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Guideline

World association for the advancement of veterinary parasitology (WAAVP) guideline for the evaluation of the efficacy of anthelmintics in food-producing and companion animals: general guidelines☆

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ABSTRACT

The general WAAVP (World Association for the Advancement of Veterinary Parasitology) guideline on anthelmintic efficacy were prepared to assist researchers with the planning, conduct and interpretation of studies to assess the efficacy of anthelmintic drugs in food-producing and companion animals. General principles are outlined herein to assist in the preparation and execution of dosage determination, dosage confirmation and field studies, which are applicable to all animal host species. These general guidelines are complemented by revised species-specific guidelines, which provide more specific, updated and detailed guidance for each animal host species.

1. Introduction

The World Association for the Advancement of Veterinary Parasitology (WAAVP) previously published guidelines to harmonise the evaluation of anthelmintic efficacy in dogs and cats (Jacobs et al., 1994), ruminants (Powers et al., 1982; revised by Wood et al., 1995), swine (Düwel et al., 1986; revised by Hennessey et al., 2006), horses (Duncan et al., 1988; revised by Duncan et al., 2002), and poultry (Yazwinski et al., 2003). Adherence to uniform methods of study design, study conduct, and data evaluation enables comparisons between studies and synthesis of data across studies or study groups, facilitates the development of evidence-based recommendations, reduces animal usage (with consequent benefits to animal welfare), and reduces drug evaluation costs. The revised guidelines do not include regulatory guidance as this is the remit of VICH (International Co-operation for the Harmonisation of the Technical Requirements for the Registration of Veterinary Medicinal Products) and individual regulatory agencies. Furthermore, the revised WAAVP guidelines no longer provide guidance for the evaluation of generic anthelmintic drugs through blood level bioequivalence or pharmacokinetic behaviour because the anthelmintic efficacy evaluation requires a good understanding of drug behaviour in a multi-compartmental system, suggesting that blood level bioequivalence alone might not be sufficient to confirm anthelmintic efficacy of a new anthelmintic drug or drug formulation (Wicks et al., 1993; Lifschitz et al., 1999; Hennessy et al., 2000; Leathwick et al., 2020). The principles outlined in the revised WAAVP anthelmintic guidelines are applicable to either single compound or combination anthelmintic drugs, with additional recommendations on the latter provided in Geary et al. (2012).

The revision of the WAAVP anthelmintic guidelines included an assessment of scientific and technological advancements with an evaluation of the benefit of these advancements to the revised guidelines, while taking the historical context, the level of validation and animal welfare considerations into account. Factors inherent to the determination of anthelmintic efficacy that were considered but were not included in the revised guidelines, are discussed in the WAAVP

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anthelmintic efficacy guidelines reflection paper (Geurden et al., in preparation). The newly established general anthelmintic guideline aim to update and harmonise the general principles regarding anthelmintic efficacy evaluation relevant for all animal host species (highlights in Box 1), and are complemented by revised species-specific guidelines for dogs and cats (Beugnet et al., in preparation), ruminants (Burden et al., in preparation), swine (Rehbein et al., in preparation), horses (Nielsen et al., 2022) and poultry (Yazwinski et al., in preparation). These species-specific guidelines provide more specific and detailed guidance for each animal host species with focus on the most common and relevant helminth parasites in the respective animal host species. While the WAAVP guidelines provide recommendations to enhance harmonisation, these should not be considered as prescriptive, and alternative approaches can be used if scientifically justified.

2. General principles regarding anthelmintic efficacy studies

While it is recommended to uphold the highest standards of study conduct for all anthelmintic efficacy studies, the principles of good clinical practice (GCP) are outside of the scope of the WAAVP anthelmintic efficacy guidelines and are discussed in detail in VICH Guideline 9 (VICH). In all anthelmintic efficacy studies, data should be Attributable, Legible, Contemporaneous, Original and Accurate (ALCOA), as described in the relevant WHO guidance World Health Organisation (WHO),. All anthelmintic efficacy studies should be conducted in accordance with local regulations regarding animal welfare and experimentation. Animal ownership and informed owner consent should be documented prior to inclusion of any animal in an anthelmintic efficacy study. All study personnel should be trained on the study protocol, the study procedures, and all tools used for data collection (including electronic data capture programs, if applicable).

2.1. Study protocol

The study protocol should be finalised and signed by all relevant study personnel before initiating the study. The protocol defines the study objective(s), including the parasite genus/species and parasite stage(s) against which the anthelmintic drug will be evaluated, and should clearly describe the experimental design and the statistical analysis, including methods of randomisation and allocation to treatment groups, the definition of the experimental unit, criteria for adequacy of infection (as applicable) and a description of the efficacy calculation. As a general principle, all non *a-priori* analyses (i.e., analyses not pre-specified in the study protocol) can only be deemed hypothesis-generating and additional studies are required to evaluate these new hypotheses. The protocol also defines whether induced and/or natural infections will be used and provides the methods to infect and/or confirm parasite infections in study animals before treatment, as well as the methods to recover and enumerate the targeted parasites and stages thereof after treatment. The study protocol should also describe the control group, which is usually an untreated control group (sometimes referred to as negative control group) but may also be a treated control group (sometimes referred to as active or positive control group). An untreated control group is defined as a group that did not receive the anthelmintic drug but was either left untreated or received a placebo-treatment. In a treated control group, the study animals receive an effective anthelmintic other than the new anthelmintic drug under evaluation. If the specific aim of the study is to demonstrate efficacy of the anthelmintic against a resistant isolate, a treated control group can be included to demonstrate decreased susceptibility of the isolate to the registered anthelmintic product.

