The influence of liver fluke infection on production in sheep and cattle: a meta-analysis

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1 ABSTRACT

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3 Liver flukes (*Fasciola* spp) are important parasites of sheep and cattle across the world,
4 causing significant damage to animal health and productivity due to both acute and chronic
5 infection. Many comprehensive reviews have discussed the results of decades of research
6 into the impact of fluke infection on livestock performance traits such as weight gain and milk
7 production. While fluke are considered to be important, there have been no attempts to
8 collate previous research in a quantitative manner, and nor has there been an attempt to
9 determine why some studies find substantial effects of fluke while others conclude that
10 effects of fluke on animal performance are negligible. In this study, we used meta-analysis to
11 provide quantitative estimates of the impact of liver fluke on animal performance, and to
12 identify elements of study design that influence the conclusions of such studies. A literature
13 search provided 233 comparisons of performance in “fluke-infected” and “uninfected”
14 animals. We standardized these data as log response ratios and calculated effect size
15 variances in order to weight studies by their sample size and accuracy of their estimates. We
16 performed multi-level meta-analysis to estimate effects of fluke infection in five traits: daily
17 weight gain (N = 77); live weight (N = 47); carcass weight (N = 84); total weight gain (N = 18)
18 and milk production (N = 6). There were significant negative effects of fluke infection on daily
19 weight gain, live weight and carcass weight (9%, 6% and 0.6% reductions in performance,
20 respectively), but not total weight gain or milk production. We then used mixed-effects meta-
21 analysis to estimate the impact of moderator variables, including host, fluke, and study
22 design factors, on study outcomes. We found that, in general, studies that gave experimental
23 infections found generally larger effects of fluke than observational or drug studies; younger
24 animals were more likely to suffer the effects of fluke infection on daily weight gain; and that
25 effects on live weight increased across the course of an experiment. Our results provide the
26 first quantitative estimate of the importance of liver fluke on performance across studies and
27 highlight the elements of study design that can influence conclusions. Furthermore, our

28 literature search revealed areas of research into liver fluke that could be the subject of
29 greater effort, and types of study that could form the basis of future meta-analyses.

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56 INTRODUCTION

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58 Liver flukes (*Fasciola* spp) are amongst the most important helminth parasites of domestic
 59 sheep and cattle worldwide, causing significant financial losses to producers (Schweizer et
 60 al., 2005). They have a typical trematode parasite life-cycle: adults inhabit the host liver and
 61 bile duct system and produce eggs, which are shed in faeces. Miracidia develop within the
 62 eggs and then hatch and search for a mud snail (typically, *Galba trunculata*) intermediate
 63 host, which they penetrate, undergoing multiplication before emerging as cercariae, which
 64 encyst on vegetation as the infectious metacercariae (cysts). These are ingested by the host
 65 while grazing; immature fluke then emerge and migrate through the intestinal wall to the
 66 liver, where they develop into adults around 10-12 weeks after ingestion of cysts (Skuce and
 67 Zadoks, 2013). Liver fluke can cause acute disease associated with migration of immature
 68 fluke, which can lead to death in severe cases, especially in sheep, and chronic disease
 69 caused by the blood-feeding activity of the adults, which can live as long as the host
 70 (Kaplan, 2001). Control of liver fluke remains a challenge in all areas of the world: vaccine
 71 development has been difficult, due in part to the lack of a robust protective host immune
 72 response and a lack of understanding of which antigens to target (Molina-Hernández et al.,
 73 2015; Toet et al., 2014); increasing flukicide resistance (Brockwell et al., 2013; Kamaludeen
 74 et al., 2019; Novobilský and Höglund, 2015); the clonal amplification of the parasite in the
 75 intermediate snail host (Beesley et al., 2018); and the ability of wildlife reservoir hosts to
 76 disseminate the parasite (French et al., 2019).

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78 A large number of influential reviews have collated decades of research into the effects of
 79 fluke infection on animal performance (Charlier et al., 2013; Dargie, 1980, 1987; Elelu and
 80 Eisler, 2018; Skuce and Zadoks, 2013). Empirical work has demonstrated statistically
 81 significant and sometimes substantial effects of liver fluke infection on traits including
 82 carcass weight (Sanchez-Vazquez and Lewis, 2013), carcass conformation or fatness
 83 (Bellet et al., 2016; Sanchez-Vazquez and Lewis, 2013), age at slaughter (Mazeri et al.,

2017), weight gain (Chick et al., 1980; Genicot et al., 1991; Hope Cawdery et al., 1977; Loyacano et al., 2002; Sykes et al., 1980), milk production (El-Tahawy et al., 2017; May et al., 2020), as well as the financial costs associated with condemnation of infected livers (Nyirenda et al., 2019). Other studies, however, have found no support for effects of liver fluke infection on performance traits including carcass weight (Bellet et al., 2016; Charlier et al., 2009; Molina et al., 2005), weight gain (Bossaert et al., 2000; Echevarria et al., 1992; Forbes et al., 2015) and milk production (May et al., 2019; Randall and Bradley, 1980). These studies vary in the direction and magnitude of effects of fluke, but also in their characteristics. Firstly, there is biological variation: in parasite species (*F. hepatica* typically in temperate regions and *F. gigantica* in the tropics) and in animal breed, age and sex. There is also variation in study design: some studies compare naturally infected with uninfected animals, some compare control with experimentally-infected animals, and others compare control with flukicide-treated animals. Finally, effects may vary with location due to climatic conditions, and with the time since initial infection, as it takes around 8-12 weeks for fluke to migrate to the liver and mature (Kaplan, 2001). As such, we lack knowledge on (1) the overall impact of fluke across studies and (2) the effects of study characteristics on outcome.

