Q fever seroprevalence and risk factors in sheep and goats in northwest Italy

Francesca Rizzo[a]*, Nicoletta Vitale[b], Marco Ballardini[a§], Vitaliano Borromeo[d], Camilla Luzzago[d], Laura Chiavacci[b], Maria Lucia Mandola[a]

[a] Molecular Virology Unit, Istituto Zooprofilattico Sperimentale del Piemonte, Liguria [e] Valle d'Aosta, Via Bologna 148, 10154 Torino, Italy
[b] Epidemiological Unit, Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Via Bologna 148, 10154 Torino, Italy
[d] Department of Veterinary Medicine, University of Milan, via Celoria 10, 20133 Milano, Italy
[§] Present address: S.S. Imperia, Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Via Nizza 4, 18100 Imperia, Italy

nicoletta.vitale@izsto.it
marco.ballardini@izsto.it
vitaliano.borromeo@unimi.it
camilla.luzzago@unimi.it
laura.chiavacci@izsto.it
marialucia.mandola@izsto.it

* Corresponding author: Francesca Rizzo, Molecular Virology Unit, Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Via Bologna 148, 10154 Torino, Italy; tel. +390112686272; fax +390112475933; email: francesca.rizzo@izsto.it

Highlights

- First large-scale randomized serosurvey for Q fever in small ruminants in Italy
- True prevalence estimates based on a solid sampling frame and testing procedure
- Evidence of a higher susceptibility to infection in goats in mixed herds
- Mixed herd represents an important risk factor for the spread of Q fever in Italy

Abstract

Q fever is a zoonosis caused by the bacterium *Coxiella burnetii*; domestic ruminants, mainly goats and sheep, are the main source of Q fever outbreaks in humans. From both a public and an animal health perspective, providing reliable prevalence data is extremely relevant for the decision processes by policymakers and food producer organizations.

Information on Q fever seroprevalence in small ruminants in Italy is currently incomplete and largely based on reports of reproductive disorders in livestock farms.

To estimate animal and flock seroprevalence of *C. burnetii* in small ruminants (sheep, goats and mixed flocks), a cross-sectional study with a two-stage design was carried out in northwest Italy. Between January and December 2012, sera from 5738 animals (2553 sheep and 3185 goats) belonging to 411 flocks (206 goats, 111 sheep, and 94 mixed flocks) were examined for specific anti-*C. burnetii* IgG antibodies by a commercial ELISA kit. A questionnaire investigating possible associations between farm management and *C. burnetii* seropositivity was administered.

At the flock level, the overall true seroprevalence adjusted for test sensitivity and specificity was 31.2% (95% confidence interval [CI]: 24.8-37.7). Sheep-farm and goat-farm true seroprevalence was 38.7% (95% CI 25.5-51.9) and 19.5% (95% CI 11.5-27.6), respectively. Interestingly, the true seroprevalence (48.5%; 95% CI 34.7-62.3) was higher in the mixed flocks (sheep and goats).

At the animal level, the overall true seroprevalence was 15.9% (95% CI 15.4-16.4). No difference was found between the two species, but the true seroprevalence was significantly higher (χ^2=7.49; p<0.007) among the goats in mixed flocks (25.7%; 95% CI 24.4-27.1) than the sheep (16.3%; 95% CI 15.1-17.4), suggesting a potential difference in susceptibility between the two species or the result of factors affecting

their immune response or related to the livestock management system as the period of exposure to *C. burnetii.*

A multivariable logistic model that controlled for farm-level clustering identified five main risk factors associated with farm seropositivity ($p \leq 0.05$): flock size of more than 12 animals (odds ratio [OR] 4.2; 95% CI 2.6-6.7), contact with other flocks (OR 2.1; 95% CI 1.2-3.6), mixed flock type (OR 2.4; 95% CI 1.4-4.2), farms located in the western area (OR 2.4; 95% CI 1.4-4.2), and infertility during the previous year (OR 2.6; 95% CI 1.2-5.2).

The results of this study yielded baseline information that may be useful to set up future epidemiologic, flock management, and public health policies for the prevention and control of Q fever in Italy.

Key words: *Coxiella burnetii*, Q fever, small ruminants, sheep, goats, seroprevalence, risk factors

Introduction

Q fever is a worldwide zoonotic disease caused by *Coxiella burnetii*, an obligate intracellular Gram-negative bacterium belonging to the *Legionellales* order, *Coxiellaceae* family. *C. burnetii* can infect a wide range of animals, both aquatic and terrestrial, and can survive for prolonged periods in the environment as a highly resistant spore-like form, which favors its spread by the wind over long distances (Maurin and Raoult, 1999; Arricau-Bouvery and Rodolakis, 2005; Kersh et al., 2010; van den Brom et al., 2015).