The study protocol should also include all relevant information regarding the anthelmintic drug formulation, the dosage(s) being evaluated, methods of treatment administration, animal weighing and management, and animal and drug accountability (see appendix 1). For all host animal species, it is recommended to ensure identification of individual animals throughout the study, with the possible exception of avian host species (chickens, turkeys, etc.) which are often group-housed. In order to accurately calculate individual doses of the specific anthelmintic drug to be administered, individual study animals should be weighed using a calibrated scale with the accuracy of the scale verified before and, if applicable, after weighing of the animals. If a scale cannot be used, alternative methods (for example girth tape) should be justified after appropriate calibration. Guidance regarding dose calculation and anthelmintic drug administration should be provided in the protocol, as well as directions for how to handle cases where the treatment dose (either the full or a partial dose) is accidentally not applied or how to avoid anthelmintic drug transfer to other animals in the study (e.g., for topically applied formulations). If treatments are administered via medicated feed or water, daily feed or water consumption before and during the study should be recorded to determine dose rates and verify accurate dosing.

Information on the number, breed and age of animals to be used, as well as the housing, animal management and procedures for monitoring animal health throughout the study should be provided in the protocol. This also includes planning for an acclimation period for study animals prior to the study start, in order to allow animals to adapt to the study facility and conditions, and to collect essential pre-treatment data, including health observations, water and feed consumption, behaviour, bodyweights and infection status, as applicable. While not in the scope of the efficacy assessment described in the WAAVP anthelmintic guidelines, it is highlighted that the safety assessment is an important

Box 1
General principles of anthelmintic efficacy evaluation.

1. Anthelmintic efficacy evaluation generally starts with dosage determination studies, followed by dosage confirmation studies and, finally, field studies.
2. Anthelmintic efficacy can be defined as either therapeutic efficacy (against pre-existing infections) or persistent efficacy (protection against reinfection).
3. In dosage determination and dosage confirmation studies, natural or induced infections can be used. In dosage determination studies with induced infections, both laboratory and field isolates can be used, while in dosage confirmation studies, field isolates are generally recommended. Anthelmintic efficacy ($\geq$90% reduction) in dosage determination and dosage confirmation studies is based on the comparison of geometric mean parasite counts after necropsy of a treated and an untreated control group.
4. Animals in field studies are naturally infected. Different biomarkers of parasite infection can be used to assess anthelmintic efficacy in field studies, but in this general guideline, anthelmintic efficacy based on a reduction of faecal egg counts between pre-and post-treatment samples of the same treated animals is defined.

aspect of any study in which animals are enroled. Safety observations should be specified in the protocol, with consideration of the characteristics (e.g., absorption, distribution, metabolism and excretion, and any known toxicity) of the anthelmintic drug under evaluation. Study animals should be observed by a masked and qualified individual prior to treatment, periodically for several hours after dosing (or at intervals selected based on the known pharmacokinetics and/or toxicology of the drug), and at regular intervals thereafter for the duration of the study. If an injection or topical formulation is used, the application site should be identified for control as well as treated animals, and periodically examined for possible application site reactions. In conjunction with the drug-specific safety observations, general animal health and behavioural observations should be conducted at regular intervals (preferably daily) during the study. Animals that die during the study should be necropsied by a qualified individual and the cause of mortality determined, if possible, and documented.

Other pertinent information in the study protocol should include the methods related to masking (as a general rule all study personnel involved in data collection should be masked to animal treatment), the study location and the seasonality of the study (as this might be relevant for parasite infection levels), details regarding study personnel qualifications (e.g., education, relevant expertise and experience), along with a definition of their exact role in the study and their masking status. The methods for recording any clinical, safety, or parasitological data should be outlined in the protocol along with appropriate data capture forms. Any pre-specified changes to the protocol or departure from protocol-specified procedures should be recorded as amendments or deviations, respectively. The impact of any study protocol deviation(s) should be evaluated in the study report. A checklist to assist in drafting a study protocol is provided in Appendix 1.

2.2. Study report

The study report (sometimes referred to as a final study report) includes an evaluation of whether the study objectives as defined in the protocol were met, provides all relevant details around the study conduct, and provides sufficient detail to allow for an independent evaluation of the study design, conduct, results and conclusions. The study report should also document information about the anthelmintic drug including the trade name and common or chemical name (if the anthelmintic drug is not a marketed product), manufacturer, lot number and expiration date, product formulation, drug storage, and drug accountability during and at the end of the study. Similar details should also be described for the control product used in the study. If applicable, the study report will define new hypotheses identified, including those as per non *a-priori* analysis. As mentioned above, the observation and accurate reporting of any adverse event, including treatment application site reactions, is a critical endpoint and a key responsibility of any study investigator. Any adverse events observed after use of already authorised drugs should be reported to the manufacturer and/or relevant regulatory authorities in accordance with local pharmacovigilance regulations.

