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Evaluation of losses in carcasses of cattle naturally infected with *Fasciola hepatica*: effects on weight by age range and on carcass quality parameters

Ricardo Almeida da Costa [a], Luis Gustavo Corbellini [a,b], Eleonor Castro-Janer [c], Franklin Riet-Correa [a,*]

[a] *Instituto Nacional de Investigación Agropecuaria (INIA), Estación Experimental INIA La Estanzuela, Ruta 50 Km 11, Colonia del Sacramento, Colonia, Uruguay*
[b] *Laboratório de Epidemiologia Veterinária (Epilab), Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, UFRGS, Porto Alegre, RS, Brazil*
[c] *Departamento de Parasitología Veterinaria, Facultad de Veterinaria, UDELAR, Montevideo, Uruguay*

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ABSTRACT

Although fasciolosis is a relatively common disease, the productive and economic losses resulting from cattle with chronic fasciolosis are unclear. This paper aims to investigate the effect of fasciolosis on the parameters of carcass quality and discuss the hypothesis that the effects on weight differ among age ranges of cattle. For this, we analysed abattoir data of 30,151 bovines, from 928 farms, slaughtered in Uruguay in 2016, of which 33.9% (95% confidence interval (CI): 27.3–41.1%) had *Fasciola hepatica* (liver fluke). A mixed model was built to assess whether the effect of fasciolosis on weight differs depending on the age range, using the interaction term 'age*F. hepatica'. The effect on the carcass parameters was tested using a proportional logistic regression. The interaction of age and *F. hepatica* was statistically significant ($P < 0.001$). Differences in carcass weights between infected and non-infected animals were observed mostly at younger ages (up to 30 months), with the highest difference observed in the 23–30 months age range (estimated marginal mean difference of 6.34 kg). Overall, the presence of *F. hepatica* was positively associated with poor conformations and lower fat scores of carcasses ($P < 0.001$). The carcasses of cattle infected with *F. hepatica* had 0.16 times greater odds of having worse conformation scores than carcasses of cattle without *F. hepatica* (proportional odds ratio (POR) = 1.16; 95% CI: 1.07–1.26). Similarly, carcasses of cattle with *F. hepatica* had 0.30 times (POR = 1.30, 95% CI: 1.23–1.39) greater odds of having poorer fat scores than carcasses of cattle without *F. hepatica*. Therefore, infection with *F. hepatica* is associated with poorer carcass quality parameters and lower weights, and the effect on weight differs across age ranges.

1. Introduction

Fasciolosis is a parasitic disease caused by trematodes of the genus *Fasciola*, affecting various species of animals such as cattle, sheep, horses, pigs and wild animals (Urquhart et al., 1996). The occurrence of this disease is dependent on an intermediate host. The intermediate host found in Uruguay is *Lymnea viatrix* and there are no reports of natural infections by *Lymnea columella* (Nari et al., 1983). However, recently, molecular biological studies in Uruguay reclassified *L. viatrix* (=*Lymnea viator*) as *Lymnea neotropica* (Bargues et al., 2017). The climatic conditions are linked to the high dependence of both fasciolid larval stages and their freshwater lymnaeid snail vectors. In addition, some climatic factors such as temperature (10–25 °C) and low altitudes are directly related to the prevalence and spread of fasciolosis, as well as areas with high humidity such as wetlands and those for irrigated agriculture or extensive livestock production (Andrews, 1999). In cattle, *F. hepatica* causes liver damage due to immature fluke migration and obstruction of the bile ducts by adult parasites (Urquhart et al., 1996; Olaechea et al., 2013; Cullen and Stalker, 2015).

Over the past 10 years, there has been a global increase in the prevalence of fasciolosis (Borgsteede et al., 2005), which makes the evaluation of the impact of the disease on livestock production an important subject. The reasons for this situation are not yet clear, but may be associated with various global changes including climatic changes, imbalances of the ecosystem, and other anthropogenic changes, as well as changes in cattle management with the displacement of livestock to more suitable areas for the development of the intermediate host, and increased resistance of *F. hepatica* to fasciolicides (Mas-Coma and Bargues, 2009; Bargues

* Corresponding author.
E-mail address: frcorrea@inia.org.uy (F. Riet-Correa).