In this study, we present a meta-analysis of the impact of liver fluke infection on performance in sheep and cattle. Meta-analysis aims to collate data from published and unpublished sources addressing the same question and then puts data from these studies onto a standardized scale ("effect sizes"), enabling statistical analysis of the overall effect and causes of variation in outcomes (Gurevitch et al., 2018). The term "meta-analysis" was coined in 1976 (Glass, 1976) and the techniques were quickly embraced by medical and social sciences, with studies in ecology and evolution beginning in the early 1990s (Lau et al., 2013). Only more recently has this approach been applied in veterinary science (Lean et al., 2009). We followed the PICO (population; intervention; comparator; outcome) approach to formulate our research questions (Stewart et al., 2013), aiming to compare different measures of weight and milk production in sheep and cattle infected with fluke against those

designated as uninfected. We first estimated an overall effect size using random-effects meta-analysis and then assessed the impact of biological and study design factors (“moderators”) on study outcomes. Our results reveal that fluke infection has a particularly strong influence on weight gain, and that animal age and experimental design are important factors influencing study outcome.

METHODS

Literature search

We searched the scientific literature in order to identify studies that investigated the impact of liver fluke on performance of sheep and cattle. A *Web of Science* search was conducted on 18/10/2019 with the search terms (Fasciola OR fluke) AND (Cattle OR cow* OR calf OR calves OR sheep) AND (producti* OR weight OR grow* OR milk OR performance OR fertility OR carcas*) and the search yielded 662 papers. To these, we added all papers cited in a number of influential reviews on the impacts of fluke on livestock productivity (Charlier et al 2014A; Charlier et al 2014B; Dargie 1987; Elelu & Eiser 2018; Skuce & Zadoks 2013; Dargie 1980). We then added all papers citing these articles using the *Publish or Perish* software (Harzing 2016). Finally, we added a paper by da Costa et al (2019) that was published in late October 2019. We also added data provided by Scotbeef Ltd (Hayward et al., in prep) and McIntosh-Donald Ltd (Skuce et al., in prep). This resulted in a total of 1582 data sources.

We reviewed the titles and abstracts of these publications, sifting out publications that were clearly unsuitable for a variety of reasons (**Figure S1**). Once duplicates were removed, this initial sift resulted in 106 publications that were fully reviewed. Specifically, we searched for papers that compared performance in groups of animals that were infected with fluke (naturally or through experimental infection) versus animals that were uninfected (naturally

or through flukicide treatment). We collected data on the mean, standard deviation, standard error and number of animals with performance measured in each group. Where data were presented in figures but not in tables or text, we used the R package 'metaDigitise' (Pick et al 2018) to extract data. Where it seemed that relevant data may been collected but not reported in the publication or supporting information, we contacted authors in order to request data. Once the full review was complete and unsuitable publications removed (**Figure S2**), our final dataset consisted of 233 effect sizes from 28 sources (**Table 1**).

Data synthesis

We analysed the influence of liver fluke infection on five performance traits, as follows:

Daily weight gain: the calculated average increase in body weight per day. In some studies – mostly experimental – this is live weight gain (weight of the live animal divided by time in days), but in abattoir studies, this is usually dead weight gain (carcass weight divided by age in days). These effects are considered the same trait due to (1) the generally close correlation between live weight and carcass weight and (2) the way in which we tested for effects of study design in our analysis.

Live weight: the weight of the live animal, generally reported multiple times across the course of experimental studies.

Carcass weight: the weight of the animal's carcass in abattoir studies.

Total weight gain: the amount of weight gained by the animal from the start of an experiment to the time of measurement.

Milk production: the weight of milk produced, generally expressed as a daily rate.

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169 Our included studies reported raw mean performance in animals that were deemed to be
 170 fluke-infected versus animals that were deemed to be uninfected, plus sample sizes for both
 171 groups and standard deviation. Where standard errors were reported, we estimated the
 172 standard deviation as $SD = SE \times \sqrt{N}$. We calculated the ratio of means between fluke-
 173 infected and uninfected animals as our standardized measure of effect size and used log-
 174 transformation to normalize the values. Thus, we calculated log response ratio as $\ln(RR) =$
 175 $\ln(\bar{y}_{inf}) / \ln(\bar{y}_{uninf})$, where $\bar{y}_{inf}$ and $\bar{y}_{uninf}$ are the means of the trait y in infected and
 176 uninfected animals respectively. We calculated the variance for each effect as $v_{lnR} =$
 177 $(s_{inf}^2 / (n_{inf} \times \bar{y}_{inf}^2)) + (s_{uninf}^2 / (n_{uninf} \times \bar{y}_{uninf}^2))$ where s^2 , n , and $\bar{y}^2$ are the squared
 178 standard deviation, sample size and squared mean for infected or uninfected individuals
 179 (Koricheva et al 2013).

