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Bayesian estimation of the true prevalence of *Mycobacterium avium* subsp. *paratuberculosis* infection in Cypriot dairy sheep and goat flocks

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ABSTRACT

We estimated the prevalence of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infected sheep and goats, older than 2 years, reared in the region effectively controlled by the Government of the Republic of Cyprus, in September 2005. The sera collected from 8011 animals (3429 sheep and 4582 goats) from 83 flocks, non-vaccinated against MAP, were examined for antibodies with a commercially available enzyme linked immunosorbent assay (Pourquier® paratuberculosis antibody screening ELISA). The true prevalence of MAP infection was calculated, in a Bayesian framework, separately in sheep and goats, with models that adjusted for the misclassification of animals because of the imperfect accuracy of the ELISA. The within-flock mean seroprevalence in sheep and goats was 9.9% (95% CI: 8.9; 10.9%) and 7.9% (7.2; 8.7%), respectively. There was at least one seropositive sheep in 52% (38; 66%) of flocks with sheep and one seropositive goat in 50% (39; 62%) of flocks with goats. Within the seropositive flocks the mean seroprevalence was 12.1% (10.9; 13.4%) and 10.3% (9.3; 11.4%) in sheep and goats, respectively. The calculated mean true prevalence of infected sheep and goats was 15.0 and 11.1%, respectively. There was at least one infected sheep in 60.8% (95% credible interval: 42.3; 78.8%) and at least an infected goat in 48.6% (30.4; 68.5%) of the flocks. In the infected flocks, the mean within flock true prevalence of infection in sheep and goats was estimated at 24.6% (16.3; 33.3%) and 23.1% (15.5; 33.6%), respectively.

1. Introduction

Paratuberculosis is a chronic infection, mainly of ruminants, which is caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). The infection is responsible for major economic losses in the primary industry and adversely affects international and national animal trade; therefore, there is increasing interest in control programs (Kennedy and Benedictus, 2001; Nielsen, 2009). In Cyprus, paratuberculosis was first diagnosed in sheep in 1965 (Crowther et al., 1976). From 1979 onwards, an average of 19 outbreaks of paratuberculosis per year, usually with severe clinical symptoms, is officially reported in sheep or goat flocks [Diagnostic Veterinary Agency (DVA) 1979–2008].

Valid estimation of the true prevalence of MAP infection in sheep and goats, both at the flock- and at the animal-level, is the starting point for decision makers to determine whether the infection should be considered important or not, and which measures to apply. Such measures

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could pertain to eradication in case of low prevalence, control in case of high prevalence, and surveillance in case of the likely absence of the infection. The latency and slow progression of MAP infection makes diagnosis a challenge and tests with high sensitivity (Se) are lacking, therefore, seriously impeding successful control efforts for paratuberculosis. Due to the serious misclassification rate, particularly in sub-clinically infected animals, lack of knowledge of or disregard for test errors could lead to bias in prevalence estimation surveys. Thus, it is imperative that studies aiming to estimate the true herd prevalence and within herd prevalence of MAP infection should adjust for the imperfect Se and Sp of the diagnostic procedure. Importantly, Se and Sp estimates to be incorporated in the estimation procedure should originate from studies relevant to the study population because the Se of the available tests depends on the distribution of the infection stages in the test population (Collins and Sockett, 1993), whilst Sps are likely to differ between areas with different environmental mycobacteria (Nielsen et al., 2002).

Recently, Nielsen and Toft (2009) conducted a critical comparative review of the reported within- and between-herd prevalence estimates of MAP infection among farmed animals in Europe. They found no interpretable animal-level and only very scarce flock-level estimates for sheep and goats due to the variability in the (a) study design, (b) target population, (c) target disorder detected by the diagnostic procedure and (d) the variability or absence of Se and Sp estimates. In this study we aimed to estimate the true prevalence of MAP infection in Cypriot sheep and goat flocks. The true prevalence was estimated, in a Bayesian framework, separately in sheep and goats with models that adjusted for the misclassification of animals because of the imperfect accuracy of the ELISA.

