Livestock Science 177 (2015) 151–158

Contents lists available at ScienceDirect

Livestock Science

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

Effect of *Mycobacterium avium* subsp. *paratuberculosis* infection status on culling and calving difficulty in dairy cattle

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ARTICLE INFO

Article history:
Received 22 December 2014
Received in revised form
15 April 2015
Accepted 19 April 2015

Keywords:
Paratuberculosis
Culling
Calving difficulty
Dairy cattle

ABSTRACT

The present paper was designed to assess the effect of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection status (antibody positive *vs* antibody negative, as measured by ELISA) on time to culling and calving difficulty in dairy cows. The study was carried out in 8 dairy farms in Galicia (north-west Spain). All of them were taking part in an ongoing paratuberculosis control program, as well as in a dairy herd improvement program. In order to estimate the relation between time to culling and MAP serological *status* of dairy cows, the present study followed the Andersen–Gill model for survival analysis. Similarly, in order to evaluate the influence on calving difficulty an ordinal logistic regression model was applied. The results indicated that seropositive cows were more likely to be culled due to death/urgent slaughter (hazard ratio=1.88), low productivity (hazard ratio=2.55), infertility (hazard ratio=4.64) and other causes (hazard ratio=1.67). Additionally, the probability of difficulties at calving time was 2.74 times higher for seropositive cows. The estimated effects could determine the economic benefits of a paratuberculosis control program.

1. Introduction

Mycobacterium avium subspecies *paratuberculosis* (MAP) is the causative agent of paratuberculosis (Johne's disease), a chronic granulomatous enteric disease that affects domestic and wild ruminants. Johne's disease (JD) has become a widespread infectious disease problem for cattle herds in developed countries (Maning and Collins, 2001).

JD has a long incubation period and a slow course. The clinical signs typically appear only after two to five years. The main signs include weight loss and chronic watery diarrhea. Animals may be culled due to the onset of these clinical signs or, more often, in the pre-clinical stage for other reasons indirectly related to JD. These reasons include a decrease in milk production (Kudahl et al., 2004; Beaudeau et al., 2007; Aly et al., 2010) and a greater susceptibility to other diseases, especially mammary infections (Tiwari et al., 2005; Villarino and Jordan, 2005). JD has also been related to a reduction in fertility rates (Tiwari et al., 2005; Diéguez et al., 2008);

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http://dx.doi.org/10.1016/j.livsci.2015.04.015
1871-1413/

antibody positive animals had significantly higher number of days open (Elzo et al., 2009). Additionally, Raizman et al. (2007a) indicated that JD was also associated with pneumonia.

Premature culling might lead to the disposal of the animals before reaching their production potential, subsequently increasing the replacement costs. Without any management changes aiming at reducing the farm-level prevalence of MAP infection, it will continue to reduce farm income by increasing premature removal from the herd (Lombard, 2011). Some previous papers have measured the overall effect of JD on culling (Smith et al., 2010; Raizman et al., 2007b), but they did not estimate the concrete impact on each of the different causes for loss.

Due to the chronic and debilitating nature of the disease, other consequences not typically attributed to JD could affect animal performance. In that respect, no previous papers have addressed such topics as the possible influence of JD on calving difficulty.

Biosecurity measures to reduce opportunities for transmission are essential to control the disease. Due to the recommended practices being often laborious and the difficulties to understand the negative implications of JD on the part of the farmers, they often fail to comply with the recommended practices and end up giving up the control measures.

For that reason, quantification of the financial effect of JD on cow performance is essential to encourage dairy cattle producers to take part in control programs. In addition, reliable information in this regard is crucial to produce models for cost–benefit analysis of different control strategies.

The aim of this study was to analyze the effect of MAP infection status (antibody positive vs antibody negative, as measured by ELISA) on time to culling as well as on calving difficulty. The models obtained can provide support to JD economic impact analysis.

2. Material and methods

2.1. Studied area

The study was carried out in Galicia. Galicia is the major cattle-farming region of Spain. It is responsible for 35% of the milk produced in Spain, constituting approximately 1.7% of the milk produced in the European Union (MAGRAMA, 2012). In Galicia, 35% of the herds are enrolled in the Dairy Herd Improvement Program (DHIP), which represents 82% of the milk produced in this region (AFRICOR, 2013). Galicia was the first region in Spain to establish a voluntary JD control program that started in 2004. The percentage of Galician herds involved increased from 4.6% in 2004 to 45% in 2013. From the laboratory point of view, the control program consists of running ELISA antibody tests on animals over 12 months of age at 1 year intervals in order to determine the serological profile of the herds and to identify cows most likely shedding the organism. Fecal samples of all ELISA-positives were cultured for the presence of MAP. Culture does not produce false-positive results (100% specificity). However, occasional passive shedding can occur when an animal has a positive fecal test as a consequence of the ingestion of MAP but such animal is not truly infected ("pass-through" phenomenon). The sensitivity is 70% for affected cattle (OIE, 2008).

2.2. Herds surveyed

The data used in the study were obtained from 8 dairy farms (all of which are Holstein breed) included both in the JD control program and in the DHIP. The mean herd size (cows ≥ 1 year) in the studied farms was 274.2 (maximum of 377 and minimum of 197). In these herds, blood samples were six-monthly collected since 2004 and analyzed by antibody ELISA. Fecal samples of ELISA positive animals were cultured as indicated. Blood was collected by tail vein venopuncture into anticoagulant-free Vacutainer tubes. After collecting the samples they were refrigerated at 4 °C and submitted to the "Animal Health and Production Laboratory of Galicia" on the same day. In the laboratory, the serum was separated by centrifugation (5000 g, 5 min) and aspiration. They were stored at −70 °C until analysis.

The commercial ELISA used was "Paratuberculosis Antibody Screening" (Institute Pourquier, France). The number of false-positive results was minimized by pre-absorbing the samples with sonicates of the environmental mycobacterium *Mycobacterium phlei*. This assay transforms ELISA reader optical density values into sample-to-positive *(S/P)* percentage. Samples were considered positive at a *S/P* percentage of 55% or higher. According to the manufacturer's validation report, the sensitivity of the test was 40.8% and its specificity 99.8%. Fig. 1 shows the frequency distribution of within-herd seroprevalences in the herds which were the object of study, obtained using this ELISA, for each of the 20 samplings performed from 2004 to 2013.

Culling and calving records from the 8 farms were provided by the monthly visits by the DHIP, during which the supervising technician inquired about the reason for animal losses and the calving ease of cows since the previous visit. The reasons for losses were then coded according to the Royal Decree 368/2005 (BOE, 2005), which regulates the program according to some specific rules:

1. Death/urgent slaughter: animals are discarded when they are found prostrate or dead on the farm/animals sent to emergency slaughter (in cases such as metabolic disorders, accident, toxemia, peritonitis, pericarditis, and systemic infection).
2. Lack of productivity: animals are discarded because of low production.
3. Mastitis: animals are discarded because of udder problems (such as mastitis, loss of quarters of the udder, and sagging udder).
4. Infertility: animals are discarded because of reproductive problems (such as abortions, metritis, infertility, sterility, and mummified fetuses).
5. Loss in official disease eradication programs (zoonoses).
6. Others: animals are discarded either for some reasons which are not included in the classification above or for multiple causes.
7. Lameness: animals are discarded because of musculoskeletal problems (such as lameness, hoof infection).