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181 The 233 effect sizes were unevenly distributed across our 28 sources, with 4 studies
 182 contributing just one effect size, 5 contributing more than 10 and 3 studies contributing 40
 183 effect sizes each. These last three were abattoir studies where we were provided with raw
 184 data by the authors or the abattoirs themselves and we calculated breed- and sex-specific
 185 statistics for the ten commonest breeds in each data set, in order to better account for these
 186 factors and to maximise our number of effect sizes. Multiple effect sizes were contributed for
 187 a number of reasons including comparisons being made across many weeks of an
 188 experimental study; fluke status being manipulated as well as another experimental
 189 treatment such as diet; data being subdivided by sex and/or breed. We categorized our
 190 effect sizes according to a number of biological and experimental factors, many of which we
 191 included in our meta-regression analyses (see below).

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193 *Host species:* our data contained more effect sizes from cattle (205, 88%) than sheep (28,
 194 12%).

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196 *Host breed*: data came from 4 breeds of sheep and 29 breeds of cattle. Among these were
197 crosses and cattle denoted simply as “dairy”, “beef” or “mixed”, each of which was included
198 as a separate breed in our analyses.

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200 *Host sex*: our data contained 88 effect sizes from females (38%), 106 from males (45%), 30
201 from mixed-sex groups (13%) and 9 where animal sex was not recorded (4%).

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203 *Host age group*: animals were divided into three age categories, namely adults (12% of
204 effect sizes), young (35%) and mixed (53%). For cattle, young animals were ≤ 12 months of
205 age and adults ≥ 23 months of age. For sheep, young animals were ≤ 12 -13 months of age
206 and adults were year old (yearlings) or older.

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208 *Parasite*: the data were dominated by *F. hepatica*, which accounted for 90% of effect sizes,
209 with 9% contributed by *F. gigantica* and 1% from a single study recording a mixed *Fasciola*
210 species burden.

211

212 *Experimental design*: 29 effect sizes (12%) came from 6 studies where flukicidal drugs were
213 used to remove fluke from an experimental group, performance in which was compared with
214 a control group. In the drug-treated (i.e. “uninfected”) groups, the maximum mean fluke FEC
215 was 3 eggs/gram and the maximum live fluke burden at post-mortem was 3. While some of
216 these animals defined as “uninfected” clearly carried fluke, we consider these burdens low
217 enough as to be negligible. These studies were denoted as “drug”. 56 (24%) effect sizes
218 were from studies (denoted as “infection”) where animals were experimentally infected with
219 fluke and compared with uninfected controls. The remaining 148 (64%), denoted “natural”,
220 were largely from abattoirs and animals that acquired infection (or not) naturally.

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Week post-infection: for experimental studies, data were collected from 4-54 weeks post-infection.

Absolute latitude: our effect sizes were predominantly from studies conducted in the UK (64%) with others coming from studies conducted in the Americas (12%), Asia (9%), Europe (8%), Australasia (4%), and Africa (3%). Absolute latitude ranged from 1.25-57.3.

Multi-level meta-analysis

Meta-analyses were performed using the `rma.mv` function of the R package 'metafor' (Viechtbauer 2010), version 2.1-0. We performed separate random-effects meta-analyses in order to estimate the mean effect of fluke infection on five traits: daily weight gain, live weight, carcass weight, total weight gain and milk yield.

Global effects: First, we determined the mean effect of fluke infection on each of the traits. We used multi-level analyses: in order to account for non-independence of effect sizes derived from the same studies and from animals of the same breed, we fitted random effects of study and breed, as well as an observation-level random effect in order to estimate the residual variance.

Meta-regression: Next, we used a meta-regression approach in order to investigate whether the effect size depended upon a number of factors related to host and parasite biology or study design. These included host species (cattle or sheep), host sex, host age group (adult, young, or mixed), parasite species (*F. hepatica* or *F. gigantica*), study design (observational study of natural infections, infection experiment, drug treatment experiment), absolute latitude (continuous), and week post-infection. Each of these moderating variables was investigated in a separate meta-regression model. In each model, we determined whether the moderator was supported (i.e. whether the effect size varied according to the moderator)

using Wald-type chi-square tests (Q_M). We examined whether each level within categorical moderators was significantly different from zero using z-tests. We did not test all of the moderators for each of the five traits, since not all traits had variation in all moderators (e.g. daily weight gain data all came from cattle and so it was not possible to test the ‘host species’ moderator).

Details on the terms fitted for each trait are shown in **Table 2**.

Analysis of heterogeneity and bias

Heterogeneity in effect sizes may be generated through variation between studies and variation within studies. In order to quantify this, we calculated the proportion of total variance that was due to variation in effect sizes (the I^2 statistic), where the remainder is accounted for by sampling error. Since we fitted random effects to account for expected similarity between effect sizes from the same study and from the same breed of animal, we calculated modified I^2 (Nakagawa & Santos 2012). Hence, for each meta-analysis, we calculated I^2 values for the between-study effect and between-breed effect (where these were fitted (**Table 2**), and the residual effect.

The causes of bias in meta-analyses can include publication bias (the possibility that studies finding non-significant effects will not be published) and changes in effect size across time, e.g. if literature searches are less able to sample early sources. To test for publication bias, we generated funnel plots and performed regression of meta-analytic model residuals of each effect size (corrected for random effects and significant moderators) on the variance in each effect size (Egger, 1997; Nakagawa & Santos 2012). Where the intercept was significantly different from zero, we concluded that there was significant bias (Nakagawa & Santos, 2012). Where our data included both published and unpublished studies, we performed this test on both the full dataset, and then on the published studies only.