If the study is later submitted for publication, researchers are encouraged to utilise current reporting guidelines such as described by the REFLECT (Reporting guidelines for Randomised Controlled Trials for Livestock and Food safety) statement (O'Connor et al., 2010) or ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guidelines (Percie du Sert et al., 2020) in the preparation of their manuscripts.

2.3. Animal selection and allocation

As a general principle, anthelmintic efficacy is demonstrated in the respective target animal species. Infection models using non-target animal species or pharmacokinetic/pharmacodynamic studies can support specific aspects of anthelmintic efficacy assessment (for example dosage selection, or as an adjunct to the evaluation of persistent efficacy), but these non-target animal host studies are not within the scope of the WAAVP guidelines.

To avoid unnecessary repetition of animal studies, the selection of animals that are representative of the target animal population is critical to generate meaningful data. Dosage determination and dosage confirmation studies are conducted in either purpose-bred animals (to ensure uniformity) or representative target animals, while field studies are conducted in a broader animal population within the range of targeted animals. Animals should be suitable for inclusion in an efficacy study and free of any clinical disease that might compromise animal welfare, confound the assessment of anthelmintic efficacy or otherwise influence the study outcome.

A completely randomised study design, in which animals are randomly assigned to the different treatment groups, is the foundation for inferential value. It is acknowledged that large variability in parasite burdens is commonly observed in animal host populations, including overdispersion of parasites among otherwise comparable animals. However, the use of biomarkers of parasite infection, such as faecal egg counts (FEC), faecal larval counts, or microfilarial counts to block animals before randomisation to treatment group is not generally recommended for studies in which effectiveness is estimated based on parasite counts following necropsy, as these biomarkers in general do not reliably correlate with the underlying parasite burdens. Biomarkers of parasite infection may be appropriate as a blocking factor in studies directly using these biomarkers as a primary or secondary effectiveness outcome. As appropriate, the animal selection and allocation procedure should ensure that animal host characteristics potentially influencing parasite burdens (e.g., animal age, breed, physiological status, prior infection status) have minimal variation and are randomly distributed between experimental groups. The use of stratification and blocking should be discussed with a statistician during protocol development, if alternatives to complete randomisation are considered.

3. Studies supporting the assessment of anthelmintic efficacy

Anthelmintic efficacy can be defined as either therapeutic efficacy (against pre-existing helminth infections) or persistent efficacy (protection against re-infections). Treatment efficacy can be evaluated in animals after induced or natural infection. Persistent efficacy is evaluated by induced infections at a predefined timepoint after treatment or by exposing the treated animals to natural infections for prescribed periods of time after treatment. Persistent efficacy can be claimed for any defined time period after treatment, yet efficacy against re-infections acquired less than 7 days after treatment should not be considered as persistent anthelmintic efficacy.

Efficacy against resistant isolates for a new anthelmintic drug cannot be assumed based on a difference in mechanism of action because biochemical changes and/or genetic mutations can lead to cross resistance between anthelmintic drugs. Efficacy of a new anthelmintic drug against an isolate that has documented anthelmintic resistance towards another anthelmintic drug should be demonstrated through studies against the resistant isolate(s).

The process of anthelmintic efficacy evaluation is a stepwise and structured process, generally starting with dosage determination (sometimes referred to as dose determination or dose titration) studies, followed by dosage confirmation (sometimes referred to as dose confirmation) studies, and culminating with field studies (sometimes referred to as clinical trials or field-use studies). The assessment of anthelmintic efficacy in these studies, including persistent efficacy, is primarily based on parasite counts following necropsy. In field studies, anthelmintic efficacy is typically evaluated based on biomarkers of parasite infections in live animals, such as FEC (as described in Section 3.2.2), faecal larval counts or other appropriately justified biomarkers. Clinical parameters (e.g., weight loss or clinical signs of parasitism) do not always correlate with parasite burdens and are therefore not generally considered reliable outcomes for the evaluation of

anthelmintic efficacy.

3.1. Dosage determination and dosage confirmation studies (anthelmintic efficacy based on worm counts following necropsy)

3.1.1. Source of parasite infections

In previous guidelines (Wood et al., 1995; Hennessey et al., 2006), induced infections (sometimes referred to as artificial or experimental infections) with laboratory or field isolates were recommended for dosage determination studies and natural infections for dosage confirmation studies. In Vercruysse et al. (2001), it was recommended that induced infections be used to evaluate efficacy against some larval stages and that natural infections be used to evaluate efficacy against adult parasites and inhibited larval stages. While applicable for certain parasites and animal host species (e.g. *Ancylostoma* spp. or *Toxocara* spp. in companion animals), an induced infection model might not be available for other helminth infections (e.g. Cyathostominae in horses) and natural infections are therefore used for both larval and adult worm efficacy evaluation. The revised WAAVP guidelines acknowledge that the selection of either natural or induced infections in dosage determination and dosage confirmation studies will depend on the specific helminth genus, species or stage being evaluated. More specific guidance is provided in the species-specific guidelines.

