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Implications of Endectocide Residues on the Survival of Aphodiine Dung Beetles: A Meta-Analysis

Domhnall Finch,[a] Henry Schofield,[b] Kevin D. Floate,[c] Laura M. Kubasiewicz,[d] and Fiona Mathews[a,*]

[a]School of Life Sciences, University of Sussex, Falmer, East Sussex, United Kingdom
[b]Vincent Wildlife Trust, Bronsil Courtyard, Eastnor, Ledbury, Herefordshire, United Kingdom
[c]Agriculture and Agri-Food Canada, Lethbridge Research and Development Centre, Lethbridge, Alberta, Canada
[d]Research and Operational Support Department, The Donkey Sanctuary, Sidmouth, Devon, United Kingdom

Abstract: It is often difficult to compare studies examining the effects of endectocides on dung fauna because of different experimental approaches, for example, active ingredients (eprinomectin, doramectin, ivermectin, moxidectin) and formulations (injectable, pour-on, spiked). To gain a better understanding, we performed a quantitative meta-analysis using 22 studies to assess the overall effect of endectocide residues on the occurrence (presence or absence) and abundance of aphodiine dung beetles. Our results document a positive effect on the occurrence of adult beetles, indicating that adults tend to be attracted to dung with residues. Conversely, larvae are less likely to occur in the presence of residues. Thus, either adults that colonize dung with residues do not lay eggs or, more likely, the larvae that hatch from these eggs die early in development. Abundance of adult and larval stages was shown to be significantly reduced in dung containing residues. When individual endectocides were compared, only ivermectin demonstrated a significantly negative effect on the abundance of both adults and larvae, possibly owing to a small sample size for other agents. In laboratory studies, only dung "spiked" with endectocides reduced the abundance of larvae, whereas during field research, only pour-on applications were shown to reduce the abundance of larvae. The present study further documents the nontarget effects of endectocide residues on dung-dwelling organisms, provides robust evidence on the consequences of different application methods, and emphasizes the need for standardized methodological techniques in future studies. *Environ Toxicol Chem* 2020;00:1–10. © 2020 SETAC

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INTRODUCTION

Endectocides are among the world's most widely sold veterinary pharmaceuticals and have global application for the control of external and internal parasites affecting livestock. There is growing concern about resistance by target organisms to endectocides and the consequent implications for farming (Rose et al. 2015). Much less attention has focused on the potential environmental impacts of endectocides. Some endectocides can be poorly metabolized by the gut of livestock, with between 62 and 98% of the active ingredient being excreted as residue in dung (Canga et al. 2009). These residues can persist in the environment, with a half-life of 240 d in laboratory conditions (Lumaret et al. 2012); under field conditions, no degradation was detected for up to 45 d postapplication (Sommer et al. 1992). This is concerning because residues can have significant impacts on both flora (Eichberg et al. 2016) and fauna (Iglesias et al. 2006) in the natural environment.

Under phase II environmental risk assessment guidelines (European Union 2009), the risk of veterinary pharmaceuticals to nontarget species of dung-breeding organisms is assessed in single-species laboratory studies (tier A testing; Veterinary International Conference on Harmonization 2004). If a specific risk threshold is exceeded in tier A testing, additional testing is mandatory, using multispecies communities of dung-breeding organisms under more realistic field or field-like conditions (tier B testing; Floate et al. 2016). Specific risk thresholds to the dung fauna can include mortality, reduced fecundity, impaired behavior, and delayed development.

Other than this broad requirement, there is no standard methodology for tier B tests (Jochmann et al. 2011). Researchers may use dung pats that differ in size and number, and are derived from different species of animals fed on different

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* Address correspondence to f.mathews@sussex.ac.uk
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diets. Studies may be performed at different times of the year with taxa identified to different levels of taxonomic resolution (e.g., family vs genus vs species). In addition, endectocides include both avermectins (e.g., doramectin, eprinomectin, ivermectin) and milbemycins (e.g., moxidectin) that can be formulated and administered to livestock as oral pastes, injections, extended-release injections, pour-ons, and sustained-release boluses (Herd et al. 1996). All of these factors influence fecal concentrations of endectocide residues entering the environment (Lumaret et al. 2012) and the interpretation of results (Jochmann et al. 2011). Relatively few studies have directly compared the nontarget effects of different endectocides (Hempel et al. 2006; Webb et al. 2010) or of the same endectocide in different formulations (Herd et al. 1996).

Depending on their concentration, fecal residues may be lethal to the organisms that colonize the dung and their offspring that develop within the dung. They may also affect behavior, fecundity, and developmental times. Residues also have been variously reported to attract or repel insects from contaminated dung (Holter et al. 1993a; Floate 2007; Rodríguez-Vivas et al. 2019). Generally, however, fecal residues are reported to reduce the richness and abundance of diverse insects (especially species of Coleoptera, Diptera, and Hymenoptera) and other organisms in dung (Lumaret et al. 2012; Nieman et al. 2018). Nevertheless, there can be considerable variation between studies in terms of the size and direction of the effects (Webb et al. 2010; Rodríguez-Vivas et al. 2019).