Finally, for each meta-analysis, we tested the effect of year of study as a continuous moderator in order to determine whether there has been a linear change in effect size across time, assessing significance using Wald-type chi-square tests (Q_M).

RESULTS

Daily weight gain

Multi-level meta-analysis of daily weight gain revealed an overall negative effect of fluke infection ($\beta_{global} = -0.0981$, 95%CI = -0.1554 - -0.0408, $z = -3.36$, $P < 0.001$), suggesting that infected animals gained 9.5% less weight per day than uninfected animals (Figure 1). Meta-regression analyses revealed that the moderators of parasite species, experimental type, sex, latitude and week had no influence on the effect size (Table 3), but that there was a significant effect of the moderator of age ($Q_M = 5.29$, $P = 0.021$), suggesting that young animals, but not mixed-aged groups, experienced negative effects of fluke. Our results also revealed that there were negative effects of fluke both in animals infected with *F. gigantica* and *F. hepatica*, studies that used experimental infections but not drug studies or studies using natural infections, and in both sexes (Figure 1). Total variation in effect sizes was high ($I^2 = 99\%$) and largely due to variation between studies ($I^2 = 91\%$), with only a small amount of residual variation ($I^2 = 8\%$). There was no evidence of changes in effect sizes across time ($\beta_{year} = 0.0036$, 95%CI = -0.0012 – 0.0084, $z = 1.46$, $P = 0.145$). Finally, there was no evidence of publication bias through inspection of funnel plots (Figure S3) or regression of model residuals on effect size variances in either the full data set ($t = -0.30$, $DF = 74$, $P = 0.763$) or published data ($t = -0.03$, $DF = 14$, $P = 0.975$).

Live weight

Multi-level meta-analysis of live weight revealed a negative effect of fluke infection ($\beta_{global} = -0.0611$, 95%CI = -0.0877 - -0.0344, $z = -4.48$, $P < 0.001$), suggesting that infected animals weighed around 6% less than uninfected animals (Figure 2). Meta-regression revealed that the moderators of parasite species, host species, host age, experimental type, and latitude had no influence on the effect size (Table 4). There was, however, a significant effect of sex ($Q_M = 16.04$, $P < 0.001$), with males and especially mixed-sex groups of animals showing a greater impact of fluke than female only groups (Figure 2). There was also support for week post-infection ($Q_M = 7.66$, $P = 0.006$), with effects of fluke increasing across time in experimental fluke challenge studies (Figure S4). Fluke had a negative impact on live weight in animals infected with *F. hepatica* and *F. gigantica* (although the latter effect was marginally not supported), in both cattle and sheep, in young animals but not adults, in studies that administered experimental infections rather than those that cleared infections with flukicides and in male and mixed-sex groups but not females (Table 4). Total variation in effect sizes was moderate ($R^2 = 71\%$) and largely due to residual variation ($R^2 = 50\%$), with smaller variation due to between-study effects ($R^2 = 22\%$). Effect sizes did not change with time ($\beta_{year} = -0.0006$, 95%CI = -0.0023 – 0.0012, $z = -0.61$, $P = 0.542$). Finally, there was no evidence of publication bias through inspection of funnel plots (Figure S5) or regression of model residuals on effect size variances ($t = -0.41$, $P = 0.683$).

Carcass weight

Analysis of carcass weight supported a significant negative effect of fluke infection ($\beta_{global} = -0.0060$, 95%CI = -0.0099 – -0.0021, $z = -3.00$, $P = 0.003$), although the effect size was negligible, suggesting a 0.6% reduction in carcass weight in infected animals (Figure 3). Meta-regression revealed that the moderators of age, experimental type, and latitude had no influence on the effect size (Table 5). There was, however, a significant effect of parasite species ($Q_M = 6.44$, $P = 0.011$), with animals infected with *F. gigantica* having greater carcass weight than uninfected animals, and animals infected with *F. hepatica* having

marginally lower carcass weight than uninfected animals (Figure 3). Total variation in effect sizes was high ($I^2 = 82\%$), with a relatively small influence of between-study effects (23%) and a large contribution of residual variation (59%). Effect sizes increased significantly with time ($\beta_{year} = 0.0013$, 95%CI = 0.0005 – 0.0021, $z = 3.21$, $P = 0.001$). There was no evidence of publication bias through inspection of funnel plots (Figure S6) or regression analysis in either the full dataset ($t = 0.06$, $DF = 82$, $P = 0.951$) or the published data ($t = 0.39$, $DF = 22$, $P = 0.701$).

Total weight gain

There was an overall negative influence of fluke infection on total weight gain, and while the effect did not reach statistical significance, it was of considerable magnitude ($\beta_{global} = -0.1541$, 95%CI = -0.3258 – 0.0176, $z = -1.76$, $P = 0.079$), with infected animals gaining 14% less weight than uninfected animals (Figure S7). Meta-regression revealed that the moderators of parasite species, experimental type, sex, latitude and week had no influence on the effect size (Table S1). Negative effects of fluke were detected in studies that administered experimental infections rather than drug treatments, and in males but not mixed-sex groups (Figure S7). Total variation in effect sizes was high ($I^2 = 79\%$) and largely due to variation between breeds ($I^2 = 72\%$), with smaller amounts of between-study ($I^2 = 4\%$) and residual variation ($I^2 = 2\%$). There was no evidence of changes in effect sizes across time ($\beta_{year} = 0.0040$, 95%CI = -0.0086 – 0.0166, $z = 0.62$, $P = 0.537$) and no evidence of publication bias through funnel plots (Figure S8) or regression analysis ($t = 0.85$, $P = 0.409$).

