Preventive Veterinary Medicine 117 (2014) 233–241

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed

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

E. Angelidou*, P. Kostoulas, L. Leontides

Laboratory of Epidemiology, Biostatistics and Animal Health Economics, Faculty of Veterinary Medicine, University of Thessaly, Trikalon 224, GR-43100 Karditsa, Greece

ARTICLE INFO

Article history:
Received 12 June 2014
Received in revised form 3 September 2014
Accepted 3 September 2014

Keywords:
Paratuberculosis
Goat flock
Milk ELISA
Risk factor

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.

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.

* Corresponding author. Tel.: +30 2441066005; fax: +30 2441066041.
E-mail addresses: eaggel@vet.uth.gr, elisavetnikol@hotmail.com (E. Angelidou).

http://dx.doi.org/10.1016/j.prevetmed.2014.09.002
0167-5877/

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