The effect of residues on dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae, Aphodiinae) is of particular interest. They are typically among the most prominent insects present in fresh dung in terms of both abundance and biomass. Their feeding and breeding activities accelerate the removal of dung from pastures (Wall and Strong 1987), thereby increasing grazing area and encouraging the growth of healthy grass through soil aeration and nutrient recycling. Consequently, they help to increase the carrying capacity of pastures and reduce the risk of disease transmission (Herd 1995; Nichols et al. 2008; Beynon et al. 2012). The presence of dung beetles has been shown to reduce numbers of pest flies breeding in dung by 58% (Beynon et al. 2015) and to reduce the prevalence of cattle nematode infections by 55 to 89% (Fincher 1975). The resulting economic benefits have been estimated to equate to £367 million a year in the United Kingdom alone (Beynon et al. 2015). The impacts of endectocide residues are therefore important to the global agricultural economy.

Studies that report on the effects of fecal residues on dung beetles often include data for species in the subfamily Aphodiinae. This is likely because they are common in livestock dung across North America, Europe, Asia, and northern Africa, with most aphodiines easy to identify to the species level. In addition, *Aphodius constans* Duftschmid has been approved by the Organisation for Economic Co-operation and Development (OECD) as a model test organism to assess the toxicity of fecal residues on dung-breeding organisms (Hempel et al. 2006; Organisation for Economic Co-operation and Development 2010). The collective body of literature on the nontarget effects of fecal residues to aphodiines includes diverse—and occasionally contradictory—results, possibly owing to differences in experimental design (e.g., Floate et al. 2002; Webb et al. 2010).

In the present study, we conducted a meta-analysis to understand better the overall responses of aphodiine beetles to endectocide residues in livestock dung. This subfamily was specifically chosen as a model group for our meta-analysis because of the considerable amount of raw data already available in the literature and also because they are one of the few dung-breeding insects for which an OECD guidance document has been produced. The analyses were specifically designed to assess the effect of different endectocides on the occurrence (presence or absence) and abundance of individuals, with consideration given to both larval and adult life stages. The analysis of beetle abundance incorporated and examined data from multiple studies representing both field and laboratory experiments, which used different formulations (pour-on, injectable [in cattle], mixed in [i.e., "spiked" dung]).

METHODS

Data source and selection

Literature published in any language between 1990 and 2016 that reported the impact of endectocides on the abundance and occurrence (presence or absence) of aphodiine species was identified using the databases ISI Web of Knowledge and Google Scholar. The Medical Subject Headings search terms were as follows: (aphodiine OR species taxonomic names) AND (endectocide* OR anthelmintic* OR specific name of an endectocide) AND (cattle OR cow OR sheep OR livestock). These terms are an example of what was searched; exact search terms are given in the Supplemental Data (S1). The abstracts of each paper were reviewed to identify studies that 1) reported data for aphodiine beetles, 2) examined endectocides, and 3) incorporated use of control (uncontaminated) dung.

The literature search identified 149 papers, of which 27 matched the above 3 criteria. The papers that they cited, plus the papers that cited them, were cross-checked to identify 10 further papers suitable for inclusion. Requests for raw experimental data were then sent to the authors of these 37 papers. Responses received for 11 papers provided >25 000 individual rows of raw data. Additional responses identified papers for which data were no longer available, or which were not available in a suitable format.

To qualify for inclusion in the analysis, studies had to present key summary data (mean abundance per treatment type, standard deviation (SD), number of samples, and/or p value), provide information that permitted the calculation of these values, or provide raw data. For statistical rigor, we limited our focus to compounds represented in at least 2 data sets. At the end of this screening process, the data used in our meta-analysis comprised 31 individual data sets from 22 studies spanning 13 countries (Table 1).

Each data set assessed the effect of endectocide products on aphodiine beetles in cattle dung but were otherwise diverse in nature (Table 1). Most data sets examined the effects of

