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# Effects of paratuberculosis on Friesian cattle carcass weight and age at culling

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## Abstract

Bovine paratuberculosis (PTB) causes major economic losses to dairy farmers because of decreased milk production, poor body condition, weight loss and early culling. The aim of this study was to evaluate the effect of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) infection on carcass weight and age at slaughter in Friesian cattle. A total of 1,014 adult cows slaughtered at two local abattoirs in the Basque Country were included in this study. MAP infection was determined by different methods: indirect ELISA on blood samples, detection of MAP in tissues by culture and real time PCR (rtPCR), and histopathological examination. Serial and parallel interpretations of these methods were also considered for setting the upper and lower infection rates. MAP infection was confirmed by at least one test in 58.9% of animals. Most infected cows were detected by histopathology (46.9%) and rtPCR (29.6%). Overall estimates of carcass mean weight losses ranged from 3.7% for cases identified by the presence of microscopic specific PTB inflammatory lesions to 12.4% for cases with positive results in the paratuberculosis 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. The life expectancy of seropositive cows and those showing diffuse lesions was reduced by nearly one year compared to that of non-infected ones. Our results provide consistent evidences of PTB effect on the reduction of slaughter weight and lifespan of dairy cows, which could be considered as surrogates of clinical disease.

**Additional key words:** age; dairy cattle; Johne's disease, lifespan; meat; *Mycobacterium*; slaughter weight.

## Resumen

**Efectos de la paratuberculosis bovina sobre el peso de la canal y la edad de desvieje en ganado Frison**

La paratuberculosis bovina (PTB) ocasiona grandes pérdidas económicas en las explotaciones de vacuno lechero a causa del descenso de la producción láctea, la pérdida de peso y el sacrificio prematuro de los animales infectados. El objetivo de este estudio fue evaluar el efecto de la infección por *Mycobacterium avium* subspecies *paratuberculosis* (MAP) en el peso neto canal y la edad media al desvieje en el ganado Frisón. En el estudio se incluyeron 1014 vacas adultas sacrificadas en dos mataderos del País Vasco. La infección por MAP fue evaluada mediante varias técnicas: ELISA indirecto a partir de muestras de sangre, aislamiento de MAP de tejidos mediante cultivo y PCR a tiempo real (rtPCR) y examen histopatológico. Las interpretaciones en serie y en paralelo fueron estimadas para determinar las tasas de infección mínimas y máximas. En el 58,9% de los animales se detectó la infección por MAP mediante alguna de estas técnicas. La histopatología (46,9%) y la rtPCR (29,6%) detectaron la mayoría de las vacas infectadas. Las pérdidas de peso neto canal se estimaron en el 3,7% si se observaban lesiones inflamatorias compatibles con PTB y 12,4% si se detectaban anticuerpos anti-MAP. La presencia de altas cargas bacterianas en tejidos y la observación de formas lesionales difusas se asociaron con las mayores estimaciones de pérdida de peso, 22,2% y 26,0%; respectivamente. La vida productiva en vacas seropositivas y en aquellas con enteritis granulomatosa de tipo difuso se redujo aproximadamente un año. Nuestros resultados indican que las formas paratuberculosas más patógenas en el vacuno lechero conllevan un proceso consuntivo de reducida vida productiva, probablemente también asociado con la aparición de signos clínicos.

**Palabras clave adicionales:** carne; edad; enfermedad de Johne, *Mycobacterium*; peso neto canal; vacuno de leche; vida productiva.

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Abbreviations used: BCS (body condition score); CJ-LN (caudal jejunal limph-node); DI (distal ileum); HPC (hexa-decyl pyridinium chloride); I-LN (ileal lymph-node); ICV (ileocecal valve); JD (Johne's disease); LJ (Loewestein-Jensen); MAP (*Mycobacterium avium* subspecies *paratuberculosis*); PTB (paratuberculosis); TNFα (Tumor necrosis factor α).

