Prevalence and Factors Associated with Anthelmintic Resistance in Gastrointestinal Nematodes of Cattle: A Systematic Review and Meta-analysis

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Abstract

A systematic review and meta-analysis was conducted with the aim to measure the prevalence of anthelmintic resistance (AR) in cattle gastrointestinal nematodes (GIN) and potential management factors associated with development of such resistance. A search algorithm was constructed and a comprehensive search of the primary literature was conducted in: CAB abstracts (1990-2016), Medline (1860-2016), Agricola (1924-2016) and Lilacs (1985-2016). Prevalence estimates were combined through meta-analysis (MA) using the logit prevalence and between-study heterogeneity was quantified. Twenty-nine publications (5 cross-sectional studies; 14 prevalence surveys and 10 field trials) were included in this review. Random effects MA resulted in an overall AR prevalence of 72.0% (95% CI: 58.4% to 80.0%). However, a high heterogeneity was observed (I²=55.9%). From studies reporting the nematode genera involved in the AR, Cooperia spp were present in 91.7% of the studies (n=24); Ostertagia sp. in 44.5% (n=22); Haemonchus sp. in 47.8% (n=23); Trichostrongylus sp. in 36.4% (n=22) and Oesophagostomum spp. in 23.8% (n=21). The included cross sectional studies suggested that frequency of treatments, age of cattle and type of management were potential management factors associated with AR in bovine GINs. However, more detailed studies are necessary to fully evaluate management guidelines for implementation of sustainable GIN control strategies.

Keywords: Ruminants; Parasites; Resistance establishment; Risk factors; Meta-analysis

Introduction

Gastrointestinal nematodes (GIN) are an important cause of production losses in young grazing cattle, particularly in intensive production systems. The prevention of GIN infections and parasitic gastroenteritis relies on broad spectrum anthelmintic drugs. At present, the major classes of anthelmintics available for cattle belong to the families of the imidazothiazoles (levamisole), benzimidazoles (albendazole, febendazole, and oxfendazole) and macrocyclic lactones (avermectins and milbemycins).

For testing drug efficacy, the two most widely accepted tests are the fecal egg count reduction test (FECRT) and the controlled efficacy test [1,2]. The "International harmonization of anthelmintic efficacy guidelines", indicate that an acceptable product should be at least 90% effective [3]. Even when anthelmintic resistance (AR) occurs at the parasite level, it is diagnosed through the parasitized animals and the outcome expressed at the farm level.

Anthelmintic resistance has been recognized in small ruminants worldwide, and this phenomenon was initially reported in cattle in New Zealand [4]; Australia [5]; South America [6-8]; North America [2] and Europe [9-11].

A systematic review (SR) follows a structured methodology in which each step is conducted by two independent reviewers to minimize bias. Meta-analysis (MA) refers to the statistical methodology for combining results from similar independent studies, with the aim to produce a more precise overall estimate of effect [12]. This methodology allows identification and quantification of factors that can explain variability between studies of the outcome of interest.

The objective of this study was to conduct a SR and MA of the available literature to assess the prevalence of AR in cattle farms and to identify management factors associated with occurrence of AR.

Materials and Methods

Review question, definitions and protocol

This SR studied the farm prevalence of AR in bovine GINs and potential risk factors associated with its occurrence. The PRISMA guidelines (Preferred Reporting items for Systematic reviews and Meta-Analyses statement; [13]) were followed and adapted to a prevalence/exposure SR-MA.

The review question was structured to simultaneously gather information on AR prevalence among bovine GINs and the factors associated with its occurrence. The population was defined as the bovine species. The exposures were:

1. The farm prevalence of AR of the GINs Haemonchus placei, H. contortus or H. similis; Ostertagia ostertagi or O. leptospicularis; Trichostrongylus colubriformis, T. axei or T. longispicularis; Cooperia oncophora, C. macmasteri, C. surfonada, C. punctata or pectinata; Nematodirus battus, N. helvetianus or N. spathiger; Oesophagostomum radiatum; Trichuris globulosa or discolor or Bunostomum phlebotomum. The methodology to diagnose AR was registered without...
importance in bovine species.

research investigating AR and/or risk factors on GIN of economical between respective reviewers. At this stage, we included primary good reviewer agreement). Conflicts were resolved by consensus "pass" (yes or no) to perform the kappa test (kappa>0.8 was considered 

reviewer agreement was evaluated using 30 abstracts using the variable standardized and pre-tested form (Supplementary material S1). The 

search.

