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Title: Ostertagia spp., rumen fluke and liver fluke single- and poly-infections in cattle: an abattoir study of prevalence and production impacts in England and Wales

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Abstract: This study aims at investigating the occurrence, risk factors and production impacts on beef carcass parameters of three of the most important cattle helminth infections in England and Wales. Abomasa, reticulorumen and livers from healthy cattle were collected and examined post-mortem quarterly over a one year period in an abattoir in South-West England. Specific viscera from 974 cattle were collected, examined and scored for Ostertagia spp., adult rumen fluke and liver fluke lesions/presence. A total of 89%, 25% and 29% of the carcasses had lesions/presence of Ostertagia spp., rumen fluke and liver fluke, respectively, and 39% had presence of helminth co-infection. Animal demographic and carcass parameters associated with helminth infections were investigated using multilevel multinomial and multilevel linear mixed models respectively. After adjusting for other factors, significant differences in the distribution of helminth infections were observed among cattle by type of breed, animal category (cow, heifer, steer, young bull), age, season and concurrent helminth infections. Compared to carcasses free of helminths, carcasses presenting solely Ostertagia Spp. lesions or adult rumen fluke had significantly lower cold carcass weight (coef.: -30.58 [-50.92;-10.24] and -50.34 [-88.50;-12.18]) and fat coverage (coef.: -3.28 [-5.56;-1.00] and -5.49 [-10.28;-0.69]) and carcasses presenting solely liver fluke lesions had significantly lower conformation grade (coef.: -3.65 [-6.98;-0.32]). Presence of helminth poly-infections was negatively associated with cold carcass weight.

1   *Ostertagia spp*__**., rumen fluke and liver fluke single- and poly-infections in cattle: an**__

2   **abattoir study of prevalence and production impacts in England and Wales**

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28

29   **ABSTRACT**

30   This study aims at investigating the occurrence, risk factors and production impacts on beef

31   carcass parameters of three of the most important cattle helminth infections in England and

32   Wales. Abomasa, reticulorumen and livers from healthy cattle were collected and examined

33   post-mortem quarterly over a one year period in an abattoir in South-West England. Specific

34   viscera from 974 cattle were collected, examined and scored for *Ostertagia spp.*, adult rumen

35   fluke and liver fluke lesions/presence. A total of 89%, 25% and 29% of the carcasses had

36   lesions/presence of *Ostertagia spp.*, rumen fluke and liver fluke, respectively, and 39% had

37   presence of helminth co-infection. Animal demographic and carcass parameters associated

38   with helminth infections were investigated using multilevel multinomial and multilevel linear

39   mixed models respectively. After adjusting for other factors, significant differences in the

40   distribution of helminth infections were observed among cattle by type of breed, animal

41   category (cow, heifer, steer and young bull), age, season and concurrent helminth infections.

42   Compared to carcasses free of helminths, carcasses presenting solely *Ostertagia Spp*. lesions

43   or adult rumen fluke had significantly lower cold carcass weight (coef.: -30.58 [-50.92;-

44   10.24] and -50.34 [-88.50;-12.18]) and fat coverage (coef.: -3.28 [-5.56;-1.00] and -5.49 [-

45   10.28;-0.69]) and carcasses presenting solely liver fluke lesions had significantly lower

46 conformation grade (coef.: -3.65 [-6.98;-0.32]). Presence of helminth poly-infections was

47 negatively associated with cold carcass weight.

48

51

52 1. Introduction

53   Recent projections of the world population's growth have emphasized the urgent need to

54 increase worldwide food production, especially annual meat production (FAO, 2009), while

55 reducing environmental impacts and maintaining high levels of animal health and welfare. In

56 the United Kingdom (UK), parameters such as increased growth rate, higher carcass weight

57 and low-cost grazing systems will be key in enhancing production, given that animal numbers

58 are expected to decline (Thornton, 2010). In this context, production limiting diseases such as

59 helminth infections are of major concern. In temperate areas, helminth infections in grazing

60 livestock are not only an important cause of reduced productivity, but can also lead to poor

61 welfare and contribute to increases in net greenhouse gas emissions (Sargison, 2014).

62 Evidence of increases in prevalence and spread of endemic helminths have already been

63 reported in the UK (Sargison, 2014). Helminth infections are seasonal, ubiquitous on

64 livestock farms and responsible for major impacts on both animal production and

65 reproduction (Charlier et al., 2014). Beef cattle, are particularly susceptible to such chronic

66 and insidious production limiting diseases because the majority of UK production systems are

67 pasture-based (AHDB, 2009; Sargison, 2014). To date however, very few abattoir studies

68 have been published on the epidemiology and impact of helminth infection in beef cattle

69 (Charlier et al., 2009). In the UK especially, no published abattoir survey on prevalence of

3

70   helminths in cattle were conducted since the eighties (Froyd, 1975; Bairden and Armour,

71   1981).

72   In temperate areas such as the UK, two of the most economically important helminth

73   parasites affecting cattle are the abomasal nematode, *Ostertagia ostertagi,* and the liver fluke,

74   *Fasciola hepatica* (Charlier et al., 2014). The recent increasing number of rumen fluke cases

75   in cattle that have been reported in Western Europe also raises concerns about the potential

76   production impact this parasite could have. However, data remain scarce, especially in the

77   UK, and the true prevalence of the rumen fluke in cattle is unknown (Gordon et al., 2013) .

78   Although several diagnostic tools have been developed to detect host exposure to

79   helminths, current methods often have poor specificity and a lack of correlation over time

80   with the actual impact on the host (Charlier et al., 2014). Specific gross examinations of

81   parasitized organs post-mortem is considered the 'gold standard' for assessing prevalence and

82   pathology (Rapsch et al., 2006; Larraillet et al., 2012; Sanchez-Vazquez and Lewis, 2013;

83   Toolan et al., 2015) and could aid in widening and refining our current knowledge on cattle

84   helminth infections.

85   Very few studies have been published on poly-parasitism in adult cattle and none on the

86   impact of such poly-parasitism on cattle production,, especially in the case of co-infections

87   with *Ostertagia spp.*, *F. hepatica* and rumen fluke (Murphy et al., 2006). The aims of this

88   study were to: (1) estimate the prevalence and severity of helminth single and poly-infections

89   in cattle (beef and dairy) at slaughter in England and Wales, focussing on abomasal lesions

90   typical of *Ostertagia spp.*, rumen fluke and liver fluke; 2) investigate if helminth prevalence

91   and severity differed between animal demography and (3) evaluate their production impacts

92   on prime beef carcass weight and classification.

93

2. Materials and Methods

2.1. Sample collection and viscera scoring

Abomasa, reticulorumens and livers from commercial cattle were collected and examined post-mortem quarterly over a twelve month period from March 2014 to January 2015 in an abattoir slaughtering up to 1500 cattle per week in South-West England. On each visit at slaughter, specific viscera from all cattle were inspected on the slaughter line. Livers were examined on-line with the meat inspectors at the abattoir. The liver was examined and scored for the presence of typical cholangiohepatitis lesions ("pipe stem" appearance) and its surface incised as deemed appropriate to detect the presence of liver fluke. Reticulorumens and abomasa were examined in the "gut room", where they were excised and the contents expelled. The internal surfaces of the reticulorumen were visually assessed for the presence of adult rumen fluke and, if present, for their numbers. The abomasum was dissected from the omasum, everted and rinsed to expose the mucosal surface and estimate the number of characteristic lesions of *Ostertagia spp.* on the fundus and pylorus of each abomasum.

Abomasum gross lesions were classified into four categories (scores 0-3) based on the number of gastric gland lesions typical of *Ostertagia spp.* (Larraillet et al., 2012): 0- no lesions; 1- less than 100 lesions; 2- between 100-1000 lesions; 3- more than 1000 lesions. Each reticulum and rumen were thoroughly examined and classified on a numerical scale according to the number of adult rumen fluke (scores 0-3): 0- no fluke; 1- between 1 and 10; 2- between 11 and 100; 3- between 101 and 200; 4- more than 200 fluke. The presence of liver fluke (0- no fluke (i.e. neither fluke nor liver fluke lesions); 1- actual presence (i.e. presence of fluke and liver fluke lesions); 3- historical presence (i.e. no fluke but presence of liver fluke lesions)) and the severity of the liver lesions due to liver fluke (0- no lesions; 1- moderate lesions; 2- severe lesions) were also scored, based on gross-pathological scales used

118   in previous studies (Sanchez-Vazquez and Lewis, 2013). The scoring of gross lesions was

119   conducted by the same group of operators at each visit, who were blinded to the identity of

120   the animal or farm.

121   Before the commencement of the study, the scoring system was pilot-tested in the same

122   abattoir as a feasibility check. At the same time, a sample of adult rumen fluke specimens

123   were collected from two animals and preserved in 70% methanol and were sent for speciation

124   (Moredun Research Institute, UK), applying PCR amplification and DNA sequencing of the

125   ITS-2 region using generic primers (Rinaldi et al., 2005) with subsequent sequencing of

126   purified PCR amplicons (Gordon et al., 2013).

127

128   *2.2. Animal demographic and carcass parameters*

129   Data from the abattoir information management system were used to provide additional

130   information on each animal, using the kill number as the unique identifier. The following

131   demographic information was extracted: date of birth, date of slaughter, farm, breed, sex

132   (male/female), category (mature bull, cow, heifer, steer and young bull), cold carcass weight

133   (CCW) (kg), carcass conformation and fat classifications and liver condemnations (yes/no).