TABLE 1: Description of the 22 studies included in the meta-analysis

Source of data	Endectocide	Application method	Parameter(s) tested for adults (Ad) or larvae (L) in lab or field	Abundance (Ab) and/or occurrence (O) analyses	Country of study
Beynon et al. (2012)	Ivermectin	Pour-on	Attraction (Ad)—field (pitfall traps) Toxicity (L)—field and lab (dung pats)[a]	Ab	UK
Errouissi and Lumaret (2010)	Ivermectin	Sustained-release bolus	Attraction (Ad)—field (pitfall traps)	Ab	France
Errouissi et al. (2001)	Ivermectin	Sustained-release bolus	Toxicity (L)—lab	Ab	France
Floate (1998a)	Ivermectin	Pour-on	Attraction (Ad)—field (pitfall traps)	Ab	Canada
Floate (1998b)	Ivermectin	Pour-on	Toxicity (L)—field and lab (dung pats)[a]	Ab and O	Canada
Floate et al. (2002)	Ivermectin, doramectin, eprinomectin, moxidectin	Pour-on	Toxicity (L)—field and lab (dung pats)[a]	Ab and O	Canada
Floate (2007)	Ivermectin, doramectin, eprinomectin, moxidectin	Pour-on	Attraction (Ad)—field (pitfall traps)	Ab	Canada
Floate et al. (2016)	Ivermectin	Pour-on	Toxicity (L)—field and lab (dung pats)[a]	Ab and O	Canada and The Netherlands
Hempel et al. (2006)	Ivermectin, moxidectin	Spiked	Toxicity (L)—lab	Ab and O	Germany
Holter et al. (1993a)	Ivermectin	Injection[b]	Attraction (Ad)—field (pitfall traps)	Ab	Denmark
Holter et al. (1993b)	Ivermectin	Injection	Attraction (Ad)—field (pitfall traps)	Ab	Denmark
Jochmann et al. (2016)	Ivermectin	Spiked	Toxicity (L)—field and lab (dung pats)[a]	Ab and O	Switzerland
Krüger and Scholtz (1998a)	Ivermectin	Injection	Toxicity (Ad)—field and lab	Ab	South Africa
Krüger and Scholtz (1998b)	Ivermectin	Injection	Toxicity (Ad)—field and lab	Ab	South Africa
Madsen et al. (1990)	Ivermectin	Injection	Toxicity (L)—field and lab (dung pats)[a]	Ab	Denmark
McCracken and Foster (1993)	Ivermectin	Spiked	Toxicity (Ad and L)—field	Ab and O	Scotland
Nunome et al. (2009)	Ivermectin	Pour-on	Attraction (Ad)—field (pitfall traps)	Ab	Japan
O'Hea et al. (2010)	Ivermectin	Injection	Toxicity (Ad and L)—lab	Ab and O	Republic of Ireland
Römbke et al. (2007)	Ivermectin	Spiked	Toxicity (L)—lab	Ab and O	Germany
Römbke et al. (2010)	Ivermectin	Injection Spiked	Attraction (Ad)—field	Ab and O	Spain
Strong and Wall (1994)	Ivermectin, moxidectin	Injection	Toxicity (Ad and L)—field	Ab and O	England
Webb et al. (2010)	Ivermectin, doramectin	Pour-on	Attraction (Ad)—field (pitfall traps)	Ab and O	Scotland

[a]Dung pats exposed in the field to egg-laying adults and then held in the laboratory; toxicity assessments based on counts of emergent adults.
[b]Owing to lack of clarity in the method descriptions, only data collected from exposure via injection of livestock, and not through spiked dung, were used from this study in this meta-analysis.

ivermectin ($n = 22$), with much less data being available for moxidectin ($n = 4$), doramectin ($n = 3$), and eprinomectin ($n = 2$). In some cases, endectocides were added directly to the dung ("spiked" dung) rather than using dung collected from treated animals. Some studies placed known numbers of beetles into dung to assess the insecticidal toxicity of residues under laboratory conditions, whereas other studies used pitfall traps in the field to test the attraction or repulsion of beetles to residues. A further group exposed dung in the field to egg-laying adult beetles and then recorded subsequent numbers of larval or adult beetles recovered from the dung. There was also variation in the postapplication period during which dung was collected from treated animals, the species of aphodiine beetles examined, time of year, and environmental conditions (humidity, soil pH, temperature). Additional information on variation of cattle breeds, diet, and endectocide dosage used in each study can be found in the Supplemental Data (S2). Thus, an aim of the meta-analysis was to detect general patterns of endectocidal effects on aphodiine beetles that might be otherwise masked by variation across individual studies, through the use of covariate analysis.

Data synthesis: Occurrence

The effects of endectocides on adult beetle and larvae occurrence were tested using raw data contributed by authors (relating to $n = 11$ papers). "Occurrence" was defined as the presence of at least one individual in a given dung sample. Generalized linear mixed effects models with a binomial error structure (link = logit) were built in R (Ver 3.3.0; R Development Core Team 2016) using the package lme4 (Bates et al. 2015). Separate models were built for adult beetles and larvae. Study identity, species, and individual dung pat identity were specified as random effects, with treatment type (control or endectocides) set as a fixed effect. Presence was coded as 1 and absence as 0. Dung-baited pitfall trap studies are best suited to assess the attraction or repulsion of residues to beetles. However, there was no material difference in the results for numbers of adult beetles when analyses were based on this subset of studies rather than the entire data set. Therefore, to maximize statistical power, all available data were used.

Data synthesis: Abundance

To test the effect of endectocide exposure on the abundance of aphodiine beetles, we used the standardized mean difference (Hedges' adjusted *g*) between endectocide-treated and control samples to calculate the effect size and 95% confidence interval. Hedges' *g* is a variation of Cohen's *d* that determines the posttest difference in means between 2 treatments. The mean difference is then divided by the pooled SD to correct for small sample bias (Hedges and Olkin 1985; Hedges and Vevea 1996). Effects sizes can cautiously be interpreted as small (0.2), medium (0.5), or large (0.8 or greater; Cohen 1988). Analyses were performed using Comprehensive Meta-Analysis (Ver 3.3.070; Biostat).

Owing to random errors within studies and the variation between studies, we expected high heterogeneity and therefore chose a priori to apply random-effects models (REM) as the most appropriate method to calculate mean effect size. In addition, REMs are more applicable when the aim is to generalize beyond the scope of solely those studies used in the meta-analysis (Hedges and Vevea 1998). To determine the level of heterogeneity between studies, we calculated I^2 (level of heterogeneity as a percentage) and then tested whether the level of heterogeneity was significant using Cochran's heterogeneity statistic (*Q*). Higgins et al. (2003) tentatively assign categories of low, medium, and high heterogeneity to I^2 values of 25, 50, and 75%, respectively. The sensitivity of the results to the exclusion of individual studies was tested using a sequential leave-one-out approach.