Milk production

There was no support for a significant overall influence of fluke infection on milk production ($\beta_{global} = -0.0500$, 95%CI = -0.1065 – 0.0066, $z = -1.73$, $P = 0.083$; Figure S9). Meta-regression revealed that the moderators of experimental type and latitude had no influence

on the effect size (Table S2). Total variation in effect sizes was high ($I^2 = 88\%$), but we did not fit random effects of study or breed because they were found to be zero. There was no evidence of changes in effect sizes across time ($\beta_{year} = -0.0004$, 95%CI = $-0.0040 - 0.0031$, $z = -0.23$, $P = 0.815$) and no evidence of publication bias through inspection of a funnel plot (Figure S10) and regression analysis ($t = -0.17$, $P = 0.875$).

DISCUSSION

In this study, we used a meta-analytic approach in order to estimate the overall influence of liver fluke infection on performance traits in sheep and cattle. We found large amounts of variation between studies in the effects they estimated for liver fluke in each trait, and identified moderator variables that were associated with effect sizes. For each trait, we initially ran a random-effects meta-analysis in order to estimate an overall effect size. We found significant and substantial effects of fluke infection on two traits, with a 9% reduction in daily weight gain (DWG) and a 6% reduction in live weight. Moreover, we found a significant reduction in carcass weight in fluke-infected animals, although the overall effect was negligible, with infected animals having 0.6% lower carcass weight than fluke-free animals. Reductions in total weight gain and milk production in fluke-infected animals were not statistically supported. We also found that, although moderators were generally not supported, there was evidence to suggest that studies were more likely to find significant effects of fluke if they used experimental infection compared to natural infections or infected versus flukicide-treated animals, and if they studied young animals rather than adults.

The overall effect of a reduced DWG of 9% is likely to have a significant impact on several parameters: for the welfare of the animal, experiencing chronic disease (Howell and Williams, 2020); for the producer, in terms of financial costs (Mehmood et al., 2017; Schweizer et al., 2005); and for the environment, with the increased greenhouse gas emissions emitted by less efficient animals (Skuce et al., in prep). There was support for the

moderator of age group: the effect of fluke was significantly greater in young animals compared to mixed-age animals. This is potentially related to the fact that young animals are likely to be growing, while older animals may be putting on weight rather than growing *per se*; as such, younger animals are more likely to suffer the effects of fluke infection with regard to their ability to increase in weight. Further, it is possible that this may reflect age-dependent effects of immunity to the parasite, with younger animals less able to control fluke infection – although evidence for effective immunity in adults is scant (Hoyle et al., 2003) – or responding in a way which induces more immunopathology. While the moderator of experimental design was not supported, infection studies showed an effect of fluke that was significantly different from zero (Table 3), but this was not the case for drug studies or studies observing the effects of natural infections. Infection studies may be associated with larger effect sizes because they tended to involve administration of a single large infective dose of metacercariae at the start of the experiment; of the 15 experimental infection studies across our whole dataset, only two used trickle infections. It may well be that a large influx of immature fluke caused more damage than a natural infection due to the migration of a large number of larvae simultaneously (Boray, 1967; Ross and Dow, 1966). Another explanation is that we may have seen smaller effects in drug studies because they may not have fully cleared fluke infections (even though mean burdens in treated animals were very low), and in natural infection studies because unlike in experimental challenge studies, there would be variation in fluke challenge and duration of infection within infected groups resulting in a more variable response to infection. Finally, we saw significant effects of both *F. hepatica* and *F. gigantica* on DWG, and very similar effects in both sexes. There was, however, no variation in effect size with latitude or across time in experimental studies.

The difference in live weight between infected and uninfected animals of 6% is, as with DWG, likely to prove relevant to the efficiency of production. Two moderators were supported. First, there was variation between sex classes, with stronger effects of fluke in males and mixed-sex groups than in females. This is likely to be due to the fact that all effect

sizes of males and mixed-sex groups came from studies on young animals, while most of those on females (12/14) came from adults, and as with DWG, there were stronger effects of fluke in young animals. There was also a significant effect of duration of infection, with effect sizes increasing with week post-infection in experimental infection studies; this result is consistent with our finding of lower DWG in infected animals, since a difference in DWG would lead to an increasing divergence in live weight across time. As with DWG, there were stronger effects of fluke in young animals compared to adults, and in infection experiments compared to drug experiments, presumably for the same reasons as discussed above for DWG. A final striking observation was the very similar magnitude of the average effect size in cattle and sheep, which is potentially surprising given the generally greater capacity for fluke to cause more severe disease in sheep than cattle (Howell and Williams, 2020).

While we found a significant impact of fluke infection on carcass weight, the overall effect was negligible, with infected animals having carcass weights only 0.6% lower than uninfected animals. The statistical significance of such a small effect size is likely related to the fact that the majority of the data came from abattoir studies, with very large sample sizes and very small effect size variances. The utility of carcass weight as a metric for assessing performance may be questionable, given that most producers will take animals to slaughter only when they have reached a target weight – and indeed will be penalised for not doing so – yet carcass weight is still commonly reported as a measure of performance. The only noteworthy moderator variable was that of fluke species: animals with *F. gigantica* actually had higher carcass weights than uninfected animals. That said, all of the effect sizes concerning *F. gigantica* came from a single study (Molina, 2005), in which three groups of cattle of different ages were heavier when infected with *F. gigantica* compared to their uninfected counterparts. In the same abattoir study, water buffalo (*Bubalus bubalis*, which were not included in the meta-analysis) had lighter carcasses when infected with fluke, and no explanation was apparent or discussed (Molina, 2005). The overall effect size for total weight gain was substantial, with 14% lower total weight gain in fluke-infected animals,

although this was marginally non-significant. Once again, we saw a considerably stronger effect in infection experiments compared to drug studies.