134   No additional information on the history of the animals in relation to previous grazing,

135   housing and anthelmintic treatments was available. To determine the geographic origin of the

136   farm the animals were submitted from, the postcodes of each farm were used and related

137   latitude, longitude and altitude extracted from "Google Maps" (Map data ©2016 Google).

138   The breed information was classified in four categories: pure-dairy, dairy-cross, pure-beef

139   and beef-cross, using the information provided on the passport and DEFRA (Department for

140   Environment, Food and Rural Affairs) breed classification list (DEFRA, 2014). The animal

141   slaughter-age in months was calculated from the date of birth to the kill date. Carcass

142 conformation and fat classifications were evaluated referring to the EUROP scale (Pritchard

143 et al., 2013).

144

145 *2.3. Statistical analysis*

146 Data were coded, checked and entered into a database (Microsoft Excel 2010). A

147 preliminary descriptive analysis was conducted using STATA 12.1 (STATA Inc., Texas,

148 USA) to summarize the data. Three sets of analysis were conducted, as described below:

149

150 *2.3.1. Prevalence and severity of helminth infections*

151 Descriptive statistics were conducted to summarise the prevalence of *Ostertagia spp.*,

152 adult rumen fluke and liver fluke at farm level and at cattle level, based on abomasal lesions,

153 presence of adult rumen fluke and *F. hepatica* presence and lesions respectively. For each

154 helminth, the carcasses were summarised based on severity scores of the helminths, season

155 and category of animal. Where scores were available for all three helminths, the percentage

156 of co-infected animals was calculated.

157

158 *2.3.2. Factors associated with presence and severity of helminth infections*

159 Multinomial logistic regression was used to investigate the relationship between the

160 carcass categorical severity scores for helminths and the general demographic and other

161 collected variables (Dohoo et al., 2009). Three models (one for each helminth) were built.

162 Since several carcasses originated from the same farm, observations could not be considered

163 independent; hence a multilevel mixed-effect model was built accounting for the hierarchy in

164 the data. The three models incorporated two hierarchical levels: level 1 (i), the cattle-level,

165 level 2 (j) the herd-level. The outcome variable was: for model 1, the scores of *Ostertagia*

166   *spp.* lesions (0- no lesions, 1- less than 100 lesions; 2- between 100-1000 lesions; 3- more

167   than 1000 lesions), for model 2, the scores of adult rumen fluke (0- no fluke; 1- between 1

168   and 100 fluke; 2- more than 100 fluke) and for model 3, the scores of liver fluke lesions (0-

169   no lesions; 1- moderate lesions; 2- severe lesions). For all three models the reference category

170   for the outcome was score 0 and the predictor variables were: breed, category, age, month of

171   sampling, altitude and presence of co-infection. The model was built using a stepwise

172   approach, combining both forward selection and backward elimination of predictor variables.

173   The evaluation of the effects of significant factors on the three outcomes was based on Wald

174   tests. P-value ≤ 0.05 was considered significant. Confounding variables also remained in the

175   final model. The multilevel multinomial models 1, 2 and 3 used a logit link function to

176   express the ratio probability of a given helminth score to the probability of the reference

177   score, as shown in equation (1) (Rasbash et al., 2009):

178
$$log\left(\frac{\pi_{ij}^{(s)}}{\pi_{ij}^{(0)}}\right) = \beta_0^{(s)} + \beta_1^{(s)}x_{ij} + u_{0j}^{(s)} \quad (1)$$

179   Where: $\pi_{ij}^{(s)}$ was the probability of the *i*th carcass of the *j*th herd to have a score "s" (s=1, 2,

180   3, for model 1; s=1, 2, for model 2 and 3) compared to the score 0; $\beta_0^{(s)}$ was the score-specific

181   intercept of the model; $\beta_1^{(s)}$ represents the vector of coefficients; x_{ij} was the vector of

182   predictor variables and $u_{0j}^{(s)}$ was the herd-level random effect, assumed to be normally

183   distributed. All statistical analyses were performed using MLwiN v2.30. All the calculations

184   were based on a Restricted Iterative Generalized Least Squares (RIGLS) procedure and a

185   second-order approximation by penalized quasi-likelihood (Rasbash et al., 2009). Models

186   were checked for any influential observations or outliers.

187

188 *2.3.3. Impact of helminths on carcass parameters*

189   The impact of helminth past/current infections on beef production carcass parameters was

190 estimated using three multilevel mixed-effect linear regression models with outcomes: (1) the

191 cold carcass weight (CCW), (2) the carcass conformation and (3) the carcass fat

192 classification. Since several carcass originated from the same herd, the model had carcasses

193 nested within herds. Only steers, heifers and young bulls from 12 to less than 36 months were

194 included in this analysis, as these represent the population of cattle reared for prime beef in

195 the UK (AHDB, 2009). The predictor variables for the three models were: breed, category,

196 age, carcass parameters, month, altitude and an eight-level categorical variable for presence

197 of co-infection (i.e. no helminths; *Ostertagia spp.* lesions only; adult rumen fluke only; liver

198 fluke lesions only; *Ostertagia spp.* lesions and adult rumen fluke; *Ostertagia spp.* lesions and

199 liver fluke lesions; adult rumen fluke and liver fluke lesions; *Ostertagia spp.* lesions, adult

200 rumen fluke and liver fluke lesions). Models were developed using a Restricted Generalised

201 Iterative Least Squares (RIGLS) algorithm in MLwiN 2.30 (Rasbash et al., 2009). Both

202 conformation and fat classifications were converted into a 15-numerical scale (Pritchard et

203 al., 2013). The models were built following the stepwise approach and took the form of

204 equation (2) (Rasbash et al., 2009):

205 $$y_{ij} = \beta_0 + \beta_1 x_{ij} + u_{0j} + e_{ij} \qquad (2)$$

206 Where: y_{ij} was the outcome (CCW/Carcass conformation/Carcass fat classification) of the

207 *i*th carcass from the *j*th herd; β_0 is the intercept; β_1 was the coefficient for the effect of a unit

208 increase of the predictor x_{ij} on the outcome y_{ij}; u_{0j} is the herd-effect and e_{ij} was the bottom

209 level residual, both assumed to be normally distributed. Model goodness-of-fit was assessed

210 at each hierarchical level by the examination of the normal probability and the leverage plots

211 of residuals (Dohoo et al., 2009; Rasbash et al., 2009).

212

3. Results

3.1. Description of animal and carcass parameters

A total of 974 carcasses were sampled from March 2014 to January 2015: 298 (31%) in March, 233 (24%) in June, 230 (24%) in October and 213 (22%) in January. The carcasses originated from 156 UK farms, localised in 23 counties. A total of 134 (86%) farms could be geo-localised, of which 82% (110/134) were from England and 18% (24/134) from Wales. The median [25th percentile (p25) - 75th percentile (p75)] number of carcasses per farm was 4 [2-8]. The sample included 64% males and 36% females, of which 53% (518/974) were steers, 20% (193/974) cows, 16% (155/974) heifers, 11% (106/974) young bulls and less than 1% (2/974) mature bulls. Fifty percent (484/974) of the carcasses were from beef-cross breeds, 36% (353/974) from pure-dairy breeds, 9% (83/974) from pure-beef breeds and 4% (42/974) from dairy-cross breeds; the rest (12/974) belonging to either dual-purpose or other breeds. Table 1 presents, by cattle category, the sample median [p25-p75] of age, CCW, conformation and fat classifications, and percentage of liver condemnations.

3.2. Description of carcass parasites' presence/lesions

3.2.1. Prevalence and severity of helminth infections as defined by scores

Adult rumen fluke specimens isolated from the two carcasses sampled in the pilot study were identified as *Calicophoron daubneyi*.

Out of 972 carcasses (mature bulls excluded), a total of 933 abomasa, 936 reticulorumen and 951 livers were scored for *Ostertagia spp.* lesions, presence of adult rumen fluke and liver fluke lesions, respectively; the others being either condemned or lost. There was a large variation in the prevalence of helminths with, at cattle-level, 89% (828/933), 25% (231/936)

236 and 29% (272/951) of the carcasses and, at farm-level, 97% (149/154), 48% (73/153) and

237 64% (98/152) of the producers with at least one carcass with signs of ostertagiasis, adult

238 rumen fluke and liver fluke lesions respectively. Distribution of carcasses by severity score

239 for each category of animal is presented in Table 2.