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et al., 2017). In Uruguay, the prevalence of bovine fasciolosis in abattoirs was reported to be 52.8% in 1974, with variations across different departments of the country (Nari and Cardozo, 1976). However, since that date, there have been important production system changes in the country, with a significant increase in the areas designated for agriculture and forestation, and a reduction in the areas designated for livestock with an increase in productivity and stoking rate (Miraballes and Riet-Correa, 2018).

Losses caused by fasciolosis include the condemnation of livers from slaughtered cattle (Mwabonimana et al., 2009; Ezatpour et al., 2015; Mochankana and Robertson, 2016; Vidal et al., 2016), cost of treatments for the disease (Howell et al., 2015), reduced fertility and milk production (Howell et al., 2015), secondary infections (Tessele et al., 2013) and deaths, mainly of young cattle in rare outbreaks of the disease (Adrien et al., 2013). However, the productive and economic losses in relation to reduced weight gain in cattle due to fasciolosis are still a matter of discussion (Mwabonimana et al., 2009; Sanchez-Vazquez and Lewis, 2013; Howell et al., 2015; Mazeri et al., 2017).

The estimated cost of condemned livers in Uruguay is approximately US $6.5 million per year (PLANISA, 2009), but losses due to other causes have not been estimated. There are few studies evaluating the productive losses in carcasses from cattle with chronic fasciolosis throughout the world. Mazeri et al. (2017) reported that slaughter weight is not the best variable to evaluate the effect of fasciolosis on carcasses because the animals are sent to the slaughterhouse when they reach a certain weight, regardless of age. Therefore, parasitised animals are sent to slaughter at a similar weight to non-parasitised animals, but at a different age. Consequently, the effect of fasciolosis, using weight as a response variable, is underestimated (Mazeri et al., 2017). Therefore, we hypothesised that for a more accurate assessment of the effect of fasciolosis on the weight of bovine carcasses, it was important to test whether the effect of fasciolosis differs across age ranges.

The objectives of this study were as follows: (i) to assess the effect of fasciolosis on carcass quality parameters (carcass conformation and fat score) and weights of carcasses from cattle infected by *F. hepatica* and non-infected cattle, and (ii) to evaluate whether the effect of fasciolosis on the weights of the carcasses differs across age ranges, by adding an interaction term in the model.

2. Materials and methods

2.1. Abattoir data

For this study, available data from animals slaughtered in 2016 from one abattoir were analysed; the abattoir was among the five largest exporters of bovine meat in Uruguay. It received cattle from 18 of 19 departments in the country, except for the department of Montevideo, in which there is no significant number of bovines. The slaughterhouse had a daily slaughter of 450 to 700 cattle and met the international standards for exportation with inspection by veterinarians of the Ministry of Livestock, Agriculture and Fisheries (MGAP), Uruguay. During 2016, the abattoir slaughtered 116,206 bovines; a total of 2,266,764 bovines were slaughtered in Uruguay in 2016 (Instituto Nacional de Carnes (INAC), Uruguay. 2017. Anuario Estadístico. https://www.inac.uy/innovaportal/file/15850/1/anuario-estadistico-2017_web_v03.pdf (Accessed December 12, 2018)). In Uruguay, post-mortem inspection at the slaughterhouses must be carried out by the official veterinary service and is carried out by visualisation, palpation and various incisions in the livers of all animals in which they are inspected individually. The inspectors are well trained which allows them to correctly identify the fasciolosis lesions with a low margin of error. In the slaughterhouses of Uruguay, it is not necessary to establish the degree of intensity of injury.

To obtain a representative sample of all seasons of the year, the data used in this study were from the first week of each month in 2016. In that period, 30,151 bovine carcasses (26.8% of the total slaughtered) from 928 farms were evaluated individually. At slaughter, animal carcasses were classified as positive or negative for *F. hepatica.* Positive animals were considered those in which the liver was condemned due to the presence of *F. hepatica* in the bile ducts or the animal presented lesions compatible with the parasitosis according to the rules established by Decree 369–983 of the MGAP. Livers were evaluated individually by the following procedures: visualisation; palpation and incisions in the gallbladder and retrohepatic ganglia; longitudinal incision in the main bile duct; and deep incisions to the hepatic parenchyma and bile ducts.