C. burnetii can induce reproductive disorders in domestic ruminants (Arricau-Bouvery and Rodolakis, 2005; Berri et al. 2005, Berri et al. 2007; Muskens et al. 2011;

Agerholm, 2013). It is documented as a major cause of ovine abortion in flocks throughout Europe (Berri et al., 2002; Masala et al., 2004; Rodolakis et al., 2006; Garcia-Perez et al., 2009; Ruiz-Fons et al., 2010; Kennerman et al., 2010). Small domestic ruminants, because they shed the bacterium in urine, feces, milk, birth products, and vaginal mucus, are largely recognized as the most important source of outbreaks in humans (Berri et al., 2001; Berri et al., 2005; Arricau-Bouvery and Rodolakis, 2005; Guatteo et al., 2006; Guatteo et al., 2007; Nielsen et al., 2013). To date, Q fever in humans is considered an endemic, mostly occupational, disease in several Mediterranean countries, with a peculiar epidemiological trend consisting of both sporadic cases and epidemic outbreaks (Million and Rault 2015; van den Brom et al., 2015). In particular, dairy goat farms were identified as the primary source of Q fever infection during the recent epidemic in the Netherlands, which caused outbreaks of unprecedented size in humans, with over 4000 acute Q fever cases recorded between 2007 and 2010 (EFSA, 2010; Schimmer et al., 2011).

In response to the rising concerns about the risks of Q fever for humans and animals, the European Commission requested a scientific opinion and risk assessment for Europe. In its report, the European Food Safety Authority (EFSA) outlined the need for harmonized schemes for the passive and active monitoring/reporting of Q fever in animals so that its prevalence/incidence could be compared over time and between countries (Sidi-Boumedine et al., 2010; EFSA, 2010). Reliable prevalence data to inform policymakers in the context of an evidence-based decision scheme remain scarce, however, despite an overall apparent mean flock prevalence of 37% and 25% in cattle and small ruminants, respectively (Guatteo et al., 2011).

In the years since the Dutch epidemics, the correlation between risk factors for infection and *C. burnetii* seropositivity in sheep and goat flocks has been investigated

in several European countries (Ruiz-Fons et al., 2010; Schimmer et al., 2011; Anastacio et al., 2013; Shimmer et al., 2014). Farm-level factors, such as farm location, density and proximity to infected ruminant farms or contacts with professional farm visitors, were found to be associated with higher Q fever seroprevalence. Although comparisons between flocks belonging to different types of production and geographic areas turned up critical differences, the real drivers of Q fever infection in a flock were intrinsic farm factors, such as production systems and management in most cases (Schimmer et al., 2014).

In Italy, Q fever surveys in animals are very scarce and have been focused on reproductive disorders and particularly on abortion as the major clinical problem (Cabassi et al., 2006; Parisi et al., 2006; Natale et al., 2009; Vicari et al., 2013). The only extensive investigation conducted to date was carried out in Sardinia among flocks with previous abortion notification, which revealed a seroprevalence of 38% and 47% on sheep and goat farms, respectively (Masala et al., 2004).

Northwest Italy (Piedmont) is surrounded by the Alps on three sides and borders with France and Switzerland where outbreaks in both animals and humans have been reported (Frankel et al., 2011; Bellini et al., 2014). Except for preliminary surveys performed in 2009, which reported a 49% (49/100) PCR positivity rate in samples from raw milk vending machines (Gallina *et al*. Vendita diretta di latte crudo: valutazione di *E. sakazakii*, *Coxiella burnetii* e *M. paratuberolosis* nell'esperienza piemontese. *In* Proceedings of the XIX Congresso A.I.V.I., 2009, pp. 53-55) and seropositivity in ovine and chamois sharing the same grazing area (Viganò *et al.* Sieropositività per *Coxiella burnetii* in camosci e ovini in Val D'Ossola (Prov. VB). *In* Proceedings of the 28émes Rencontres du G.E.E.F.S.M., 2010, pp. 43; Mandola *et al*. Monitoraggio attivo per Febbre Q in ovicaprini transumanti. *In* Proceedings of the XII Congresso Nazionale S.I.Di.L.V., 2010, pp. 276), no other epidemiological data on Q

fever prevalence in northwest Italy are available. Therefore, we investigated pathogen exposure among sheep and goats in Piedmont. To do this, an active monitoring plan was carried out to accurately assess the true seroprevalence of *C. burnetii* infection at the animal and flock level by species. Epidemiological criteria were applied to identifythe risk factors for seropositivity and generate comparable data. Associations between seropositivity and management practices, as well as farm characteristics, were also assessed.

Methods, techniques

Study area

Piedmont (45°0'N 08°0'E, 25,399 km^2) is located in the northwestern side of Italy. It is divided into eight provinces: Torino (TO), Cuneo (CN), Asti (AT), Alessandria (AL), Novara (NO), Vercelli (VC), Biella (BI), and Verbano-Cusio-Ossola (VCO). The regional geography is marked by alpine, hilly, and lowland areas. The region is strongly devoted to animal production and its sheep and goat livestock may be considered representative of prealpine sheep and goat production. According to the Italian National Livestock registration database (VetInfo) as of 31/12/2012, the regional small ruminant population was composed of 108,995 sheep and 72,505 goats belonging to 8288 farms, corresponding to 1647 sheep, 4573 goats, and 2078 mixed flocks. Most goat flocks are small, while sheep and mixed farms are larger by comparison (see Table 1 for the distribution of animals per farm according to the VetInfo database).