To permit the inclusion of data for studies where the SD of some treatment groups was zero (i.e., no traps recovered beetles), a small value (0.001) was substituted for zero. There were no material differences in the results when the analyses were repeated using the averaged SD obtained across all treatment groups within a particular study. Within this meta-analysis, the relative sample sizes for each study were weighted according to the number of dung pats examined and the number of years over which the experiments were conducted. In addition, the number of exposure days and individual species identities were incorporated within this analysis, to account for random variation that might occur within and between studies and to enable a generalized meta-analysis to be performed. If a study did not report results for species individually, then the species was recorded as an "aphodiine species."

Analyses were first performed on all studies combined, to assess the overall effects of endectocides on dung beetle abundance. Additional analyses considered outcomes for adult beetles and larvae separately. For these analyses, beetles were defined as adult individuals that had colonized fresh dung pats naturally. In contrast, larvae were immature individuals that either had been directly placed into dung by the researcher or had developed from eggs laid in dung colonized by adults (i.e., "progeny"; Floate 1998b).

The initial analysis used data for all endectocides combined (i.e., ivermectin, doramectin, eprinomectin, moxidectin). Each endectocide was then assessed individually when data were available for at least 2 studies; using this criterion doramectin and eprinomectin were not examined for larvae because no data were available. The interaction between formulations (i.e., injectable [in cattle], pour-on, spiked) and experiment type (i.e., field, laboratory) was also assessed for all models. Insufficient data prevented analyses of these interactions for 1) adults in laboratory conditions, 2) adults under field conditions using spiked dung, 3) larvae under laboratory conditions using pour-on formulation, and 4) studies using a sustained-release bolus formulation.

Publication bias

We explored the possibility of publication bias for the overall analysis of the impact of endectocides on the abundance of aphodiine beetles. Two methods were used: 1) construction of

a funnel plot (Sterne and Egger 2001), and 2) the computation of the fail-safe *n* test. The former permits a visual assessment to assess whether studies with small effect sizes are underrepresented in the literature. The latter method is used to calculate the number of nonsignificant, unpublished studies required to nullify the overall effect size (Rosenthal 1979, 1984).

RESULTS

Occurrence

Endectocide-treated dung was significantly more likely to have at least one adult aphodiine beetle than was control dung (odds ratio 1.59, confidence interval [CI] 1.41–1.79, $p < 0.001$; Figure 1). The opposite effect was found for larvae (Figure 1; odds ratio 0.64, CI 0.58–0.70, $p < 0.001$).

Abundance

A significant negative relationship was detected between endectocide exposure and the total abundance of aphodiine beetles (adults plus larvae; 22 studies, Hedge's $g = 0.46$, 95% CI 0.21–0.71, $p < 0.001$; Figure 2). The heterogeneity of the effect sizes among these studies was high ($I^2 = 96.95\%$, $Q = 689.60$). However, sensitivity analyses showed that the exclusion of individual studies had little impact on the effect size (Supplemental Data S2). A significant negative relationship between treatment and abundance was detected for adults (14 studies; Hedge's $g = 0.34$, 95% CI 0.05–0.62, $p = 0.022$; Figure 3), and a stronger effect was detected for larvae (12 studies; Hedge's $g = 0.52$, 95% CI 0.21–0.84, $p = 0.001$; Figure 4). There was high heterogeneity of effect sizes for both life stages (adults: $I^2 = 93.05\%$, $Q = 187.13$; larvae: $I^2 = 96.34\%$, $Q = 301.29$), but sensitivity analyses showed that the results were robust to the exclusion of individual studies (Supplemental Data S2).

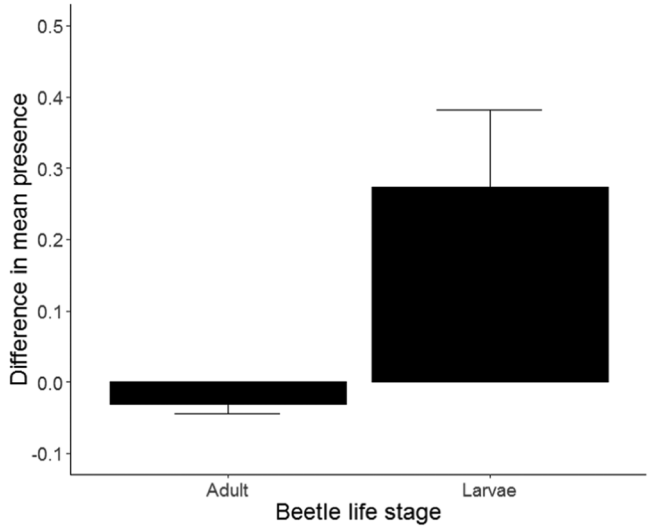

FIGURE 1: Mean difference in the proportion of aphodiine beetle occurrence between endectocide and control dung, with upper confidence intervals. Scale and direction of effect are from −1 (endectocide) to 1 (control).

