Flock-level factors associated with the risk of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection in Greek dairy goat flocks

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ABSTRACT

In this cross-sectional study we identified flock-level risk factors for *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection, in Greek dairy goat flocks. We collected 1599 milk samples from does that were at the last stage of lactation in 58 randomly selected dairy goat flocks, during May to September 2012. The collected samples were tested with a commercial milk ELISA (IdexxPourquier, Montpellier, France) and the results were interpreted at a cut-off that optimized the accuracy of the diagnostic process. For the analysis of the data we used Bayesian models that adjusted for the imperfect Se and Sp of the milk-ELISA. Flock was included as a random effect. Does in flocks that used common water troughs and communal grazing grounds had 4.6 [95% credible interval (CI): 1.5; 17.4] times higher odds of being MAP-infected compared to does in flocks that had no contact with other flocks. Does of flocks supplied with surface water from either streams or shallow wells had 3.7 (1.4; 10.4) times higher odds of being infected compared to those in flocks watered by underground and piped water sources. When kids were spending equal to or more than 10 h per day with their dams they had 2.6 (1.1; 6.4) times higher odds of being MAP infected compared to kids that were separated from their dams for less than 10 h per day. Finally, does in flocks that continuously used the same anti-parasitic compound had 2.2 (1.0; 4.6) times higher odds of MAP infection compared to those in flocks alternating anti-parasitic compounds. These results should be considered in the development of a nationwide future control program fOr caprine paratuberculosis in Greece.

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1. Introduction

Paratuberculosis (Johne's disease) is a chronic intestinal infection of global importance in mainly domestic and wild ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). MAP infection of small ruminants has worldwide distribution, recognized in sheep and goats in many countries, including the southern hemisphere in Australia, New Zealand and South Africa, numerous northern hemisphere countries, particularly Great Britain, Norway and Austria, with increasing recognition in Mediterranean countries including Greece, Spain, Portugal, Morocco and Jordan (Benazzi et al., 1995; Djønne, 2010; Hailat et al., 2010). Caprine paratuberculosis is also recognized in Turkey, France, Norway, Switzerland, Croatia, Canada, the USA and Chile (Barkema et al., 2010). MAP infection mostly results from fecal-oral route exposure.

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Fecal-oral route exposure may occur from: (1) ingestion of fecal material from an infected animal, particularly on the teat of an infected dam, plus exposure to manure contaminated pasture, water, supplements or hay contaminated with fecal material from an infected adult animal (Windsor and Whittington, 2010) and (2) the drinking of contaminated colostrum or milk as MAP is also excreted in the colostrum and milk of sheep and goats (Lambeth et al., 2004; Nebbia et al., 2006). Pre-natal infection is also now well described (Lambeth et al., 2004; Whittington and Windsor, 2009). The clinical manifestations of paratuberculosis in goats include progressive wasting and decrease in milk production, which are followed by the manifestation of advanced clinical disease: flafy skin, poor hair coat, progressive emaciation, dehydration, anemia with submandibular edema, depression, and diarrhea (Stehman, 1996). Paratuberculosis was first recognized in Greek goats in 1975 (Leontides et al., 1975). Today, the majority of Greek goat flocks are endemically infected with MAP (Ikonomopoulos et al., 2007; Dimareli-Malli et al., 2013).

Greece has the largest goat herd in the EU accounting for around 50% of the EU total and is self-sufficient in goat-meat (http://lhu.emu.ee/downloads/Welfood/WP1T2L4.pdf). The Greek national herd comprises of approximately 6 million goats, which are reared primarily for milk production (Zygogiannis and Katsaounis, 1992). The main reason why there are so many goats in Greece is because there is a strong tradition of cheese consumption in the Greek gastronomy; cheese is not a food supplement, it is food. Contrary to its European counterparts of France, Italy and Spain, Greeks consume cheese at all times, i.e. for breakfast, lunch, dinner, alone or with other food, having the highest consumption in EU of 23 kg per person per year. A plethora of protected destination of origin (e.g. feta) or protected geographical indication cheeses of Greece are dependent on the production of goat milk. In a study on the prevalence of MAP in retail feta cheese (produced from sheep and goat milk) the authors reported 50% (21/42) and 4.7% (2/42) PCR- and culture-positivity, respectively, for MAP (Ikonomopoulos et al., 2005). A potential zoonotic link between MAP and human inflammatory bowel diseases including Crohn's disease has been suggested but remains unclear (Over et al., 2011). If MAP is confirmed as a zoonotic pathogen, public confidence in products of the dairy industries is very likely to decline.