While traits relating to body weight were well represented, we had a much smaller dataset for milk yield. Many studies have tested the impact of fluke infection on milk yield and have found substantial effects (Arenal et al., 2018; Charlier et al., 2007; Howell et al., 2015; Köstenberger et al., 2017; Mezo et al., 2011), but these have studied effects at the herd level using bulk tank milk samples and so were not included in our analysis. Other studies of individual animals did not report the necessary statistics for us to calculate effect size variance (Khan et al., 2010). Perhaps due to the small number of studies included in our analyses, we found no overall influence for an effect of fluke on milk yield. A future meta-analysis of the many bulk tank studies could prove informative in determining overall herd-level effects.

The effect of fluke infection has been measured with respect to other performance traits, as reviewed recently (Charlier et al., 2013; Skuce and Zadoks, 2013), but for these there were insufficient studies in our initial scoping search for them to be included in the full search and analysis. For example, there have been many studies of the influence of fluke infection on wool weight, but during our initial searches, we found that most of these did not report the standard deviation (or standard errors and sample sizes) that were required to calculate effect size variance. These older studies did typically report substantial effects of fluke infection on wool yield: two studies administering a range of infection doses reported lower yields in infected animals, particularly at higher infectious doses (Edwards et al., 1976; Hawkins and Morris, 1978) and a study testing the effect of flukicide treatment found that treatment enhanced wool growth (Hawkins, 1984). Two further studies reported increasing effects of fluke infection on wool yield over time (Hagh-Nazari and Dalimi, 2000; Roseby, 1970), suggesting that larger effects are seen once adult fluke are established in the liver from 11-12 weeks post-infection (Kaplan, 2001). In addition, several studies report a

negative influence of fluke on reproduction (Marley et al., 1994), including a 39 day delay in first oestrus in cattle (López-Díaz et al., 1998), a 4.7 day increase in calving interval (Charlier et al., 2007) and an increase in pregnancy rate from 57% in control ewes to 80% in flukicide-treated ewes (Hope Cawdery, 1976). Some studies, however, report no significant influence of fluke infection on reproductive traits (Loyacano et al., 2002; Mezo et al., 2011). Another performance trait that we did not consider was time to reach slaughter weight, which has been found to be delayed by 33-93 days in fluke-infected animals, depending on the severity of infection (Mazeri et al., 2017), but once again, there were too few studies to consider this as an outcome trait.

Only a small number of meta-analyses have provided quantitative reviews of the impacts of disease on performance in livestock and in general have reported stronger effects than we saw here. A meta-analysis of 75 trials studying the impact of anthelmintic treatment on milk production in dairy cows reported an increased daily yield of 0.35kg in treated compared to untreated animals, suggesting a negative influence of helminth infection (almost exclusively gastrointestinal nematodes based on the studies included) on performance (Sanchez et al., 2004). A meta-analysis of 18 studies examining the influence of endoparasite – largely nematode – infection on weight gain in pigs reported a 31% reduction in infected animals, which was largely due to reduced feed intake in infected animals (Kipper et al., 2011). More closely related to our study, a meta-analysis of 94 effect sizes examining effects of nematode infections on sheep performance across Europe found that nematode infection was associated with a 15% reduction in weight gain, 10% lower wool production and 22% lower milk yield (Mavrot et al., 2015). While this latter study was unable to estimate the impact of moderator variables on outcomes, it was able to show that increased parasite burden, measured as faecal egg count, was associated with a greater negative impact of infection in “highly parasitized” compared to “low parasitized” individuals (Mavrot et al., 2015). We were unable to estimate the impact of fluke burden as a moderator in our study because of the large variation between studies in their design: in most studies, individuals

were simply assigned as “infected” or “uninfected”, and in experimental infection studies, a range of infection doses were given, either as single infections or as trickles across varying time scales.

CONCLUSION

This meta-analysis has provided quantitative estimates of the impact of liver fluke infection on the performance of sheep and cattle by collating relevant data from both published and unpublished sources. We found that effects on live weight gain and live weight were the most pronounced, while effects on carcass weight and milk production were either negligible or non-significant. Since we focused on individual-based assessments of infection, there remains a great deal of data to be exploited for meta-analysis of influences of fluke at the herd level, particularly regarding bulk-tank milk samples. Our results also reveal that studies administering experimental infections, and studies of younger animals, are more likely to reveal effects of fluke that differ from zero, and that detecting effects of natural infection may be more difficult. Improved diagnostics of fluke infection, particularly those which can quantify the level of infection, may be required to gain a more accurate estimate of the impact of subclinical fluke infection on performance in the future, aiding understanding of between-host variation in resistance and tolerance of infection and aiding the effort to design improved mitigation strategies for fluke infection.

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DECLARATION OF INTERESTS

The authors have no competing interests to declare.