A semi-extensive production management system for sheep and mixed flocks is predominant (65%) and is characterized by housing in winter months until earlyspring, when parturition occurs, and seasonal grazing for the rest of the year over open pastoral systems in hilly and mountainous areas. More often, goats are raised for

milk production and usually kept in stables all year long.

Among the sheep and goat farms in Piedmont, the predominant productive type is meat, about 65%, followed by 27% mixed meat and milk type production, and 8% only milk type production, mostly of goats. According to the VetInfo database about 17.1% of sheep belong to the Biellese breed, which is particularly appreciated for its meat, while 43.13% of sheep and goats are crossbreed. The predominant goat breeds are: Alpine (8.38%), Saanen (5.85%), and Camosciata delle Alpi (5.15%). Raw milk production is an ancient tradition specific to Piedmont; one example is the milk of the Roccaverano goat breed (1.22% of goats) used for the production of a Protected Designation of Origin (PDO) cheese exported worldwide.

In the population, 8.8% of the animals were between 6 months and 1 year of age, 16.4% were 1 to 2 years, and 74.8% were > 2 years (VetInfo database).

Study design

This cross-sectional study with a two-stage design (flock and a constant number of elements per cluster at a second level) was carried out from January to December 2012. The total number of ovine and goat flocks (n=411) to be sampled was set according to Cannon and Roe (1982), considering an expected prevalence of 35% according to a pilot survey conducted in VCO province in 2010 (unpublished data) with 4.5% precision at the 95% confidence level (e.g. since no other epidemiological data were available). *C. burnetii* vaccination was not applied to the target population. The study area was divided into four macroareas, and the sample size of 411 flocks was proportionally stratified according to the number of flocks per area: western area

(TO province, n=107); southern area (CN province, n=101), eastern area (AL and AT provinces, n=96), northern area (VC, NO, VCO, and BI provinces, n=107). At the animal level, the number of animals per flock was selected using random sampling. For flocks with ≥ 30 heads, the number was calculated using an expected seroprevalence of 10% (unpublished data from a pilot survey conducted in VCO province in 2010) with 95% confidence level to detect at least one seropositive animal by means of the formula *(1)* by Martin et al. (1987):

$$(1) \qquad k=[1-1(1-\alpha^{1/d})]\ [N-1/2(d-1)] = 30$$

where k is the number of animals to sample within each flock; α the probability of observing at least one seropositive animal in the sample when the infection affects at least d/N of the animals in the flock; d the expected number of infected animals in a flock; N the average flock size. In small flocks (<30 heads), all animals >6 months of age were sampled. For mixed flocks with more than 30 goats and 30 sheep, a maximum of 30 animals per species were randomly selected.

The flock sampling frame was the VetInfo database for the identification and registration of livestock. The PROC FREQ procedure in SAS® version 9.2 (SAS Institute) was used to stratify random sampling.

Blood samples were collected by government veterinarians during annual mandatory brucellosis testing of breed animals > 6 months of age. Information on potential risk factors for *C. burnetii* at the flock level was recorded through a questionnaire administered to farmers by the veterinarians at the time of sampling. The questionnaire was prepared by a focus group composed of epidemiologists and field veterinarians and approved by the regional veterinary officers. Questions were prepared in closed format and divided into sections, such as general information, production, flock management, contacts (with other animals or professionals),

reproductive disorders, and health treatment. Detailed written instructions for questionnaire administration and compilation were distributed to each local veterinary service. All data were entered into a dedicated database (Microsoft ACCESS®).

Serological test

Blood samples were collected from each animal into 10 ml vacuum transparent polystyrene tubes (Vacuette ®, Greiner Bio-one, Austria), immediately stored at +4°C and delivered to the laboratory by the sample transportation service of the local health unit within 48 hours of collection. At the laboratory, the tubes were centrifuged (10 min, 1600 × *g*), and the sera obtained after centrifugation were collected and stored at -20°±5° C until analysis. Ovine and caprine sera were tested for specific anti-*C. burnetii* IgG antibodies by a commercial indirect enzyme-linked immunosorbent assay (ELISA) kit (LSIVet^{TM} Ruminant Q Fever-Serum-Milk, LSI, Lissieu, France) according to the manufacturer's instructions. The assay was performed on microtiter plates pre-coated with a cocktail of inactivated antigens phases I and II *C. burnetii* strain recovered from ruminant (ovine CbO1) and isolated by INRA (French National Institute for Agricultural Research, Montpellier, France). The ELISA kit sensitivity (se) and specificity (sp), as stated in the manufacturer's validation report performed by the Central Veterinary Institute (The Netherlands), are 88.8% and 98.5% in sheep, 91.6% and 98.9% in goats, respectively.

The results were expressed as optical density (OD), measured at 450 nm and the titer of the sample expressed as sample to positive (S/P) ratio%, which was calculated according to the formula: $(OD_{sample}-OD_{negatice\ control})/(OD_{positivecontrol}-OD_{negative\ control})$ x 100. The manufacturer's recommended test cut-off value to consider a sample aspositive was a serum titer >40%.