No language or other restrictions were imposed at this stage of the 

missing from electronic searches, were added into the review process. 

Duplicated references were manually removed. Search verification was 

library. Additionally, we manually searched the proceedings of the 

Journal.

in Veterinary Parasitology (WAAVP) and the Veterinary Parasitology 

International Conference of the World Association for Advancement 

parasitological databases (CAB abstracts (1990-2016), Medline (1860-2016) and 

Lilacs (1985-2016), Medline, Cab Direct and LILACS: (bovine OR cattle OR steer OR heifer OR calves NOT (sheep OR ovine OR goat)) AND ((gastrointestinal OR internal) and (parasite* OR nematode*)) OR helmin* or haemonchus OR ostertagia OR cooperia OR trichostrongylus) AND ((anthelmintic OR drench or "macrocyclic lactone*" OR benzimidazol* OR levamisol* OR ivermectin) AND ((gastrointestinal OR internal) and (parasite* OR nematode*)) OR helmin* or haemonchus OR ostertagia OR cooperia OR trichostrongylus) AND ((anthelmintic OR drench or "macrocyclic lactone*" OR benzimidazol* OR levamisol* OR ivermectin) AND (resistance OR resistant*) AND prevalence). Adding the RF search 

terms did not retrieve a new citation beyond those already captured by the anthelmintic resistance terms, therefore, the risk factor search 
terms were removed. These search terms were adapted to search the 
database Agricola (1924-2016) from the National Agricultural 

library. Additionally, we manually searched the proceedings of the 

International Conference of the World Association for Advancement 
in Veterinary Parasitology (WAAVP) and the Veterinary Parasitology 

journal.

Citations retrieved from databases and manual searches were 

imported into a reference management software ("RefWorks-COS"). Duplicated references were manually removed. Search verification was 

performed by hand-searching of 4 literature reviews [14,15,19,20]. All 

relevant citations identified through manual searching, which were 

missing from electronic searches, were added into the review process. 

No language or other restrictions were imposed at this stage of the 

search.

Relevance screening

Abstract-based relevance screening was conducted using a 

standardized and pre-tested form (Supplementary material S1). The 

reviewer agreement was evaluated using 30 abstracts using the variable "pass" (yes or no) to perform the kappa test (kappa>0.8 was considered 
good reviewer agreement). Conflicts were resolved by consensus 
between respective reviewers. At this stage, we included primary 
research investigating AR and/or risk factors on GIN of economical 

importance in bovine species.

Methodological assessment and data extraction

A protocol form was developed and adapted from a previously 

form used by the first author (AM) which is included as supplementary 
online material (S2). This process included three reviewers and three 

full-text primary research articles for the risk of bias assessment and 
data extraction step of the pre-test.

Before methodological assessment (BA) and data extraction (DE) 

were performed, the relevance of articles selected through abstract 
screening was confirmed using the full-text papers to determine whether:

1. The article was published in English, Spanish, Portuguese, 

Italian or French.

2. The study designs used cross-sectional, prevalence surveys, 

longitudinal prevalence surveys, cohort, case-control or field 

trial.

3. The study reported that the methodology employed to detect 

AR at the farm level had an appropriate control group when 

using "in vivo" tests (e.g. FECRT or worm count reduction test).

4. The results reported sufficient detail to provide quantitative 
data for use in the MA.

The information extracted from each study included variables 
grouped in:

1. Characteristics of the cattle population and study settings

2. Type of anthelmintic drugs evaluated

3. Type of outcome measured

4. Risk factors evaluated

5. Laboratory method

6. Study results.

Management factors reported in cross-sectional studies associated 

with AR development were grouped according to the main factors 

reported in the searched literature as surrogates of potential causes of 

AR: treatment frequency, grazing management and refugia, age and 

breed.

The overall methodological quality was assessed using the following 
criteria:

Method of selection of participants

1. Sampling strategy

2. Follow-up

3. Assessment of confounders

4. Clustering adjustment

5. Sufficiently reported (referenced) laboratory protocols.

Several publication tools or guidelines to conduct observational or 

experimental trials were followed to build the quality assessment form 

[21-23].

Further, risk of systematic bias was assessed using guidelines for 

observational studies or experimental trial studies. The domains for 

observational studies were selection of participants, confounding 

variables, measurement of exposure, blinding of outcome assessment,
incomplete outcome data and selective outcome reporting according with the RoBANS tool [24]. For experimental studies, the domains were sequence generation, allocation concealment, blinding of outcome assessment, incomplete outcome data and selective reporting [25].