# Introduction

Paratuberculosis (PTB) or Johne's disease (JD) is a chronic granulomatous enteritis caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP) that affects both domestic and wild ruminants all over the world, and for which currently there is not known an efficient treatment (Chiodini *et al.,* 1984; Fecteau & Whitlock, 2011).

It is generally accepted that paratuberculosis infection occurs within the first 6-12 months of life, mainly by ingestion of milk, water or food contaminated with faeces containing viable MAP bacilli from infectious animals. Intra-uterine transmission and ingestion of directly shed in milk or colostrum MAP cells are considered less frequent routes (Sweeney, 1996). In dairy cattle, clinical disease typically appears between 2 and 5 years of age (first and third calving) and is characterized by intermittent diarrhea, poor body condition and decreased milk production, leading to premature culling and death (Chiodini *et al.,* 1984; Stabel 1998).

On average, the economic losses caused by bovine PTB have been estimated to be higher than those for other bovine diseases such as bovine viral diarrhea, enzootic bovine leucosis and neosporosis (Chi *et al.,* 2002). Worsening of productive performance and early culling (unrealized future income) represent the two major economic impacts of PTB. In fact, the economic consequences of these two items have been estimated to account for nearly 70% of overall direct economic losses occurring in MAP infections (Ott *et al.,* 1999; Groenendaal *et al.,* 2002). Consistent results regarding the relationship between lower milk yields and PTB are easily found in the literature (Buergelt & Duncan, 1978; Ott *et al.,* 1999; Kudahl *et al.,* 2004; Hendrick *et al.,* 2005; Tiwari *et al.,* 2007; Smith *et al.,* 2009; Aly *et al.,* 2010). In addition to this, some researchers have suggested that PTB is associated with increased mortality rates, higher infertility rates (Merkal *et al.,* 1975; Buergelt & Duncan, 1978; Johnson-Ifearulundu *et al.,* 1999), greater susceptibility to other secondary pathologies such as mastitis (Buergelt & Duncan, 1978) and decreased slaughter value.

Malabsorption and protein losing enteropathy occurring in MAP infections (Patterson *et al.,* 1967; Patterson & Berret, 1968) have been considered as the causes of weight loss. However, there are field evidences pointing out that improved nutritional levels can result in substantial weight recovery and that no differences in digestibility can be found in paratuberculous and non-paratuberculous animals (Juste *et al.*, 1991a), implying that other more general wasting mechanisms involving tumor necrosis factor α (TNFα) are in play in paratuberculosis, as well as in other chronic diseases. As a consequence, carcass values have been estimated to be reduced by 5% for cows harboring subclinical infections and 20-30% in cows showing clinical signs of disease (Benedictus *et al.,* 1987; Groenendaal *et al.,* 2002). However, when enteritis or enteric edema were also considered, the value of carcasses appeared to be reduced up to 48% because of the negative effect on both weight and meat quality (Kudahl & Nielsen, 2009). In contrast, a study on body condition scores at slaughter concluded that there were no differences between positive and negative tissue culture individuals (McKenna *et al.,* 2004). This should be interpreted in the sense that infection alone is not a good indicator of damage which instead would depend more on the stage of infection.

Although it is widely assumed that PTB (Kennedy & Benedictus, 2001) leads to a higher likelihood of early culling and thereby, to a decreased productive life expectancy (Wilson *et al.,* 1993; Groenendaal & Galligan, 2003; Raizman *et al.,* 2009), few estimates on the average age at culling are available. Additionally, some authors have concluded that there is no effect of PTB on dairy cows lifespan (Whitlock *et al.*, 1985; Benedictus *et al.,* 1987; Lombard *et al.*, 2005), whereas others have shown that MAP infected dairy cows were replaced at a younger age than non-infected ones (Buergelt & Duncan, 1978; Alonso-Hearn *et al.,* 2012). Likewise, indirect costs of surveillance and control measures to avoid MAP transmission also constitute an important issue to deal with (Kennedy & Benedictus, 2001) which, on the whole, appear to result acceptable for the industry given the balance against their benefits (Groenendaal & Galligan, 2003).