Within an infected flock most animals acquire MAP early in their life. Susceptibility to infection decreases over time, while environmental (Tiwari et al., 2009) and genetic (Koets et al., 2000) factors, which have not been fully conceptualized yet, playing a critical role on whether initial entrance and persistence of MAP will lead to clinical manifestations, be restrained during the productive life of infected animals or even be cleared out (Kostoulas et al., 2010). Although they are important for the development of national control programs, few studies aiming to identify risk factors for caprine paratuberculosis have been carried out. Ideally, the programs should depend on a risk-based system with a framework for identification of high risk, for the spread of MAP infection, flocks and regions. A Spanish study reported that factors related to intensive management such as herd size, foreign breeds and high replacement rate were associated with MAP infection (Mainar-Jaime and Vázquez-Boland, 1998). Addition of new animals and mixed farming were also found as factors associated with increased risk of paratuberculosis in goats (Al-Majali et al., 2008). However, in a recent study no associations were detected (Martínez-Herrera et al., 2012). Unfortunately, these studies ignored the fact that diagnostics for MAP are imperfect. Their estimates were not adjusted for the Sp and, most importantly, the low to average Se of MAP diagnostics. In the absence of perfect diagnostic tests and when the misclassification is non-differential odds ratio estimates are usually biased toward the null unless the analysis corrects for test accuracy (Copeland et al., 1977). Methods exist for obtaining corrected odds ratios by incorporating prior information from external estimates on the tests' Se and Sp (McInturff et al., 2004).

We conducted this cross sectional study in order to identify factors associated with the risk of MAP infection in Greek dairy goat flocks. Sampling was conducted during a period for which we demonstrated that the overlap between the distributions of the ELISA responses – the sample to positive ratio – in milk of the healthy and the MAP-infected does is the smallest (Angelidou et al., 2014). In the analysis, we employed Bayesian models to account for the imperfect Se and Sp of the diagnostic test.

2. Materials and methods

2.1. Target population and sampling scheme

Goat farming in Greece is a sector of animal production that is generally friendly to the environment usually taking place in disadvantaged for agriculture, hilly and mountainous areas. The animals are kept under semi-intensive management for milk production. The farmers select replacements among the daughters of high-yielding does. The males bought into the flocks originate from high-yielding animals from other flocks. The animals graze on communal pastures throughout most of the year and are additionally fed concentrates. They spend most of the day outside and are moved into the shed during the night. They are mated to bucks, in an unsupervised manner, in June–August and deliver from November to January of the following year. The kids are weaned 15–30 days after birth; subsequently the dams are mechanically or manually milked, twice daily. The milking duration is approximately 5 months; it is ceased gradually or abruptly when the farmer decides that the yield is low to justify the milking routine. The annual replacement risk is approximately 25%, which is the same as the culling risk because the farmers receive European Union-subsidies on the basis of flock size.

The target population included flocks in the region of Thessaly, at the center of the Greek mainland, which were managed semi-intensively for milk production. The animals belonged either to indigenous breeds (i.e. Vlahiki, Eghoria, Paggaio, Skopelos) or crosses of the indigenous with foreign breeds (i.e. Alpine, Zaanen, Damascus, Maltese). All the does of the flocks were unvaccinated against MAP. The sample size employed in this study was selected to detect an expected difference of 6% in the prevalence

between the exposed group (11%) and non-exposed group (5%) to communal grazing/watering with other flocks (based on unpublished data). The sample size was estimated assuming a 95% confidence interval (type I error = 5%) and 80% power (type II error = 20%) and an intra-class correlation coefficient 0.05, adding 20% to the minimum required sample size (of 1200 does, obtained by sampling 48 flocks with 25 does in each flock) to account for the loss of power associated with controlling for confounders (Hintze, 2014).

