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RESEARCH ARTICLE

Effect of *Mycobacterium avium* subsp. *paratuberculosis* serostatus on carcass weight and conformation and fat cover scores

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Abstract

The paper was designed to assess the influence of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection status of cows at culling (antibody positive *vs* negative) on carcass traits: weight and conformation and fat cover scores. A generalized least squares linear model was used to assess the influence of MAP on weight. Subsequently, a random effects logistic regression was completed in order to model the relation between MAP and conformation score (fair *vs* poor carcasses). A random effects ordered logistic model was used to estimate the influence on fat cover. The results indicated that seropositives had carcass weights 58.45 (34.65-83.35) kg lower than seronegatives. Regarding conformation score, the odds of having poor conformation instead of fair was 3.85 (1.35-11.85) times higher in seropositives. The odds of seropositives achieving a higher fat cover was approximately 5 (1.41-9.09) times lower than the odds for seronegatives. The estimated effects could be useful when assessing the economic benefits of a paratuberculosis control program.

Additional key words: Johne´s disease, carcass traits, serology, ELISA, economic losses.

Abbreviations used: DIM (days in milk); MAP (*Mycobacterium avium* subsp. *paratuberculosis*); JD (Johne's disease), Se (sensibilidad).

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Introduction

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

JD causes serious economic losses in dairy farming, mainly as a result of reduced milk production (Kudalh *et al.,* 2004; Beaudeau *et al.,* 2007; Aly *et al.,* 2010), increased susceptibility to other diseases, especially mammary infections (Tiwari *et al.,* 2005; Villarino & Jordan, 2005), loss of body weight (Johnson-Ifearulundu *et al.,* 2001), and consequential premature culling and increased replacement costs. Paratuberculosis has also been related to reduced fertility rates (Tiwari *et al.,* 2005; Dieguez *et al.,* 2008; Elzo *et al.,* 2009). Raizman *et al.* (2007) indicated that MAP was also associated with pneumonia.

The disease has a long incubation period and a slow course; the clinical signs typically appear only after two to five years. The main signs include progressive weight loss and chronic watery diarrhea in the most advanced stages; MAP bacteria slowly damage the intestines of infected animals and therefore signs get worse with time because the digestive tissue becomes increasingly injured. Enteritis causes malabsorption of protein and essential nutrients across the intestinal epithelium and in the later stages results in net protein loss.

As a consequence, the infection with MAP has been related to important losses of slaughter value (Benedictus *et al.,* 1987; Chi *et al.,* 2002). More recently, Kudahl & Nielsen (2009) demonstrated weight losses of up to 10% and slaughter value losses up to 17% in seropositive cows. In a study performed in Spain, Vazquez *et al.* (2012) estimated that mean weight losses ranged from 3.7% for cases identified by the presence of microscopic specific JD inflammatory lesions to 12.4% for cases with positive results in the antibody ELISA test. Isolation of high bacterial loads in tissues and occurrence of diffuse granulomatous enteritis were associated with the highest weight loses, 22.2% and 26.0% respectively.

A study on immunization against MAP of adult dairy cattle suggested that vaccination have a positive effect on carcass weights of the animals with severe histopathological lesions (Alonso-Hearn *et al.,* 2012).

In economic terms, the reduction in mean weight of culled cows attributable to paratuberculosis represented a loss of approximately 1,150 US$ paratuberculosis (Johnson-Ifearulundu *et al.,* 1999).

Another previous study showed that there was no association between body condition score and MAP infection status, with the majority of the culture-positive cows being in good condition; although in this case no carcass data were assessed (McKenna *et al.,* 2004).

However, there are fewer studies that consider the main carcass quality traits altogether in a multivariate approach: weight and conformation and fat cover scores. Providing accurate estimates on the economic impact of MAP, along with information regarding the incidence and epidemiology of the infection, an economic analysis could be conducted to measure the financial losses in dairy industry. This would provide a solid base to assess the feasibility of control programs.