2.2. Carcass characteristics

Six variables were used in the study: (1) 'weight', which was the final weight of the carcass; (2) 'age', (3) 'productive purpose', (4) 'sex', (5) 'carcass conformation score', and (6) 'fat score'. The variable 'age' was estimated by the number of permanent incisor teeth (PIT), according to Whiting et al. (2013). Bovines that had zero PIT were up to 23 months old; those that had two PIT were 23–30 months old; those that had four PIT were 30–37 months old; those that had six PIT were 37–42 months old; and those that had eight PIT were more than 42 months old. The variable 'productive purpose' classified carcasses according to the purpose of the original breeds, defined during inspection as meat (mainly Angus and Hereford, and some Charolais, Braford and Brangus), milk (mainly Holstein and some Jersey) or crosses of dairy and beef breeds. Conformation of the carcass was categorised into three levels according to the standards of the National Meat Institute of Uruguay, which are identified with the acronym INACUR. Carcasses of low quality were categorised as score 1 ("U" and "R"), those of regular and good quality as score 2 ("A" and "C") and those of excellent quality as score 3 ("I" and "N"). Finally, the variable 'fat score' was categorised from "0" (very low-fat coverage) to "4" (very good or excessive fat coverage) (INAC, 2018. Clasificación y tipificación carne vacuna. https://www.inac.uy/innovaportal/v/1776/2/innova.front/clasificacion-y-tipificacion-carne-vacuna (Accessed December 12, 2018).

2.3. Statistical analysis

Statistical analyses were carried out to test the hypothesis whether the effect of fasciolosis on carcass weights differs depending on the age ranges and to evaluate the effects of fasciolosis on carcass conformations and fat scores. The frequency of weight distribution was determined using a distribution and probability plot for weight using the PROC UNIVARIATE procedure in SAS Studio. Extreme low (carcasses of 74–100 kg) and high (carcasses of 540–600 kg) values were interpreted as probably an information error or extreme cases of a bull being slaughtered, for example. Therefore, extreme values (outliers) were excluded from these analyses by calculating the interquartile weight range as Q3 (283.4 kg) minus Q1 (222.7 kg) multiplied by 1.5 (i.e., $60.7*1.5 = \sim 91$ kg); this amount was added to Q3 (upper limit) and subtracted from Q1 (lower limit); therefore, values greater than 374.4 kg and below 131.6 kg were excluded.

To evaluate the effect of fasciolosis on carcass weight, a mixed model was used. Farms and slaughter date were used as random effects. The 'weight' of the carcass (kg) was the response variable, and the interaction term between the presence of *F. hepatica* and age range (PIT) was the variable of interest (fixed effect), adjusted for productive purpose (milk, meat and crosses) and sex (male and female). The mixed model was constructed with data from 27,242 carcasses from 921 farms with complete information using the following equation:

$$\text{Weight} = \beta_0 + \beta_1 \times F.\,hepatica + \beta_2 \times \text{age} + \beta_3 \times F.\,hepatica{*}\text{age} + \beta_4 \times \text{productive purpose} + \beta_5 \times \text{sexo} + \mu_1 \times \text{farm} + \mu_2 \times \text{date} \quad (1)$$

where β_0 is the intercept, $\beta_{1\ldots}\ \beta_k$ are the fixed coefficients and μ_1 and μ_2 are the random coefficients.

An analysis of the residuals was performed to verify the assumption of normality and homoscedasticity of the model. The intraclass correlation coefficient (ICC), which is the proportion of the variance explained by the groups, was calculated by:

$$ICC = \frac{Population\ variance\ among\ macrounits}{Total\ variance} \quad (2)$$

The macrounits in this model are the farms and the date (random effects), and the total variance is given by the sum of population variance among macrounits and the residuals. The same model described in (1) but without the interaction terms was fitted to compare the results of the model with the interaction terms.