From 58 flocks we sampled milk from 1599 does from May to September 2012. The sampled flocks were selected with simple random sampling (with the aid of computer-generated random numbers) from the sampling frame of flock identification numbers in the region. Within the flocks the does were selected with systematic random sample while the animals entered the milking parlor.

The mode within flock sample size was 48 does but ranged between 20 and 50 does depending on the size of the flock and the number of non-dry animals at the sampling day. All samples were collected during the late stage of lactation because we recently demonstrated that although in Greek dairy goats both serum and milk ELISA, in all lactation stages, have similar overall discriminatory ability, the smallest overlap between the distributions of the ELISA responses – the sample to positive ratio- in milk of the healthy and MAP-infected does was detected in late lactation (Angelidou et al., 2014).

2.2. Diagnostic tests

The milk samples were centrifuged ($1200 \times g$ for 20 min), skimmed and stored at $-21\,^{\circ}C$ until testing with a commercial indirect ELISA kit (IdexxPourquier, Montpellier, France IDEXX®) using the manufacturer's protocol for bovine milk (Salgado et al., 2005). The recorded optical densities (OD) were transformed to the sample-to-positive (S/P) ratio and were interpreted at the cut-off of 0.35 (Angelidou et al., 2014).

2.3. Questionnaire

We developed a questionnaire, in order to collect data on factors that could be associated with the risk of MAP infection in goats. Questionnaire development was based on previously published work in sheep (Lugton, 2004) – due to the absence of relevant reports in dairy goats – and expert opinion. Questionnaire data included information on flock size, housing conditions, breed type, production parameters, managerial strategies, manure management, biosecurity measures, disease prevention and nutrition (Appendix B).

Seventy two questions were included on flock-level factors. Twenty six were closed (e.g. yes/no, always/frequently/seldom/never or pre-set options), thirty were semi-closed (e.g. information on number of days, application frequencies of certain procedures) and the remaining were open-ended (e.g. product names, descriptions) questions. The questionnaire (Appendix B) was administered and filled through a face-to-face interview of the farmers by the first author who had no prior knowledge of the

MAP infection status of the flocks. Whenever possible, the interviewer checked the accuracy of the information provided by the owner, such as shelter ventilation, by inspecting the facilities.

2.4. Statistical analyses

2.4.1. Definition of infection status

Bayesian mixture models create their own probabilistic definition of infection, which implicitly assumes a biological definition that has to be explicitly described. Essentially, this is determined by the target condition that the analytes and biomarkers of the test under consideration measure (Gardner et al., 2011). In our case, to describe MAP infection in biological terms, we mean that goats carry MAP intracellularly; substantial replication need not take place because the infection can be latent. Entrance and persistence of MAP have lasted long enough to give a detectable humoral immune response at any time during their life; we assumed that once an animal has an established infection, the infection persists for life (Angelidou et al., 2014; Kostoulas et al., 2006; Nielsen and Grùnb, 2002).

2.4.2. Bayesian model specification

We employed a Bayesian logistic regression model that adjusted for imperfect Se and Sp of the diagnostic test. Let the variable r_i indicate the number of positive does out of the n tested does with milk ELISA of the ith flock. We assume that r_i is distributed binomially,

$$r_i \sim \text{Binomial}(Ap_i, n_i), \tag{1}$$

where Ap_i is the apparent seroprevalence of the ith flock. Let $T+$ denote that a milk sample of a doe has tested positive and let $D+$ denote that the doe has the target condition. We define Se and Sp of the milk ELISA to be, $Se = \Pr(T+/D+)$, and $Sp = \Pr(T-/D-)$, respectively. We also let Tp_i denote the true prevalence of MAP infection in the ith flock. Adjusting for the Se and the Sp of the milk ELISA the apparent seroprevalence of the ith flock is

$$Ap_i = Se \times Tp_i + (1 - Sp) \times (1 - Tp_i) \tag{2}$$

Then, we model the Tp_i as the logit function of the vector, X_{ij}^T, where j is the number of the predictor variables including the intercept in the model:

$$Logit(Tp_i) = X_{ji}^T \beta_j + u_i \tag{3}$$

The term $X_{ij}^T \beta_j$ is referred to as the linear predictor (McCullagh and Nelder, 1989) and u_i is indicating the flock random effect. Further, we consider the normally distributed random effect level u_i, with zero mean and a random effects variance σ_u^2.