In the present study, MAP infection was assessed in a sample of adult Friesian cows slaughtered for human consumption according to serological, microbiological and histopathological methods or a combination of them, so as to analyze the overall impact of PTB on the carcass weight (as a measure of wasting) and on the age at slaughter (as a measure of lifespan shortening).

# Material and methods

## Animals and sample collection

Blood and tissue samples from 1,014 cows over 24 months of age were collected at two local slaugh-

terhouses in the Basque Country (Bilbao and Donostia-San Sebastián) between March 2007 and May 2010. In each weekly sampling, veterinary inspectors chose the first 4 to 8 cows of Friesian breed older than 24 months and preferably between 3 and 6 years of age, without regard of external aspect or origin. This yielded an average of 5 and 3 animals per week at Bilbao and Donostia-San Sebastián slaughterhouses, respectively. Duplicate whole blood samples were collected from the jugular vein into sterile Vacutainer EDTA tubes (Becton Dickinson and Company, Franklin Lakes, NJ, USA) at the beginning of the slaughter line whereas the intestinal tissue and associated mesenteric lymph nodes from each animal were collected at the gut and tripe room. Tissues were identified and taken to NEIKER-Tecnalia necropsy room where samples were aseptically taken from the caudal jejunal lymph node (CJ-LN), ileal lymph node (I-LN), distal ileum (DI) and ileocecal valve (ICV) within 24 hours and split into two for microbiological processing in fresh, and for histopathological processing after formalin fixation.

## Serological testing

Blood samples were centrifuged 15 min at 2,800 rpm to separate the serum. Two 1-mL plasma aliquots were stored at −20 ± 5 °C until serological testing that was carried out approximately every six months in order to minimize inter-day variability. Serum samples were tested for antibodies against MAP by a commercial ELISA kit (Pourquier® ELISA paratuberculosis antibody screening and Pourquier® ELISA paratuberculosis antibody verification (Institut Pourquier, Montpellier, France), according to the instructions of the manufacturer. Optical density (OD) values were transformed to sample to positive (S/P) ratios as prescribed by the manufacturer. Cows were scored as seropositive when S/P ratios in the verification ELISA yielded doubtful or positive results according to the manufacturer.

## MAP isolation from tissues: culture and real time PCR (rtPCR)

A pool consisting in scraped-off mucosa from distal ileum, ICV area and minced CJ-LN, in the same proportions, up to a total of 2 g was weighted and used for MAP isolation by culture and real time PCR (rtPCR).

Each 2 g pool was decontaminated overnight with 0.75% hexadecyl pyridinium chloride (HPC) and afterwards 4 drops were inoculated in home-made Herrold Egg Yolk (HEYM) and Löwestein-Jensen (LJ) (Difco, Detroit, MI, USA) media, both supplemented with Mycobactin J (Allied Monitor, Fayette, MO, USA) in duplicate, as previously described (Juste *et al.,* 1991b). Inoculated tubes were incubated at 37°C for up to 20 weeks. From the 8$^{th}$ week onwards, tubes were examined for bacterial growth every four weeks. Positive samples were considered if one or more colonies forming units (CFU) consistent with MAP were noticed in any of the four tubes. Animals were classified according to the bacterial load in tissues as follow: low if < 10 CFU/tube, medium if 10-50 CFU/tube and high if > 50 CFU/tube. Quantitative results were not recorded for three animals (lost data). In all positive tubes, presence of MAP specific IS900 insertion sequence was confirmed by colony PCR as previously described (Moss *et al.,* 1992).