In dosage determination and dosage confirmation studies using induced infections, field isolates (<10 years since it was isolated from the field) are generally recommended although the use of laboratory isolates (> 10 years since isolation from the field or isolates that have been subjected to further selection by anthelmintic exposure in the laboratory) can be justified in studies with specific objectives, for example efficacy evaluation against rare parasites or efficacy evaluation against documented resistant or susceptible isolates. The details regarding the isolate source (year and location of initial isolation), previous drug exposure (if known), resistance status (if known) and maintenance after isolation should be documented. In general, young and/or parasite-naive animals are best suited for induced infections because they are most susceptible. For certain animal hosts and specific parasites, the establishment rate in the animal host may be enhanced with the use of repeated inoculations as opposed to a single inoculation. Considering the often-standardised timing between inoculation and treatment in induced infection studies when specific developmental stages are targeted by the anthelmintic treatment, these repeated inoculation scenarios must be planned carefully. No recommendations are provided in the general guideline regarding the infection dose, as this is discussed in more detail in the species-specific guidelines.

In studies with natural infections, animals are exposed to parasites reflecting the current sensitivity profile of the target parasite(s). Especially with naturally infected animals there might be considerable variation in the numbers of individual parasite species and stages of the parasite(s) present in otherwise similar animals despite originating from a single source and demonstrating a comparable background. This should be considered when defining the number of animals per treatment group, to ensure adequate infections for reliable efficacy evaluation. After naturally infected animals are brought to the study site from their source(s), a similar acclimation period is recommended for all study animals. The acclimation period should provide for adequate time between exposure to the natural infection and treatment to allow development to the parasite stage targeted by the treatment.

3.1.2. Study design and objectives

The primary objective of dosage determination studies is to define the minimum dosage of the anthelmintic drug necessary to achieve the desired efficacy against each parasite within the intended spectrum of activity.A widely accepted study design utilises animals treated with the proposed target dosage (1X treatment group), and additional groups treated with at least one lower dosage (0.5X treatment group) and one higher dosage (2X treatment group), in addition to an untreated control group (Geurden et al., in preparation). If the anthelmintic drug is administered on a single occasion, the above study design is appropriate. If the proposed treatment regimen expands over multiple days, the dosage determination studies should potentially also evaluate different treatment durations bracketing the target treatment duration using only the target dose. When conducting a series of dosage determination studies, it is recommended to use the same anthelmintic drug formulation, route of administration, and dose ranges to avoid potential bias between studies. The dosage determination studies should be carried out in the target animal species preferably using the final or near-final formulation, and treatment should occur via the intended route of administration. Dosage determination studies that used the final formulation and dose of the anthelmintic drug may provide confirmatory efficacy results if infections are adequate. A secondary objective of dosage determination studies might be to define the dose-limiting parasite(s) if broad-spectrum anthelmintic activity is anticipated. The dose-limiting parasite is the specific targeted parasite and/or parasite stage (larval or adult) requiring the highest dosage to achieve efficacy. When a dose-limiting parasite within the envisaged spectrum of activity is identified and efficacy has been confirmed in dosage confirmation studies, future research with the drug (e.g. changes in formulation, combinations, etc.) may be amenable to a streamlined approach to the confirmation of efficacy against the dose-limiting parasite, as such indirectly providing evidence of efficacy for the remainder of the parasite species and stages in the spectrum of activity.

In order to confirm the efficacy of the selected dosage, at least two dosage confirmation studies following identical protocols are recommended per parasite species and developmental stage for which efficacy is to be evaluated. As appropriate to the parasite species or developmental stage and target animal, consideration should be given to the use of different isolates (for induced infections), animal sources, study locations, and/or investigators to increase the inferential value of the studies to the wider intended animal population. For rare parasite species, a single dosage confirmation study may be acceptable. In addition, instead of a field isolate, an induced infection with a laboratory isolate is deemed acceptable to support efficacy. In general, the final formulation, proposed dosage, dosage regimen and route of administration is used in dosage confirmation studies.

3.1.3. Anthelmintic efficacy evaluation based on worm counts following necropsy

In dosage determination studies and dosage confirmation studies, anthelmintic efficacy evaluation is based on the difference in parasite populations between the treated and untreated control groups following necropsy. An anthelmintic drug is considered effective if there is:

1. An adequate infection of the targeted parasite population(s) in at least 6 untreated control animals
2. A statistically significant ($P < 0.05$) difference in parasite counts between the treated group and the untreated control group
3. A calculated percent efficacy (reduction of parasite counts of treated group vs. untreated control group) of 90% or more.

3.1.3.1. Adequacy of infection. An important pre-requisite for the evaluation of anthelmintic efficacy is the conduct of studies with a sufficient number of adequately infected animals (or experimental units) for each targeted parasite. At the animal level, adequacy of infection is defined by a minimum infection level in induced or natural infection studies allowing the evaluation of anthelmintic efficacy when comparing the number of parasites in treated and untreated control animals. Because of the diverse magnitudes of helminth infections, this general WAAVP guideline does not define a general measure for adequate infection across parasite and animal host species. In the VICH anthelmintic efficacy guidelines (VICH), minimal thresholds for

adequacy of infection have been defined for specific parasite populations per animal host species (Vercruysse et al., 2001, 2002), mainly considering historical data on parasite infection burdens. Within the scope of the revised WAAVP guidelines, it is however recommended to define an adequate infection (i.e., the minimum number of parasites in an individual control animal) in the protocol based on the expected efficacy, the sample size (number of animals in each study group), the expected infection level, and the expected distribution among individual control animals. Information about expected parasite counts under conditions of natural infection and within induced infection models, and distribution of infection among individual animals (i.e., variability in infection levels) is typically obtained from previous studies or expert opinion.