$$u_i \sim N(0, \sigma_u^2) \tag{4}$$

The standard method for specifying priors on β's is to use a multivariate normal distribution (Spiegelhalter et al., 2003). We preferred to obtain conditional mean priors (CMPs) as described by Bedrick et al. (1996). CMPs are constructed from the success probability of different covariate patterns. Briefly, instead of eliciting independent prior information about β's directly we specify uncertainty about

Table 1

Priors for the sensitivity (Se) and specificity (Sp) of the milk ELISA at the selected cutoff (0.35) and conditional mean priors (CMPs) on the expected risk of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection for specific combinations of the fitted covariates (covariate patterns) in the final model.

Covariate pattern					Prior specification		Mode
Intercept	Surface water	Contact with other flocks	Kids' spending ≥10 h per day	Alternating use of antiparasitic compounds	Se	Be (20.3, 10.08)	0.68
					Sp	Be (315.32,1.6)	0.99
1	1	1	0	1	CMP_1	Be (2.20, 27.15)	0.04
1	0	1	0	1	CMP_2	Be (1.42, 29.22)	0.01
1	1	1	0	0	CMP_3	Be (1.68, 07.95)	0.09
1	1	0	1	1	CMP_4	Be (1.40, 21.06)	0.02
1	1	0	0	1	CMP_5	Be (1.23, 26.90)	0.01

probabilities of the disease/infection state being present for various covariate patterns. For j regression coefficients (including the intercept), we specify prior information about j probabilities of success (disease/infection state being present) for j distinct covariate patterns. Subsequently, the priors on b were induced from the inverse covariance matrix (see Appendix A for a WinBUGS implementation).

Finally we use the Markov chain Monte Carlo samples from the posterior distribution of the β's to make inferences for the odds ratios. Thus we calculate the odds ratio as the exponential function of the regression coeficients (see Appendix A for a WinBUGS implementation).

2.4.3. Prior specification

We subsequently specified CMPs about the probability of an animal being sub-clinically infected for each level of the predictor and the intercept. We incorporated prior information about the prevalence of five combinations of covariate patterns, based on the expert opinion of the authors PK and LL, because there were five predictors in the final model (including the intercept). The specified covariate patterns with the corresponding input probabilities are in Table 1. In the absence of available information, non-informative, uniform beta distributions can be defined for the probabilities of success of the distinct covariate patterns.

The prior information about the Se and the Sp of the test is incorporated in the model in the form of beta distributions (Table 1):

$$Se \sim \text{beta}(\alpha_{Se}, \beta_{Se}), \quad Sp \sim \text{beta}(\alpha_{Sp}, \beta_{Sp}) \qquad (5)$$

Finally, we specify a non-informative prior on the inverse of the random effect variance:

$$\frac{1}{\sigma_u^2} \sim \text{gamma}(0.001, 0.001) \qquad (6)$$

2.4.4. Model building

For model building, seventy eight candidate variables were initially examined. When pairs of highly correlated variables were encountered, selection of the variable to be included in the model was based on biological plausibility. Twenty five variables were dropped due to high correlations. The remaining twenty variables were screened, one-by-one, using a univariable approach (Martin, 1997) in the Bayesian logistic regression model specified in Section 2.4.2. We incorporated, non-informative, uniform beta distributions for the probability of success of the distinct covariate patterns. During this screening phase, a significance level of $P < 0.25$ was used (Mickey and Greenland, 1987). We approximated the classical P-values in the Bayesian framework using the posterior densities of the beta distributions.

All twenty variables found significant, were simultaneously offered to a full model which was, subsequently, reduced by backwards elimination (Hosmer and Lemeshow, 1989), until only those significant at $P < 0.05$ remained. Finally, a stepwise forward selection process was done by offering previously excluded variables to the final model one at a time. During the model building, we incorporated non-informative, uniform beta distributions for the probability of success of the distinct covariate patterns.

2.4.5. Assessment of convergence

To assess the convergence of the Markov Chain Monte Carlo (MCMC), we checked the autocorrelations and the trace plots. We also checked the parameter summary statistics of 50,000 iterations after a burn-in phase of 50,000 iterations.