Another 2.5 g pool was used to directly detect the presence of MAP specific IS900 DNA. Prior to DNA extraction each pool was homogenized in 10 mL of sterile water for one minute in a Stomacher® 80 Biomaster (Seward, Worthing, UK). The whole homogenate was transferred to 15 mL sterile tubes. Then, an adapted protocol for tissue samples was performed using Adiapure® MAP DNA extraction and purification kit (Adiagene, Saint Brieuc, France). The protocol was carried out as described for fecal samples (Juste *et al.,* 2009) but including an additional chemical lysis stage. Briefly, 300 µL of the supernatant obtained after the cell disruption stage were transferred to Eppendorf tubes containing 20 µL of Lysis 2 buffer, firstly incubated at 70 ± 2 ºC for 10 min and next at 95 ± 2 °C for 15 min. The amplification of specific IS900 MAP DNA by using Adiavet® Map DNA extraction and detection kit (Adiagene, Saint Brieuc, France) and the ABI Prism 7000 Sequence Detection System (Applied Biosystems, Forter City, CA, USA) was carried out as previously described (Juste *et al.,* 2009). Samples were scored as positive when reaching a threshold cycle (Ct) value of 40.00 or lower.

Since bacterial isolation and rtPCR are closely related microbiological variables, but reflecting a critical difference in terms of cell viability, it was hypothesized that the lower rate of positive results in culture in relationship with rtPCR could be associated with the host response type. Therefore, a ratio between both microbiological methods results was defined as indicative of bacterial viability. This variable was calculated as fol-

lows: MAP viability = total number of positive tissue cultures / total number of positive tissue rtPCRs.

*Histopathological examination*

Formalin-fixed samples from CJ-LN, I-LN, DI and ICV were routinely embedded in paraffin, cut into 4 µm sections, and stained with haematoxylin-eosin (HE). These preparations were examined by light microscopy for lesions consistent with PTB. If these lesions were present, the Ziehl-Neelsen (ZN) staining was performed for identifying acid-fast bacilli. Lesions were classified into focal, multifocal, diffuse lymphocytic, diffuse intermediate and diffuse multibacillary lesions according to González *et al.* (2005). Because of advanced autolysis or important loss of the ileal mucosa layer, histopathological examination was not performed in 44 cows.

### Data collection: animal information and slaughter weight records

Data on breed and age were compiled from the EU bovine identification documents (Council Regulation (EC) No 820/97). Net carcass weight records at slaughter were obtained from Bilbao and Donostia-San Sebastián slaughterhouses and kindly transferred by IKT Nekazal Teknologia S.A. (Arkaute, Araba, Spain).

### Statistical analysis

Several general linear models were built with the procedure GLM of the SAS statistical package (SAS Institute, Cary, NC, USA), where diagnostic variables seropositivity, MAP detection, lesion forms and their combinations (in series and in parallel) and interactions were the classification independent variables, and slaughter weight and age were considered the dependent variables. Differences between groups were assessed with the Tukey-Kramer multiple comparisons adjusted Student's t test in the LSMEANS statement of the mentioned procedure. Statistical significance threshold was set at a standard $p < 0.05$ value. In addition to the straight associations between one infection classification variable and one clinical (wasting or lifespan) indicator, an attempt at validating MAP DNA detection results was made by analyzing the interactions of rtPCR results with bacterial isolation and immunopathological forms.

## Results

### Overall PTB prevalence

MAP infection was confirmed by all diagnostic methods. The apparent prevalence of animals carrying MAP-specific antibodies was estimated at 7.4% (75/1014). MAP detection rates ranged from 15.3% (155/1014) for culture to 29.6% (300/1014) for rtPCR. Most tissues in which MAP bacilli were isolated showed low bacterial loads (46.7%; 71/152) whereas positive tissue cultures showing medium and high bacterial loads accounted for 23.7% (36/152) and 29.6% (45/152), respectively. Microscopic lesions consistent with PTB in the intestine or associated lymph-nodes were present in 46.9% (455/970) of the cases. With regard to the immunopathological forms, delimited forms (focal and multifocal) were the most common. Focal lesions were observed in 83.7% (381/455) of animals with paratuberculosis tissue lesions whereas multifocal lesions occurred in 7.3% (33/455). Diffuse forms frequencies were as follows: 0.7% (3/455) diffuse lymphocytic, 1.3% (6/455) diffuse intermediate and 7.0% (32/455) diffuse multibacillary.

According to serial and parallel interpretation of the different methods, PTB apparent prevalence in the sampled population of adult Friesian cattle slaughtered for human consumption ranged between 5.6% (57/1014) and 58.9% (597/1014).