Statistical analyses

Descriptive statistics were applied to determine the frequency of seropositive flocks for antibodies against *C. burnetii*. A flock was considered positive when at least one animal was positive to the ELISA test. True seroprevalence was estimated by frequentist approach using the formula of Rogan and Gladen (1978) (2):

(2) $$\dot{P} = \tau+\beta-1/\alpha+\beta-1$$

$\dot{P}$ is true seroprevalence, τ is apparent seroprevalence, β is specificity, and α is sensitivity. flock level sensitivity (HSE) and specificity (HSP) were adjusted using these formulas (3, 4) (Cameron and Baldock, 1998):

(3) $$HSE=1-(1-Se *n/N)^{\wedge d}$$

(4) $$HSP=Sp^{m}$$

where Se and Sp are animal-level sensitivity and specificity, respectively, *n* is the number of animals tested in a flock, *N* the number of animals present in a flock, *d* is the expected number of infected animals, and *m* is the number of animals tested.

A primary screening test to identify exposure variables significantly correlated with *C.burnetii* seropositivity was calculated with 2×K contingency tables using χ^2 analysis for categorical variables to test whether or not a variable was associated with

the presence or absence of antibodies against *C.burnetii*, here arbitrarily defined as coxiella serological status. All variables with $p<0.15$ (two-sided) in univariate analysis were analyzed in a logistic binomial regression model of fixed effects using the loglog distribution function of the logit procedure to obtain adjusted odd ratios (OR).

Categorical variables with more than two categories were transformed into two or more dummy variables and compared by χ^2 analysis of coxiella serological status. This was done to determine whether distinction among the categories was important. Based on these results, two or more categories could be combined with minimal loss in predictive ability, and the final categorical variables consisted of only two categories.

Collinearity between the variables was investigated by χ^2 analysis. The risk factors initially offered to the model were excluded from the model with a conditional backward elimination procedure; the possible interaction terms were then investigated with a forward conditional selection procedure. A factor was entered in the model at $p\leq0.05$ and removed at $p\geq0.10$. The likelihood ratio test was used to assess the overall significance of the model (two-tailed significance level $p\leq0.05$). Confounding was monitored by evaluating the change in the coefficient of a factor after removing another factor; if the change exceeds 25% of the coefficient value, the removed factor is considered a potential confounder. The significance of each term in the model was tested by Wald's χ^2. In the final model, biologically plausible interaction between factors was investigated by significance. Estimated OR and 95% Wald's confidence interval (CI) were obtained as measures of predictor effect. The Hosmer–Lemeshow test was performed to assess the model's goodness-of-fit (Hosmer and Lemeshow, 1989). All the analyses were done using SAS® version 9.2.

Results

According to the experimental design, 5738 animals (2553 sheep and 3185 goats) belonging to 411 flocks (206 goat, 111 sheep, and 94 mixed flocks) were examined for antibodies against *C. burnetii*. In our sample set, 44.7% of the animals were crossbreed, 16.5% were Biellese, 8.5% Alpine, 5.4% Saanen, and 5.6% Camosciata delle Alpi; 93.3% were female. The median age was 4.2 years (first quartile 2.0, third quartile 5.7 years); 7.2% of the sampled animals were between 6 months and 1 year, 17.4% between 1 and 2 years, 30.0% between 2 and 4 years, and 45.5% > 4 years old.

Seroprevalence at flock and animal level

At the farm level, the overall true seroprevalence, adjusted for test flock sensitivity and specificity, was estimated to be 31.2% (95% CI 24.8-37.7). True seroprevalence at the farm level was 38.7% (95% CI 25.5-51.9) for sheep and 19.5% (95% CI 11.5-27.6) for goats. In the mixed flocks, the true seroprevalence was 48.5% (95% CI 34.7-62.3). Table 2 presents the seroprevalence adjusted for test sensitivity and specificity at the farm and animal levels. No adjustment for sampling proportion is made to provide overall prevalence estimate.

The spatial distribution of seropositive flocks is presented in Figure 1. According to the sampling frame, the true seroprevalence at the farm level in the four macroareas was: 45.7% (95% CI 32.5-58.8) in the western area, 31.5% (95% CI 18.2-44.7) in the southern area, 35.9% (95% CI 23.1-48.7) in the northern area, and 12.1% (95% CI 1.5-22.7) in the eastern area.

At the animal level (Table 2), the total true seroprevalence was 15.9% (95% CI 15.4-16.4) with no significant difference observed between sheep and goats when the

result was analysed at the species level (p<0.2978) (Table 3). True seroprevalence was higher among the goats in the mixed flocks (25.7%; 95% CI 24.4-27.1) than among the sheep (16.3%; 95% CI 15.1-17.4); the difference was statistically significant (χ^2 =7.49; p<0.007). A chi square test was applied to check whether without the mixed flocks the true seroprevalence at the animal level between goats and sheep remained non significant. There were 171/1498 seropositive sheep (11.42%; 95% CI 9.85-13.14%) and 219/2118 seropositive goats (10.34%; 95% CI 9.08-11.72%) (χ^2 1.05; p=0.3045). The difference was still not significant.