As a general rule, a minimum of six control animals with adequate infections is recommended for dosage determination and dosage confirmation studies. While historical group sizes generally range from 6 to 10 animals, the minimum number of animals to be enroled per treatment group to achieve adequacy of infection should take into account previous infection rates in similar study conditions and should be discussed with a statistician during the study protocol design to avoid unnecessary repetition of animal studies. In order to increase the likelihood of achieving adequacy of infection in at least 6 animals at necropsy, confirming the infection status by parasite counts in sentinel animals may be considered prior to treatment. The use of sentinel animals can be considered if determinations made from coprology or other screening techniques do not reliably predict the infection status, but the increased use of study animals should be balanced against the principle of reduction in use of experimental animals.

3.1.3.2. Statistical analysis and calculated percent efficacy of 90% or more. If the parasite burden is known and/or assumed to be normally distributed between study animals, standard parametric statistical procedures can be applied. As parasite counts are often not normally distributed, either the use of non-parametric procedures, parametric procedures with transformation of parasite counts, or alternative statistical models might be indicated for hypothesis testing. The selection of statistical analyses should be described and justified in the protocol. Alternative statistical models have been explored utilising specific data distributions (Alexander, 2012). All statistical analyses should be performed with a *P*-value of 0.05, unless otherwise justified.

If possible, efficacy against adult helminths is calculated for each species separately. Efficacy against larval stages is calculated for each individual parasite stage and species separately. For larval stages which cannot be identified to species based on their morphology and where there is more than one species in that genus, efficacy can be calculated at the genus or subfamily level. In an effort to promote standardisation across reported research, one method of calculating group mean parasite counts is desirable for the calculation of percent efficacy, and geometric means are recommended for anthelmintic efficacy calculations based on parasite counts after necropsy. The Controlled Test (Moskey and Harwood, 1941) is used with the anthelmintic efficacy calculated based on the geometric mean worm counts as follows:

$$\text{Percentage Efficacy (\%)} = 100 \text{ x } [(\text{control} - \text{treated}) / (\text{control})]$$

Consultation with a statistician is advised to determine the most appropriate method to estimate the group mean parasite counts, but in general it is recommended to use the mean group estimates derived from the statistical model for the efficacy calculation. Acceptance of efficacy below 90% may be justifiable when the claimed parasites do not have any other effective treatment unless the reduced efficacy is due to a drug-induced decrease of sensitivity of the parasite isolate.

3.1.3.3. Specific considerations for studies based on worm counts following necropsy. If necropsy for all study animals cannot be finalised in one day, then an equal number of randomly selected animals (or blocks of animals, if applicable) from each treatment group should be necropsied per day. Preferably, parasite counts are based on examination of the entire sample collected, but if the total amount of materials (e.g., gastrointestinal content) is too extensive to examine or if the parasite numbers are too high, parasite counts can be based on an appropriately obtained aliquot. Depending upon the amount of collected material or parasite numbers encountered, different aliquot sizes can be recommended, and this is discussed in more detail in the species-specific guidelines. If low infection burdens are expected, it is recommended to increase the aliquot size.

Readers are referred to the species-specific guidelines for specific recommendations on optimum timing of necropsy. In general, necropsy should be planned within a pre-defined period (historically often ranging from 3 to 14 days) after treatment or withdrawal of the anthelmintic drug. A necropsy performed too early might result in potential bias by prolonged expulsion of parasites after treatment (potentially underestimating efficacy), and necropsy performed too late might result in natural expulsion of certain helminths from their animal hosts (potentially overestimating efficacy and/or compromising adequacy of infection) or replacement of the targeted parasite population by resumed development of earlier parasite stages (potentially underestimating efficacy). Often, parasite counts after necropsy are based on adult worms as these allow for an easier counting process and more reliable parasite identification compared to larval stages. If so, the time after treatment required for the development into adult worms should be considered when scheduling the necropsy. The planning of the necropsy is also dependent on the time required for the anthelmintic drug to exert its anthelmintic activity against the targeted parasite stage. This exposure time might be different for larvae compared to adult worms.

When using induced infections, the efficacy against a specific parasite stage (larval stages or immature/mature adult worms) can be based on the timing of the treatment relative to the known development time after induced infection. When using natural infections, the efficacy against specific parasite stages can, in certain circumstances, also be confirmed by direct identification of the specific developmental stage(s) at necropsy in the control animals when compared to the treated animals. When using this study approach, the necropsy should be planned shortly after treatment, in order to avoid the development of any earlier developmental parasite stages, potentially leading to a bias in the efficacy assessment.

3.2. Field studies (anthelmintic efficacy based on biomarkers of parasite infection)

3.2.1. Study design and objectives

Field studies are conducted primarily to further evaluate the efficacy and safety of the final formulation of the anthelmintic drug when used at the intended dosage in larger and more diverse populations of animals that fall within the spectrum of intended use. These diversities may include different physiological statuses, breeds, and/or ages within the target animal population. Field studies should be conducted as multicentre studies in at least two different regions in which the target parasites naturally occur. These regions should reflect different epidemiological conditions (*e.g.*, prevalence and occurrence of resistance), climates (if applicable), species composition in the parasite population (if broad-spectrum efficacy is pursued), or husbandry conditions. The selection of field study sites should allow for the evaluation of the anthelmintic drug efficacy against representative, naturally occurring infections. As a general principle, the same diagnostic method and methodology for species/genus identification should be used at all sites enroled in the field study.