### Slaughter weight and MAP infection

Mean slaughter weight records according to each classificatory variable are summarized in Table 1. Significant reductions in the slaughter weight values were associated with positive results for MAP infection, regardless of the diagnostic method. Overall estimates of carcass weight losses ranged from 11.97 kg (3.7%; $p = 0.0092$) for the occurrence of inflammatory lesions consistent with PTB to 39.78 kg (12.4%; $p < 0.0001$) for the detection of specific antibodies against MAP in serum. Likewise, if serial combination was considered, MAP infected cows showed reduced slaughter weights which amounted to nearly 3.5 times those of parallel interpretation.

**Table 1.** Summary of mean carcass weight (CW) values and ages according to serological, microbiological and histopathological diagnosis of bovine paratuberculosis (PTB) in 1,014 adult dairy cows. CV = coefficient of variation; *p* values are shown in brackets for every comparison

| PTB Status | | Number of animals | Mean CW (kg) | CV (%) | Reduction in CW (%) | Mean age (months) | CV (%) | Reduction in age (%) |
|---|---|---|---|---|---|---|---|---|
| ELISA | NEG | 939 | 321.62 | 21.7 | 12.4 | 67.82 | 40.6 | 13.1 |
| | POS | 75 | 281.84 | 28.9 | (< 0.0001) | 58.94 | 38.0 | (0.0016) |
| Tissue culture | NEG | 859 | 322.49 | 21.3 | 7.8 | 67.85 | 40.1 | 6.6 |
| | POS | 155 | 297.50 | 27.6 | (0.0005) | 63.38 | 43.2 | (0.0607) |
| Tissue rtPCR | NEG | 714 | 323.01 | 21.1 | 4.5 | 67.46 | 40.9 | 1.5 |
| | POS | 300 | 308.36 | 25.4 | (0.0050) | 66.46 | 40.1 | (0.5920) |
| Histology | NEG | 515 | 323.01 | 21.5 | 3.7 | 67.81 | 42.3 | 2.3 |
| | POS | 455 | 311.04 | 23.5 | (0.0092) | 66.27 | 38.4 | (0.3770) |
| Serial combination | NEG | 957 | 322.10 | 21.7 | 18.9 | 67.91 | 40.6 | 19.5 |
| | POS | 57 | 261.23 | 29.2 | (< 0.0001) | 54.66 | 34.2 | (< 0.0001) |
| Parallel combination | NEG | 417 | 329.14 | 20.3 | 5.4 | 67.08 | 42.9 | –0.2 |
| | POS | 597 | 311.36 | 23.7 | (< 0.0001) | 67.23 | 39.1 | (0.9320) |

Overall, weight was associated with bacterial load. It was strongly reduced in cows with high bacterial loads (251.07 kg), compared with animals negative to tissue culture (322.49 kg; $p < 0.0001$) and with those showing low (317.98 kg; $p < 0.0001$) or medium (321.54 kg; $p < 0.0001$) MAP recoveries. Conversely, no differences were found between the slaughter weights of cows with low or medium load and those from in which MAP was not isolated.

Association of PTB lesion forms with slaughter weight values is shown in Table 2. Cows with diffuse forms had a carcass weight 83.98 kg lower than healthy ones (reduction of 26.0%; $p < 0.0001$). In fact, this weight difference was essentially due to the diffuse multibacillary forms, in which difference reached up to 91.20 kg (reduction of 28.2%; $p < 0.0001$). Focal and multifocal forms did not show any significant difference.

## Age at culling and MAP infection

At slaughter, seropositive cows were 9 months younger than seronegative ones ($p = 0.0016$). No differences on age were observed according to MAP isolation from tissues by culture or rtPCR. Likewise, the bacterial load in tissues did not associate with a significant difference in age. When results were combined, cows in which MAP infection was confirmed by all tests were culled on average 1.2 years-old earlier than non-infected ones ($p < 0.0001$) whereas no differences were detected between positive and negative cows according to the parallel interpretation (Table 1).

