003
Chlamydia abortus	Season	Autumn	Reference	–	Deworming or not	No	Reference	–
		Winter	0.39 (0.15–1.00)	0.05		Yes	3.89 (1.53–9.89)	0.005
		Spring	0.45 (0.20–0.97)	0.04				
	Artificial insemination or natural mating	Natural mating	Reference	–				
		Artificial insemination	0.27 (0.13–0.56)	<0.001				
Toxoplasma gondii	Month of gestation	Third month	Reference	–	Watering	Well	Reference	-
		Fourth month	22.68 (1.38–392.97)	0.03		Drilling	7.50 (2.11–26.69)	<0.001
		Fifth month	25.51 (1.47–442.11)	0.03				
	Number of gestations	1	Reference	–				
		3	0.38 (0.16–0.92)	0.03				

OR: odds ratio
CI: confidence interval

*Likelihood ratio (LR) of models:
for *C. burnetii* (LR $chi^2_{(22)} = 70.42$; $P < 0.0001$)
for *C. abortus* (LR $chi^2_{(10)} = 36.29$; $P = 0.0001$) and
for *T. gondii* (LR $chi^2_{9)} = 40.25$; $P < 0.0001$)

#Likelihood ratio (LR) of models:
for *C. burnetii* (LR $chi^2_{(5)} = 21.21$; $P < 0.0007$)
for *C. abortus* (LR $chi^2_{(2)} = 12.88$; $P = 0.0016$) and
for *T. gondii* (LR $chi^2_{3)} = 11.11$; $P < 0.01$)

Prevalence and factors associated with a higher or lower risk of exposure to *Chlamydia abortus*

Seroprevalence

The individual seroprevalence of *C. abortus* was estimated at 12.2%. This can be compared with that found in other serological studies conducted on cattle abortions. It is similar to the 12.6% obtained in Hungary (34), but lower than the 37.1% for Tunisia (27). It is also lower than the 18.18% found in the People's Republic of China in sera analysed using an indirect haemagglutination test (35), and lower than the 71.4% obtained for Chinese Taipei in sera analysed using an ELISA test (10). At herd level, the seroprevalence of *C. abortus* was 29.8%, a value well below the 66.7% observed in Tunisia on sera analysed using an ELISA test (27). This disparity is probably due to the differing contexts and protocols of the studies.

Factors associated with a higher or lower risk of exposure to *Chlamydia abortus*

At an individual level, winter and spring were identified as factors associated with a reduced risk of exposure to *C. abortus*. This result is inconsistent with reports from Mexico (36), where the risk of abortion in general was found to be higher during rainy and windy periods in winter than during the dry season on cross-bred beef cattle farms. An increase in the abortion rate in winter has also been reported in Ireland (37).

The use of artificial insemination was identified as a factor for a reduced risk of exposure to *C. abortus*. This corroborates the results of a study carried out in Germany (38), where natural mating was found to be a risk factor due to the frequent presence of *C. abortus* in bulls suffering from vesiculitis (39), or even in clinically healthy animals (40). They can become infected and act as vectors for chlamydiosis; the risk of abortion is 6.6 times higher in seropositive cows (41).

At the herd level, deworming of animals was associated with an increased risk of exposure to *C. abortus*. It is possible that this effect, at first sight paradoxical, could be caused by the antimitotic action of benzimidazoles, which could be a source of malformation or developmental anomalies of the foetus, resulting in its expulsion (42).

Prevalence and factors associated with a higher or lower risk of exposure to *Toxoplasma gondii*

Seroprevalence

The individual seroprevalence of *T. gondii*, estimated at 13.8%, was very similar to that reported in other studies, all of which used the ELISA test. This value is very close to the 15.2% observed in western Algeria (26) and 13.3% in Sudan (16). It is higher than the 5.9% reported in Iran in sera from aborted cows tested using ELISA (43).

At herd level, the seroprevalence of *T. gondii* was estimated at 29.8%, which is significantly lower than the 44.8% found in Sudan (16).

Factors associated with a higher or lower risk of exposure to *Toxoplasma gondii*

At the individual level, the fourth and fifth months of gestation were associated with an increased risk of exposure to *T. gondii*. This could be related to the fact that abortion caused by *T. gondii* is seen in the first and sixth months of gestation (44), approximately one month after initial contamination. However, farmers are not likely to report clinical abortion until the third month of gestation.

A reduction in the risk of exposure to *T. gondii* has been established during the third month of gestation, which suggests the development of acquired immunity. According to some reports, the seroprevalence of *T. gondii* is higher in cattle under the age of one (16) and in older cattle over 4 years old (23) and over 5 years old (45). Note, however, that this study does not concern young animals.

At herd level, the source of drinking water was identified as a factor that increased the risk of exposure to *T. gondii*. The water can be contaminated by *T. gondii* oocysts in faecal matter or the urine of infected cats (23, 46). In Algeria, cats are frequently kept on farms to help control rodents, which increases the risk of their droppings contaminating feed, silage and water sources. In addition, cats bury their excrement, which contaminates the soil for a long period because the *T. gondii* oocysts can survive for two years in the ground (47). This type of drinking water contamination has already been reported in the People's Republic of China (24) and Brazil (46, 48).

Conclusion

This study determined the serological prevalences (using the ELISA method) of Q fever, chlamydia abortion and toxoplasmosis, at an individual and herd level, on dairy farms in northern Algeria. This characterisation and the identification of factors associated with a higher or lower risk of exposure to the pathogens of interest provides a useful first step towards drafting recommendations aimed at reducing the frequency of abortions. It would be worthwhile to follow up this study with larger-scale epidemiological studies, using PCR for more specific identification of the agents involved in abortion.