240   Of the abomasa with lesions of ostertagiasis, 40% had scores of 3 (>1000 lesions). There

241 was a similar percentage of carcasses with ≤100 and >100 adult rumen fluke (51% and 49%

242 respectively). Live *F. hepatica* were present in approximately 86% of the livers with liver

243 fluke lesions. A seasonal variation was present for the prevalence of helminth in carcasses,

244 with highest prevalence of *Ostertagia spp.* lesions observed in January (98%), compared with

245 84% in March, 85% in June and 89% in October. A similar pattern was observed for liver

246 fluke lesions and adult rumen fluke with the lowest relative prevalence in March (22% and

247 17% respectively) and highest prevalence in January (34% and 28% respectively) and

248 October (33% and 31% respectively). The prevalence of liver fluke and adult rumen fluke in

249 June was 28% and 25% respectively.

250

251 *3.2.2 Presence of co-infection*

252   Out of the 972 carcasses, 909 (94%) had a score available for all three helminths. Of these,

253 92% (837/909) had at least one helminth presence/lesion. A total of 39% (351/909) of the

254 animals had co-infection, of which 15% (138/909) with *Ostertagia spp.* lesions and adult

255 rumen fluke, 12% (111/909) with *Ostertagia spp.* and liver fluke lesions, 11% (97/909) with

256 all the three helminths presence/lesions and 1% (5/909) with only adult rumen fluke and liver

257 fluke lesions. Presence of adult rumen fluke and liver fluke lesions were mainly concurrent

258 with other infections, with only 3% (6/219) and 6 % (15/255) of infected animals having

259 single-infection with adult rumen fluke and liver fluke respectively, compared to 57%

11

260   (465/811) with only *Ostertagia spp.* Out of 219 animals (24%) infected with adult rumen

261   fluke, 47% (102/219) also had signs of liver fluke lesions. The prevalence of co-infected

262   animals was highest in October with 50% (104/206) of the carcasses presenting signs of at

263   least two parasites, compared with 44% (83/189) in January, 35% (81/229) in June and 29%

264   (83/285) in March. The highest prevalence of co-infection was observed in cows with 51%

265   (83/162) of the carcasses infected with at least two helminths, compared with 42% (210/502)

266   for steers, 35% (51/145) for heifers and 7% (7/100) for young bulls.

267

268   *3.3. Factors associated with helminth presence/lesions and carcass infection severity*

269   The number of observations for predictor variables per model is presented in Table 3. The

270   three final multilevel multinomial models are presented in Table 4. All significant variables

271   and potential confounders were retained in the model to estimate the independent effect of

272   variables (i.e. effect of variable presented is after adjusting for the effects of variables in the

273   model).

274

275   *3.3.1. Model 1 (abomasal lesions due to Ostertagia spp.)*

276   Compared with pure-dairy breeds, dairy-cross breeds were significantly more likely to

277   have *Ostertagia spp.* lesions of all severities (Odds Ratios [OR]: 7.29; 8.63; 6.20). Whereas

278   beef-cross breeds were significantly less likely to have *Ostertagia spp.* lesions of higher

279   severity ($\geq$100 lesions) (OR: 0.49; 0.45). Compared to cows, heifers were significantly more

280   likely to have *Ostertagia spp.* lesions of all severities (OR: 2.16; 4.34; 7.11), steers were

281   more likely to have lesions of >100 (OR: 2.06; 2.54) and young bull between 100-1000 (OR:

282   3.15). There was a significant effect of age: compared to animals slaughtered at <24 months

283   of age, animals slaughtered at >30 months were at significantly higher risk of having

284 *Ostertagia spp.* lesions with all severities (OR: 2.72; 2.27; 4.40) and animals slaughtered

285 between 24-30 months more likely to have >1000 lesions (OR: 2.82). Compared to January,

286 there were significant reduced numbers of *Ostertagia spp.* lesions of all severities in March

287 (OR: 0.06 to 0.08), June (OR: 0.04 to 0.11) and October (OR: 0.06 to 0.20). The presence of

288 adult rumen fluke was significantly associated with all severities (OR: 1.92 to 3.01) of

289 abomasal lesions due to *Ostertagia spp*. There was no significant association between the

290 presence of *Ostertagia spp.* lesions and the presence of liver fluke.

291

292 *3.3.2. Model 2 (presence of adult rumen fluke)*

293   There was no significant association between the presence of adult rumen fluke and the

294 different breeds. Compared to cows, steers were significantly more likely to have adult rumen

295 fluke infestation of all severities (OR: 2.51 to 3.95) and heifers more likely to have 1 to 100

296 rumen fluke (OR: 2.55). Animals slaughtered older than 30 months were significantly more

297 likely to be heavily infected with adult rumen fluke (>100) than animals slaughtered younger

298 than 24 months (OR: 5.48). Compared with March, there were increased numbers of >100

299 adult rumen fluke infested animals in June (OR: 2.32), October (OR: 2.82) and January (OR:

300 4.45). Carcasses originating from higher altitude farms (>60m) were significantly less likely

301 to have adult rumen fluke compared to carcasses originating from lower altitude farms

302 (≤60m) (OR: 0.44 to 0.58). Presence of liver fluke lesion was significantly associated with

303 adult rumen fluke infestation of all severities (OR: 1.79 to 5.34). There was no significant

304 association between the presence of abomasal lesions due to *Ostertagia spp.* and the

305 likelihood/severity of adult rumen fluke.

306

307 *3.3.3. Model 3 (liver lesions due to liver fluke)*

308   Compared to pure-dairy breeds, beef-cross breeds were significantly more likely to have

309   both moderate and severe liver lesions due to liver fluke (OR: 2.30 to 3.18). Compared to

310   cows, heifers were significantly less likely to have liver fluke lesions (moderate and severe)

311   (OR: 0.08 to 0.43), steers less likely to have severe liver fluke lesions (OR: 0.13) and young

312   bulls less likely to have moderate liver fluke lesions (OR: 0.04). After controlling for the

313   other variables, there was no significant association between the age the animal was

314   slaughtered and the presence of liver fluke lesions. Compared with March, there were

315   significantly higher numbers of carcasses with liver fluke lesions of all severities in January

316   (OR: 1.75 to 3.20) and of moderate severity in October (OR: 2.06). Carcasses originating

317   from higher altitude farms (>60m) were significantly less likely to have moderate liver fluke

318   lesions compared to carcasses originating from lower altitude farms (≤60m) (OR: 0.56).

319   Presence of adult rumen fluke was significantly associated with presence of liver fluke

320   lesions with all severities (OR: 2.71 to 4.08). There was no significant association between

321   the presence of liver fluke lesions and *Ostertagia spp.* lesions.

322

323   *3.4. Impact of helminth presence/lesions on carcass parameters*

324   The final multilevel linear regression models are summarized Table 5. The total of

325   variance explained by the different final models was: for Model 1 (CCW), 50%, for Model 2

326   (conformation), 33%, for Model 3 (fat classification), 64%.

327   After controlling for the effects of breed, category, age and season, animals with single-

328   infection of either ostertagiasis or adult rumen fluke had, on average, significantly lower

329   CCW [Coef. (95% CI): -30.58 (-50.92;-10.24) and -50.34 (-88.50;-12.18)] and lower fat class

330   [Coef. (95% CI): -3.28 (-5.56;-1.00) and -5.49 (-10.28;-0.69)] respectively than carcasses

331   from helminth-free animals. The presence of liver fluke lesions had no significant impact on

332 CCW except when present along with both abomasal lesions due to *Ostertagia spp.* and adult

333 rumen fluke, leading to significantly lower CCW [Coef. (95% CI): -48.28 (-88.35;-8.21)]

334 compared to carcasses free of the three helminths. Carcasses with both *Ostertagia spp.*

335 lesions and adult rumen fluke had significantly lower CCW [Coef. (95% CI): -39.99 (-73.09;-

336 6.88)] compared to carcasses free of the three helminths. The presence of liver fluke lesions

337 on its own had a significant negative impact on carcass conformation by a 3.65 (-6.98;-0.32)

338 point decrease in the class numerical scale compared to carcasses free of the 3 helminths.

339 Visual examinations of the three models final residuals at each hierarchical level

340 suggested the model fits were good (data not shown).

341

342 **4. Discussion**

343 To the authors' knowledge, this is not only the first abattoir study since the eighties on

344 *Ostertagia spp.* and liver fluke prevalence in cattle in England and Wales (Froyd, 1975;

345 Burrows et al., 1980; Bairden and Armour, 1981; Hong et al., 1981), but also the first abattoir

346 survey on cattle helminths to include rumen fluke and co-infection in this region.

347 Although interpretation of these data should be cautious given the absence of information

348 on previous anthelmintic treatment and past grazing history, the prevalence of cattle

349 ostertagiasis reported in the current study was 89%, which is quite similar to that recorded in

350 previous European abattoir surveys (86% to 97%) (Agneessens et al., 2000; Borgsteede et al.,

351 2000) and much higher than that observed in the current study for *F. hepatica* and adult

352 rumen fluke (29% and 25% respectively). Very few farms (3%) in the current study had cattle

353 with no evidence of abomasal lesions due to *Ostertagia spp.* compared with 52% and 36% of

354 farms without any presence of adult rumen fluke and liver fluke lesions, respectively. These

355 results confirm the predominance and ubiquity of *Ostertagia spp.* infection among cattle

356   farms in England and Wales (Hong et al., 1981), mainly related to the relatively simple direct

357   life-cycle of this parasite compared with the indirect life-cycles of the two trematodes

358   (McCann et al., 2010b; Gordon et al., 2013). The estimate of prevalence of adult rumen fluke

359   in the current study at 25% is quite similar to that previously recorded in cattle at slaughter in

360   mainland Europe (Szmidt-Adjide et al., 2000; Gonzalez-Warleta et al., 2013; Malrait et al.,

361   2015) and confirms the establishment of this trematode in the UK (Gordon et al., 2013). A

362   higher prevalence (52%) of adult rumen fluke was recently recorded in a similar study in the

363   Republic of Ireland (ROI) (Toolan et al., 2015) and could be attributed to differences in

364   environment and cattle production systems (Murphy et al., 2006; Toolan et al., 2015).