Regarding histopathological findings, animals with diffuse forms had a reduced lifespan, over one-year, in comparison with those without any tissue change ($p = 0.0019$) and those with focal forms ($p = 0.0016$) (Table 2). A difference in age was also detected, even though less markedly, between cows with diffuse multibacillary form and those without lesions ($p = 0.0403$) or with focal forms ($p = 0.0356$). As seen in Table 2, within the animals showing diffuse lesions, the three cows with the diffuse lymphocytic form evidenced the lowest age and a minor coefficient of variation. In fact, the three cows with diffuse lymphocytic lesions were replaced over one year earlier than those with diffuse intermediate or multibacillary forms (not statistically significant).

## Interactions of rtPCR results with MAP isolation and immunopathological forms: effect on age and carcass weight

When the observations were classified according their rtPCR results, it was noticed that among rtPCR-negative animals, there was no significant difference in age between animals with and without lesions, nor between with or without MAP isolation. However, there was a significant difference in weight (36.9 kg;

**Table 2.** Relationship between bovine paratuberculosis (PTB) lesion forms, bacterial loads of MAP in tissues, mean carcass weight (CW) records and age in adult Friesian cows. High bacterial load was considered if > 50 CFU/tube. CV = coefficient of variation

| Histological examination | Number of animals | High bacterial load in tissues (%) | Mean CW (kg) | CV (%) | Mean age (months) | CV (%) |
|---|---|---|---|---|---|---|
| No lesions | 515 | 0.58 | 323.01 | 21.5 | 67.81 | 42.3 |
| Delimited | 414 | 2.66 | 318.17 | 22.3 | 67.69 | 37.9 |
| Focal | 381 | 1.31 | 317.42 | 22.2 | 68.17 | 38.5 |
| Multifocal | 33 | 18.18 | 326.77 | 22.6 | 62.11 | 27.6 |
| Diffuse | 41 | 68.18 | 239.03 | 23.9 | 51.99 | 34.9 |
| Diffuse lymphocytic | 3 | 33.33 | 247.83 | 42.5 | 37.51 | 12.0 |
| Diffuse intermediate | 6 | 66.67 | 273.18 | 29.7 | 51.94 | 37.0 |
| Diffuse multibacillary | 32 | 78.13 | 231.81 | 20.0 | 53.36 | 34.7 |

$p = 0.0044$) between animals with or without lesions, but not between those with or without isolation (data not shown).

When the subsample of cows testing positive to the rtPCR was evaluated, significant effects on age and slaughter weight were observed both for immunopathological forms and for MAP isolation (Table 3). Weight losses estimated for cows showing diffuse lesions were statistically significant if compared with their mates without lesions or MAP isolation (68.02 kg; $p < 0.0001$), with focal forms and isolation (99.19 kg; $p < 0.0001$) or not (90.41 kg; $p < 0.0001$), and with multifocal with isolation (82.55 kg; $p = 0.0009$) or without it (125.24 kg; $p = 0.0006$). The differences among animals with tissue culture isolation, but without observed histopathological lesions, had the same sign, but did not reach the significance threshold due to their small number. In general, the worst weight results corresponded to animals with positive culture results, but with a curious paradox in focal and multifocal forms among which isolation was not associated with a significant loss of weight. Similar effects of isolation and immunopathological forms were observed on age (Table 3). Animals with diffuse lesions were

**Table 3.** Effect of PTB lesions and MAP viability on mean carcass weight (CW) values and age for adult Friesian cows testing positive to tissue rtPCR. HP = histopathology. Differences in mean slaughter weights and age between pathological forms of MAP infections are shown (*p* values in brackets). Mean CW and age for each pathological form of PTB appear in bold