AUTHOR CREDIT STATEMENT

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TABLES

Table 1. A summary of the studies from which data was used for the meta-analysis. ‘Experiments’ are as described in the main text, while traits are as follows: CW = carcass weight; DWG = daily weight gain; LW = live weight; milk = milk production; TWG = total weight gain. The data source was either the publication (taken from the text, tables of figures in the publication) or the authors (send upon request by authors). ‘N’ is the number of effect sizes contributed by each study.

Reference	Host	Fluke	Country	Experiment	Traits	Data source	N
(Bellet et al., 2016)	Cattle	<i>F. hepatica</i>	UK	Natural	CW	Authors	4
(Berry, 1977)	Sheep	<i>F. hepatica</i>	UK	Infection	LW	Publication	3
(Bossaert et al., 2000)	Cattle	<i>F. hepatica</i>	Belgium	Infection	DWG	Publication	2
(Chick et al., 1980)	Cattle	<i>F. hepatica</i>	Australia	Infection	LW	Publication	8
(Crossland et al., 1977)	Sheep	<i>F. hepatica</i>	UK	Drug	LW	Publication	12
(da Costa et al., 2019)	Cattle	<i>F. hepatica</i>	Uruguay	Natural	CW	Authors	12
(Echevarria et al., 1992)	Cattle	<i>F. hepatica</i>	Brazil	Drug	CW, DWG, TWG	Publication	9
(El-Tahawy et al., 2017)	Cattle	<i>Fasciola</i> spp.	Egypt	Natural	LW, Milk	Publication	2
(Forbes et al., 2015)	Cattle	<i>F. hepatica</i>	UK	Drug	TWG	Publication	2
(Genicot et al., 1991)	Cattle	<i>F. hepatica</i>	Belgium	Natural, Drug	DWG	Publication	4
(Hope Cawdery et al., 1977)	Cattle	<i>F. hepatica</i>	UK	Infection	LW, TWG	Publication	6
(Jacob et al., 2015)	Cattle	<i>F. gigantica</i>	India	Infection	DWG, LW, TWG	Publication	6
(Jacob et al., 2014)	Cattle	<i>F. gigantica</i>	India	Infection	DWG, LW, TWG	Publication	6
(López-Abán et al., 2007)	Sheep	<i>F. hepatica</i>	Spain	Infection	DWG	Publication	1
(López-Díaz et al., 1998)	Cattle	<i>F. hepatica</i>	Spain	Infection	LW	Publication	1
(Mage et al., 1993)	Cattle	<i>F. hepatica</i>	France	Drug	DWG, TWG	Publication	2
(May et al., 2019)	Cattle	<i>F. hepatica</i>	Germany	Natural	Milk	Publication	1
McIntosh-Donald Ltd	Cattle	<i>F. hepatica</i>	UK	Natural	CW, DWG	Authors	40
(Molina et al., 2005)	Cattle	<i>F. gigantica</i>	Philippines	Natural	CW	Publication	3
(Paczkowski, 2005)	Cattle	<i>F. hepatica</i>	USA	Infection	LW	Publication	1

Table 2. Summary of parameters included in meta-analysis of six production traits. 'N' and 'studies' refers to the maximum number of effect sizes available for each trait, and the number of studies measuring each trait.

Trait	N	Studies	Random effects	Moderators tested in meta-regression
Daily weight gain (DWG)	77	11	Study + Residual	Parasite, Age, Experiment, Sex, Latitude, Week
Live weight	47	11	Study + Residual	Parasite, Host, Age, Experiment, Sex, Latitude, Week
Carcass weight	84	8	Study + Residual	Parasite, Age, Experiment, Sex, Latitude
Total weight gain (TWG)	18	8	Study + Breed + Residual	Parasite, Experiment, Sex, Latitude, Week
Milk production	6	4	Residual	Experiment, Latitude

Table 3: Results from meta-analysis of daily weight gain (DWG). 'N' is the number of effects sizes available for analysis. 'Global' indicates the overall effect size from the initial random effects model, while the subsequent moderator effects are from mixed-effects models where each moderator was fitted in turn. 'Effect sizes' and 'Studies' varies because information was not available for some of the effect sizes. 'Q_M' and the associated 'DF' and 'P' values refer to the Wald-type test for significant differences in effect size due to moderator, while 'z' and 'P' values refer to tests for differences from zero for different levels of each moderator.

Moderator	N	Studies	Q _M	DF	P	Level	Estimate (logRR)	Lower 95%CI	Upper 95%CI	z	P
Global	77	11					-0.0981	-0.1554	-0.0408	-3.36	<0.001
Parasite	77	11	1.27	1	0.259	<i>F. gigantica</i>	-0.1622	-0.2859	-0.0384	-2.57	0.010
						<i>F. hepatica</i>	-0.0800	-0.1511	-0.0089	-2.21	0.027
Age	76	10	5.29	1	0.021	Juvenile	-0.1512	-0.2177	-0.0846	-4.45	<0.001
						Mixed	-0.0404	-0.1073	0.0264	-1.19	0.236
Experiment	77	11	1.61	2	0.446	Drug	-0.0818	-0.2147	0.0512	-1.21	0.228
						Infection	-0.1516	-0.2527	-0.0505	-2.94	0.003
						Natural	-0.0658	-0.1531	0.0215	-1.48	0.139
Sex	74	10	2.77	1	0.096	Female	-0.0933	-0.1541	-0.0324	-3.01	0.003
						Male	-0.1039	-0.1641	-0.0437	-3.38	<0.001
Latitude	77	11	2.22	1	0.149	Continuous	0.0043	-0.0003	0.0090	1.82	0.149
Week	13	6	2.09	1	0.148	Continuous	0.0061	-0.0022	0.0144	1.45	0.148