365   Overall, 29% of the cattle were infected with liver fluke. The only similar abattoir survey

366   conducted in Great Britain was more than forty years ago (Froyd, 1975). Given the expected

367   huge variability in climate conditions and the important changes that occurred in UK

368   livestock farming since the eighties, comparison of both studies is difficult. However, there

369   has been evidence of a recent spread in the UK of liver fluke infection in cattle (Pritchard et

370   al., 2005).

371   All the specimens of adult rumen fluke isolated were identified as *C. daubneyi* and not *P.*

372   *cervi,* which was previously assumed to be the predominant rumen fluke species in the

373   British Isles (Gordon et al., 2013). Despite this, the possibility of other species being present

374   in England and Wales cannot be excluded, given that only two carcasses were sampled for

375   adult rumen fluke speciation. However, this result complements previous work conducted in

376   Scotland and Ireland (Gordon et al., 2013; Zintl et al., 2014) and emphasizes the importance,

377   if not predominance, of *C. daubneyi* in the UK, as it is in mainland Europe (Szmidt-Adjide et

378   al., 2000; Gonzalez-Warleta et al., 2013; Gordon et al., 2013).

379   In the current study, 39% of the carcasses had signs of co-infection. The similar

380   environmental requirements and common microclimate and microhabitat shared by the three

381   helminths and their intermediate hosts may explain some of the animals' co-infection, but

382   not entirely (Viney and Graham, 2013). As for instance, cattle anthelmintic or management

383   practices on farms may generate different patterns of co-infection (Gordon et al., 2013).

384   However, this information was not currently available to explore any patterns. The presence

385   of adult rumen fluke was significantly associated with the presence of liver fluke lesions.

386   Because both helminths have very similar life cycles and both *F. hepatica* and *C. daubneyi*

387   can share the same intermediary host *Galba truncatula* (Zintl et al., 2014), it has been

388   suggested that cattle infected with one fluke could simultaneously be infected with the other

389   (Gordon et al., 2013). Although the presence of both fluke species was associated, only half

390   of the animals (102/219) infected in the current study with adult rumen fluke had signs of

391   liver fluke lesions. As reported previously, different lymnaeid communities can act as

392   intermediate hosts for the two helminths and in the UK snails other than *Galba truncatula*

393   may play an important role as intermediate host (Dreyfuss et al., 2014). Under these

394   circumstances, competition between either the parasites or the intermediate hosts, especially

395   for food in colonized habitat, could explain the predominance of such fluke single-infections

396   (Dreyfuss et al., 2014). These results raise questions on the current dynamic of helminth

397   infections in cattle in the UK and the need to fully understand host-helminths interactions and

398   co-evolution, especially in the context of specific helminth poly-infections (Gasbarre, 1997;

399   Viney and Graham, 2013).

400   As previously reported in the literature (Myers and Taylor, 1989; McCann et al., 2010a),

401   there was a significantly higher risk of carcass helminth infection/lesions in October-January,

402   compared to March-June, which could be related to the specific life cycles of the three

403   helminths. It is also possible that exposure of animals slaughtered in March-June was

404   reduced, given, in the UK, animals are often housed in the winter and beef cattle often

405   undergo a two-month fattening period while housed before slaughter (AHDB, 2009). Unlike

406   this study, the seasonality of *Ostertagia spp.* was not reported in a similar beef study

407   (Charlier et al., 2009), which we could be attributable to its study design and lack of test

408   specificity of the diagnostic ELISA test used.

409   After controlling for breed, cows were less likely to present *Ostertagia spp.* lesions and

410   adult rumen fluke, but more likely to present liver fluke lesions compared to heifers and

411   steers. In both cases, this is likely to be related to the development of some host immunity

412   that, for both *Ostertagia spp.* (Gasbarre, 1997) and rumen fluke (Diaz et al., 2006), would

413   reduce the worm burden and for liver fluke would cause liver fibrosis, enabling the

414   maintenance of the infection (Mendes et al., 2013).

415   Presence of liver fluke lesion solely compared with no lesion was only significantly

416   associated with lower conformation, but neither CCW nor fat classification as reported in

417   previous similar study (Sanchez-Vazquez and Lewis, 2013). There are several studies that

418   have failed to demonstrate effect of liver fluke infection on cattle growth rate and there is a

419   possibility that *F. hepatica* may alter host performance through mechanisms other than body

420   weight (Loyacano et al., 2002; Charlier et al., 2009). The study by Sanchez-Vazquez and

421   Lewis (2013) reported small significant negative effects of liver fluke on CCW and fat

422   classification. There is possibility that this effect observed in their study could be attributed to

423   the impact of presence of other co-infections that were not investigated, especially, given in

424   the current study, liver fluke in combination with *Ostertagia spp.* and rumen fluke did have

425   an impact on CCW. The current results on *Ostertagia spp.* single effect on CCW and fat

426   classification agree with previous intervention studies on beef cattle (Suarez et al., 1991;

18

427   Loyacano et al., 2002) but contradict a recent abattoir survey in which no similar association

428   was reported, though there was an effect on conformation (Charlier et al., 2009). It is likely

429   in this case that the lower specificity of *O. ostertagi* ELISA used in the latter study, combined

430   with the inclusion of only adult cows and the non-control of other helminth infections in the

431   model may explain such differences. Our model results suggest that compared to no lesion

432   negative impact of *Ostertagia spp* on CCW was higher on average (coefficient values) when

433   present along with the other two parasites. It is possible, as reported in a previous study that

434   gastro-intestinal nematodes and liver fluke impact on host performance through different

435   mechanisms and that if present simultaneously the resulting effect might be additive on the

436   CCW (Loyacano et al., 2002). Further research would need to be conducted to confirm this

437   hypothesis.

438   To our knowledge, there has not been any study on the effect of adult rumen fluke on

439   carcass weight and classification. In the current study, there was significant negative

440   association between rumen fluke and CCW and fat classification. Compared to carcasses with

441   no lesion this effect was seen when rumen fluke was present on its own or along with both

442   *Ostertagia spp* and liver fluke. These results bring into question the widely held view in

443   Europe that adult rumen fluke are relatively benign and well tolerated by their host, contrary

444   to tropical regions where its high pathogenicity was confirmed (Zintl et al., 2014; Fuertes et

445   al., 2015). Given in the current study there were only few animals solely infected by rumen

446   fluke, there is a need of further investigations into pathogenicity of adult rumen fluke in

447   cattle. In addition, what cannot be ascertained in the current study is whether any of the

448   animals that were positive for adult rumen fluke may also have been infected with juvenile

449   fluke in the duodenum; these stages are known to be highly pathogenic when present in large

450   numbers (Millar et al., 2012).

451 Although highly specific, meat inspection is considered as a poorly sensitive diagnostic

452 tool (Rapsch et al., 2006; Sanchez-Vazquez and Lewis, 2013), which is likely to

453 underestimate the prevalence estimates. Moreover, only the presence/lesions of adult

454 parasites but not juveniles were screened in the current study, which also may have led to

455 underestimation of prevalence. However, this underestimation is less likely to effect the

456 observed associations and co-infection patterns. This cross-sectional study provides us with

457 associations between various factors and presence of helminths but does not infer causality.

458 During this study, steps were taken to minimise bias by validating the feasibility and

459 reliability of the scoring system in a pilot study and by maintaining throughout the study the

460 same group of operators for scoring. Though the study was only conducted on one abattoir

461 limiting its generalisability, this abattoir is one of the largest abattoir in England with a

462 relatively high throughput. The farms were localised in 23 counties and given the study

463 sampling occurred throughout the year, it was possible to include different types of cattle

464 production systems. Finally, the study sample demographic agreed with a recent survey on

465 the general characteristics of the British beef production cattle (Pritchard et al., 2013). As a

466 conclusion, the current study provided a good picture of *Ostertagia spp.,* rumen fluke and

467 liver fluke prevalence/intensity, associated factors and production impacts on cattle in

468 England and Wales.

469

470 **Conflict of interest**

471 The authors declare that they have no competing interests.

472

473 **Acknowledgments**

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477 **References**

478 Agneessens, J., Claerebout, E., Dorny, P., Borgsteede, F.H., Vercruysse, J., 2000. Nematode

479   parasitism in adult dairy cows in Belgium. Vet. Parasitol. 90, 83-92.

480 AHDB, 2009. In the balance: the future of the English beef industry. AHDB Beef and Lamb

481   Report. 24 pp.

482 Bairden, K., Armour, J., 1981. A survey of abomasal parasitism in dairy and beef cows in

483   south-west Scotland. Vet. Rec. 109, 153-155.

484 Borgsteede, F.H., Tibben, J., Cornelissen, J.B., Agneessens, J., Gaasenbeek, C.P., 2000.

485   Nematode parasites of adult dairy cattle in the Netherlands. Vet. Parasitol. 89, 287-

486   296.

487 Burrows, R.O., Davison, C.C., Best, P.J., 1980. Survey of abomasal parasitism of culled dairy

488   cows in southern Britain. Vet. Rec. 107, 289-290.

489 Charlier, J., De Cat, A., Forbes, A., Vercruysse, J., 2009. Measurement of antibodies to

490   gastrointestinal nematodes and liver fluke in meat juice of beef cattle and associations

491   with carcass parameters. Vet. Parasitol. 166, 235-240.

