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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

49 **Keywords: *Ostertagia spp.*; rumen fluke; *F. hepatica*; co-infection; beef production**
50 **impact; multilevel modelling.**

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

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

94 **2. Materials and Methods**

95 *2.1. Sample collection and viscera scoring*

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

108 Abomasum gross lesions were classified into four categories (scores 0-3) based on the
109 number of gastric gland lesions typical of *Ostertagia spp.* (Larraillet et al., 2012): 0- no
110 lesions; 1- less than 100 lesions; 2- between 100-1000 lesions; 3- more than 1000 lesions.
111 Each reticulum and rumen were thoroughly examined and classified on a numerical scale
112 according to the number of adult rumen fluke (scores 0-3): 0- no fluke; 1- between 1 and 10;
113 2- between 11 and 100; 3- between 101 and 200; 4- more than 200 fluke. The presence of
114 liver fluke (0- no fluke (i.e. neither fluke nor liver fluke lesions); 1- actual presence (i.e.
115 presence of fluke and liver fluke lesions); 3- historical presence (i.e. no fluke but presence of
116 liver fluke lesions)) and the severity of the liver lesions due to liver fluke (0- no lesions; 1-
117 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):

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

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

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

213 **3. Results**

214 *3.1. Description of animal and carcass parameters*

215 A total of 974 carcasses were sampled from March 2014 to January 2015: 298 (31%) in
216 March, 233 (24%) in June, 230 (24%) in October and 213 (22%) in January. The carcasses
217 originated from 156 UK farms, **localised in 23 counties**. A total of 134 (86%) farms could be
218 geo-localised, of which 82% (110/134) were from England and 18% (24/134) from Wales.
219 The median [25th percentile (p25) - 75th percentile (p75)] number of carcasses per farm was 4
220 [2-8]. The sample included 64% males and 36% females, of which 53% (518/974) were
221 steers, 20% (193/974) cows, 16% (155/974) heifers, 11% (106/974) young bulls and less than
222 1% (2/974) mature bulls. Fifty percent (484/974) of the carcasses were from beef-cross
223 breeds, 36% (353/974) from pure-dairy breeds, 9% (83/974) from pure-beef breeds and 4%
224 (42/974) from dairy-cross breeds; the rest (12/974) belonging to either dual-purpose or other
225 breeds. Table 1 presents, by cattle category, the sample median [p25-p75] of age, CCW,
226 conformation and fat classifications, and percentage of liver condemnations.

227

228 *3.2. Description of carcass parasites' presence/lesions*

229 *3.2.1. Prevalence and severity of helminth infections as defined by scores*

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

232 Out of 972 carcasses (mature bulls excluded), a total of 933 abomasa, 936 reticulorumen
233 and 951 livers were scored for *Ostertagia spp.* lesions, presence of adult rumen fluke and
234 liver fluke lesions, respectively; the others being either condemned or lost. There was a large
235 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%

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 (≥ 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 ($\leq 60\text{m}$) (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 (\leq 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;

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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585

586

587

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

591

592

593 **Table 2.**

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

	Cows	Heifers	Steers	Young	Bulls	TOTAL
<i>Ostertagia spp.</i> 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)
<i>F. hepatica</i> 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)

596

597

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: <i>Ostertagia spp.</i> 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)
Category*	Dairy X	0 (0)	8 (3)	14 (6)	19 (6)	29 (4)	7 (6)	5 (5)	30 (5)	9 (4)	2 (4)
	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)
Age (Month)	Young Bull	12 (11)	33 (13)	37 (15)	20 (6)	97 (14)	4 (3)	1 (1)	102 (15)	1 (1)	1 (2)
	<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)
Month	>30	30 (28)	110 (43)	99 (41)	158 (47)	277 (39)	49 (41)	274 (88)	240 (35)	129 (57)	40 (82)
	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)
Altitude (m)	October	3 (3)	46 (18)	59 (25)	92 (27)	147 (21)	28 (23)	29 (26)	133 (20)	57 (25)	12 (25)
	≤60	-	-	-	-	194 (31)	49 (45)	43 (48)	187 (30)	79 (43)	18 (46)
O^(#)	>60	-	-	-	-	438 (69)	60 (55)	46 (52)	432 (70)	104 (57)	21 (54)
	None	-	-	-	-	89 (13)	5 (4)	6 (5)	79 (12)	23 (11)	1 (2)
RF^(#)	Present	-	-	-	-	613 (87)	111 (96)	106 (95)	576 (88)	193 (89)	44 (98)
	None	89 (89)	180 (71)	185 (77)	248 (74)	-	-	-	540 (82)	128(60)	27 (59)
F. hepatica^(#)	Present	11 (11)	74 (39)	55 (33)	88 (26)	-	-	-	120 (18)	85 (40)	19 (41)
	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: <i>Ostertagia spp.</i> 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	<i>Baseline</i>						<i>Baseline</i>				<i>Baseline</i>			
	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	<i>Baseline</i>						<i>Baseline</i>				<i>Baseline</i>			
	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	<i>Baseline</i>						<i>Baseline</i>				<i>Baseline</i>			
	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	<i>Baseline</i>				<i>Baseline</i>			
	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	<i>Baseline</i>						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	-	-	-	-	-	-	<i>Baseline</i>				<i>Baseline</i>			

O (*)	>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
	None	-	-	-	-	-	-	<i>Baseline</i>				<i>Baseline</i>			
RF (*)	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
	None	<i>Baseline</i>						<i>Baseline</i>				<i>Baseline</i>			
F. hepatica (*)	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
	None	<i>Baseline</i>						<i>Baseline</i>				<i>Baseline</i>			
	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 Obs.)		
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. ^(*#)			<i>Baseline</i>		<i>Baseline</i>			<i>Baseline</i>		
	None	64			64			64		
	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

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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
<i>Ostertagia</i> 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)
<i>F. hepatica</i> 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

35 Table 3.

		Model 1: <i>Ostertagia spp.</i> 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)
Category*	Dairy X	0 (0)	8 (3)	14 (6)	19 (6)	29 (4)	7 (6)	5 (5)	30 (5)	9 (4)	2 (4)
	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)
Age (Month)	Young Bull	12 (11)	33 (13)	37 (15)	20 (6)	97 (14)	4 (3)	1 (1)	102 (15)	1 (1)	1 (2)
	<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)
<i>F. hepatica</i>^(#)	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: <i>Ostertagia spp.</i> 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	<i>Baseline</i>						<i>Baseline</i>				<i>Baseline</i>			
	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	<i>Baseline</i>						<i>Baseline</i>				<i>Baseline</i>			
	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	<i>Baseline</i>						<i>Baseline</i>				<i>Baseline</i>			
	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	<i>Baseline</i>				<i>Baseline</i>			
	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	<i>Baseline</i>						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	-	-	-	-	-	-	<i>Baseline</i>				<i>Baseline</i>			
	>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	-	-	-	-	-	-	<i>Baseline</i>				<i>Baseline</i>			
	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	<i>Baseline</i>						-	-	-	-	<i>Baseline</i>			
	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	<i>Baseline</i>						<i>Baseline</i>				-	-	-	-
	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.

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.)			
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	<i>Baseline</i>		64	<i>Baseline</i>		64	<i>Baseline</i>	
	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