Further analyses were conducted to assess the effect of individual endectocides on the abundance of different life stages. Ivermectin was associated with a significant negative effect on the abundance of both larvae (Hedge's $g = 0.57$, 95% CI 0.18–0.86, $p = 0.002$) and adults (Hedge's $g = 0.15$, 95% CI 0.04–0.62, $p = 0.028$) relative to controls. Similar negative patterns were observed for doramectin (Hedge's $g = 0.30$, 95% CI –0.33 to 0.93, $p = 0.351$) and eprinomectin (Hedge's $g = 0.06$, 95% CI –0.05 to 0.17, $p = 0.281$); though not significant, the results could not exclude the possibility of no effect, and further research is required. For moxidectin residues, sample sizes were relatively low, and patterns consistent with either a positive or negative effect were observed for adults (Hedge's $g = –0.19$, 95% CI –1.25 to 0.86, $p = 0.721$) and larvae (Hedge's $g = 0.36$, 95% CI –1.34 to 2.05, $p = 0.680$).

Exploration of the interaction between study type (field vs laboratory) and formulation (pour-on, injectable, spiked), showed that pour-on formulations showed a clear negative association with larval abundance in field experiments (Hedge's $g = 0.26$, 95% CI 0.19–0.34, $p < 0.001$). Results for injectable formulation and spiked dung on larvae were equivocal, with more data being required to assess the direction of these effects (injectable: Hedge's $g = 0.49$, 95% CI –0.98 to 1.96, $p = 0.513$; spiked: Hedge's $g = 0.32$, 95% CI –0.39 to 1.04, $p = 0.372$). In laboratory studies, a reduction in larval abundance was detected in spiked dung compared with controls (Hedge's $g = 1.20$, 95% CI 0.65–1.74, $p < 0.001$). With the available data, we were unable to detect a clear positive or negative association when using the injectable formulation (Hedge's $g = 0.25$, 95% CI –0.13 to 0.64, $p = 0.201$). For adults no clear conclusion can be drawn from the field experiments using pour-on and injectable formulations (pour-on: Hedge's $g = 0.35$, 95% CI –0.14 to 0.82, $p = 0.160$; injectable: Hedge's $g = 0.47$, 95% CI –0.09 to 1.03, $p = 0.100$; no data were available for spiked formulations).

Publication bias

The asymmetry of the funnel plot computed for the total analysis suggested the presence of small-study bias or unexplained heterogeneity (Figure 5). It was calculated that correcting for this asymmetry would require 4 studies (black dots in Figure 5) to fall on the right of the mean effect size; these are studies which show significant positive effects of endectocides on aphodiine beetles. Using an REM including the imputed values for these 4 missing studies, we demonstrated that the new mean effect size for the symmetrical total analysis is very similar to the original estimate (Hedge's $g = 0.54$, 95% CI 0.31–0.93 compared with the original: Hedge's $g = 0.46$, 95% CI 0.21–0.71), suggesting that publication bias is unlikely to explain the results. Using the fail-safe *n* method for the total analysis, 1188 additional unpublished or undiscovered studies would be required to nullify the results. Rosenthal (1984) states that effect sizes are robust if the fail-safe *n* number is 5-fold greater than the number of studies used in the meta-analysis plus 10. Thus, for every data set used in the present study, an