2.4.6. Statistical software

All models were built and run in the freeware program WinBUGS (Spiegelhalter et al., 2003). WinBUGS code with detailed step-by-step explanations and the CMPs specification can be found in the Appendix A. WinBUGS was also used for checking the autocorrelation plots. To calculate the parameters of the beta prior distributions we utilized the Betabuster software, which is public domain software available at http://www.epi.ucdavis.edu/diagnostictests.

3. Results

Flock sized ranged from 45 to 650 does (median 160). In 14/58 (24.1%) flocks there was at least one test-positive doe. In these test-positive flocks the mean within-herd prevalence was 10% (0.08; 0.12).

After uni-variable screening and pairwise correlation analysis the variables with $P < 0.25$ further considered in multivariable analysis included the information from the administrated questionnaire (Appendix B): (1) Housing conditions; flooring, altitude, kind of roof, (2) water supplied to the flock; origin of the water from surface, (3) exposure of the kids post partum; where the does of the flock usually deliver, applied disinfectant to the maternity paddock, (4) exposure of the kids during suckling; kids' spending hours per day with their does, food and

Table 2
The frequency distributions of the significant variables offered to the final Bayesian logistic regression model. Results were based on the analysis of data from 1599 does in 58 Greek dairy goats flocks adjusting for the imperfect Se and Sp of the milk ELISA.

Variable	Category	Milk-ELISA	
		Neg%	Pos%
Origin of the water from surface	No	60.4	5.2
	Yes	30.8	3.6
Contact with other flocks	No	8.0	57.7
	Yes	2.4	31.9
Kids' spending hours per day with their does	<10 h	9.1	57.3
	≥10 h	3.5	30.1
Alternating use of antiparasitic compounds	No	37.8	28.4
	Yes	21.6	12.3

water sharing of the kids with the does, (5) production parameters; culling rate per year, age category at culling, (6) biosecurity; replacing rate, communal grazing with other flocks, contact with wildlife during grazing, (7) gastrointestinal parasite control; compound combination, alternating use of antiparasitic compounds, (8) nutrition; type of additional bulk food providing in the shed, additional supplements containing minerals providing to the does and (9) soil PH – manure management; disinfection applied per year with limestone, frequency of cleaning, disposal location of the manure.

The final model included four factors: the origin of the water from surfaces, the contact with other flocks, the kids' spending equal to or more than 10 h per day with the dams and the alternating use of different anti-parasitic compounds. The frequency distributions of the significant variables offered to the final Bayesian logistic regression model are in Table 2. The odds ratios estimated under the Bayesian model that accounted for the imperfect Se and Sp of the milk ELISA are in Table 3. Specifically, does of flocks which used common water troughs and communal grazing

grounds had 4.6 [95% Credible Interval (CI): 1.5; 17.4] times higher odds of being MAP-infected compared to does of flocks that had no contact with other flocks. Does of flocks supplied with surface water from either streams or shallow wells had 3.7 (1.4; 10.4) times higher odds of being infected compared to those in flocks which were watered by underground and piped water sources. Does in flocks where the kids were spending equal to or more than 10 h per day with their dams had 2.6 (1.1; 6.4) times higher odds of MAP infection than those in flocks where the kids were separated from their dams for less than 10 h per day. Finally, does in herds that continuously used the same anti-parasitic compound had 2.2 (1.0; 4.6) times higher odds of MAP infection compared to those in flocks commonly alternating anti-parasitic compounds (the inverse association is in Table 3). Finally, the flock level variance was 0.8 (0.1; 2.0).

4. Discussion

In this cross-sectional study we found that communal grazing and the use of common water troughs with other flocks was associated with higher odds of MAP infection. This agrees with similar results elsewhere reported, suggesting that contact between flocks is a risk factor for the spread of MAP infection. Mixed farming was a risk factor for caprine paratuberculosis in Jordan (Al-Majali et al., 2008). The only non-infected Chilean dairy goat flocks were the ones that did not import goats from other flocks and were located in geographical areas where no mixed grazing with other susceptible ruminant species took place (Kruze et al., 2007). In Australia, sharing of roads between neighboring farms was also associated with higher paratuberculosis infection in sheep flocks (Dhand et al., 2007). Evidently, in areas that are endemically infected with MAP, especially in high agricultural density areas, increased biosecurity measures that would prevent contact between flocks must be part of a control program in order to prevent reintroduction and spread of the same or different MAP strains.