Risk factor analyses

The univariate analysis at the animal level indicated two factors associated with *C. burnetii* seropositivity: age (χ^2=19.5; p<0.0002) and breed (χ^2= 8.2; p<0.0042), as older and crossbreed animals were more likely to be seropositive, while no correlation was found for sex and species (Table 3). These results were also confirmed at multivariate analysis (Wald chi square 27.6924; p value 0.0001). In particular, first kidding animals (1-2 years) (OR 1.9; 95% CI 1.2-3.1) showed a twofold increased probability to be *C. burnetii* seropositive as compared with replacement animals (6 months-1 year) (OR 1).

The factors associated with Q fever seropositivity at the flock level are shown in Table 4. The main factors were: flock type, flock size, seasonal grazing, roaming grazing, flock location, manure treatment, introduction of animals, and contacts with other flocks, wildlife, and farmers or professionals. Other factors related to health status that were found to be associated with *C. burnetii* seropositivity were history of reproductive disorders and use of anti-parasite and antimicrobial treatments.
Biosecurity factors were not associated with coxiella serological status. The analysis

showed collinearity between geographic location, flock size, seasonal grazing, roaming grazing, and animal introduction.

In the final logistic regression model (likelihood ratio χ^2=75.8, degrees of freedom=5, p<0.001), only five factors were retained as being significantly associated with *C. burnetii* seropositivity: flock size (i.e., farms with >12 heads) increased the probability fourfold (OR 4.2; 95% CI 2.6-6.7); contact with other flocks increased the probability nearly twofold (OR 2.1; 95% CI 1.2-3.6) as compared with flocks without contacts, mixed flock increased the probability nearly twofold (OR 2.4; 95% CI 1.4-4.2) related to farms with only sheep or only goats, flock location in the western area increased the probability to be seropositive twofold (OR 2.4; 95% CI 1.4-4.2). Among the reproductive disorders, flocks with infertility during the previous 12 months had a twofold higher probability (OR 2.6; 95% CI 1.2-5.2) to be seropositive than the others. The Hosmer and Lemeshow test showed no evidence of poor fit (7.26, degrees of freedom=6, p=0.30) (Table 5).

Discussion

Exposure of sheep and goats to *C. burnetii* was evaluated by testing for the presence of antibodies with an indirect ELISA test. The study design provided data on true prevalence at the flock and the animal level by species in an area particularly exposed to the infection because it borders with countries with recurrent and documented Q fever outbreaks in both animals and humans (ECDC, 2014; Magouras et al., 2015). The semi-extensive management system for sheep and goats is predominant in Piedmont and is largely applied in the rest of northern Italy.

The overall results showed a true flock seroprevalence of 31.2% and an animal prevalence of 15.9%, indicating an epidemiological situation similar to that reported

for other European countries (Guatteo et al., 2011). Considering the results by species, the differences between ruminant species at the flock level were statistically significant (χ^2=29.1; p< 0.0001), with a higher seroprevalence in sheep flocks than in goat ones (38.7% *vs.* 19.5%), consistent with the rates reported for Spain and Portugal (Ruiz-Fons et al., 2010; Anastacio et al., 2013). This situation can be explained by the local farming system in Piedmont, which is characterized by semi-extensive grazing.
Because sheep flocks are usually larger than goat flocks and more familiar with seasonal grazing, they are more likely to be exposed to an increased risk of infection. A different situation was reported during the Q fever outbreaks in the Netherlands, where the flock seroprevalence was 14.5% for sheep and 17.9% for goats, respectively, with significantly higher values recorded in dairy than non-dairy farms for both species, likely related to the intensive dairy husbandry systems and the flock size there (van den Brom et al., 2013).

As outlined in the study, the highest flock true seroprevalence was recorded in the mixed flock (48.5%) and confirmed by logistic analysis, indicating that this flock type, characterized by larger size and copresence of the two species, more likely represents a risk factor for the spread of the infection. Although no differences in seroprevalence between goats and sheep were observed at the animal level, the goats kept in mixed flocks were more likely to test positive for *C. burnetii* infection (true prevalence of 25.7% vs. 16.3% in sheep). A similar trend, albeit with lower values, has been recently reported in small ruminant mixed flocks in Portugal, where both the higher animal prevalence and the lower flock prevalence may be indicative of a higher within-flock prevalence among goats (Anastacio et al., 2013).

In light of our data on mixed flocks and assuming that the animals were exposed to fairly similar levels of risk of infection, a potential difference in susceptibility to *C. burnetii* between sheep and goats needs to be investigated

to verify whether an increased susceptibility of goats to the infection actually exists, as has beensuggested, but not reported, in the literature so far (Klaasen et al., 2014). Besides species susceptibility, livestock management practices might also contribute to this difference. For example, as compared with sheep, the longer permanence of adult goats on the farm, and consequently their increased exposure to infection, may contribute to the higher seroprevalence in this species, since the animals can remain seropositive for years. Moreover, the grazing habits of mixed flocks, as compared to flocks with only goats, exposes goats to more contacts, and hence to a higher risk of infection.