| | Age (months) | HP-Culture- | HP-Culture+ | Focal Culture- | Focal Culture+ | Multifocal Culture- | Multifocal Culture+ | Diffuse Culture+ | MAP viability ratio (%) | Number of animals |
|---|---|---|---|---|---|---|---|---|---|---|
| **CW (kg)** | | **307.05** | **276.49** | **329.44** | **338.22** | **364.27** | **321.58** | **239.03** | | |
| **HP-Culture-** | **71.38** | — | 30.56 (0.4976) | –22.39 (0.4338) | –31.17 (0.2902) | –57.22 (0.4014) | –14.53 (0.9850) | 68.02 (< 0.0001) | — | 90 |
| **HP-Culture+** | **73.51** | –2.13 (0.9998) | — | –52.95 (0.0276) | –61.73 (0.0180) | –87.78 (0.0694) | –45.09 (0.3816) | 37.46 (0.3860) | 11.63 | 25 |
| **Focal Culture-** | **68.73** | 2.65 (0.9953) | 4.78 (0.9861) | — | –8.78 (0.9967) | -34.83 (0.8852) | 7.86 (0.9996) | 90.41 (< 0.0001) | — | 73 |
| **Focal Culture+** | **67.90** | 3.48 (0.9935) | 5.61 (0.9819) | 0.83 (1.0000) | — | –26.05 (0.9757) | 16.64 (0.9829) | 99.19 (< 0.0001) | 33.64 | 37 |
| **Multifocal Culture-** | **61.40** | 9.98 (0.9596) | 12.11 (0.9330) | 7.33 (0.9920) | 6.50 (0.9967) | — | 42.69 (0.8322) | 125.24 (0.0006) | — | 7 |
| **Multifocal Culture+** | **57.18** | 14.2 (0.3272) | 16.33 (0.3859) | 11.55 (0.6074) | 10.72 (0.7736) | 4.22 (0.9998) | — | 82.55 (0.0009) | 73.08 | 19 |
| **Diffuse Culture+** | **51.99** | 19.39 (0.0020) | 21.52 (0.0224) | 16.74 (0.0199) | 15.91 (0.1070) | 9.41 (0.9755) | 5.19 (0.9916) | — | 100.00 | 41 |

culled up to 1.8 years earlier than those without lesions, regardless tissue culture results (negative, $p = 0.0020$; positive, $p = 0.0224$).

## Discussion

Results from this study support previous findings suggesting that MAP infection in adult dairy cattle leads to economic losses because of decreased mean slaughter weight and reduced lifespan. However, the estimates for both variables varied depending on the diagnostic method and therefore, various approaches for evaluation of the impact of natural MAP infection forms were taken.

Our study found that adult cows showing high mycobacterial loads in tissues or advanced granulomatous inflammatory lesions consistent with PTB had reduced slaughter weights in comparison with the individuals testing negative to these tests; 22.2% and 26.0%, respectively. Conversely, no significant reductions were observed between those with lower bacterial loads or developing focal lesions in the small intestine or associated lymph-nodes, related to healthy ones. These results indicate that the economic impact of bovine PTB is mostly related to the more advanced forms, while the subclinical infections assumedly related to the milder immunopathological forms do not associate with direct losses in weight or lifespan. These results are in agreement with those reported for Danish Holstein cows with concomitant high antibody levels in milk and enteritis or enteric edema whose slaughter weights were reduced by 29.6% (Kudahl & Nielsen, 2009). These results, however, seem to be in disagreement with earlier findings estimating a 10% loss of weight in subclinical dairy cattle shedding MAP (Whitlock *et al.,* 1985), although it has to be taken into account that different methodology was used in both studies. Anyway, all these results, as well as those suggesting smaller slaughter values for dairy cows harboring clinical MAP infections compared to those subclinically infected (Benedictus *et al.,* 1987), follow that like other mycobacterial diseases, PTB impact on dairy cattle industry is not just a matter of infection or not, but that it depends on the location of each animal on that specific mycobacteriosis immunopathological spectrum.

It is striking that other authors failed to detect any association between tissue culture-positive cows and poor body condition scores (BCS), according to a numeric scale for morphological traits (McKenna *et al.,* 2004). In this sense, since the bacterial load was not considered it could be hypothesized that the potential effect of MAP isolation on the deteriorated body condition was underestimated by using this methodology. As presented herein, low and medium bacterial loads in tissues were not directly related to significant carcass weight reductions, similarly to the effect of focal and multifocal lesions. Hence, it could be likely that poor BCS would have been only associated with animals showing high bacterial loads.