The aim of the present paper was to assess the influence of MAP infection status at culling (as measured by antibody ELISA) on carcass traits: weight and conformation and fat cover scores.

Material and methods

Area description

The study was carried out in the region of Galicia (North-West of Spain). Galicia is the major cattle area of Spain, responsible for 40% and 1.9% of the milk produced in Spain and the European Union, respectively. Galicia was the first region of Spain to establish a voluntary paratuberculosis control program in 2004. In Galicia, vaccination against MAP is not permitted since the region is not tuberculosis-free.

Concerning the laboratory, the program was based, among other aspects, on testing every adult animal at 12-month intervals with antibody ELISAs in order to determine the serological profile of herds and to identify the cows which were most likely to shed the organism.

Study design and data collection

The same serological testing strategy was used for the present study. It was conducted on 64 Holstein animals, from 30 dairy herds (mean herd size=67.5 cows > 1 year; standard deviation=56.4). These herds had been taking part in the paratuberculosis control program since 2005 and such herds had at least one seropositive animal by that time. Besides, in all those herds the presence of MAP had been confirmed by means of fecal culture.

The 64 cows included in the study were culled for different reasons from 2010 to 2013 but without showing specific signs consistent with paratuberculosis (emaciation, diarrhea or edema). This was determined by clinical examination performed by the veterinary responsible for the sanitary program of the farms, prior to transport to the slaughterhouse. The mean age for these animals at culling was 7.5 years (minimum 3 years, maximum 14.5 years).

Thirty-two out of 64 cows were ELISA positive at culling. Besides, as a previous condition before being included in the study, those animals must have had at least two positive ELISA results before culling and such results must be consistent (always positive after the first positive test).

The other 32 were animals with negative results in all the ELISA tests performed annually, matched by age and herd with the seropositives and again, must have been tested at least twice.

In spite of the fact that over the course of the study a total of 246 animals from the surveyed herds tested ELISA positive, only 32 of them were considered object of study on the grounds that only those animals met all the required criteria (involving clinical examination and laboratory tests). Other factors such as ready availability of information from the slaughter house and the possibility of counting on data from their respective seronegative control animals were also taken into account.

The following information was collected from those animals in the slaughterhouse: cold carcass weight, conformation score (graded according to the SEUROP classification; S=superior, E=excellent, U= very good, R=good, O=fair and P=poor) and fat cover score (measured also by the EU classification, with a 5-point scale; 1=low, 2=slight, 3=average, 4=high, 5=very high). The conformation class is determined by a visual appraisal of shape, taking into account carcass blockiness and development of muscle in the hind quarters. The fat cover score is also determined by a visual appraisal of external fat development (EC, 2008). All the studied animals were scored O or P (conformation score) and 1 to 4 (fat cover score).

Additionally, the birth and culling dates for each animal and the days in milk (DIM) at culling were also recorded. For analysis, DIM at culling were divided into five categories: (1) fresh, from 0 to 21 DIM, (2) early lactation, from 22 to 80 DIM, (3) mid lactation, from 81 to 200 DIM, (4) late lactation, >200 DIM, and (5) dry cow. Among the studied animals there were no cows culled with less than 22 DIM (fresh).

Antibody ELISA

A commercial antibody ELISA (Paratuberculosis Antibody Screening, Institute Pourquier, France) was used in this study. False-positive results were reduced by pre-absorbing the samples with sonicates of the environmental mycobacterium *Mycobacterium phlei*. Samples were considered positive at a% sample positive ratio of 55% or more.

Statistical analysis

Statistical analyses were performed with Stata 10.1 (StataCorp LP, Collegue Station, TX, USA). Initially, in a univariate approach, a t-test was performed to evaluate the effect of seropositivity on carcass weight. Conformation and fat cover scores were tested by comparing proportions in animals with different serological status using chi-squared test (conformation) and Fisher's exact test (fat cover, since the expected frequencies in some categories were less than 5).