3.2.2. Anthelmintic efficacy evaluation based on biomarkers of parasite infection

3.2.2.1. Use of biomarkers in field studies. In contrast to dosage determination and dosage confirmation studies, anthelmintic efficacy in field studies is generally not determined by comparing parasite populations after necropsy of treated and control animals, except for poultry field efficacy studies (Yazwinski et al., in preparation). Anthelmintic efficacy in field studies is typically estimated based on biomarkers of parasite infection, such as FECs, faecal larval counts, microfilarial counts, and antigen or antibody measurements, as it allows for measurement live animals. As monitoring of these biomarkers only measures the effect of treatment on the specific biomarker and may not provide reliable information on the efficacy against the underlying parasite population, it is recommended to phrase the study conclusions accordingly. If for example, the anthelmintic treatment results in a reduction of faecal egg excretion, the conclusion should state that the anthelmintic drug reduces faecal egg excretion for *n* weeks or days after treatment.

The FECs are a commonly used parasite biomarker to evaluate anthelmintic efficacy against gastrointestinal parasites in the field. For recommendations regarding field studies based on biomarkers other than FECs, we refer to the species-specific guidelines. Previous guidelines describe an evaluation of field efficacy based on a comparison of FECs from treated and control animals after treatment (Wood et al., 1995; Vercruysse et al., 2001; Hennessey et al., 2006). This general anthelmintic guideline recommends that efficacy evaluation in field studies is based primarily on the percentage reduction between pre-and post-treatment FEC of treated animals only (FEC reduction) and should be estimated separately for each species or genus being evaluated, where possible.

3.2.2.2. Use of FEC reduction to evaluate field efficacy. The recommendation to use FEC reduction (FECR) to evaluate field efficacy of a new anthelmintic drug (to establish baseline field efficacy) is largely based on the specific methodology on how to conduct a faecal egg count reduction test (FECRT) for the detection of anthelmintic resistance in ruminants, horses, and swine (Kaplan et al., in preparation). When establishing the baseline field efficacy for any new anthelmintic drug, the principles for the faecal sampling/handling, choice of a FEC method, and the use of a Bayesian approach that has been developed for the statistical analysis of FECR data should follow the guideline given by Kaplan et al. (in preparation). However, group size estimates provided for FECRT research protocols in Kaplan et al., in preparation do not apply to field efficacy studies. The treatment group size for field efficacy studies should be determined by the following 3 factors: 1) desired threshold efficacy (typically $\geq$90%), 2) the expected raw egg counts pre-treatment (see below), and 3) an appropriate representation of animals that fall within the spectrum of intended use. The treatment group size at each site should be chosen to maximise the chances of determining FECR with 95% confidence above the threshold efficacy. The overall number of animals and sites should also be sufficient to fulfil the objectives described in Section 3.2.1.

3.2.2.3. Study design and treatment groups in field studies. Although the field efficacy evaluation is based on the pre-and post-treatment FEC of the same animals in the treated group only, there are circumstances that may warrant the inclusion of a control group to provide a second calculation of efficacy based on a post-treatment comparison of the treated group to a control group. The inclusion of an untreated control group may provide useful information about the parasite infection dynamics under the specific study conditions and assist with the interpretation of the results of the study. Changes in parasite infection level over the duration of the study might affect the efficacy evaluation, which is especially relevant when evaluating efficacy over longer durations of time. However, this information is only reliable if an appropriate number of untreated animals is included in the study (Kaplan et al., in preparation). In studies evaluating the efficacy over a prolonged time after treatment, exposure to infection should be similar for the untreated and treated groups at all timepoints throughout the study, and evidence of this exposure confirmed in the untreated group. The use of a treated control group in field studies can also be considered in order to demonstrate non-inferiority to an approved reference product, which has mainly been described in companion animal field studies (Altreuther et al., 2011; Rehbein et al., 2017). If persistent efficacy is compared with that of an approved reference product, it is recommended to generate evidence that the animals in the field study were exposed to infection after treatment. Exposure to continued infection during the field study can be documented through recent and historical infection data, pasture larval counts, by a parasite biomarker, tracer animals, a concurrent prevalence study in a similar target population, or a combination thereof. While a minimum of 25% of the number of animals (selected at random) in the treated group has previously been advocated for the size of the control group (Wood et al., 1995; Vercruysse et al., 2001; Hennessey et al., 2006), it is recommended that the number of animals in the control group versus the treated group(s) be discussed with a statistician during the design of the field study. The appropriate size of the control group can substantially differ between targeted parasites, animal host species, and study design.