Data from 29,873 carcasses were used to evaluate the effect of fasciolosis on the carcass conformation (ordered from lowest (1) to highest (3) quality) and fat scores (ordered from low (0) to excessive (4) fat coverage); these models were both adjusted by the variables 'productive purpose', 'sex', 'age' and 'slaughter date'. Logistic regression models for ordinal data were used to estimate the proportional odds ratio (POR). Additionally, an intercept-only multilevel logistic model that predicts the overall prevalence (Khan and Shaw, 2011) for the sampling universe (i.e. animals slaughtered in the studied period) using the departments as random effect was made. The predicted prevalence for each department was extracted from the model.

Data were analysed with SAS Studio assuming a level of significance of 5% using PROC MIXED, PROC LOGISTIC for ordinal data and PROC GLIMMIX for the multi-level logistic regression. The estimated marginal means (i.e., the adjusted mean of the weight) were reported using the SAS command LSMEANS of PROC MIXED and the commands OUTPUT OUT and PRED (BLUP ILINK) of the GLIMMIX procedure were used to obtain the predicted prevalence for each department (Supplementary Data S1). In addition, the association between age range and *F. hepatica*, adjusted by sex and productive purpose, was assessed by a robust Poisson regression to estimate the prevalence ratio (PR) using PROC GENMOD. The map with estimated prevalence was performed using the open source (Quantum GIS) QGIS (version 2.14.3 Essen), a free and open source Geographic Information System software (GIS).

2.4. Data availability

The database is available at Corbellini, L.G., Costa, R.A., Riet-Correa, F., Castro-Janer, E., 2019. Bovine carcasses Uruguay. Mendeley Data, (https://doi.org/10.17632/3jnn876my4.3).

3. Results

The expected log-odds of a positive carcass was −0.67, corresponding to odds of exp(−0.67) = 0.51, which means the estimated sampling ratio of positive and negative carcass. This corresponds to a predicted prevalence of 1/(1 + exp(0.67)) = 0.339. Therefore, the overall estimated prevalence of *F. hepatica* was 33.9% (95% CI: 27.3–41.1%); the ICC obtained from the multilevel logistic model was 11%, which means a between-level (department) variability in the prevalence (Fig. 1). Of the 928 farms included, 730 (78.7%) had at least one *F. hepatica*-positive carcass. Descriptive statistics of the observed data from the slaughtered cattle are shown in Table 1. There was a significant association between the age of cattle and *F. hepatica* prevalence ($P < 0.05$); considering the youngest age range as a reference, the prevalence ratio (PR) tended to increase according to the age range. For example, the prevalence of *F. hepatica* in animals older than 42 months was 0.97 times greater than the prevalence of *F. hepatica* in animals up to 23 months (PR = 1.97, 95% CI: 1.7–2.3; Supplementary Table S1).

The results of the mixed model showed that the interaction between age and *F. hepatica* was statistically significant ($P < 0.001$) (Supplementary Table S2), suggesting that the effect of fasciolosis on carcass weight differed across the ages of the animals. Differences in carcass weight between carcasses of animals with and without *F. hepatica* were observed mostly at younger ages (up to 30 months.), with the highest difference observed in the age range of 23–30 months. In that age range, the difference of the estimated (marginal) mean weights between *F. hepatica*-positive and *F. hepatica*-negative animals was on the order of 6.34 kg. Marginal means estimated by the model (i.e., the mean weight between *F. hepatica*-positive and *F. hepatica*-negative animals across the age range adjusted by covariates) are illustrated in Table 2 (Supplementary Table S3 contains the model estimates). The ICC of the model was 54.2%. The proportion of the variance of the weight due to the farms was 48.1% and due to the date of slaughter was 6.1% (Supplementary Table S4 contains the covariance parameters estimate). The effect of *F. hepatica* on weight without considering the interaction was significant ($P = 0.04$), but the difference in the estimated marginal weight between positive and negative *F. hepatica* animals was only 890 grams (Table 2). The residual panel showed that random errors in the regression model are homoscedastic (i.e., have constant variance over the weight) and are normally distributed.

Overall, the presence of *F. hepatica* was positively associated with poor conformation and lower fat scores of carcasses ($P < 0.001$). The carcasses from animals with *F. hepatica* had 0.16 times (POR = 1.16; 95% CI: 1.07–1.26) greater odds of having a level 1 and 2 (low and regular-to-good quality carcass) relative to a level 3 (excellent quality carcass) in comparison with the *F. hepatica*-negative carcasses. In the same way, carcasses from animals with *F. hepatica* had 0.30 times (POR = 1.30, 95% CI: 1.23–1.39) greater odds of having a level 0, 1, 2, and 3 (lower) fat score relative to a level 4 (very good or excessive fat coverage) fat score in comparison with liver fluke-negative carcasses.