Afterwards, to evaluate the influence of MAP serological status on carcass weight in a multivariate approach, a generalized least squares linear regression model was used (xtreg procedure in Stata). The explanatory variables "ELISA result" at culling (positive *vs* negative), age at culling (in months) and DIM at culling (categorized as mentioned) were included in the model. The interaction terms between seropositive and lactating (*vs* dry) at culling and between seropositive and parity ≥ 3 (*vs* < 3) were evaluated; these are two binary variables indicating that and animal is lactating and seropositive at culling or in parity ≥ 3 and seropositive at culling. Also, herd was included as a random effect variable to account for clustering at herd level.

Regarding conformation, since only O and P carcasses were found, a multivariate analysis using logistic regression was completed, in order to assess the relation between conformation score and the independent variables and interaction terms already mentioned in the previous model. The xtlogit command was used and herd cluster effects were managed as previously explained.

Finally, a random effects ordered logistic model (xtologit command), was used to estimate the influence of MAP serological status on fat cover score. Once more, the same independent variables and first order interactions were considered and herd was included as a random effect variable. The following odds were modeled:

Fat score 1, 2, 3 *vs* 4
Fat score 1 and 2 *vs* 3 and 4
Fat score 1 *vs* 2, 3, and 4

Results

Descriptive analysis of data showed that the mean observed carcass weight was 296.41 kg for seronegative animals and 231.28 for seropositive ones; the difference was statistically significant according to the t-test ($p<0.001$) (Table 1). Besides, 17 out 32 seronegative animals (53.1%) were scored O, while the remaining 15 (46.9%) were scored P; otherwise, 9 out of 32 seropositive animals (28.1%) were scored O while the remaining 23 (71.9%) were scored P. Regarding the fat cover score, among the seronegative animals, 13 out of 32 animals (40.6%) were scored 1, 4 animals (12.5%) were scored 2, 5 cows (15.6%) were scored 3 and 10 (31.3%) were scored 4. Among the seropositives, 22 out of 32 (68.8%) were scored 1, 7 (21.9%) were scored 2, 2 (6.3%) were scored 3 and 1 (3.1%) were scored 4. The chi-squared test and Fisher`s test also indicated significant differences in the distribution of both conformation and fat cover scores according to the animal´s serological status ($p=0.039$ and $p=0.014$, respectively) (Table 1).

Subsequently, the multivariate linear model showed that seropositive animals had, on average, carcass weights 58.45 kg lower than the seronegative ones ($p<0.001$; Table 2). Cows culled at any stage of lactation also had lower weights than those slaughtered during the dry period ($p=0.006$, $p=0.003$ and $p=0.007$ when comparing cows culled in early, mild and late lactation to those culled during the dry period; Table 2). The interaction term between seropositive and lactating at culling was significant when added ($p<0.001$; Table 2). Age at culling did not seem to have an effect on carcass weight and was removed from the model.

As regards conformation score, the logistic model indicated that the odds of having P instead of O conformation was 3.85 times higher in seropositive cows ($p<0.003$; Table 3). The odds was also higher

Table 1. Descriptive statistics for carcass traits by MAP serological status.

Traits		Antibody negative animals	Antibody positive animals
Mean carcass weight,kg[a]		296.41	231.28
Conformation score,n/total (%)[b]	O	17 (53.1%)	9 (28.1%)
	P	15 (46.9%)	23 (71.9%)
Fat cover score, n/total (%)[c]	1	13 (40.6%)	22 (68.8%)
	2	4 (12.5%)	7 (21.9%)
	3	5 (15.6%)	2 (6.3%)
	4	10 (31.3%)	1 (3.1%)

[a]$p<0.001$, [b]$p=0.039$, [c]$p<0.014$

Table 2. Results of a linear model for the effect of MAP serological status on cold carcass weight of Holstein cows.