**Systematic review management**

An electronic SRS nexus review format (Möbius Analytics, Ottawa, Ontario, Canada) was used for all steps of the SR. Each abstract or full paper was assessed by two independent reviewers against each eligibility criteria (Relevance Screening, Bias Assessment and Data Extraction) and any conflicts were resolved through consultation.

**Summary measures**

**Prevalence:** The % FECR or percentage total worm count reduction (%WCR) and, when available, a 95% confidence interval (CI), were extracted for each anthelmintic drug for each farm studied. A farm was classified as AR positive for at least one anthelmintic drug with a % FECR or %WCR less than 95 or 90 according to the author cut off. The mean anthelmintic resistance proportion for each study and 95% CI, when not provided by the authors, were estimated using the number of positive farms for AR and the total number of sampled farms reported in the study.

**Risk factors:** Adjusted odd ratios (OR) were extracted when presented either from a Mantel-Haenszel or logistic regression analysis and 95% CI. When raw data was available (e.g. total sample size, number of farms with anthelmintic resistance with the risk factor present and absent), ORs and 95% CI were estimated. The variables extracted, specified by our a priori categories were:

1. Frequency of treatments (number of annual treatments, number of summer treatments and using only more than 75% of avermectins in the past)
2. Management (grazing management, refugia index and type of control plan)
3. Breed
4. Age.

**Meta-analysis**

The mean proportion of AR in cattle GINs was analyzed using the logit prevalence to fulfill the assumption of normal distribution to perform a MA of continuous data. Logit prevalence and the standard error were computed using the formula [26]:

\[ \text{logit prevalence} = \ln \left( \frac{p}{1-p} \right) \text{ and } S.E = \sqrt{\frac{1}{n * p * (1-p)}} \]

where \( p \) is prevalence, S.E is standard error and \( n \) is the sample size.

When there was no evidence of AR (e.g. AR=0) a correction of 0.01 was added before logit prevalence estimation [27].

The random effects MA was carried out given the a priori assumption that between study heterogeneity was present. A pooled logit prevalence and 95%CI was generated (forest plots) by the Dersimonian-Lair method stratified by study type, and pooled estimates were back-transformed to prevalence using the formula:

\[ \text{Prevalence} = \frac{1}{(1 + \exp(-\text{coefficient}))} \]

Between studies heterogeneity was estimated using the \( F \) statistic, which describes the percentage of variation between studies that is due to heterogeneity rather than chance [12].

Heterogeneity was evaluated using sub-group analysis and univariable meta-regression, a weighted regression of the study results based on study characteristics thought to be a source of variation that may influence the response of subjects to treatment [12].

The study level variables used in the meta-regressions were:
1. Study design (cross-sectional, prevalence survey, field trial)
2. Sampling design (random, convenience or purposive, not applicable)
3. Sample size
4. Continent (Europe, Americas, Oceania, Asia, Africa
5. Clustering (yes, no)
6. Language (English, Spanish or Portuguese)
7. Drug type (benzimidazole, imidazothiazole, macrocyclic lactone).

All the analyses were conducted in STATA V 12.

**Results**

**Study selection and characteristics**

Using the format suggested by [13] the numbers of studies included at each stage of the review with reasons for exclusion are reported in Figure 1. In total, 29 studies were included in the MA, and from these

**Figure 1:** Number of studies identified, screened, assessed for eligibility, excluded and included in the systematic review-meta-analysis of the prevalence of anthelmintic resistance in cattle gastrointestinal nematodes and risk factors [15].
five were qualitatively assessed for risk factors associated with bovine GINs anthelmintic resistance occurrence (Table 1). A table with included articles is presented in Appendix A.

Most studies evaluated the efficacy of the three major anthelmintic drug families benzimidazole (n=25), imidazothiazole (n=18) and macrocyclic lactones (n=25). Therefore, we extracted data and analyzed the efficacy of the major drug families and not combinations or narrow-spectrum drugs such as closantel.

A total of 518 farms were investigated in the 29 publications included in this systematic review-meta-analysis (SR-MA). The countries where the studies were conducted were Argentina (n=5), the United States of America (n=3), the United Kingdom (n=3), Brazil (n=4), Belgium and Germany (n=2), New Zealand (n=2), Australia (n=3), Mexico (n=2), Greece (n=1), Nicaragua (n=1), Venezuela (n=1), Bangladesh (n=1) and Cameroon (n=1). The results of the main characteristics and the methodological assessment of the included studies are presented in Tables 2 and 3 respectively.