4. Discussion

Inspection of abattoirs is a useful tool for the diagnosis of incidental or asymptomatic parasitosis (Sanchez-Vazquez and Lewis, 2013). The systematic investigation of a large sample of animals with and without lesions has served as a model for the study of various diseases and has already been used successfully in several countries (Mwabonimana et al., 2009; Dutra et al., 2010; Tessele et al., 2013; Ezatpour et al., 2015; Murthy and D'Souza, 2015; Mochankana and Robertson, 2016; Vidal et al., 2016). In 2015, 2,204,391 cattle were slaughtered in Uruguay, with a monthly average of 183,699 heads, generating approximately US $1.5 billion in exports, corresponding to 83.69% of the total exports of the national meat sector (INAC, 2015). Anuario Estadístico, existencia/faenas/precios/exportación. http://www.inac.gub.uy/innovaportal/file/13042/1/anuario-estadistico-2015_web-2.pdf (Accessed December 12, 2018). According to the study of the National Animal Health Research Plan (PLANISA, 2009), 81% of the liver condemnations in Uruguay are due to *F. hepatica*. Estimating an average loss of US $2.64 per liver, losses for this condition total US $6.5 million a year. Since *F. hepatica* infection was found

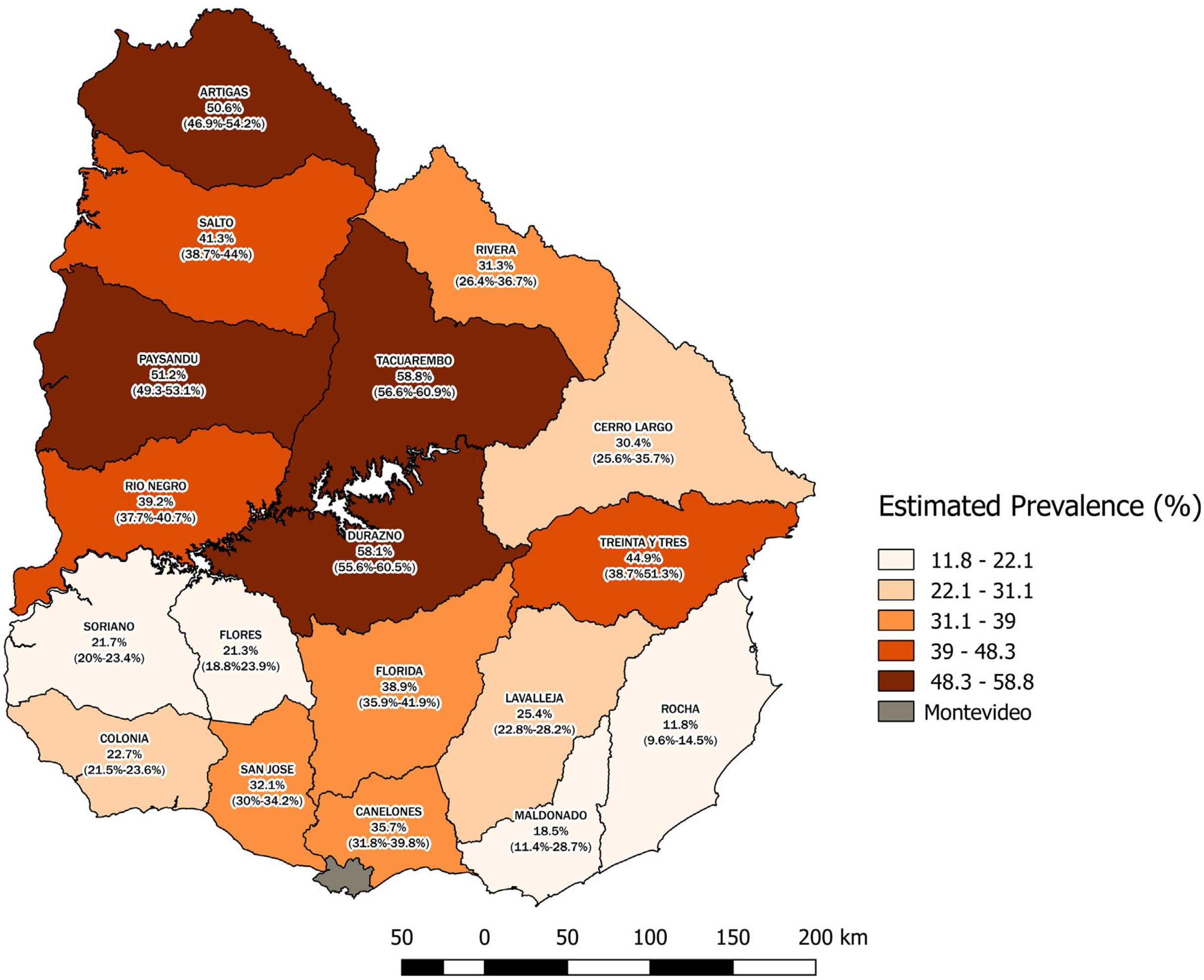

Fig. 1. Marginal prevalence (95% confidence interval) of *Fasciola hepatica* by department in cattle slaughtered in 2016 in a slaughterhouse in Uruguay.

Table 1
Descriptive statistics of data used in the study to assess the effect of liver fluke on carcass parameters from a sample of 30,151 bovines slaughtered in a slaughterhouse in Uruguay in 2016.

Variable	Cattle type/age	No.	Negative for fluke (%)	Positive for fluke (%)
Productive purpose	Milk	7301	5050 (69.2)	2251 (30.8)
	Beef	12,839	8193 (63.8)	4646 (36.2)