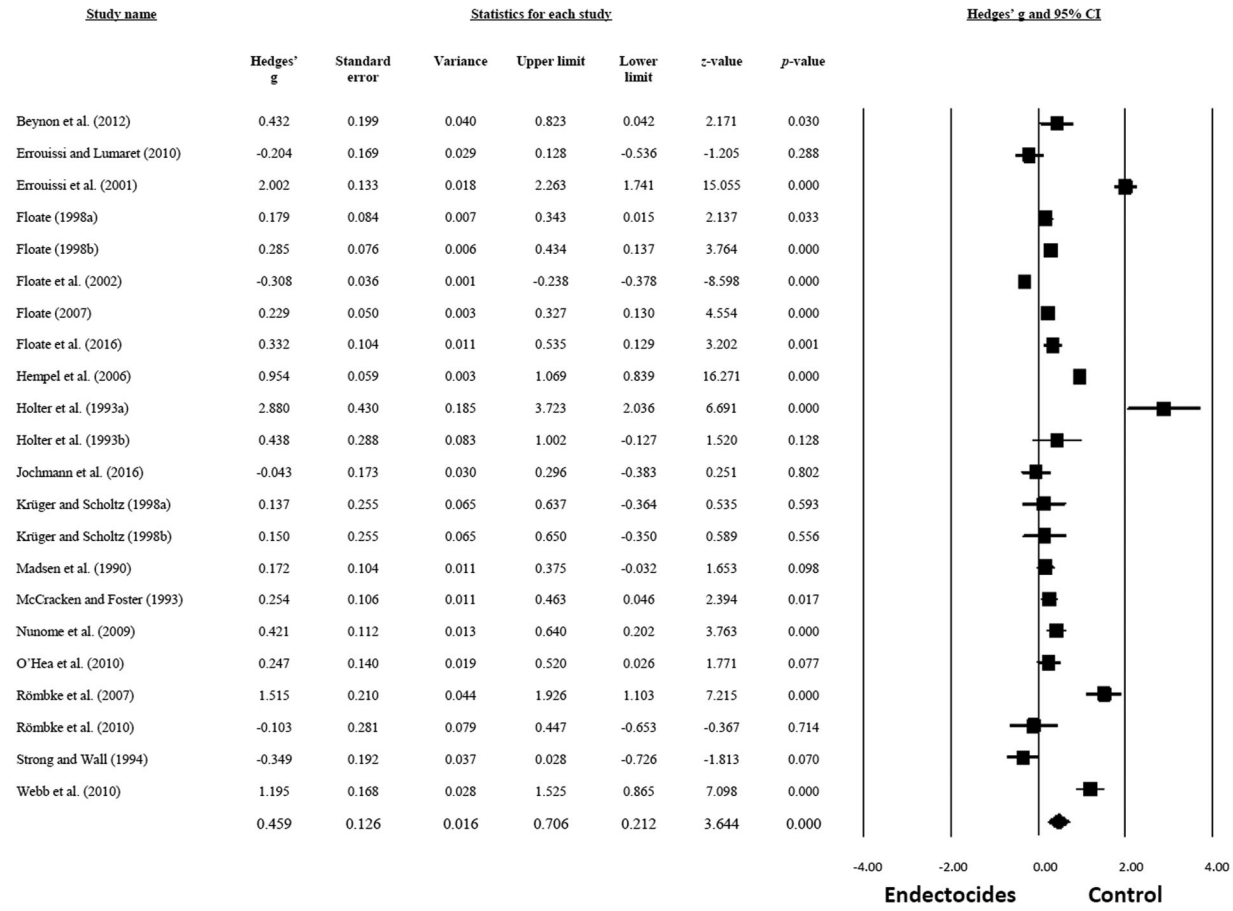

FIGURE 2: Forest plot illustrating the impact of endectocides on total abundance of aphodiine beetles (larvae and adults). Boxes represent Hedges' *g* estimates of effect size for individual studies within the overall meta-analysis, and lines represent their 95% confidence intervals (CIs). The diamond represents the combined mean Hedges' *g* estimate of all studies, with its width representing its 95% CI. If an effect size is positive (to the right of zero), the data have greater association with "control" dung rather than those exposed to "endectocides" (negative; to the left of zero), thus highlighting the direction of the effect for each study.

additional 54 data sets showing no effect of endectocide residues would be needed to counter the effect of our findings. It can therefore be concluded that the estimated effect sizes in the meta-analysis, are robust and unbiased, can be interpreted in a meaningful way.

DISCUSSION

Our results indicate a significant overall negative effect of endectocide fecal residues on the abundance of both larval and adult aphodiine beetles. The high heterogeneity (I^2) associated with study-specific factors (e.g., time of year, temperature, species, endectocide product, formulation) confirm the value of developing a standardized procedure for tier B testing (Jochmann et al. 2011).

The outcomes from this meta-analysis resolve the conflict between studies showing higher abundance of certain aphodiine beetle species in treatment dung (e.g., Errouissi and Lumaret 2010; Webb et al. 2010; Jochmann et al. 2016) and those that show the opposite effect (e.g., Floate et al. 2002; Floate 2007). Our results show that endectocides lower the abundance of aphodiine beetles, with the effect size being larger for larvae than for adults. Hence, even if adult dung beetles are observed in dung contaminated with endectocide residues, the survival of offspring developing in that dung is significantly reduced compared to offspring developing in untreated dung. Ivermectin was determined to be particularly toxic, but consistent negative patterns were also detected for the other endectocides considered. The exception was the combination of adult beetles and moxidectin, though the data were limited and are consistent with the possibility of a negative effect. However, recent research has illustrated that moxidectin did not impact adult survival or reproductive success but did impact larval survival rates (Martínez et al. 2018). Confounding factors across studies could also influence these results, including variation in dose and length of exposure. It is therefore appropriate to apply a precautionary principle until further data become available; this is particularly true for all endectocides other than ivermectin. Importantly, all of the endectocides tested were linked with some form of negative impact, so it would be unwise to classify any as environmentally safe based on current evidence. It would be valuable for future research to assess other veterinary parasiticides that may have a more limited impact on the environment.