The ELISA test appeared as an indicator of major economic losses at slaughter because humoral responses were well correlated with advanced MAP infection forms. If so, over three-fifths of seropositive cows (61.3%) associated with diffuse enteritis and/or high bacterial loads would have been easily identified by a single immunological testing. Moreover, the detection of two or more positive results for a milk ELISA has been also proposed as an indicator of higher risk of decreased carcass weights (Kudahl & Nielsen, 2009).

The other noteworthy finding from this slaughterhouse study was that lifespan of MAP infected cows was reduced by nearly one year if MAP-specific antibodies or diffuse lesions in tissues were detected, but no differences were observed associated with just MAP detection. Interestingly, cows showing granulomatous enteritis lesions classified as diffuse lymphocytic forms were culled on average at the age of three years, one year earlier than the other two diffuse forms and nearly two earlier than their uninfected mates, and with an even narrower degree of variability than any other immunopathological form. Although few animals showed this form, it may be possible that this type of lesion, characterized by an inflammatory infiltrate with a much higher proportion of lymphoid cells relative to macrophagic cells, would represent an individual higher susceptibility to progression to clinical disease than other forms of PTB.

Since cows testing positive to PTB have been observed to be more likely replaced by one year after testing (Lombard *et al.,* 2005), it could be suggested that most animals resulted positive during second lactations and were on average culled during third lactations. This fact is fully consistent with the traditionally accepted pattern of clinical disease onset.

Even though the same tendency has been observed in two previous studies considering a lower number of slaughtered dairy cows, some differential features were observed in our data. In the earlier study (Buergelt

& Duncan, 1978), regardless the development of clinical disease, infected cows were culled on average at 4 years-old, three years earlier than non-infected ones. Likewise, cows showing diffuse PTB lesions were culled around 4 years-old, at least two years earlier than those that showed no inflammatory damage or focal lesions (Alonso-Hearn *et al.,* 2012). Thus, both estimations of reduced lifespans were nearly twice or three times higher than the figures reported here. However, it should be taken into account that this study was mainly focused on sampling 3-6 years-old animals (70% of total animals). This sampling bias could account for the smaller differences between infected and healthy cows in comparison with previous studies. In general, our results confirm that PTB diagnosis is strongly determined by variables such as the design of the study, the animals (age, species, breed, culled, etc.) or the criteria for detecting and classifying diverse forms of infection, and, therefore, stress the need to consider all these variable when comparing estimates of losses caused by MAP infections in different studies.

In summary, neither wasting nor life expectancy shortening appeared to be associated with delimited forms of paratuberculosis with low viability bacteria, while strong effects for both variables were associated with the occurrence of diffuse lesions and high MAP viability rates. These observations support a slow infection spectral model of pathogenesis of paratuberculosis (Bastida & Juste, 2011) and suggest that like in other infectious or parasitic models a low level of infection could sustain a kind of premunition status where further progression to clinical disease is efficiently blocked. These forms could, therefore be considered as indicative of resistance and, since associated with low viability of the excreted bacilli, have a minimal negative or even a positive impact in the epidemiology of paratuberculosis in a large part of the cattle population provided that environmental conditions do not alter this premunition status.

In conclusion, these results support that bovine PTB is a wasting disease having a larger impact on weight than on lifespan expectancy. Significant weight losses were observed among MAP infected dairy cows regardless of the diagnostic method, whereas reduced ages, which accounted for nearly one year, were only related to seropositive cows, those showing diffuse lesions and the ones considered infected according to serial combination of humoral, microbiological and histopathological results. Adult cows showing high bacterial loads or severe granulomatous enteritis in tissues were associated with the strongest decreased carcass weights, 22.2% and 26.0% respectively. However, milder forms, even though it cannot be fully ruled out that are related to minor losses, could represent a state of relative resistance.

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