		Coefficient β (CI[c] for β)	Significance
MAP status[a]	Seropositive	-58.45 (-83.35 / -34.65)	<0.001
Lactation stage[b]	Early	-50.22 (-86.28 / -14.23)	0.006
	Mid	-60.61 (-100.19 / -21.01)	0.003
	Late	-41.96 (-72.14 / -11.75)	0.007
Seropositive×Lactating at culling		-77.11 (-113.33 / -40.91)	<0.001

[a]MAP status: seronegative is the base. [b]Lactation stage: dry is the base. [c]CI: 95% confidence interval.

Table 3. Results of a logistic model for the effect of MAP serological status on conformation score (defined as binary trait: O or P) of Holstein cows. All the animals culled in early lactation (both seropositives and seronegatives) were scored P.

		OR (CI[c] for OR)	Significance
MAP status[a]	Seropositive	3.85 (1.35 / 11.85)	0.003
Lactation stage[b]	Early	—	—
	Mid	19.12 (1.81 / 197.74)	0.013
	Late	4.71 (1.14 / 19.38)	0.033
Seropositve×Lactating at culling		21.51 (2.92 / 40.10)	0.007

[a]MAP status: seronegative is the base. [b]Lactation stage: dry is the base. [c]CI: 95% confidence interval.

Table 4. Results of an ordinal model for the effect of MAP serological status on fatness score of Holstein cows. The following odds were modeled: (1) fat score 1,2,3 *vs* 4; (2) fat score 1 and 2 *vs* 3 and 4; and (3) fat score 1 *vs* 2,3, and 4.

		OR (CI[c] for OR)	Significance
MAP status[a]	Seropositive	0.21 (0.21 / 0.71)	0.013
Lactation stage[b]	Early	0.12 (0.01 / 0.88)	0.032
	Mid	0.11 (0.01 / 1.02)	0.057
	Late	0.23 (0.14 / 0.86)	0.022

[a]MAP status: seronegative is the base. [b]Lactation stage: dry is the base. [c]CI: 95% confidence interval.

when comparing cows in mid or late lactation with those culled during the dry period (p=0.013 and p=0.033; Table 3); all the animals culled in early lactation (both seropositives and seronegatives) were scored P. The interaction term between seropositive and lactating at culling was again significant (p=0.007; Table 3). As in the previous case, no association between conformation score and age at culling could be demonstrated.

Finally, ordinal regression indicated that the fat cover score was lower in seropositive animals (Table 4) or in those culled during the lactation period. This score was not related to the age at culling. Thereby, the odds of seropositive animals achieving a higher score was approximately five times lower than the odds for seronegatives (Odds ratio=0.21, p=0.013, Table 4). The lowest scores were also observed most frequently in early (p=0.032) and mid lactation (although not significant) (p=0.057), followed by late lactation (p=0.022) when compared to the reference category (dry cows) (Table 4).

Discussion

Each animal affected by JD falls into one of the four stages of disease (Whitlock, 1992). Stage one is silent infection. Stage two is the pre-clinical stage, which consist of unspecific clinical signs (such as increased susceptibility to other diseases, reduced milk production or mild weigh loss), which may develop into stages 3 and 4, characterized by severe weight loss, intermittent diarrhea that can become permanent and possibly edema as a consequence of hypoproteinemia. The present paper aims to assess the effect of the infection on carcass traits before the appearance of specific signs (emaciation, diarrhea or edema).

The results observed in the present paper imply a reduction of slaughter value as a result of lower carcass weight and worse conformation and fat cover scores, which indicates that the cows suffered some weight loss, although not severe according to clinical observation; therefore none of the studied animals was eliminated for this reason. The decrease in weight and the worsening conformation and fat cover were different expressions of poor condition due to MAP infection, but considering that the carcasses' value is dependent upon the three aforementioned traits, evaluating them independently would provide a more accurate estimation of the potential economic losses.

A possible limitation of the study is the use of antibody ELISA as the indicator of MAP infection. ELISA sensitivity (*Se*) is a direct function of the distribution of the infection stages in the test population (Collins & Sockett, 1993). False negative results are more likely to occur in the initial stage of the infection; as the infection progresses the *Se* of the ELISA increases (Sweeney *et al.,* 2006).