492 Charlier, J., van der Voort, M., Kenyon, F., Skuce, P., Vercruysse, J., 2014. Chasing

493   helminths and their economic impact on farmed ruminants. Trends Parasitol. 30, 361-

494   367.

495 DEFRA, 2014. CTS updated breed code list. Guidance on keeping cattle, bison and buffalo in

496   Great Britain. 68 pp.

497 Diaz, P., Lomba, C., Pedreira, J., Arias, M., Sanchez-Andrade, R., Suarez, J.L., Diez-Banos,

498   P., Morrondo, P., Paz-Silva, A., 2006. Analysis of the IgG antibody response against

499   *Paramphistomidae trematoda* in naturally infected cattle. Application to serological

500   surveys. Vet. Parasitol. 140, 281-288.

501   Dohoo, I., Martin, W., Stryhn, H., 2009. Veterinary epidemiologic research VER Inc., 865

502   pp.

503   Dreyfuss, G., Vignoles, P., Rondelaud, D., 2014. *Fasciola hepatica* and *Paramphistomum*

504   *daubneyi*: decrease in prevalence of natural infection in habitats colonized by *Galba*

505   *truncatula* and *Lymnaea glabra*. Rev. Med. Vet 165, 160-166.

506   FAO, 2009. How to feed the world in 2050. 35 pp.

507   Froyd, G., 1975. Liver fluke in Great Britain: a survey of affected livers. Vet. Rec. 97, 492-

508   495.

509   Fuertes, M., Perez, V., Benavides, J., Gonzalez-Lanza, M.C., Mezo, M., Gonzalez-Warleta,

510   M., Giraldez, F.J., Fernandez, M., Manga-Gonzalez, M.Y., Ferreras, M.C., 2015.

511   Pathological changes in cattle naturally infected by *Calicophoron daubneyi* adult

512   flukes. Vet. Parasitol. 209, 188-196.

513   Gasbarre, L.C., 1997. Effects of gastrointestinal nematode infection on the ruminant immune

514   system. Vet. Parasitol. 72, 327-337; discussion 337-343.

515   Gonzalez-Warleta, M., Lladosa, S., Castro-Hermida, J.A., Martinez-Ibeas, A.M., Conesa, D.,

516   Munoz, F., Lopez-Quilez, A., Manga-Gonzalez, Y., Mezo, M., 2013. Bovine

517   paramphistomosis in Galicia (Spain): prevalence, intensity, aetiology and geospatial

518   distribution of the infection. Vet. Parasitol. 191, 252-263.

519 Gordon, D.K., Roberts, L.C., Lean, N., Zadoks, R.N., Sargison, N.D., Skuce, P.J., 2013.

520   Identification of the rumen fluke, *Calicophoron daubneyi*, in GB livestock: possible

521   implications for liver fluke diagnosis. Vet. Parasitol. 195, 65-71.

522 Hong, C., Lancaster, M.B., Michel, J.F., 1981. Worm burdens of dairy heifers in England and

523   Wales. Vet. Rec. 109, 12-14.

524 Larraillet, L., Forbes, A.B., Pravieux, J.J., 2012. Abattoir survey of abomasal lesions

525   associated with ostertagiosis in adult cattle. Vet. Rec. 171, 299.

526 Loyacano, A.F., Williams, J.C., Gurie, J., DeRosa, A.A., 2002. Effect of gastrointestinal

527   nematode and liver fluke infections on weight gain and reproductive performance of

528   beef heifers. Vet. Parasitol. 107, 227-234.

529 Malrait, K., Verschave, S., Skuce, P., Van Loo, H., Vercruysse, J., Charlier, J., 2015. Novel

530   insights into the pathogenic importance, diagnosis and treatment of the rumen fluke

531   (*Calicophoron daubneyi*) in cattle. Vet. Parasitol. 207, 134-139.

532 McCann, C.M., Baylis, M., Williams, D.J., 2010a. The development of linear regression

533   models using environmental variables to explain the spatial distribution of *Fasciola*

534   *hepatica* infection in dairy herds in England and Wales. Int. J. Parasitol. 40, 1021-

535   1028.

536 McCann, C.M., Baylis, M., Williams, D.J., 2010b. Seroprevalence and spatial distribution of

537   *Fasciola hepatica*-infected dairy herds in England and Wales. Vet. Rec. 166, 612-617.

538 Mendes, E.A., Mendes, T.A., dos Santos, S.L., Menezes-Souza, D., Bartholomeu, D.C.,

539   Martins, I.V., Silva, L.M., Lima Wdos, S., 2013. Expression of IL-4, IL-10 and IFN-

540   gamma in the liver tissue of cattle that are naturally infected with *Fasciola hepatica*.

541   Vet. Parasitol. 195, 177-182.

542   Millar, M., Colloff, A., Scholes, S., 2012. Disease associated with immature paramphistome

543   infection. Vet. Rec. 171, 509-510.

544   Murphy, T.M., Fahy, K.N., McAuliffe, A., Forbes, A.B., Clegg, T.A., O'Brien, D.J., 2006. A

545   study of helminth parasites in culled cows from Ireland. Prev. Vet. Med. 76, 1-10.

546   Myers, G.H., Taylor, R.F., 1989. Ostertagiasis in cattle. J. Vet. Diagn. Invest. : official

547   publication of the American Association of Veterinary Laboratory Diagnosticians, Inc

548   1, 195-200.

549   Pritchard, G.C., Forbes, A.B., Williams, D.J., Salimi-Bejestani, M.R., Daniel, R.G., 2005.

550   Emergence of fasciolosis in cattle in East Anglia. Vet. Rec. 157, 578-582.

551   Pritchard, T., Wall, E., Moore, K., Coffey, M., 2013. Feasibility of using abattoir generated

552   data and BCMS records for carcass trait evaluations (Carcass Trait Evaluations).

553   SRUC Project.

554   Rapsch, C., Schweizer, G., Grimm, F., Kohler, L., Bauer, C., Deplazes, P., Braun, U.,

555   Torgerson, P.R., 2006. Estimating the true prevalence of *Fasciola hepatica* in cattle

556   slaughtered in Switzerland in the absence of an absolute diagnostic test. Int. J.

557   Parasitol. 36, 1153-1158.

558   Rasbash, J., Steele, F., Browne, W.J., Goldstein, H., 2009. A User's Guide to MLwiN.

559   Manual, Centre for Multilevel Modelling, University of Bristol. 306 pp.

560   Rinaldi, L., Perugini, A.G., Capuano, F., Fenizia, D., Musella, V., Veneziano, V., Cringoli,

561   G., 2005. Characterization of the second internal transcribed spacer of ribosomal

562   DNA of *Calicophoron daubneyi* from various hosts and locations in southern Italy.

563   Vet. Parasitol. 131, 247-253.

564   Sanchez-Vazquez, M.J., Lewis, F.I., 2013. Investigating the impact of fasciolosis on cattle

565   carcase performance. Vet. Parasitol. 193, 307-311.

566   Sargison, N.D., 2014. Sustainable helminth control practices in the United Kingdom. Small

567   Rumin. Res. 118, 35-40.

568   Suarez, V.H., Bedotti, D.O., Larrea, S., Busetti, M.R., Garriz, C.A., 1991. Effects of an

569   integrated control programme with ivermectin on growth, carcase composition and

570   nematode infection of beef cattle in Argentina's western pampas. Res. Vet. Sci. 50,

571   195-199.

572   Szmidt-Adjide, V., Abrous, M., Adjide, C.C., Dreyfuss, G., Lecompte, A., Cabaret, J.,

573   Rondelaud, D., 2000. Prevalence of *Paramphistomum daubneyi* infection in cattle in

574   central France. Vet. Parasitol. 87, 133-138.

575   Thornton, P.K., 2010. Livestock production: recent trends, future prospects. Philosophical

576   transactions of the Royal Society of London. Series B, Biolog. Sci. 365, 2853-2867.

577   Toolan, D.P., Mitchell, G., Searle, K., Sheehan, M., Skuce, P.J., Zadoks, R.N., 2015. Bovine

578   and ovine rumen fluke in Ireland-Prevalence, risk factors and species identity based

579   on passive veterinary surveillance and abattoir findings. Vet. Parasitol. 212, 168-174.

580   Viney, M.E., Graham, A.L., 2013. Patterns and processes in parasite co-infection. Adv.

581   Parasitol. 82, 321-369.

582   Zintl, A., Garcia-Campos, A., Trudgett, A., Chryssafidis, A.L., Talavera-Arce, S., Fu, Y.,

583   Egan, S., Lawlor, A., Negredo, C., Brennan, G., Hanna, R.E., De Waal, T., Mulcahy,

584   G., 2014. Bovine paramphistomes in Ireland. Vet. Parasitol. 204, 199-208.

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588   **Table 1.**

589   Cattle median [p25-p75] age, cold carcass weight (CCW), carcass conformation and fat

590   classifications and percentage of liver condemnations by category (N=972).

Variables (N)	Cows (193)	Heifers (155)	Steers (518)	Young Bulls (106)
Age (Months)	79 [56-113]	29 [26-31]	29 [26-31]	14 [14-15]
CCW (Kg)	323 [283-346]	314 [290-334]	344 [307-384]	294 [267-334]
Conformation	P^+ [P^+-O^+]	R [O^+-R]	O^+ [O^+-R]	O^+ [O^+-R]
Fat classification	3 [2-4L]	4L [3-4L]	3 [3-4L]	2 [2-3]
Liver condemnation	31.6	12.9	14.1	9.4

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593   **Table 2.**

594   Stratification of abomasa, reticulorumen and livers scoring percentages by cattle category

595   (N=972).