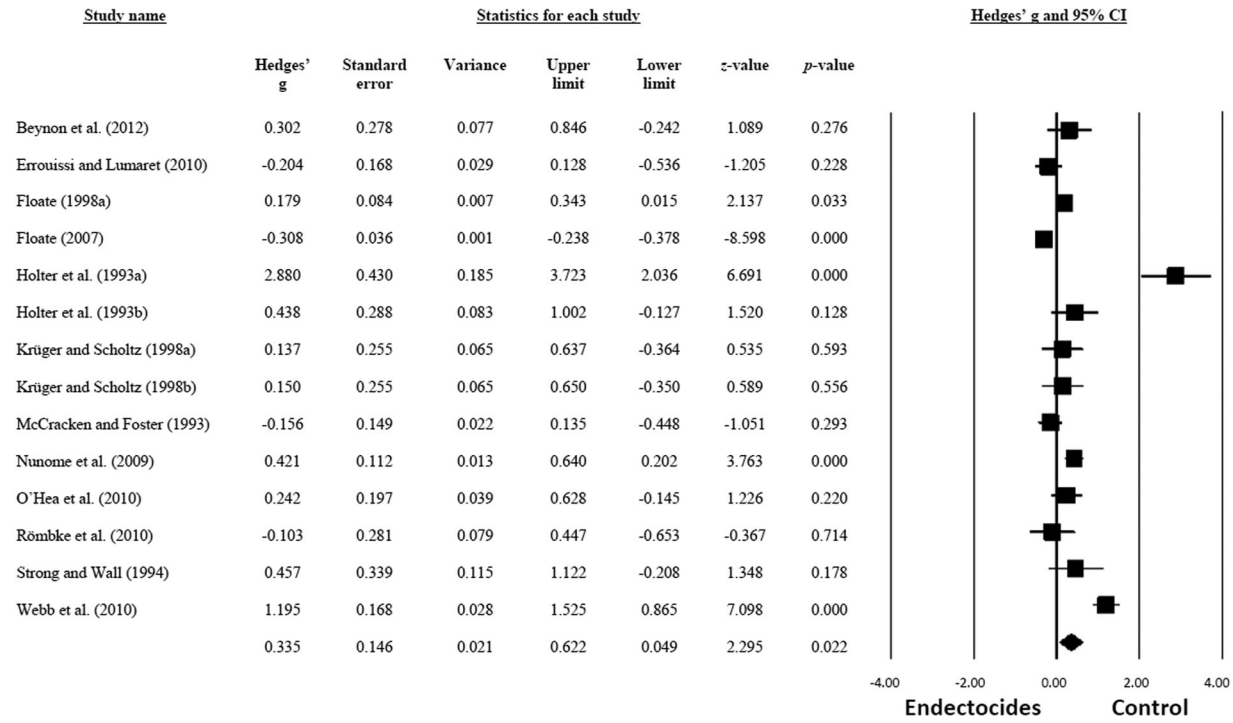

FIGURE 3: Forest plot illustrating the impact of endectocides on adult aphodiine beetle abundance. Boxes represent Hedges' *g* estimates of effect size for individual studies within the overall meta-analysis, and lines represent their 95% confidence intervals (CIs). The diamond represents the combined mean Hedges' *g* estimate of all studies, with its width representing its 95% CI. If an effect size is positive (to the right of zero), the data have greater association with "control" dung rather than those exposed to "endectocides" (negative; to the left of zero), thus highlighting the direction of the effect for each study.

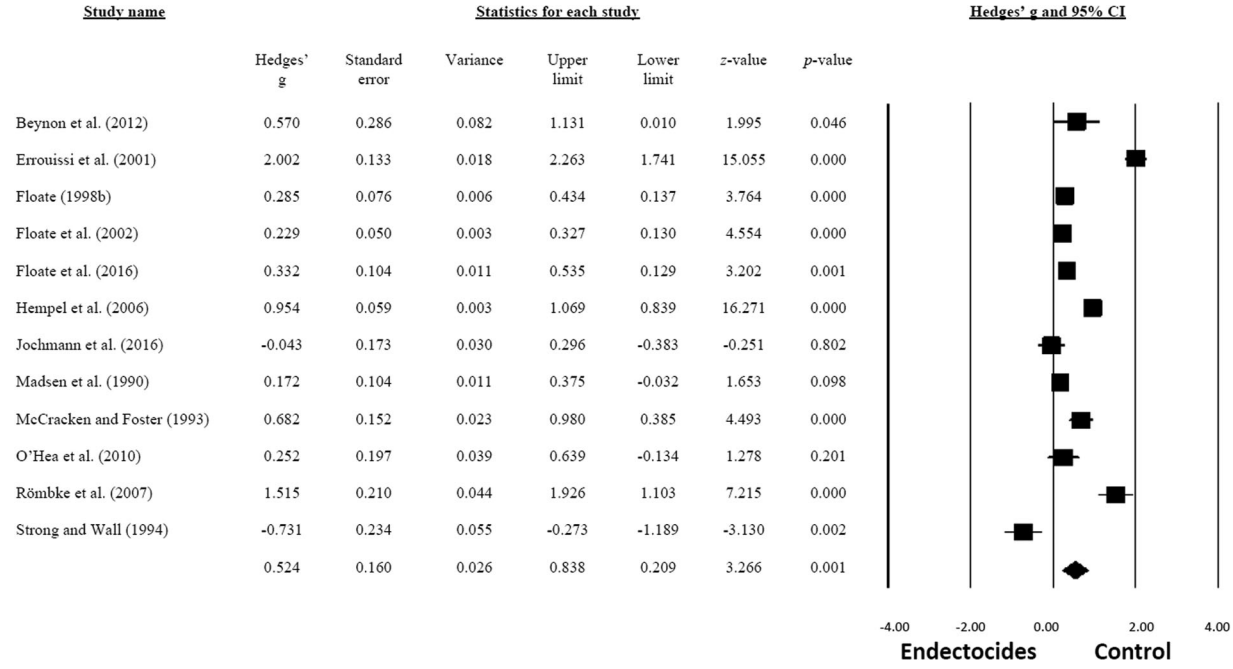

FIGURE 4: Forest plot illustrating the impact of endectocides on larval aphodiine beetle abundance. Boxes represent Hedges' *g* estimates of effect size for individual studies within the overall meta-analysis, and lines represent their 95% confidence intervals (CIs). The diamond represents the combined mean Hedges' *g* estimate of all studies, with its width representing its 95% CI. If an effect size is positive (to the right of zero), the data have greater association with "control" dung rather than those exposed to "endectocides" (negative; to the left of zero), thus highlighting the direction of the effect for each study.