	Cross-breed	10,011	6222 (62.2)	7301 (37.8)
Permanent incisor teeth (age)[a]	0 (0–23 months)	744	598 (80.4)	146 (19.6)
	2 (23–30 months)	3825	3159 (82.6)	666 (17.4)
	4 (30–37 months)	4491	3471 (79.3)	1020 (20.7)
	6 (37–42 months)	3959	2691 (68.0)	1268 (32.0)
	8 (>42 months)	17,132	9546 (55.7)	7586 (44.3)[b]
Conformation	1 (bad)	1396	641 (45.9)	775 (54.1)
	2 (regular)	25,058	16,008 (63.9)	9050 (36.1)
	3 (good)	3697	2818 (76.2)	881 (23.8)
Fat score	0 (very low)	2060	1047 (50.8)	1013 (49.2)
	1(low)	6734	4655 (69.1)	2079 (30.9)
	2 (regular)	20,535	1306 (67.8)	7429 (36.2)
	3–4 (good/excessive)	821	657 (79.9)	165 (20.1)

[a] Estimated age in months.
[b] There is an association between age and prevalence ($P < 0.05$).

in the 18 departments that raise cattle in Uruguay and in ~70% to 100% of the farms in those departments, this disease can certainly cause important economic losses in addition to losses caused by the condemnation of livers. Other economic losses including lower weight gains, decreased carcass quality and expenses for treatments and labour, need further investigation.

This work established the negative impact of *F. hepatica* on the meat industry and demonstrated the importance of analysing the interaction between *F. hepatica* and age to better assess the impact of the infection on carcass weights. These results are in accordance with previous reports noting that *F. hepatica* is associated with less weight gain (Mazeri et al., 2017), poorer conformations of

Table 2
Estimated marginal means of weights from liver fluke-positive and -negative bovine carcasses across age ranges. The weight was estimated using a mixed model with the interaction term (fluke*age) adjusted by the covariates sex and purpose. For comparison purposes, the same model was fitted without including the interaction term.