Goats in flocks supplied surface water (from streams or shallow wells) had higher odds to be MAP infected compared to those that were watered by underground and piped water sources. In consistency, an association

Table 3
Estimated Bayesian odds ratios and associated 95% credible intervals (CI) for factors associated with the risk of MAP infection after adjusting for the imperfect Se and Sp of the milk ELISA. Flock was included as a random effect.

Variable	Category	Odds ratios (CI)	P
Origin of the water from surface	No	1	
	Yes	3.7(1.4; 10.4)	0.005
Contact with other flocks	No	1	
	Yes	4.6(1.5; 17.4)	0.003
Kids' spending hours per day with their does	<10 h	1	
	≥10 h	2.6(1.1; 6.4)	0.016
Alternating use of antiparasitic compounds	No	1	
	Yes	0.5(0.2; 0.9)	0.020
$\sigma_u^{2\,a}$		0.8 (0.1; 2.0)	

[a] The variance of the random effect. u_t at the flock level.

between lower seroprevalence and presence of piped water was found in a cross-sectional study of small ruminants (Mainar-Jaime and Vázquez-Boland, 1998). However, the access to open water, though believed to aid transmission, was not found to be influential in sheep flocks (Lugton, 2004). Generally open source water is liable to MAP contamination from both domestic and wildlife species. Wildlife could be implicated in paratuberculosis transmission cycles in Greece (Florou et al., 2008). MAP can circulate among wildlife hosts including deer species and rabbits and a possible contamination of the pasture could infect sheep and cattle (Carta et al., 2013). However, MAP excretion by wildlife host is lower than excretion by clinically affected animals (Daniels et al., 2003). Thus, the contamination of the water from the affected goats in the flock should play the major role – compared to contamination due to wildlife – to the spread of MAP infection in endemically infected areas.

Goats in flocks were the kids' were allowed to spend equal to or more than 10 h per day with their dams had higher odds of MAP infection. Within an infected flock most animals acquire MAP early in their life. Because infection primarily occurs via the fecal oral route, the major source of MAP for the kids is the contaminated with feces udder. Calves that had suckled a foster cow during calfhood had a very high risk of testing ELISA positive compared with calves fed milk replacer indirectly (Nielsen et al., 2008). The direct contact with contaminated milk and colostrum is a major source of MAP infection for suckling ruminants. Under the semi-intensive management system of the Greek dairy flocks, kids directly suckle milk and colostrum from their does. Currently, a program of feeding milk replacement products or pasteurized milk is not applied. Hence, the longer they stay with their dams the more likely they are to ingest higher loads of MAP.

Poor control of intestinal parasites could affect the incidence of paratuberculosis. We found that, the use of the same anti-parasitic compounds rather than the alteration between different anti-parasitic treatments was associated with higher odds of MAP infection. In consistency to our result, a risk factor study in sheep flocks revealed that the use of ivermectin as the only anti-parasitic treatment was the factor with the strongest association with paratuberculosis seroprevalence (Coelho et al., 2010). Not alternating parasitic treatments or using a single anti-parasitic may contribute to the risk of MAP infection by increasing the probability of goats having higher parasitic loads and enduring longer exposure to parasitic infections. The use of the same antiparasitic compound is associated with increased antiparasitic resistance (Sangster and Gill, 1999; Köhler, 2001). Further, at the early stages of paratuberculosis, a cell-mediated immune response acts protectively against MAP. A concurrent parasitic infection could cause an easier shift to the humoral immune response (Stabel, 2000). However, once this shift occurred, the effect of insufficient antiparasitic treatment in the course of MAP infection is expected to be minimal at the late stages of paratuberculosis (Lugton, 2004). The latter authors found no association between the control of parasites and late clinical paratuberculosis in sheep,

since drenching of clinical cases simply delayed death. In our study, we adjusted for all the latent stages of infection by incorporating Se and Sp in the models and the observed association primarily concerns the subclinically infected goats because those clinically affected are low yielding animals not maintained for a full lactation period.