A statistical analysis was also performed to identify exposure variables significantly correlated with coxiella seropositivity. At the animal level, the increase in seroprevalence with age was expected, given the more relevant probability of repeated contacts with the pathogen proportionally to life span. Moreover, when we examined the age at onset of puberty, the relevant increase in seroprevalence between replacement animals and first kidding animals confirms the high exposure to the bacteria during the lambing season (Garcia-Perez et al., 2009).

In general, the risk for farms to acquire *C. burnetii* infection seems to be a complex issue. The multivariate analysis confirmed that age and breed were statistically significant at the animal level, but also that other critical factors lie at the flock level. Several studies on small ruminants have pointed out that the degree of exposure to infection differs with the management system operated; therefore, it may be assumed that the local productive characteristics will have a major effect on *C. burnetii* prevalence in livestock from different geographic areas.

Although the univariate analysis at the flock and animal levels showed several factors associated with coxiella seropositivity, the multivariate logistic regression identified five main factors: infertility, flock size, mixed flock type, contact between

flocks, and geographic area. Indeed, infertility is a recognized warning sign of *C. burnetii* infection in both small ruminants and cattle (Cabassi et al., 2006; Garcia-Perez et al., 2009; Rodolakis et al., 2009). Moreover, flock size and contact with other flocks are considered the most important risk factors for coxiella seropositivity in small ruminants and cattle (McCaughey et al., 2010; Schimmer et al., 2011, 2012; Anastacio et al., 2013).

The correlation between a higher risk of coxiella seropositivity and flock size may be related to the greater number of lambing and kidding females at lambing season, which increases the total population at risk and, subsequently, the risk of pathogen introduction and transmission. A positive correlation also exists between seropositivity and factors related to the risk of *C. burnetii* transmission, such as contact with other flocks and seasonal grazing, since sharing the same pasture fields with other livestock increases the risk of introducing pathogens from the environment (Schimmer et al., 2014).

Based on the geographical sampling frame we used, the distribution of flocks with the highest percentage of seropositive animals was observed in the northern and western provinces bordering with Switzerland and France and characterized by medium-large size flocks grazed on summer alpine pastures. The highest seroprevalence recorded in these areas may be due to the larger size of the flocks in these provinces as compared with the rest of Piedmont and the abundance of alpine pastures shared by different flocks. Of note is that about 60 to 80 animal infections per year and recurrent Q fever outbreaks in humans have been recorded in Switzerland since 2009, the latest occurring in spring 2012 in the Canton of Vaud with 14 human cases involved (Bellini et al., 2014). Moreover, at least four human outbreaks traced to

exposure to sheep, goats, and cattle have been reported in France over the last 25 years (Frankel et al., 2011). The widespread distribution of Q fever in the French sheep and goat population may also be reflected by the high load of *C. burnetii* DNA in dairy products (64% positivities) detected in France. Nonetheless, *C. burnetii* infection in humans has never been directly correlated with the consumption of infected milk products (Eldin et al., 2013).

Conversely, a few sporadic Q fever epidemics in humans have been reported in Italy, mostly associated with direct or indirect exposure to infected sheep flocks (Selvaggi et al., 1996; Boschini et al., 1999; Santoro et al., 2004). Recently, seroprevalence rates of 50% have been reported in agricultural workers in northern Italy (Tabibi et al., 2013) and of 62.9% among occupationally exposed workers in Sicily (Fenga et al., 2015), but since most cases are mild and go unreported, the true incidence of the disease in humans remains unknown and underestimated.

The high prevalence of *C. burnetii* antibodies recorded in Piedmont poses a significant risk of professional exposure to the infection. Since 2012, local health authorities have collaborated in an information campaign about the risks for and the clinical signs of human Q fever to allow early detection of the disease. Through this effort, a Q fever human outbreak in two abattoir workers was identified in May 2014 in the western Cuneo province, two weeks after the slaughter of a group of sheep (data not published).

Conclusions

The active monitoring performed through this study revealed widespread seeroprevalence of *C. burnetii* in northwest Italy where the semi-extensive management system for sheep and goats is predominant and where goat milk is traditionally transformed into typical pieces of cheese PDO appreciated worldwide.

From both a public and an animal health perspective, this study provides baseline and essential data to set up future epidemiologic, flock management, and public health policies for the prevention and control of Q fever in Italy.

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Figure legend

Figure 1. Spatial distribution of *C. burnetii* seropositive flocks in Piedmont. Shades of grey denote the four macroareas. VDA denotes the Valle d'Aosta region; LOM denotes the Lombardy region; LIG denotes the Liguria region. No samplings were performed in the southern parts of VC and NO provinces because these areas are completely devoted to rice cultures.

Table 1: Descriptive statistics of number of animals per farms in Piedmont region according to the Italian National Livestock registration database (VetInfo) as of 31/12/2012.