Model	Variable	Estimated marginal mean, kg (95% CI)		
	Age (months)[b]	Weight of animals without fluke	Weight of animals with fluke	Difference (kg)[c]
With interaction[a]	0 (~0–23)	221.5 (217.2–225.7)	218.9 (213.1–224.7)	−2.6
	2 (~23–30)	235.4 (231.9–238.8)	229.1 (225.1–233.0)	−6.3
	4 (~30–37)	244.3 (240.9–247.6)	242.8 (239.2–246.4)	−1.5
	6 (~37–42)	252.9 (249.5–256.3)	253.4 (249.9–257.0)	0.5
	8 (>42)	262.1 (258.8–265.3)	261.6 (258.4–264.9)	−0.5
Without interaction	Fasciolosis	243.03 (239.8–246.3)	242.14 (238.8–245.4)	−0.89

[a] Intercept (245.93 kg), reference for sex "male" and reference for purpose "milk".
[b] Estimated age for permanent incisor teeth.
[c] Weight positive for fluke – weight negative for fluke

carcasses and lower fat scores (Sanchez-Vazquez and Lewis, 2013). The lower weights of the carcasses of cattle with *F. hepatica* could be due to the alterations generated by this parasite in the hepatic parenchyma, reducing its capacity for synthesis and metabolism (Okoye et al., 2013; Sanchez-Vazquez and Lewis, 2013).

According to Mazeri et al. (2017), the farmer and the market determine the weight at which an animal is sent to the abattoir, and farmers will delay sending animals infected with *F. hepatica* until they reach the desired weight. In addition, a higher prevalence of fasciolosis is found in older cattle, and it would be expected that *F. hepatica*-infected animals arrive later to the slaughterhouse. This situation is recognised as simultaneous bias (Mazeri et al., 2017), in which it is possible to underestimate the effects of fasciolosis in older animal populations with a higher prevalence and an "ideal slaughter weight". An association between age and prevalence was observed here, with a higher prevalence found in older animals, probably due to a longer exposure time (Khan et al., 2013). According to this theory, slaughter weight is not the best variable to evaluate the effects of *F. hepatica* in bovine carcasses, as suggested by the results of the model without the interaction term, where the difference in the estimated marginal mean weight between the infected and non-infected animals was only ~900 g. However, by including the interaction term of age and *F. hepatica* in the model, marked differences were found in the estimated marginal mean weight of the carcasses of the liver fluke-infected cattle compared with that of non-infected cattle, mostly in the younger age range. The differences in estimates are very small or even opposite (carcass of *F. hepatica*-infected animals are heavier than those of non-infected animals) in cattle older than 37 months. The evaluation of the interaction of age and liver fluke infection in relation to the final weight of the carcass is better evidenced in young animals (~23–37 months), possibly because they are more susceptible to the effects of parasitic infections than the older animals. Additionally, the high value of the ICC (54%; 48% due to farms and 6% due to date of slaughter) showed that there were strong effects of the farm on the variation in the weights of the carcasses due to unknown factors, which was expected. These factors may be associated with different management practices, genetics, or different *F. hepatica* control practices between farms, for example.

In addition to the differences observed in the estimated weights across the age range, there was also an association of *F. hepatica* with poor carcass conformations and lower fat scores. Fasciolosis produces biochemical changes in enzymatic activities and lower levels of total proteins, albumin, globulins, glucose, creatinine, urea, cholesterol, triglycerides, and lipoproteins (Okoye et al., 2013), which may be responsible for the poor carcass conformations, low fat scores, and low body weights of parasitised animals.

In this research the losses in the carcasses were estimated according to age using the number of PIT. This type of study gives farmers and veterinarians a clearer and more practical view of the economic and productive losses caused by *F. hepatica* in cattle of different ages. The principal novelty of this study is the scientific axiom we presented, represented by the mixed model including the interaction term between age and *F. hepatica*. While Mazeri et al. (2017) postulated that slaughter weight is not the best variable to evaluate the effects of *F. hepatica*, we are postulating that weight is important when the model includes the interaction terms between *F. hepatica* and age. However, we corroborate what was postulated by Mazeri et al. (2017), that weight is not a good variable only when *F. hepatica* is not interacted with age (as could be seen in the model without that interaction).

In conclusion, carcasses of bovines infected by *F. Hepatica* have poorer conformations, lower fat scores, and less weight gains than carcasses of non-infected cattle, with the differences in weight more notable in younger age ranges. To assess the impact of *F. hepatica* on the weights of carcasses, it is important to test the interaction between *F. hepatica* and age. Since the occurrence of *F. hepatica* seems to be nationwide, farmers and industry need to be aware of the effects of this disease.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpara.2019.06.005.

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