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Appendix 1

Detailed results of the 124 farms studied: number of females of reproductive age, number of clinical abortions observed during the study period and number of positive, doubtful and negative results by the pathogenic agent concerned

Farm		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Number of females of reproductive age		180	10	13	5	9	8	37	45	39	44	50	56	65	48	63	8	15	5	13	13	4	7	4	8	11	6	5	4	13	35	31
Number of abortions		21	1	1	1	1	1	7	7	6	8	9	5	6	5	7	1	3	1	2	2	2	1	1	2	2	1	1	2	2	4	3
Percentage of abortions		12	10	8	20	11	13	19	16	15	18	18	9	9	10	11	13	20	20	15	15	50	14	25	25	18	17	20	50	15	11	10
Coxiella burnetii	P	6							2	1										1	1											
	D	2																				1										
	N	13	1	1	1	1	1	7	5	5	8	9	5	6	5	7	1	3	1	1	1	1	1	1	2	2	1	1	2	2	4	3
Chlamydia abortus	P	1										1						1								1	1			1	2
	D															1																
	N	20	1	1	1	1	1	7	7	6	8	8	5	6	5	6	1	2	1	2	2	2	1	1	2	1		1	2	2	3	1
Toxoplasma gondii	P	2												2											1				1			
	D																					1			1			1				
	N	19	1	1	1	1	1	7	7	6	8	9	5	4	5	7	1	3	1	2	2	1	1	1		2	1		1	2	4	3

Farm		32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62
Number of females of reproductive age		45	60	25	82	32	49	22	42	23	28	32	20	17	22	13	9	29	8	24	16	30	48	19	22	26	20	18	20	15	5	32
Number of abortions		3	4	2	5	4	4	3	3	2	2	2	2	2	3	2	1	3	2	3	4	4	7	4	3	5	2	3	3	1	1	3
Percentage of abortions		7	7	8	6	13	8	14	7	9	7	6	10	12	14	15	11	10	25	13	25	13	15	21	14	19	10	17	15	7	20	9
Coxiella burnetii	P																							1	1	3					1	
	D																															
	N	3	4	2	5	4	4	3	3	2	2	2	2	2	3	2	1	3	2	3	4	4	7	3	2	2	2	3	3	1		3
Chlamydia abortus	P		1	1				1	1		1		1	1	1		1				1						1			1		
	D				2																1	1	1				1					
	N	3	3	1	3	4	4	2	2	2	1	2	1	1	2	2		3	2	3	2	3	6	4	3	5		3	3		1	3
Toxoplasma gondii	P	2		1			1				1	1			2			1					1									
	D						1		1	1						1									1								
	N	1	4	1	5	4	2	3	2	1	1	1	2	2	1	1	1	2	2	3	4	4	6	4	2	5	2	3	3	1	1	3

Farm		63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93
Number of females of reproductive age		18	15	23	15	10	11	13	39	14	23	42	12	18	52	17	9	27	15	32	8	10	33	60	6	70	7	12	18	35	24	28
Number of abortions		2	3	2	2	2	1	2	4	2	3	6	1	2	5	1	1	3	2	4	1	1	3	6	1	5	1	4	2	3	3	3
Percentage of abortions		11	20	9	13	20	9	15	10	14	13	14	8	11	10	6	11	11	13	13	13	10	9	10	17	7	14	33	11	9	13	11
Coxiella burnetii	P																								1					1	1	
	D																															
	N	2	3	2	2	2	1	2	4	2	3	6	1	2	5	1	1	3	2	4	1	1	3	6		5	1	4	2	2	2	3
Chlamydia abortus	P										1	1						1		1				1					1			
	D											1												1					1			
	N	2	3	2	2	2	1	2	4	2	2	4	1	2	5	1	1	2	2	3	1	1	3	4	1	5	1	4		3	3	3
Toxoplasma gondii	P				1				1	1		1					1		1	1		1			3						1
	D																							1								
	N	2	3	2	1	2	1	2	3	1	3	5	1	2	5	1		3	1	3	1		3	5	1	2	1	4	2	3	3	2

Farm		94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124
Number of females of reproductive age		17	42	19	25	21	12	66	7	17	32	29	6	56	25	8	15	13	29	12	7	21	43	28	16	11	61	26	86	38	6	28
Number of abortions		2	4	2	3	3	1	8	1	2	4	3	1	4	3	1	2	1	3	1	1	2	3	2	2	1	4	3	5	3	1	3
Percentage of abortions		12	10	11	12	14	8	12	14	12	13	10	17	7	12	13	13	8	10	8	14	10	7	7	13	9	7	12	6	8	17	11
Coxiella burnetii	P	1													1	1												1		1		
	D								1																		1			1		
	N	1	4	2	3	3	1	8		2	4	3	1	4	2		2	1	3	1	1	2	3	2	2	1	3	2	5	1	1	3
Chlamydia abortus	P		2			2				1			1					1								1						
	D																															1
	N	2	2	2	3	1	1	8	1	1	4	3		4	3	1	2		3	1	1	2	3	2	2		4	3	5	3	1	2
Toxoplasma gondii	P							2			2				1								2				2		1			
	D																1		1								1		1			
	N	2	4	2	3	3	1	6	1	2	2	3	1	4	2	1	1	1	2	1	1	2	1	2	2	1	1	3	3	3	1	3

P: number of positive aborted females
D: number of doubtful aborted females
N: number of negative aborted females