3.2.2.4. Recommendations for field studies. For the evaluation of anthelmintic efficacy based on FEC in field studies, the following is recommended:

- Individual faecal samples should be collected directly from the rectum of each individual animal. Fresh faecal samples can be collected from the ground when the identification of the individual animal is certain. Composite faecal samples are generally not recommended (Kaplan et al., in preparation).
- As outlined in Kaplan et al., in preparation, anthelmintic efficacy is best estimated based on the pre-and post-treatment FEC of the same animals in a treated group, as this approach accounts for potential bias by animal-specific factors, such as immunity, pharmacokinetics, or age.
- The percentage reduction in FEC may differ between different anthelmintic drug classes, but as a minimum should exceed 90% to support a claim of anthelmintic efficacy. User-friendly online interfaces (e.g., http://shiny.math.uzh.ch/user/furrer/shinyas/shiny-eggCounts/) exist for robust model-based estimations, and their use to estimate anthelmintic efficacy is generally recommended.
- As outlined above, the inclusion of an untreated control group for post-treatment comparison to the treated group in a field study may provide useful information about the parasite infection dynamics under the specific study conditions. When extraneous factors influencing FEC are expected that may bias the study results, researchers should rely on the comparison of efficacy from treated and untreated control groups post-treatment to estimate anthelmintic efficacy. The Bayesian approach developed for analysis of paired data has not been evaluated for use with unpaired data and cannot yet be recommended for this use. In addition, neither the arithmetic nor geometric mean sufficiently address the statistical challenges of FEC data sets. For studies evaluating efficacy over longer periods of time, alternative approaches such as the use of a cumulative faecal egg excretion approach may be considered. Consultation with a statistician is recommended when developing protocols for field studies utilising a control group.
- In field studies in dogs and cats, the treatment efficacy is sometimes reported as absence or presence of infection (Becskei et al., 2020). Similarly, the reduction in FEC can be interpreted as efficacious (i.e., above the efficacy threshold) or not. These binomial outcomes allow for a different analysis in which the number of animals with

treatment failure between groups is compared. However, the underlying data still consist of pre-and post-treatment FEC. Specific recommendations on how to evaluate FEC in dogs and cats are provided in Beugnet et al. (in preparation).

- A FEC method/protocol should be selected which is appropriate for the objectives of the study. In order to avoid separate recommendations for specific egg counting methods, it is recommended to establish the cumulative raw egg count (before applying a diagnostic method-specific conversion factor) across animals in the treated group (Kaplan et al., in preparation). To increase the diagnostic power of the FECR determination, the minimum number of eggs counted pre-treatment in each treatment group should exceed a level allowing for a reliable pre-and post-treatment comparison. It should be kept in mind that as per the recommendations, the predetermined minimum total number of eggs (cumulative number of eggs counted before application of a conversion factor) should be counted from each species or genus before an efficacy estimate can be made with statistical significance (Kaplan et al., in preparation). A minimum count of 200 eggs per treatment group was previously recommended for cattle, sheep, and goats (Kaplan et al., 2020). If fewer eggs are counted than recommended, a second FEC (or an additional chamber of the counting slide) from each animal in the study should be counted until the raw egg count exceeds the required pre-treatment FEC. The post-treatment FEC should be conducted using the same diagnostic method and same number of slides and/or chambers counted as for the pre-treatment FEC.
- The pre-treatment faecal sample should be collected as closely as possible to the treatment and not earlier than 7 days prior to treatment. The post-treatment faecal sample is collected within 14 days after treatment (Coles et al., 1992). While this 14-day interval is valid for short-acting drugs, a longer interval might be required for specific drugs, as some drugs inhibit nematode egg production by the female worm for more than 14 days and use of a shorter interval may potentially result in an overestimation of the true efficacy (De Graef et al., 2012). The post-treatment interval may vary by species and drug, as outlined in detail in Table 2 in Kaplan et al., in preparation. As such, it may be needed to plan for more than one FEC after treatment. If the post-treatment interval exceeds the prepatent period of the parasite, measures should be taken to avoid re-infection after treatment, including appropriate housing of the study animals. To estimate persistent efficacy, FEC are performed at regular intervals after treatment in animals exposed to re-infection.
- The genera and/or species of the eggs present in the samples should be differentiated, since helminth infections are often composed of multiple species. Ideally this should be performed both pre- and post-treatment, to determine if there are any species or genus specific differences or changes due to treatment, and to calculate species-specific FEC reductions. If identification is not possible based on egg morphology, coprocultures have historically been the primary means to achieve differentiation of helminth species/genera. However, new molecular tests such as multiplexed tandem PCR and metabarcoding assays could serve as useful alternatives (Avramenko et al., 2015; Roeber et al., 2017; Borkowski et al., 2020; Kotze et al., 2020).

4. Conclusions

The WAAVP anthelmintic guidelines were revised to ensure consistency over the different host animal species and considering recent scientific advancements. The general anthelmintic guideline provides updated and standardised guidance for the conduct of dosage determination, dosage confirmation and field efficacy studies to evaluate anthelmintic efficacy in food-producing and companion animals, and are complemented by the revised species-specific guidelines for dogs and cats (Beugnet et al., in preparation), ruminants (Burden et al., in preparation), swine (Rehbein et al., in preparation), horses (Nielsen et al., in preparation) and poultry (Yazwinski et al., in preparation). A reflection paper (Geurden et al., in preparation) provides additional background to these revised guidelines.