Species	n° farms	median of animal per farm	5^{th} of animal per farm	95^{th} of animal per farm
goat	4573	4	1	35
sheep	1647	6	1	53
mixed	2078	15	2	185

Table 2: Descriptive characteristics and estimates of *Coxiella burnetii* seroprevalence at the flock level and the animal level, expressed as total and separate for each ruminant species. At the flock level, true seroprevalence was adjusted by flock sensitivity and flock specificity, using Rogan-Gladen CL.

Variable	Frequency (*n*)	Positive (*n*)	Raw prevalence (95% CI)	True seroprevalence (95% CI)
Flock level				
Total	411	149	36.3% (31.6-41.1%)	31.2% (24.8%-37.7%)
Goat	206	51	24.8% (21.8-27.7%)	19.5% (11.5%-27.6%)
Sheep	111	45	40.5% (36.0-45.1%)	38.7% (25.5%-51.9%)
Mixed	94	53	56.4% (51.6-60.9%)	48.5% (34.7%-62.3%)
Animal level				
Goats	3185	437	13.7% (13.1-14.3%)	16.2% (15.5%-16.8%)
Sheep	2553	336	13.2% (12.5-13.9%)	15.5% (14.8%-16.2%)
Total animals	5738	773	13.5% (13.1-13.9%)	15.9% (15.4%-16.4%)
Goats in mixed flock	1086	230	21.2% (19.8-22.2%)	25.7% (24.4%-27.1%)
Sheep in mixed flock	1045	164	15.7% (14.9-17.1%)	16.3% (15.1%-17.4%)

Table 3: Association between animal characteristics and coxiella serological status with corresponding chi square (χ^2), p-value, odds ratio (OR), confidence interval (CI).

Characteristic	Percentage seropositivity (n° seropositive/ total)	χ^2	p-value χ^2	Adjusted OR (95% CI)
Age (years)		19.47	0.0002	
6 months- ≤ 1	6.6% (27/411)			1.0 (ref.)
>1 - ≤ 2	12.2% (122/998)			1.9 (1.2-3.1)
>2 - ≤ 4	15.4% (265/1721)			2.6 (1.6-4.0)
>4	13.3% (347/2607)			2.2 (1.4-3.4)
Sex		3.12	0.0773	
Male	10.1% (39/385)			1.0 (ref.)
Female	13.5% (723/5353)			1.4 (0.9- 1.9)
Species		1.08	0.2978	
Sheep	13.2%(336/2553)			1.0 (ref.)
goats	13.7% (437/3185)			0.92 (0.8-1.1)
Breed		8.21	0.0042	
crossbreed	14.8% (380/2565)			1.3 (1.1- 1.5)
pure breed	12.0% (381/3173)			1.0 (ref.)

Table 4: Univariate analysis of farm-based factors associated with coxiella serological status from 411 sampled farms. Significant p-values are given in bold.