A major strength of this study is that we countered the effect of misclassification measured by the imperfect Se and Sp of the milk-ELISA. McInturff et al. (2004) showed that adjusting for the imperfect Se and Sp of the diagnostic process leads to corrected estimates that take into account all latent stages of MAP infection. In our case, we incorporated prior information for the Se and Sp which are based on a recent and relevant validation study for the milk ELISA (Angelidou et al., 2014). Milk ELISA is an imperfect diagnostic test; assuming the opposite would incorporate bias toward to null hypothesis leading to loss of significant variables. Prior information was in the form of probability space rather than single values to capture uncertainty and the analysis was carried out in a flexible Bayesian framework. The cross-sectional nature of the study design has a built-in problem with reverse causation (Martin, 2008), i.e. cross-sectional studies capture time-point associations that could not ensure that the animals were not infected prior to the exposure of the identified factors. However, the risk factors in the final model can be considered constant over time since they represent either routine managerial practices. This minimizes the limitations arising from the cross-sectional design. Another likely study limitation is the inflation of the Type I error rate due to multiple hypothesis testing, the consequence of testing the association with outcome of numerous variables (Kleinbaum, 1994). The paucity of previous similar studies on goats made necessary the development of a rather detailed questionnaire with many factors. This concern is, however, restricted by the somewhat strong associations ($0.003 < p < 0.02$) in the final model.

5. Conclusion

The use of common water troughs, communal grazing, surface water and kids' spending equal to or more than 10 h per day with their dams were associated with higher odds of MAP infection. Finally, the alternating use of different anti-parasitic compounds was associated with lower odds of MAP infection. These results should be considered in the development of a nationwide future control program for caprine paratuberculosis in Greece.

Acknowledgments

Appendix A.

Model

```
{
for (i in 1:N){
# where r is the number of positive does
r[i] ~ dbin(Ap[i],n[i])
# Incorporation of test sensitivity and specificity
Ap[i] < −Se*Tp[i] + (1 − Sp)*(11 − Tp[i])
logit(Tp[i]) < −b[1] + b[2]*X1[i] + b[3]*X2[i] + b[4]*X3[i] + b[5]*X4[i] + u[i]
}
```

Informative priors on sensitivity and specificity
Sp ~ dbeta(315.32, 1.62)
Se ~ dbeta(20.3,10.08)
tau~dgamma(1.0E-3, 1.0E-3)
sigma < −1/sqrt(tau)
sigma2 < −1/tau
p[1] ~ dbeta(2.20, 27.15)
p[2] ~ dbeta(1.42, 29.22)
p[3] ~ dbeta(1.68, 7.95)
p[4] ~ dbeta(1.40, 21.06)
p[5] ~ dbeta(1.23, 26.9)
#Conditional mean priors specification
b[1] < −xinv[1,1]*logit(p[1]) + xinv[1,2]*logit(p[2]) + xinv[1,3]*logit(p[3]) + [1,4]*logit(p[4]) + xinv[1,5]*logit(p[5])
b[2] < −xinv[2,1]*logit(p[1]) + xinv[2,2]*logit(p[2]) + xinv[2,3]*logit(p[3]) + xinv[2,4]*logit(p[4]) + xinv[2,5]*logit(p[5])
b[3] < −xinv[3,1]*logit(p[1]) + xinv[3,2]*logit(p[2]) + xinv[3,3]*logit(p[3]) + xinv[3,4]*logit(p[4]) + xinv[3,5]*logit(p[5])
b[4] < −xinv[4,1]*logit(p[1]) + xinv[4,2]*logit(p[2]) + xinv[4,3]*logit(p[3]) + xinv[4,4]*logit(p[4]) + xinv[4,5]*logit(p[5])
b[5]xinv[5,1]*logit(p[1]) + xinv[5,2]*logit(p[2]) + xinv[5,3]*logit(p[3]) + xinv[5,4]*logit(p[4]) + xinv[5,5]*logit(p[5])

```
for(j in 1:5){
P[j] < −step(b[j])
#computation of odds
Odd[j] < −exp(x[1,j]*b[1] + x[2,j]*b[2] + x[3,j]*b[3] + x[4,j]*b[4] + x[5,j]*b[5])
}
}
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Appendix B. Questionnaire

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