### Summary measures

The overall farm proportion of AR was 85.4% (95% CI=76.2% to 94.6%). When stratified by drug class, 83.3% (95% CI=73.5% to 93.1%) of the studied farms presented resistance to macrocyclic lactones; 47.0% (95% CI=27.6% to 66.4%) to the benzimidazole and 45.1% (95% CI=19.1% to 71.2%) to imidazothiazole.

From studies reporting AR nematode genera, *Cooperia* spp. was reported in 91.7% (n=24), *Ostertagia* sp. in 45.4% (n=22), *Haemonchus* sp. in 47.8% (n=23), *Trichostrongylus* sp. in 36.4% (n=22) and *Oesophagostomum* sp. in 23.8% (n=21).

The overall mean risk of bias for observational studies included in the SR-MA is illustrated in Figure 2. Unclear (not reported or unable to assess) was found in selection of farms and blinding of outcome (80% and 100%, respectively) and high in farm sample justification (80%). Figure 3 summarizes the overall mean risk of bias of 10 experimental field trials included in the SR-MA. Except for blinding of outcome assessment (100% unclear), the included studies presented low risk of systematic bias.
**Table 2:** Descriptive characteristics of 29 publications which were included in the systematic review-meta-analysis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
<th>Categories</th>
<th>Number of studies (n=29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study design</td>
<td>Type of study design</td>
<td>Cross sectional</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prevalence survey</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Field Trial</td>
<td>10</td>
</tr>
<tr>
<td>Drug</td>
<td>Anthelmintic group that efficacy has been evaluated</td>
<td>Macrocylic lactones</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bencimidazole</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Imidazothiazole</td>
<td>13</td>
</tr>
<tr>
<td>Laboratory test</td>
<td>Test employed to assess anthelmintic efficacy</td>
<td>FECRT or ECT</td>
<td>28</td>
</tr>
<tr>
<td>Cut off</td>
<td>Cut off to define lack of efficacy</td>
<td>≤ 90%</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≤ 95%</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Not reported</td>
<td>3</td>
</tr>
<tr>
<td>Gastrointestinal Nematodes (GIN)</td>
<td>Genera of GIN reported to be resistant to the studied drug</td>
<td>Cooperia spp</td>
<td>22(n=24)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ostertagia ostertagi</td>
<td>10(n=22)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Haemonchus spp</td>
<td>11(n=23)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trichostrongylus spp.</td>
<td>8(n=22)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oesophagostomum spp</td>
<td>5(n=21)</td>
</tr>
<tr>
<td>Date published</td>
<td>Year of study publication</td>
<td>Before 2000</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After 2000</td>
<td>28</td>
</tr>
<tr>
<td>Type of cattle</td>
<td>Type of cattle studied</td>
<td>Dairy</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Beef</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mixed</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Not reported</td>
<td>9</td>
</tr>
<tr>
<td>Continent</td>
<td></td>
<td>Europe</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Americas</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oceania</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Asia</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Africa</td>
<td>1</td>
</tr>
<tr>
<td>Language</td>
<td>Language of study publication</td>
<td>English</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spanish</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Portuguese</td>
<td>2</td>
</tr>
</tbody>
</table>

**Table 3:** Summary for methodological soundness and/or reporting of 29 publications included in the systematic review-meta-analysis.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Assessment</th>
<th>Number of studies (n=29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Was the simple size justified at the farm level</td>
<td>Yes</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Not applicable</td>
<td>9</td>
</tr>
<tr>
<td>Was the simple size justified at the animal level</td>
<td>Yes</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>22</td>
</tr>
<tr>
<td>How were operations selected for the study?</td>
<td>Not reported</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Random</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Convenience or purposively</td>
<td>23</td>
</tr>
<tr>
<td>Were the laboratory methods described in sufficient detail to be replicated?</td>
<td>Yes</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Reference paper</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>Did the author report that blinding was used?</td>
<td>Yes</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>28</td>
</tr>
<tr>
<td>Based on the study design, was clustering accounted for appropriately in the analysis?</td>
<td>Yes</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Not applicable</td>
<td>25</td>
</tr>
<tr>
<td>In cross-sectional studies, was the statistical analysis described adequately so that it can be reproduced?</td>
<td>Yes</td>
<td>2(n=5)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>0(n=5)</td>
</tr>
<tr>
<td></td>
<td>Statistical analysis not done</td>
<td>3(n=5)</td>
</tr>
<tr>
<td>Measurement of exposure</td>
<td>Yes</td>
<td>4(4)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Not applicable</td>
<td>25</td>
</tr>
<tr>
<td>Incomplete outcome data</td>
<td>Yes</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>26</td>
</tr>
<tr>
<td>Selective outcome reporting</td>
<td>Yes</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>28</td>
</tr>
</tbody>
</table>
Meta-analysis