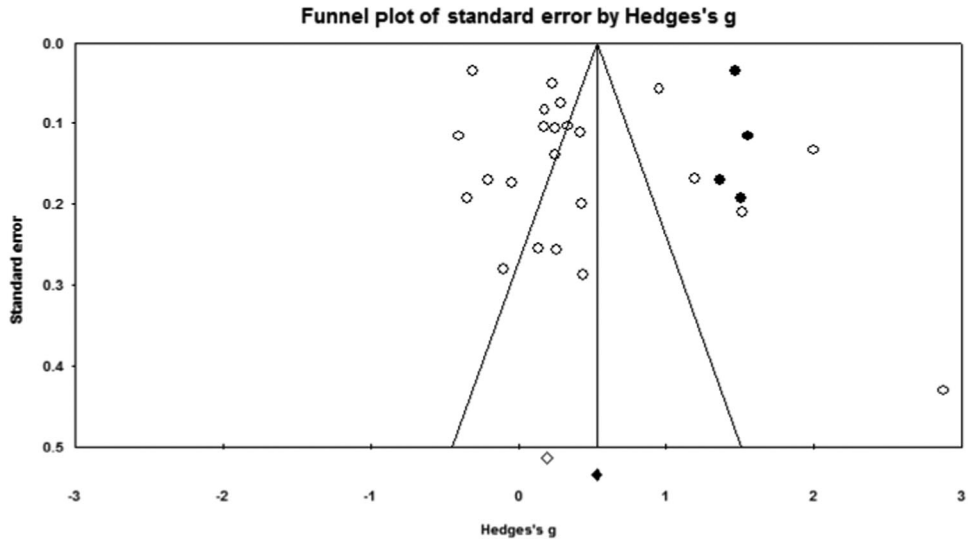

FIGURE 5: Hollow circles in the funnel plot represent individual studies from the total analysis ($n = 22$). Black circles represent imputed studies from the trim and fill method, and the black diamond represent the 95% confidence interval for the meta-analysis around the random effect model's mean adjusted for publication bias (black straight line). See text for further explanation.

Investigating the interaction between formulation and study type (field vs laboratory) identified a significant negative impact of pour-on formulations on beetle larvae in the field, whereas evidence for the other application methods was more equivocal. Laboratory experiments only showed a reduction in the abundance of larvae developing in spiked dung, but most applications of endectocides have negative associations with beetle abundance (e.g., Krüger and Scholtz 1998b; Errouissi et al. 2001). However, we caution that there is a lack of studies that directly compare the nontarget effects of different formulations, and this evidence gap should be filled as a matter of urgency. For example, we were unable to obtain data that directly compared in the same study the effect of spiked dung versus dung from treated animals. There was also a lack of data assessing the abundance of adult beetles in spiked dung in field studies and for larvae developing in dung from treated animals in laboratory studies.

Analysis of the raw occurrence data (11 papers) demonstrated that treated dung had a slightly higher probability of containing at least one adult beetle compared to untreated dung, indicating that residues can act as an attractant. The opposite pattern was detected for larvae, suggesting that residues increase egg and larval mortality. To our knowledge, this is the first report of how the occurrence of adult and larvae aphodiine beetles is affected by endectocide residues. It highlights the potential for a "snowball effect," whereby attraction to residues may increase the likelihood of adults laying their eggs in dung that is particularly toxic to their progeny. In the absence of immigration, the application of endectocides could therefore potentially contribute to the local extirpation of aphodiine populations. The attraction of dung beetles to residues has been reported previously, with variation within and among studies associated with year, season (e.g., spring vs autumn), and length of exposure (e.g. Floate 1998a; Rodríguez-Vivas et al. 2019). Römbke et al. (2010) state that the attraction can occur when acetone is used as a solvent in studies that use dung spiked with ivermectin. However, we observed the same effects with alternative application formulations. These results demonstrate the complexity of the issue of attraction behind individual studies and the local factors that need to be accounted for. When investigating the effects of endectocides, more research is needed on the occurrence of species and not just on their abundance.

Overall, our results clearly demonstrate the negative impact of endectocide residues on aphodiine beetles. We stress that a standardized methodological approach should be taken when conducting multispecies environmental impact assessments of different endectocide products (e.g., Jochmann et al. 2011, 2016; Floate et al. 2016). Critically, integrated research is needed to understand the synergies and trade-offs between veterinary pharmaceutical use and the delivery of ecosystem services, such as dung removal from pasture. As well as benefiting wildlife, more measured use of veterinary pharmaceuticals will slow the worldwide development of parasiticide resistance by target species. In Europe, nematodes on 12.5% of farms surveyed in 4 major cattle markets were recently found to be resistant to both ivermectin and moxidectin (Geurden et al. 2015). In Brazil, a study of 10 farms demonstrated that none of 4 avermectins (doramectin, eprinomectin, ivermectin, moxidectin) were effective for the control of nematodes affecting cattle (Ramos et al. 2016). In the United Kingdom, guidelines have been created to manage for parasiticide resistance (Control of Worms Sustainably 2017; Sustainable Control of Parasites in Sheep 2017); however, dissemination and application of this information can be variable. Adhering to such guidelines and using parasiticide products with limited nontarget effects may slow current declines being reported for insect populations (e.g., Hallmann et al. 2017) and will help sustain ecosystem services that annually return many millions to the global agricultural industry (e.g., Beynon et al. 2015).

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