	Cows	Heifers	Steers	Young	Bulls	TOTAL
***Ostertagia spp.* lesion (N=933)**						
0- No lesion	16 (9)	12 (8)	65 (13)	12 (12)		105 (11)
1- ≤ 100	48 (28)	36 (25)	136 (26)	33 (32)		253 (27)
2- 101-1000	43 (25)	34 (23)	126 (25)	37 (36)		240 (26)
3- > 1000	65 (38)	64 (44)	186 (36)	20 (20)		335 (36)
Adult rumen fluke presence (N=936)						
0- No fluke	135 (77)	112 (76)	361 (70)	97 (95)		705 (75)
1- ≤100	17 (10)	23 (16)	75 (15)	4 (4)		119 (13)
2- > 100	23 (13)	12 (8)	76 (15)	1 (1)		112 (12)
Liver fluke lesion (N=951)						
0- No lesion	94 (51)	116 (75)	367 (72)	102 (98)		679 (72)
1- Moderate	62 (34)	32 (21)	128 (25)	1 (1)		223 (23)
2- Severe	28 (15)	6 (4)	14 (3)	1 (1)		49 (5)
***F. hepatica* presence (N=950)**						
0- No fluke	115 (63)	119 (77)	380 (75)	103 (99)		717 (76)
1- Actual presence	22 (12)	13 (9)	82 (16)	1 (1)		118 (12)
2- Historical presence	47 (25)	22 (14)	46 (9)	0 (0)		115 (12)

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598 **Table 3.**

599 Cattle level variables in multilevel multinomial models predicting cattle carcasses intensity of

600 *Ostertagia spp.* lesions (Model 1), adult rumen fluke presence (Model 2) and liver fluke

601 lesions (Model 3).

		Model 1: *Ostertagia spp.* lesions (933 Cattle)				Model 2 : adult rumen fluke presence (936 Cattle)			Model 3 : liver fluke lesions (951 Cattle)		
		None	<100	100-1000	>1000	None	≤ 100	> 100	None	Moderate	Severe
Variables	**Categories**	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Breed	**Pure dairy**	30 (29)	76 (30)	97 (41)	135 (40)	263 (37)	38 (32)	41 (37)	255 (38)	64 (29)	20 (41)
	Pure beef	9 (9)	22 (9)	21 (9)	26 (8)	54 (8)	14 (12)	10 (9)	55 (8)	25 (11)	3 (6)
	Beef X	65 (62)	145 (58)	106 (44)	152 (46)	354 (51)	59 (50)	54 (49)	331 (49)	123 (56)	24 (49)
	Dairy X	0 (0)	8 (3)	14 (6)	19 (6)	29 (4)	7 (6)	5 (5)	30 (5)	9 (4)	2 (4)
Category*	**Cow**	16 (15)	48 (19)	43 (18)	65 (19)	135 (19)	17 (14)	23 (20)	94 (14)	62 (28)	28 (57)
	Heifer	12 (11)	36 (14)	34 (14)	64 (19)	112 (16)	23 (19)	12 (11)	116 (17)	32 (14)	6 (12)
	Steer	65 (62)	136 (54)	126 (52)	186 (55)	361 (51)	75 (63)	76 (68)	367 (54)	128 (57)	14 (29)
	Young Bull	12 (11)	33 (13)	37 (15)	20 (6)	97 (14)	4 (3)	1 (1)	102 (15)	1 (1)	1 (2)
Age (Month)	**<24**	29 (28)	53(21)	59 (25)	41 (12)	165 (23)	13 (11)	4 (1)	166 (25)	17 (8)	2 (4)
	24-30	46 (44)	91 (36)	82 (34)	137 (41)	264 (38)	58 (48)	34 (11)	273 (40)	79 (35)	7 (14)
	>30	30 (28)	110 (43)	99 (41)	158 (47)	277 (39)	49 (41)	274 (88)	240 (35)	129 (57)	40 (82)
Month	**March**	46 (44)	62 (25)	72 (30)	111 (33)	238 (34)	35 (29)	16 (14)	232 (34)	57 (25)	8 (16)
	June	33 (31)	74 (29)	49 (20)	74 (22)	173 (24)	31 (26)	27 (24)	166 (24)	58 (26)	8 (16)
	January	23 (22)	72 (28)	60 (25)	59 (18)	148 (21)	26 (22)	40 (36)	148 (22)	53 (24)	21 (43)
	October	3 (3)	46 (18)	59 (25)	92 (27)	147 (21)	28 (23)	29 (26)	133 (20)	57 (25)	12 (25)
Altitude (m)	**≤60**	-	-	-	-	194 (31)	49 (45)	43 (48)	187 (30)	79 (43)	18 (46)
	>60	-	-	-	-	438 (69)	60 (55)	46 (52)	432 (70)	104 (57)	21 (54)
O (#)	**None**	-	-	-	-	89 (13)	5 (4)	6 (5)	79 (12)	23 (11)	1 (2)
	Present	-	-	-	-	613 (87)	111 (96)	106 (95)	576 (88)	193 (89)	44 (98)
RF (#)	**None**	89 (89)	180 (71)	185 (77)	248 (74)	-	-	-	540 (82)	128(60)	27 (59)
	Present	11 (11)	74 (39)	55 (33)	88 (26)	-	-	-	120 (18)	85 (40)	19 (41)
F. hepatica **(#)**	**None**	79 (77)	170 (69)	158 (68)	248 (75)	540(78)	73 (62)	47 (44)	-	-	-
	Present	24 (23)	77 (31)	76 (32)	84 (25)	155 (22)	44 (38)	60 (56)	-	-	-

602 * Mature bull excluded; # O = *Ostertagia spp.* lesions; RF= presence of adult rumen fluke; *F. hepatica* =
603 presence of liver fluke
604

605 **Table 4.**

606 Final multilevel multinomial models predicting cattle carcasses intensity of *Ostertagia spp.* lesions (Model 1), adult rumen fluke presence

607 (Model 2) and liver fluke lesions (Model 3), containing cow and herd as random effects and general demographic and carcass parameters

608 as fixed effects with respectively no pathology (Model 1 and 3) and no worm (Model 2) as a reference [CCW = Cold Carcass Weight; X

609 = Cross].

Variables Categories		Model 1: *Ostertagia spp.* lesions (154 Herds, 933 cattle, 2697 Obs.) [a,b,c]						Model 2: Adult rumen fluke presence (153 Herds, 936 cattle, 1584 Obs.) [a,b,c]				Model 3: liver fluke lesions (153 Herds, 951 cows, 1584 Obs.) [a,b,c]			
		<100		100-1000		>1000		≤ 100		>100		Moderate		Severe	
		O.R	95% C.I.	O.R	95% C.I.	O.R	95% C.I.	O.R	95% C.I.	O.R	95% C.I.	O.R	95% C.I.	O.R	95% C.I.
Breed	**Pure**	Baseline						Baseline				Baseline			
	Pure beef	1.40	0.86-2.27	0.79	0.46-1.34	0.69	0.44-1.09	1.87	0.87-4.00	1.73	0.69-4.35	1.99	1.00-3.96	0.92	0.20-4.32
	Beef X	1.14	0.83-1.55	0.49*	0.35-0.69	0.45*	0.34-0.61	0.91	0.53-1.56	1.13	0.64-2.02	2.30*	1.46-3.64	3.18*	1.42-7.11
	Dairy X	7.29*	4.48-11.88	8.63*	5.16-14.42	6.20*	3.92-9.78	2.03	0.80-5.11	1.01	0.29-3.51	1.03	0.36-2.96	0.79	0.09-7.33
Category	**Cow**	Baseline						Baseline				Baseline			
	Heifer	2.16*	1.35-3.45	4.34*	2.52-7.46	7.11*	4.38-11.53	2.55*	1.07-6.12	2.15	0.81-5.70	0.43*	0.21-0.86	0.08*	0.01-0.41
	Steer	1.24	0.84-1.85	2.06*	1.32-3.20	2.54*	1.72-3.75	2.51*	1.20-5.28	3.95*	1.91-8.18	0.64	0.38-1.10	0.13*	0.05-0.33
	Young	2.08	0.99-4.37	3.15*	1.48-6.66	2.01	0.95-4.22	0.92	0.21-3.93	1.21	0.11-13.94	0.04*	0.01-0.38	0.14	0.01-2.80
Age (months)	**<24**	Baseline						Baseline				Baseline			
	24-30	1.59	0.93-2.71	1.60	0.95-2.70	2.82*	1.70-4.67	1.50	0.68-3.31	3.08	0.88-10.72	1.07	0.54-2.10	0.66	0.06-6.78
	>30	2.72*	1.56-4.75	2.27*	1.31-3.94	4.40*	2.59-7.46	1.35	0.57-3.16	5.48*	1.56-19.21	1.87	0.92-3.80	3.75	0.43-32.94
Month	**March**	0.08*	0.05-0.12	0.07*	0.04-0.10	0.06*	0.04-0.08	Baseline				Baseline			
	June	0.11*	0.07-0.16	0.05*	0.03-0.08	0.04*	0.03-0.05	1.24	0.69-2.23	2.32*	1.13-4.75	1.09	0.66-1.82	1.25	0.36-4.34
	January	Baseline						1.11	0.58-2.12	2.82*	1.34-5.92	1.75*	1.04-2.94	3.20*	1.16-8.86
	October	0.20*	0.13-0.29	0.09*	0.06-0.14	0.06*	0.04-0.09	2.01*	1.08-3.72	4.45*	2.12-9.38	2.06*	1.21-3.50	1.81	0.63-5.17
Altitude (m)	**≤60**	-	-	-	-	-	-	Baseline				Baseline			

		OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
	>60	-	-	-	-	-	-	0.58*	0.36-0.92	0.44*	0.26-0.72	0.56*	0.38-0.82	0.63	0.29-1.33
O [(*)]	**None**	-	-	-	-	-	-	*Baseline*				*Baseline*			
	Present	-	-	-	-	-	-	2.40	0.93-6.18	1.51	0.58-43.94	0.90	0.49-1.65	3.42	0.41-28.22
RF [(*)]	**None**	*Baseline*						-	-	-	-	*Baseline*			
	Present	3.01*	2.27-4.00	1.92*	1.38-2.67	2.27*	1.70-3.03	-	-	-	-	2.71*	1.83-4.02	4.08*	1.95-8.50
F. hepatica [(*)]	**None**	*Baseline*						*Baseline*				-	-	-	-
	Present	1.06	0.80-1.41	1.57*	1.13-2.19	0.92	0.68-1.25	1.79*	1.08-2.96	3.21*	1.93-5.34	-	-	-	-

610   OR - Odds Ratio; 95% CI - 95% Confidence Interval; [*] O = *Ostertagia spp*. lesions; RF= presence adult rumen fluke; *F. hepatica* = presence of liver fluke

611 **Table 5.**

612 Final multilevel linear regression models predicting impacts on carcass parameters,

613 respectively Cold Carcass Weight (Model 1), Conformation (Model 2) and Fat classification

614 (Model 3), containing cow and herd as random effects and cattle parameters and helminths

615 scoring as fixed effects [CCW = Cold Carcass Weight; Obs. = Observations; * =

616 Significant].

			Model 1: CCW (115 Herds, 756 cattle, 618 Obs.)			Model 2: Conformation (115 Herds, 756 cattle, 709 Obs.)			Model 3: Fat classification (115 Herds, 756 cattle, 630 Ob...		
Fixed effects											
Variables	**Categories**	**N**	**β**	**95% C.I.**		**N**	**β**	**95% C.I.**	**N**	**β**	**95% C.I.**
Intercept (SE)			295.35 (12.49)				14.15 (2.25)			28.30(1.63)	
Helminth Inf. [*#]	**None**	64	*Baseline*			64	*Baseline*		64	*Baseline*	
	O only	401	-30.58*	-50.92;-10.24		401	1.13	-0.53;2.78	401	-3.28*	-5.56;-1.00
	RF only	6	-50.34*	-88.50;-12.18		6	2.41	-1.27;6.09	6	-5.49*	-10.28;-0.69
	LF only	11	-20.39	-50.76;9.98		11	-3.65*	-6.98;-0.32	11	-1.41	-5.71;2.89
	O-RF	102	-39.99*	-73.09;-6.88		102	-1.69	-4.36;0.98	102	-1.72	-5.57;2.14
	O-LF	80	-22.94	-52.89;7.01		80	-1.26	-3.65;1.12	80	-0.35	-3.91;3.21
	RF-LF	4	-32.41	-73.06;8.24		4	3.48	-0.66;7.64	4	-4.85	-10.19;0.49
	O-RF-LF	57	-48.28*	-88.35;-8.21		57	-1.27	-4.68;2.14	57	-3.81	-8.61;0.99
Random effects											
	Level		Variance	SE			Variance	SE		Variance	SE
	Herd		561.42	101.81			2.31	0.68		4.45	1.26
	Cattle		844.80	56.10			13.34	0.803		20.98	1.34

617 [*] Breed, category, age, CCW, conformation, fat, month and altitude were included in model as confounders,
618 and results presented adjusted for these variables; [#] O = *Ostertagia spp.* lesions; RF= presence of adult rumen
619 fluke; LF= liver fluke lesions

620

621

622

623

1 **Tables**

2

3 **Table 1.**

4 Cattle median [p25-p75] age, cold carcass weight (CCW), carcass conformation and fat
5 classifications and percentage of liver condemnations by category (N=972).

6

7 **Table 2.**

8 Stratification of abomasa, reticulorumen and livers scoring percentages by cattle category
9 (N=972).

10

11 **Table 3.**

12 Cattle level variables in multilevel multinomial models predicting cattle carcasses intensity of
13 *Ostertagia spp.* lesions (Model 1), adult rumen fluke presence (Model 2) and liver fluke
14 lesions (Model 3).

15

16 **Table 4.**

17 Final multilevel multinomial models predicting cattle carcasses intensity of *Ostertagia spp.*
18 lesions (Model 1), adult rumen fluke presence (Model 2) and liver fluke lesions (Model 3),
19 containing cow and herd as random effects and general demographic and carcass parameters
20 as fixed effects with respectively no pathology (Model 1 and 3) and no worm (Model 2) as a
21 reference [CCW = Cold Carcass Weight; X = Cross].

22

23 **Table 5.**

24 Final multilevel linear regression models predicting impacts on carcass parameters,
25 respectively Cold Carcass Weight (Model 1), Conformation (Model 2) and Fat classification
26 (Model 3), containing cow and herd as random effects and cattle parameters and helminths
27 scoring as fixed effects [CCW = Cold Carcass Weight; Obs. = Observations; * =
28 Significant].

29   **Table 1.**

Variables (N)	Cows (193)	Heifers (155)	Steers (518)	Young Bulls (106)
Age (Months)	79 [56-113]	29 [26-31]	29 [26-31]	14 [14-15]
CCW (Kg)	323 [283-346]	314 [290-334]	344 [307-384]	294 [267-334]
Conformation	P$^+$ [P$^+$-O$^+$]	R [O$^+$-R]	O$^+$ [O$^+$-R]	O$^+$ [O$^+$-R]
Fat classification	3 [2-4L]	4L [3-4L]	3 [3-4L]	2 [2-3]
Liver condemnation	31.6	12.9	14.1	9.4

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31

32   **Table 2.**

	Cows	Heifers	Steers	Young	Bulls	TOTAL
***Ostertagia spp.* lesion (N=933)**						
0- No lesion	16 (9)	12 (8)	65 (13)	12 (12)		105 (11)
1- ≤ 100	48 (28)	36 (25)	136 (26)	33 (32)		253 (27)
2- 101-1000	43 (25)	34 (23)	126 (25)	37 (36)		240 (26)
3- > 1000	65 (38)	64 (44)	186 (36)	20 (20)		335 (36)
Adult rumen fluke presence (N=936)						
0- No fluke	135 (77)	112 (76)	361 (70)	97 (95)		705 (75)
1- ≤100	17 (10)	23 (16)	75 (15)	4 (4)		119 (13)
2- > 100	23 (13)	12 (8)	76 (15)	1 (1)		112 (12)
Liver fluke lesion (N=951)						
0- No lesion	94 (51)	116 (75)	367 (72)	102 (98)		679 (72)
1- Moderate	62 (34)	32 (21)	128 (25)	1 (1)		223 (23)
2- Severe	28 (15)	6 (4)	14 (3)	1 (1)		49 (5)
***F. hepatica* presence (N=950)**						
0- No fluke	115 (63)	119 (77)	380 (75)	103 (99)		717 (76)
1- Actual presence	22 (12)	13 (9)	82 (16)	1 (1)		118 (12)
2- Historical presence	47 (25)	22 (14)	46 (9)	0 (0)		115 (12)

33

34

Table 3.