Nielsen & Toft (2008) indicated that the specificity of several commercial ELISAs ranged between 91% and 98%. The proportion of false positive results is expected to be low considering that the samples were pre-absorbed with Mycobacterium phlei. In any case, in all the studied farms, the presence of bacteria had been confirmed by means of fecal culture at least in one cow. Moreover only animals with at least two positive tests were included in the present study.

The sale of animals for slaughter represents, in the studied population, 7-8% of revenue. Owing to the falling prices of milk in recent times, Spanish farmers have been gradually increasing the sale of their least productive cows for slaughter, as a way to cope with the lack of financial liquidity. According to the reference prices in Spain, the value of a carcass with the average weight, conformation and fat score of a seronegative cow, as determined in the present paper, was €490 €350 in the case of an average seropositive cow carcass, which means 28.6% less (Lonja Binefar, 2015).

Although most of the loss (77%) attributable to paratuberculosis was categorized as loss of future income as a result of suboptimal culling, 12% of the loss resulted from a lower slaughter value of infected culled cattle and treatment costs (that mainly consist of veterinary visits for diagnosis and extra farm labor cost due to disease); meanwhile, lower milk production accounted for 11% of the total loss attributable to *paratuberculosis* (Groenendaal & Galligan, 2003). This underlines the importance of including carcass traits in the estimation of the economic losses incurred to the farmer.

Previous studies have differed in terms of the reduced value of the animal depending on the parameters considered (mainly carcass weight, and less often conformation score), the test used to detect MAP and the area of the study. Benedictus *et al.* (1987) found that slaughter value of infected cows was 30% lower than usual slaughter value, but they included animals that were culled because they had specific clinical signs of paratuberculosis. More recently, Chi *et al.* (2002), assumed a 25% decrease in slaughter value for a cow affected with JD (also, by antibody ELISA) whereas Kudahl & Nielsen (2009) indicated that, compared with presumably unaffected cows, slaughter value was reduced by 17% in cows with positive ELISA at slaughter. Thereby, the reduction of slaughter value observed in the present paper was higher than that observed in other studies also based on antibody test.

Weight at slaughter was not only affected by MAP infection but also by lactation stage. After controlling by lactation stage, the present paper found an average reduction of more than 58 kg in seropositive cows, which means a 19.6% reduction compared to seronegative ones. Previously, Johnson-Ifearulundu *et al.* (1999) found that a 10% increase in the proportion of seropositive cows was associated with a 33.4 kg decrease in the mean weight of culled cows at herd level. At individual level, Kudahl & Nielsen (2009) indicated that the mean slaughter weight was reduced by 10%, in cows with positive ELISA at slaughter which is less than what has been observed in the present study. Another previous study, also carried out in Spain, estimated mean weight losses from 3.7% for cases identified by the presence of microscopic specific JD inflammatory lesions to 12.4% for cases with positive results in the paratuberculosis antibody ELISA test (Vazquez *et al.,* 2012). Unlike most previous studies, McKenna *et al.* (2004), using body condition scores, found no relationship between this trait and isolation of MAP in the intestines or lymph nodes.

A smaller number of studies have assessed the influence of MAP on conformation and fat cover scores. Kudhal & Nielsen (2009) used conformation scores to estimate the influence of MAP on the overall reduction of slaughter value. The present paper aims to measure the probability of having worse conformations or fat scores in MAP positive cows.

Recently, Whist *et al*. (2014) have studied several variables to assess whether a herd should be incorporated in a surveillance program for MAP. They concluded that the identification of slaughtered animals >3yr of age that had been classified as having low conformation and no or little fat could be considered a risk indicator for MAP.

The multivariate models indicated that detectable infection with MAP (as measured by antibody ELISA) resulted in less carcass weight, worse conformation score and thinner fat covers after controlling by lactation stage at culling and considering herd-cluster effects.

The estimated effects provide comprehensive information on losses related to lower slaughter value that could be useful for its consideration when performing cost-benefit analysis of a paratuberculosis control program.

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