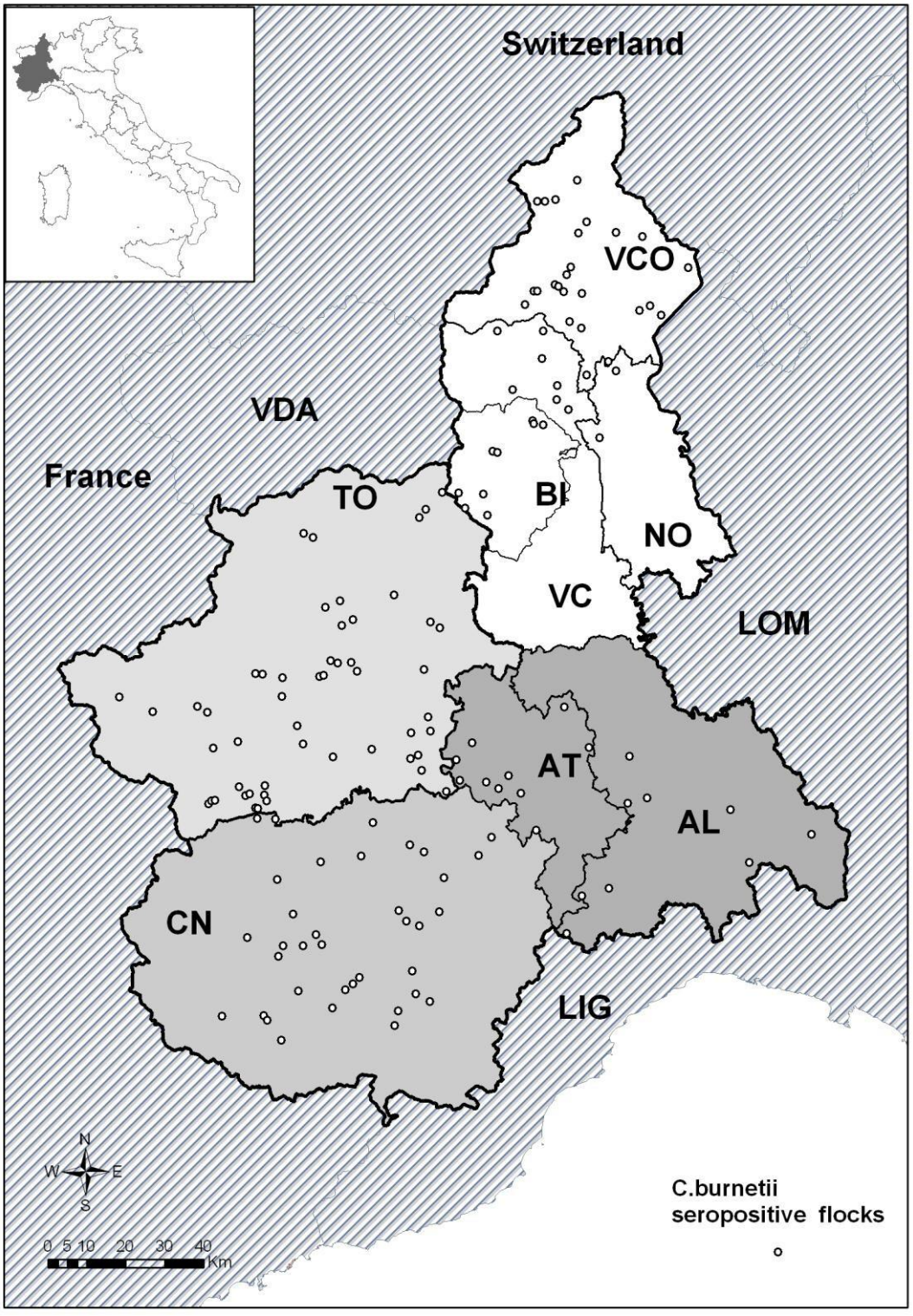
Factors	category	Frequency (N)	Sero-prevalence	p-value
General informations				
Flock type	goat	206	24.8%	ref
	sheep	111	40.5%	
	mixed	94	56.4%	**0.0001**
Flock location (Piedmont macroareas)	North	107	40.2%	ref
	East	96	19.8%	
	South	101	36.6%	
	West	107	46.7%	**0.0007**
Flock size	>12 heads	189	56.6%	ref
	no	222	18.9%	**0.0001**
Production				
Type of production	milk	11	36.4%	ref
	others	400	34.0%	0.3575
Cheese production	yes	33	48.5%	ref
	no	408	32.1%	0.4549
Raw milk cheese production	yes	22	50.0%	ref
	no	389	34.7%	0.0601
Flock management				
Roaming grazing	yes	7	85.7%	ref
	no	404	35.4%	**0.0063**
Seasonal grazing[a]	yes	209	45.9%	ref
	no	202	26.2%	**0.0001**
Animal introduction during the previous year	yes	99	44.4%	ref
	no	312	33.7%	**0.0378**
Quarantine	yes	42	35.7%	ref
	no	369	33.6%	0.1325
Estrous synchronization	yes	20	40.0%	ref
	no	391	36.1%	0.7165
Lambing pens or areas	yes	13	61.5%	ref
	no	398	35.4%	0.6953
Dealing with fetus/abortion, retained placenta	yes	183	43.7%	ref
	no	228	28.5%	**0.0063**
Manure treatment	yes	41	43.9%	ref
	no	370	34.6%	**0.0042**
Contacts				
Contact with cattle	yes	93	52.7%	ref
	no	318	30.8%	**0.0001**
Contact with other flocks	yes	107	57.9%	ref
	no	304	28.3%	**0.0001**
Contact with professionals of other flocks	yes	62	59.7%	ref
	no	349	32.1%	**0.0001**
Contact with wildlife at pasture	yes	196	46.9%	ref
	no	215	48.4%	**0.0001**
Tick infestation on animals	yes	49	49.0%	ref
	no	362	34.3%	0.8394
Presence of ticks in the environment	yes	46	39.1%	ref
	no	365	35.6%	0.6141
Presence of dogs	yes	317	37.5%	ref
	no	94	31.9%	0.5257
Reproductive disorders				
Infertility during the previous year	yes	53	66.0%	ref
	no	358	31.3%	**0.0001**
Late-term abortion during the previous year	yes	15	66.7%	ref
	no	396	35.1%	**0.0139**
Stillbirth/premature delivery	yes	13	61.5%	ref
	no	398	35.4%	0.3012
Health treatment				

Antimicrobial treatment	yes	20	70.0%	ref
	no	391	34.5%	**0.0002**
Antiparasite products for external use	yes	45	60.0%	ref
	no	366	32.5%	**0.0006**

[a] Seasonal grazing refers to flocks that were declared to graze in open pastoral systems in hilly and mountainous areas depending on the season.

Table 5: Final multivariate logistic regression model for the presence of *C. burnetii* antibody in the sera of 411 goats and sheep farms. Hosmer and Lemeshow goodness-of-fit test p=0.36**.**

Parameter	baseline	OR	95% CI OR	Wald χ^2	p-value χ^2
Flock size	>12	4.2	2.6-6.7	31.7	0.0001
Flock type	mixed	2.4	1.4-4.2	10.3	0.0013
Flock location	Western area	2.4	1.4-4.2	10.7	0.0011
Contact with other flocks	yes	2.1	1.2-3.6	7.5	0.0062
Infertility during the previous year	yes	2.6	1.2-5.2	6.6	0.0101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