Variables	Categories	Model 1: *Ostertagia spp.* lesions (933 Cattle)				Model 2 : adult rumen fluke presence (936 Cattle)			Model 3 : liver fluke lesions (951 Cattle)		
		None	<100	100-1000	>1000	None	≤ 100	> 100	None	Moderate	Severe
		N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Breed	Pure dairy	30 (29)	76 (30)	97 (41)	135 (40)	263 (37)	38 (32)	41 (37)	255 (38)	64 (29)	20 (41)
	Pure beef	9 (9)	22 (9)	21 (9)	26 (8)	54 (8)	14 (12)	10 (9)	55 (8)	25 (11)	3 (6)
	Beef X	65 (62)	145 (58)	106 (44)	152 (46)	354 (51)	59 (50)	54 (49)	331 (49)	123 (56)	24 (49)
	Dairy X	0 (0)	8 (3)	14 (6)	19 (6)	29 (4)	7 (6)	5 (5)	30 (5)	9 (4)	2 (4)
Category*	Cow	16 (15)	48 (19)	43 (18)	65 (19)	135 (19)	17 (14)	23 (20)	94 (14)	62 (28)	28 (57)
	Heifer	12 (11)	36 (14)	34 (14)	64 (19)	112 (16)	23 (19)	12 (11)	116 (17)	32 (14)	6 (12)
	Steer	65 (62)	136 (54)	126 (52)	186 (55)	361 (51)	75 (63)	76 (68)	367 (54)	128 (57)	14 (29)
	Young Bull	12 (11)	33 (13)	37 (15)	20 (6)	97 (14)	4 (3)	1 (1)	102 (15)	1 (1)	1 (2)
Age (Month)	<24	29 (28)	53 (21)	59 (25)	41 (12)	165 (23)	13 (11)	4 (1)	166 (25)	17 (8)	2 (4)
	24-30	46 (44)	91 (36)	82 (34)	137 (41)	264 (38)	58 (48)	34 (11)	273 (40)	79 (35)	7 (14)
	>30	30 (28)	110 (43)	99 (41)	158 (47)	277 (39)	49 (41)	274 (88)	240 (35)	129 (57)	40 (82)
Month	March	46 (44)	62 (25)	72 (30)	111 (33)	238 (34)	35 (29)	16 (14)	232 (34)	57 (25)	8 (16)
	June	33 (31)	74 (29)	49 (20)	74 (22)	173 (24)	31 (26)	27 (24)	166 (24)	58 (26)	8 (16)
	January	23 (22)	72 (28)	60 (25)	59 (18)	148 (21)	26 (22)	40 (36)	148 (22)	53 (24)	21 (43)
	October	3 (3)	46 (18)	59 (25)	92 (27)	147 (21)	28 (23)	29 (26)	133 (20)	57 (25)	12 (25)
Altitude (m)	≤60	-	-	-	-	194 (31)	49 (45)	43 (48)	187 (30)	79 (43)	18 (46)
	>60	-	-	-	-	438 (69)	60 (55)	46 (52)	432 (70)	104 (57)	21 (54)
O [#]	None	-	-	-	-	89 (13)	5 (4)	6 (5)	79 (12)	23 (11)	1 (2)
	Present	-	-	-	-	613 (87)	111 (96)	106 (95)	576 (88)	193 (89)	44 (98)
RF [#]	None	89 (89)	180 (71)	185 (77)	248 (74)	-	-	-	540 (82)	128 (60)	27 (59)
	Present	11 (11)	74 (39)	55 (33)	88 (26)	-	-	-	120 (18)	85 (40)	19 (41)
F. hepatica [#]	None	79 (77)	170 (69)	158 (68)	248 (75)	540 (78)	73 (62)	47 (44)	-	-	-
	Present	24 (23)	77 (31)	76 (32)	84 (25)	155 (22)	44 (38)	60 (56)	-	-	-

36   * Mature bull excluded; [#] O = *Ostertagia spp.* lesions; RF= presence of adult rumen fluke; *F. hepatica* =
37   presence of liver fluke
38

39 **Table 4.**

Variables Categories		Model 1: *Ostertagia spp.* lesions (154 Herds, 933 cattle, 2697 Obs.) [a,b,c]						Model 2: Adult rumen fluke presence (153 Herds, 936 cattle, 1584 Obs.) [a,b,c]				Model 3: liver fluke lesions (153 Herds, 951 cows, 1584 Obs.) [a,b,c]			
		<100		100-1000		>1000		≤ 100		>100		Moderate		Severe	
		O.R	95% C.I.	O.R	95% C.I.	O.R	95% C.I.	O.R	95% C.I.	O.R	95% C.I.	O.R	95% C.I.	O.R	95% C.I.
Breed	**Pure**	Baseline						Baseline				Baseline			
	Pure beef	1.40	0.86-2.27	0.79	0.46-1.34	0.69	0.44-1.09	1.87	0.87-4.00	1.73	0.69-4.35	1.99	1.00-3.96	0.92	0.20-4.32
	Beef X	1.14	0.83-1.55	0.49*	0.35-0.69	0.45*	0.34-0.61	0.91	0.53-1.56	1.13	0.64-2.02	2.30*	1.46-3.64	3.18*	1.42-7.11
	Dairy X	7.29*	4.48-11.88	8.63*	5.16-14.42	6.20*	3.92-9.78	2.03	0.80-5.11	1.01	0.29-3.51	1.03	0.36-2.96	0.79	0.09-7.33
Category	**Cow**	Baseline						Baseline				Baseline			
	Heifer	2.16*	1.35-3.45	4.34*	2.52-7.46	7.11*	4.38-11.53	2.55*	1.07-6.12	2.15	0.81-5.70	0.43*	0.21-0.86	0.08*	0.01-0.41
	Steer	1.24	0.84-1.85	2.06*	1.32-3.20	2.54*	1.72-3.75	2.51*	1.20-5.28	3.95*	1.91-8.18	0.64	0.38-1.10	0.13*	0.05-0.33
	Young	2.08	0.99-4.37	3.15*	1.48-6.66	2.01	0.95-4.22	0.92	0.21-3.93	1.21	0.11-13.94	0.04*	0.01-0.38	0.14	0.01-2.80
Age (months)	**<24**	Baseline						Baseline				Baseline			
	24-30	1.59	0.93-2.71	1.60	0.95-2.70	2.82*	1.70-4.67	1.50	0.68-3.31	3.08	0.88-10.72	1.07	0.54-2.10	0.66	0.06-6.78
	>30	2.72*	1.56-4.75	2.27*	1.31-3.94	4.40*	2.59-7.46	1.35	0.57-3.16	5.48*	1.56-19.21	1.87	0.92-3.80	3.75	0.43-32.94
Month	**March**	0.08*	0.05-0.12	0.07*	0.04-0.10	0.06*	0.04-0.08	Baseline				Baseline			
	June	0.11*	0.07-0.16	0.05*	0.03-0.08	0.04*	0.03-0.05	1.24	0.69-2.23	2.32*	1.13-4.75	1.09	0.66-1.82	1.25	0.36-4.34
	January	Baseline						1.11	0.58-2.12	2.82*	1.34-5.92	1.75*	1.04-2.94	3.20*	1.16-8.86
	October	0.20*	0.13-0.29	0.09*	0.06-0.14	0.06*	0.04-0.09	2.01*	1.08-3.72	4.45*	2.12-9.38	2.06*	1.21-3.50	1.81	0.63-5.17
Altitude (m)	**≤60**	-	-	-	-	-	-	Baseline				Baseline			
	>60	-	-	-	-	-	-	0.58*	0.36-0.92	0.44*	0.26-0.72	0.56*	0.38-0.82	0.63	0.29-1.33
O [(*)]	**None**	-	-	-	-	-	-	Baseline				Baseline			
	Present	-	-	-	-	-	-	2.40	0.93-6.18	1.51	0.58-43.94	0.90	0.49-1.65	3.42	0.41-28.22
RF [(*)]	**None**	Baseline						-	-	-	-	Baseline			
	Present	3.01*	2.27-4.00	1.92*	1.38-2.67	2.27*	1.70-3.03	-	-	-	-	2.71*	1.83-4.02	4.08*	1.95-8.50
F. hepatica [(*)]	**None**	Baseline						Baseline				-	-	-	-
	Present	1.06	0.80-1.41	1.57*	1.13-2.19	0.92	0.68-1.25	1.79*	1.08-2.96	3.21*	1.93-5.34	-	-	-	-

40 OR - Odds Ratio; 95% CI - 95% Confidence Interval; [*] O = *Ostertagia spp.* lesions; RF= presence adult rumen fluke; *F. hepatica* = presence of liver fluke

Table 5.

Fixed effects											
		Model 1: CCW (115 Herds, 756 cattle, 618 Obs.)			**Model 2: Conformation (115 Herds, 756 cattle, 709 Obs.)**			**Model 3: Fat classification (115 Herds, 756 cattle, 630 Obs.)**			
Variables	**Categories**	**N**	**β**	**95% C.I.**	**N**	**β**	**95% C.I.**	**N**	**β**	**95% C.I.**	
Intercept (SE)			295.35(12.49)			14.15(2.25)			28.30(1.63)		
Helminth Inf. [#]	**None**	64	*Baseline*		64	*Baseline*		64	*Baseline*		
	O only	401	-30.58*	-50.92;-10.24	401	1.13	-0.53;2.78	401	-3.28*	-5.56;-1.00	
	RF only	6	-50.34*	-88.50;-12.18	6	2.41	-1.27;6.09	6	-5.49*	-10.28;-0.69	
	LF only	11	-20.39	-50.76;9.98	11	-3.65*	-6.98;-0.32	11	-1.41	-5.71;2.89	
	O-RF	102	-39.99*	-73.09;-6.88	102	-1.69	-4.36;0.98	102	-1.72	-5.57;2.14	
	O-LF	80	-22.94	-52.89;7.01	80	-1.26	-3.65;1.12	80	-0.35	-3.91;3.21	
	RF-LF	4	-32.41	-73.06;8.24	4	3.48	-0.66;7.64	4	-4.85	-10.19;0.49	
	O-RF-LF	57	-48.28*	-88.35;-8.21	57	-1.27	-4.68;2.14	57	-3.81	-8.61;0.99	
Random effects											
	Level	Variance	SE		Variance	SE		Variance	SE		
	Herd	561.42	101.81		2.31	0.68		4.45	1.26		
	Cattle	844.80	56.10		13.34	0.803		20.98	1.34		

[*] Breed, category, age, CCW, conformation, fat, month and altitude were included in model as confounders, and results presented adjusted for these variables; [#] O = *Ostertagia spp.* lesions; RF= presence of adult rumen fluke; LF= liver fluke lesions