Credit author statement

All authors contributed equally to conceptualization, investigation and writing of the original draft. Thomas Geurden, Emily Smith and Martin K. Nielsen were responsible for review and editing of the manuscript. Thomas Geurden was responsible for the supervision of the initiative.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix 1. Protocol Checklist

Protocol Checklist	Checked Y/N/ NA
General study conduct	
Are the names, contact details, training and qualifications of all study personnel involved in the study specified in the protocol?	
Does the protocol state where the study will be conducted?	
Is it clearly stated which guidelines and welfare regulations apply to the study (e.g. GCP)?	
Have requirements of the Ethics Committee/ Institutional Review Board been described? Has any outcome of an ethical review procedure been addressed?	
Is a statement included regarding blinding or masking of study personnel to treatment allocation where appropriate (e.g. which personnel/roles are masked and unmasked), and steps to unmask if required?	
Does the protocol include a section on how to document amendments and deviations?	
Is it clearly stated where original data will be archived during and on completion of the study?	
In cases of blood sampling, is the individual blood volume and total blood volume over specified times defined?	
Has any sampling been discussed with ethical review board, as necessary?	
Are the assays used to analyse any samples taken in the study clearly described or referenced?	
Has the retention period for the samples been clearly defined in the protocol?	
Does the protocol include a procedure for reporting and recording Adverse Events or human exposure to the drug?	
Is the study period and year clearly defined in the protocol?	

(*continued on next page*)

(*continued*)

Protocol Checklist	Checked Y/N/ NA
Are all Standard Operating Procedures (SOPs) clearly defined in the protocol?	
Has all equipment and the methods of validation throughout the study been described in the protocol?	
How will the sponsor/investigator contacts be documented throughout the study?	
Study objective and study design (including statistical analysis)	
Is the study objective clearly defined, including targeted parasite species/stage?	
Is the study design clearly described, including number and definition of study groups and duration of the study?	
As seasonality might have an impact on parasite infections, is the time of year in which the study is conducted justified?	
Does the protocol include a sample size consideration and power calculation based on a properly chosen experimental unit (animals or pens)?	
Is the source and method of helminth infection (natural vs. induced, field or laboratory isolate, specific procedures associated with induced infections, etc.) described in the protocol?	
Is the procedure for confirmation of helminth infection described (if applicable) in the protocol?	
If induced infections are used, are the details of the isolate characterization described?	
Is the a priori hypothesis clearly described? If more than one hypothesis, make sure to capture all in the protocol.	
Are the primary and secondary variables clearly described, and how these will be measured?	
Is the method of parasite identification and quantification clearly described and is this consistent throughout the study (at all timepoints and at all study sites)?	
Are adequacy of infection criteria described, if applicable?	
Are the statistical methods described and justified, if applicable?	
Are descriptive analyses included in the protocol, for those data that are not analysed, e.g. age or sex of the study animals?	
Does the protocol describe methods of randomisation, including any blocking or grouping prior to randomization and whether replacement after randomization is allowed?	
Does the protocol describe methods for handling missing data or data outliers?	
Does the protocol describe methods of calculating percent efficacy?	
Does the protocol describe the criteria that will be used to draw conclusions on anthelmintic efficacy?	
Anthelmintic drug information and treatment details	
Is the anthelmintic drug and drug formulation clearly described in the protocol (e.g. lot number, concentration, certificate of analysis, material safety data sheet, storage conditions, special handling requirements)?	
Is the dosage, route of administration, administration instructions, number of treatments, and interval between treatments (if applicable) described in the protocol?	
Is information provided regarding accurate calculation of treatment dosages?	
Does the protocol specify the use of a calibrated scale and procedures for confirming the accuracy of the scale used for weighing animals?	
If applicable, are treatment dosages listed in dosage tables? Ensure dosage tables include all potential bodyweights.	
Does the protocol include guidance on how to handle misdosing or prevent drug transfer to other study animals after dosing?	
If applicable, is there a clear description on how to observe and record feed or water consumption to confirm adequate dosing?	
Is the frequency and details of specific post-treatment safety observations, general health observations and behavioural observations described?	
If applicable, is the application site clearly identified and are post-treatment evaluations of application site reactions described?	
Are the procedures to manage and document drug accountability (e.g. reconciliation of drugs received and used) clearly described?	
Does the protocol state whether concurrent medications and treatments are allowed during the study and how such treatments will be documented?	
If applicable, is the withholding period clearly described in the protocol?	
Study animals	
Is the target animal species (including breed and age of the animals) and the sourcing of the animals clearly described in the protocol?	
Are the animal inclusion and exclusion criteria clearly defined in the protocol, including but not limited to age, sex, physiological status, health, parasitological criteria, washout periods from previous treatments?	
Is guidance relating to method of identification and permissible treatments during the acclimation period pre-treatments of animals provided?	
Is the animal management during the study clearly described? Is the animal housing, floor and feeder space defined in the protocol and appropriate according to local animal welfare standards? Is there consideration given to all animal weights and requirements for the duration of the study?	
Is it defined in the protocol who has responsibility for the welfare of the animals during transportation to the study site and throughout the study?	
Does the protocol provide guidance on the removal of animals from the study after enrolment (criteria, examinations performed, personnel responsible, and disposition)?	
Is the ownership of study animals clearly documented in the protocol or subsequent study documentation?	
If applicable, is the method of euthanasia defined in detail including any sedation requirements? Is the method of euthanasia in compliance with relevant guidelines?	
Is the post-study fate of enrolled animals described in the protocol? Consider any withdrawal or withhold requirements e.g. milk, food chain.	
Does the protocol describe how animal accountability will be documented?	
Is an acclimation period defined in the protocol, allowing animals to adapt to the study site and, if applicable, allowing for development to the parasite stage targeted by the treatment?	
Are (daily) general health observations and specific safety observations described in the protocol?	

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