The overall logit AR proportion back-transformed was 72.0% (95% CI=60.8% to 80.8%) for the group of observational studies (n=19), with high between study heterogeneity (I²=70.9%, p<0.001) (Figure 4). The AR proportion for 10 studies following a field trial design was 99.9% (95% CI=16.3% to 99.9%).

When exploring potential sources of between-study heterogeneity, results from the univariable meta-regressions, suggested that study location (categorized as “continent”) was associated with AR proportion (p<0.05). This contributed to explain 100% of the between study variation (I²=0.0%) and part of the total variation (Adjusted R²=68.6%). Study type, language, cattle type, study sponsorship and type of nematode developing resistance, were not significantly associated with AR logit prevalence.

Risk factors

A qualitative summary of the main RFs reported in five cross-sectional studies is presented in Table 1. Only two studies had enough data to perform a multivariable logistic model [16,28] while the remaining presented univariable ORs or p-values [29,30]. The results presented from these studies suggest that frequency of treatments and cattle age are associated with the presence of AR.

Conclusions

According to the results of this SR-MA, the phenomenon of anthelmintic resistance in nematodes of cattle has been studied in many parts of the world. Cattle are particularly susceptible to parasitic gastroenteritis at a young age, and then are able to develop immune protection when reaching adult age. Therefore, the number of anthelmintic treatments administered to adult cattle is expected to be low. Nonetheless, the results from this study indicated a high number of farms with bovine GINs resistant to one or more anthelmintic drug worldwide.

However, the high number of farms presenting resistance to the macrocyclic lactones (82%) suggests that this modern and broad spectrum drug has been employed frequently to control not only internal but external parasites such as ticks or screw worms. According to [31] the use of this kind of anthelmintic drugs has been the structural basis of worm management for nearly 40 years and reaffirms that the continual use has led to the global selection of drug-resistant worms populations. From all the studies included in this SR-MA, only one reported that the studied farms were randomly selected while most of them were conveniently selected. Only five studies reported sample size justification. For the observational studies, we identified unclear risk of bias when selecting the farms (85%) and high risk of bias for sample size justification (80%). None of these studies reported blinding, either of the administration of the drugs assigned to each group or of laboratory personnel performing the tests. Because of this, the studies included in this SR-MA are likely to
represent a bias selection of farms, because farmers who were aware that anthelmintic treatments were not effective tended to be more likely to participate in the studies, or because researchers selected farms with a previous knowledge of anthelmintic efficacy failure. Thus, AR values are likely to overestimate the true AR in cattle farms.

High between-study heterogeneity was expected a priori, in part due to regional characteristics influencing the production systems, epidemiological conditions and GIN control measures applied. This was supported by the fact that “region” was the only variable associated with the outcome and contributed to explain the between-study heterogeneity. This is concordant with Higgins [32] and Ioannidis [33] who are in favor of conducting MA even when the statistics demonstrates that the true effect size varies among studies.

Unfortunately, there were not enough studies to perform a MA on potential risk factors associated with the development of AR. Although some evidence suggests that frequency of treatments, population in refugia and host age at treatment are positive associations [28,30] further research is necessary to establish the main risk factors for GIN AR development [34]. Although some of the included studies were not representative of source populations of cattle ranches, results from this SR-MA suggested that practitioners and producers should be cautious in the frequency of treatment with anthelmintic drugs [35], in order to avoid development of AR which could exacerbate parasitic gastroenteritis and production losses [36].

Acknowledgements
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Authors Contribution
A. Mederos was responsible for managing the systematic review, execution of the meta-analysis and preparation of the manuscript. A. Minho contributed as second reviewer; B. Carracelas contributed as second reviewer; S. Fernández contributed as second reviewer and J. Sánchez contributed with the meta-analysis.

Conflict of Interest
The authors have not conflicts of interest to declare.

References