Table 4. Results from meta-analysis of live weight. 'N' is the number of effects sizes available for analysis. 'Global' indicates the overall effect size from the initial random effects model, while the subsequent moderator effects are from mixed-effects models where each moderator was fitted in turn. 'Effect sizes' and 'Studies' varies because information was not available for some of the effect sizes. 'Q_M' and the associated 'DF' and 'P' values refer to the Wald-type test for significant differences in effect size due to moderator, while 'z' and 'P' values refer to tests for differences from zero for different levels of each moderator.

Moderator	N	Studies	Q _M	DF	P	Level	Estimate (logRR)	Lower 95%CI	Upper 95%CI	z	P
Global	47	11					-0.0611	-0.0877	-0.0344	-4.48	<0.001
Parasite	46	10	0.01	1	0.913	<i>F. gigantica</i>	-0.0519	-0.1109	0.0070	-1.73	0.084
						<i>F. hepatica</i>	-0.0556	-0.0860	-0.0253	-3.59	0.003
Host	47	11	0.01	1	0.910	Cattle	-0.0598	-0.0960	-0.0236	-3.24	0.001
						Sheep	-0.0631	-0.1076	-0.0186	-2.78	0.005
Age	47	11	2.38	1	0.123	Adult	-0.0222	-0.0757	0.0312	-0.81	0.415
						Juvenile	-0.0704	-0.0973	-0.0435	-5.12	<0.001
Experiment	46	10	1.84	1	0.174	Drug	-0.0225	-0.0743	0.0293	-0.85	0.395
						Infection	-0.0642	-0.0915	-0.0368	-4.60	<0.001
Sex	47	11	16.04	2	<0.001	Female	-0.0234	-0.0518	0.0050	-1.62	0.106
						Male	-0.0478	-0.0785	-0.0172	-3.06	0.002
						Mixed	-0.1042	-0.1330	-0.0755	-7.10	<0.001
Latitude	47	11	0.15	1	0.696	Continuous	-0.0003	-0.0021	0.0014	-0.39	0.696
Week	33	8	7.66	1	0.006	Continuous	-0.0023	-0.0039	-0.0007	-2.77	0.006

Table 5. Results from meta-analysis of carcass weight. 'N' is the number of effects sizes available for analysis. 'Global' indicates the overall effect size from the initial random effects model, while the subsequent moderator effects are from mixed-effects models where each moderator was fitted in turn. 'Effect sizes' and 'Studies' varies because information was not available for some of the effect sizes. 'Q_M' and the associated 'DF' and 'P' values refer to the Wald-type test for significant differences in effect size due to moderator, while 'z' and 'P' values refer to tests for differences from zero for different levels of each moderator.

Moderator	N	Studies	Q _M	DF	P	Level	Estimate (logRR)	Lower 95%CI	Upper 95%CI	z	P
Global	84	8					-0.0060	-0.0099	-0.0021	-3.00	0.003
Parasite	84	8	6.44	1	0.011	<i>F. gigantica</i>	0.1497	0.0294	0.2700	2.44	0.015
						<i>F. hepatica</i>	-0.0062	-0.0101	0.0023	-3.10	0.002
Age	84	8	0.37	2	0.831	Adult	-0.0042	-0.0127	0.0044	-0.96	0.340
						Juvenile	-0.0081	-0.0209	0.0048	-1.23	0.219
						Mixed	-0.0063	-0.0114	-0.0011	-2.40	0.017
Experiment	84	8	0.01	1	0.914	Drug	-0.0178	-0.2332	0.1975	-0.16	0.871
						Natural	-0.0060	-0.0099	-0.0021	-2.99	0.003
Sex	81	7	0.32	2	0.851	Female	-0.0056	-0.0102	-0.0009	-2.34	0.019
						Male	-0.0066	-0.0113	-0.0019	-2.78	0.006
						Mixed	-0.0043	-0.0170	0.0084	-0.67	0.506
Latitude	84	8	1.30	1	0.254	Continuous	-0.0003	-0.0008	0.0002	-1.13	0.257

FIGURE LEGENDS

Figure 1. Estimated response ratio (RR) from meta-regression analysis of fluke-infected and uninfected animals for daily weight gain (DWG). A response ratio of 1 indicates equal performance in infected and uninfected animals; values <1 indicate poorer performance in infected animals. Plots show estimated mean effect of the global effect on infection (Intercept), plus the effect of infection in different moderators, with 95%CI.

Figure 2. Estimated response ratio (RR) from meta-regression analysis of fluke-infected and uninfected animals for live weight. A response ratio of 1 indicates equal performance in infected and uninfected animals; values <1 indicate poorer performance in infected animals. Plots show estimated mean effect of the global effect on infection (Intercept), plus the effect of infection in different moderators, with 95%CI.

Figure 3. Estimated response ratio (RR) from meta-regression analysis of fluke-infected and uninfected animals for carcass weight. A response ratio of 1 indicates equal performance in infected and uninfected animals; values <1 indicate poorer performance in infected animals. Plots show estimated mean effect of the global effect on infection (Intercept), plus the effect of infection in different moderators, with 95%CI.