2. Materials and methods

2.1. Management of sheep and goats in Cyprus

The majority of Cypriot sheep and goats are cross-breeds. Sheep belong mostly to crosses between the Chios and the local breed, whereas goats are crosses between the Damascus and the local breed. The animals are kept, under intensive or semi-intensive management, for milk production, which is the primary production goal. There is no seasonal movement between pastures. Semi-intensively managed flocks graze for some hours in private pastures but their diet is mainly based on in-house feeding and watering. Approximately half of the flocks are located in 57 farming areas defined with wire strand fences. Sheep-only and goat-only flocks comprise 15 and 46%, respectively, and the remaining (39%) are mixed flocks with, on average, half sheep and half goats. Purchase of females between flocks is limited and very few animals are imported into the island. The farmers usually select replacements among the daughters of high-yielding ewes and goats, whilst males bought into the flocks usually originate from high yielding animals from other flocks. In 2008, 36629 animals were recorded as moving between flocks and, in the past 4 years, only 500 animals have been imported. These were from UK, Germany and Bulgaria. The overall number of sheep and goats are fairly constant, with a turnover rate of 20–30%.

2.2. Target population and sampling

In total, there were 339,371 sheep and goats reared in 3878 flocks. Their age-distributions are given in Fig. 1. The target population included all sheep and goats over 24 months old (according to the records of the DVA, these animals comprised an estimated 55 and 65% of the population of sheep or goats, respectively), with no history of vaccination against MAP. The region effectively controlled by the Government of the Republic of Cyprus covers an area of 5828 km^2 and is administratively divided into 5 Districts namely, Lefkosia, Lemesos, Larnaka, Paphos and Ammochostos. The sampling unit was the flock and the design was a combination of stratified and cluster sampling. The District and herd size (categorized in 3 groups, flocks with ≤100 animals, with 101–300 animals and with >300 animals) were the strata variants for stratification (15 strata). Sampled flocks were randomly selected proportionally to the District-specific herd-size distribution. Within these flocks, all sheep and goats older than 2 years were blood sampled, to increase the Se of the diagnostic procedure (Kostoulas et al., 2006a). The minimum number of flocks in order to estimate a prevalence of 65% (estimate based on DVA data) of infected flocks with a 95% level of confidence and 10% absolute precision was 83. Blood sampling was done from September 2005 until September 2006. Collected samples were centrifuged (1000 × g for 10 min) and the separated sera were stored at −20 °C until examination.

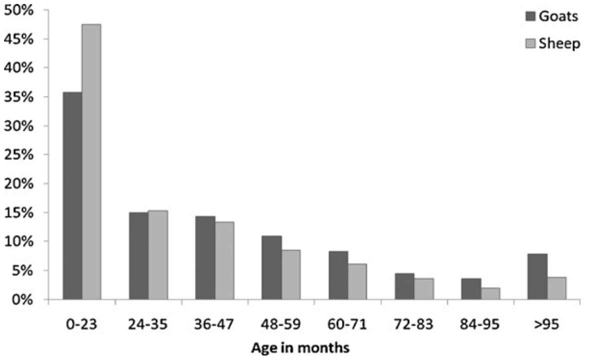

Fig. 1. Age distribution of sheep and goats in the region effectively controlled by the Government of the Republic of Cyprus in September 2005.

2.3. Serological testing

Sera were examined with a commercial indirect absorbed ELISA kit (Pourquier® Elisa paratuberculosis antibody screening) according to the manufacturer's instructions. Samples were considered positive at a sample to positive value ≥70% and negative otherwise.

2.4. Bayesian estimation of true prevalence of infection

To estimate the true prevalence of MAP infection separately in sheep and goats we applied models that adjusted for the misclassification of animals because of the imperfect accuracy of the ELISA. We chose a Bayesian approach that allowed for the incorporation of prior information in terms of probability space rather than single values (Branscum et al., 2005).

2.4.1. Definition of MAP infection

Bayesian models for prevalence estimation create their own probabilistic definition of infection, which, however, implicitly assumes a biological definition based on the fact that true prevalence estimates are obtained after adjusting for the imperfect Se and Sp of the diagnostic procedure (Enøe, 2003). For defining MAP infection in biological terms, we used the approach described by Nielsen et al. (2002) and Kostoulas et al. (2006a). Hence, by 'infection' we mean that sheep or 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 an immune response at any time during their life; we assumed that once an animal has an established infection, the infection persists for life.

2.4.2. The model

We apply a model that has been extensively described by Branscum et al. (2005). Briefly, let $y_1, y_2, \ldots, y_k$, be the numbers of test-positive animals out of n sampled animals in each of k flocks. The data y_k are assumed to be independent and follow binomial distributions:

$$y_k \sim \text{Binomial}(n, \pi_k\text{Se} + (1-\pi_k)(1-\text{Sp}))$$