

#276

Effectiveness of Anthelmintics: Specific Recommendations for Products Proposed for the Prevention of Heartworm Disease in Dogs

Guidance for Industry

Draft Guidance

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Additional copies of this draft guidance document may be requested from the Policy and Regulations Staff (HFV-6), Center for Veterinary Medicine, Food and Drug Administration, 7500 Standish Place, Rockville MD 20855, and may be viewed on the Internet at <https://www.fda.gov/animal-veterinary>, <https://www.fda.gov/regulatory-information/search-fda-guidance-documents>, or <http://www.regulations.gov>

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This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff responsible for this guidance as listed on the title page.

I. Introduction

This draft guidance, if finalized, provides recommendations for the effectiveness evaluation of drugs indicated for the prevention of heartworm disease caused by *Dirofilaria immitis* in dogs. These recommendations should be read in conjunction with Guidance for Industry (GFI) #90 (VICH GL7), “Effectiveness of Anthelmintics: General Requirements,”¹ and GFI #111 (VICH GL19), “Effectiveness of Anthelmintics: Specific Recommendations for Canines,”² and are intended to provide additional detail to elements of study design and interpretation under the recommendations laid out in these guidances.

In general, FDA’s guidance documents do not establish legally enforceable responsibilities. Instead, guidances describe the Agency’s current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

II. Recommended Approach to Demonstrate Substantial Evidence of Effectiveness

The recommended approach to demonstrate substantial evidence of effectiveness of an investigational new animal drug intended for the prevention of heartworm disease in dogs is for the sponsor to conduct two laboratory dose confirmation studies and one multi-site field effectiveness study in accordance with the principles of Good Clinical Practice (GCP) as described in GFI #85 (VICH GL9), “Good Clinical Practice.”³ Laboratory dose confirmation studies are experimentally-induced infection studies that provide known exposure to infectious *Dirofilaria immitis* (*D. immitis*) larvae (L3) due to contemporaneous experimental infection of the same number of L3 to negative control dogs and dogs administered the investigational new animal drug. Laboratory dose confirmation studies also allow for quantitative evaluation of

¹ <https://www.fda.gov/media/70349/download>

² <https://www.fda.gov/media/70091/download>

³ <https://www.fda.gov/media/70333/download>

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outcome by determining the presence of adult worms as well as the individual worm burden in each dog.

The field effectiveness study evaluates the effectiveness of the investigational new animal drug under actual conditions of use in client-owned dogs and with the current enzootic status, ecologic, and genetic factors affecting heartworm disease in dogs in each location.

FDA's recommended approach considers the laboratory dose confirmation studies and the field effectiveness study together when evaluating if substantial evidence of effectiveness (21 CFR 514.4) and inferential value to the target population have been met. We have provided certain study design elements in the recommendations for the laboratory dose confirmation studies in section [III. Laboratory Dose Confirmation Studies](#) (e.g., percent effectiveness threshold, susceptibility characterization of isolates) based on the assumption that you will conduct a field effectiveness study that incorporates the recommendations listed in section [IV. Field Effectiveness Studies](#).

III. Laboratory Dose Confirmation Studies

The laboratory dose confirmation studies are intended to confirm effectiveness of the investigational new animal drug under laboratory conditions.⁴ The studies should evaluate effectiveness of a defined minimum labeled dose (mg/kg), dosage interval, and number of doses. Timing of study procedures (infection, initiation of dosing) should be justified based on the specific properties of the investigational new animal drug.

A. Number of Studies

FDA recommends sponsors conduct two induced laboratory dose confirmation studies, each conducted at different laboratory facilities, led by different independent investigators, and using recent isolates of *D. immitis* from two separate geographic locations in the United States. For products applied topically, one of the two laboratory dose confirmation studies should include evaluation of the effect of bathing/water immersion.

B. *D. immitis* Isolates

Laboratory dose confirmation studies should be conducted with *D. immitis* isolates that are established from current *D. immitis* populations circulating in the United States and that were isolated from the field within 5 years of study initiation. The two isolates should be from two different geographic locations, and at least one of the two isolates should be from a highly endemic area, typically the southeastern United States⁵ or the Mississippi Delta Region.⁶ Isolates used in studies for investigational new animal drugs may, at the sponsor's

⁴ For additional information about the recommended design and conduct of laboratory dose confirmation studies, refer to GFI #90 (VICH GL7) and GFI #111 (VICH GL19).

⁵ We consider the southeastern United States to include the states of Alabama, Arkansas, Florida, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, and Tennessee.

⁶ We consider the Mississippi Delta Region to be the area covered by the Delta Regional Authority. Available online at <https://dra.gov/about-dra/map-room/>. (Accessed November 16, 2022)

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discretion, be characterized for susceptibility against FDA-approved products⁷ at the approved dosages, prior to conduct of the laboratory dose confirmation studies.

C. Number of Dogs per Investigational Group

The chosen sample size should support the ethical principles of replacement, reduction, and refinement,⁸ and the goal of using the minimum number of animals necessary to generate data to demonstrate substantial evidence of effectiveness.

D. Number of L3 Injected for Induced Infection

FDA recommends that 50 infective *D. immitis* L3 larvae be injected subcutaneously per dog to introduce consistent and adequate infections without overburdening the negative control group dogs.

E. Pre-existing Heartworm Infection

FDA recommends testing for pre-existing (pre-study) heartworm infection at baseline and at approximately 120 days (4 months) after the day of experimental infection with *D. immitis* L3 larvae. Dogs determined to have pre-existing infections should be removed from the effectiveness evaluation.

F. Assessment of Effectiveness

1. Parasite Counts

The pivotal effectiveness variable is the *D. immitis* worm count at necropsy. Necropsy for *D. immitis* worm counts may occur as early as 150 days after infection with the *D. immitis* L3 larvae. Individuals conducting worm counts should have sufficient training and expertise to perform the counts and should be masked to treatment group. Heartworm fragments, if any, should be counted as follows: Worm fragments containing a head and worm fragments containing a tail are counted separately. The greater of the two counts (number of fragments containing a head or number of fragments containing a tail) are included in the total worm count for effectiveness calculations. Fragments without heads or tails are not included in the worm count if fragments containing heads or tails are found. If only fragments without heads or tails are found, the fragments are collectively considered to represent one worm for inclusion in the effectiveness calculations.

⁷ Products subject to an approved new animal drug application (NADA) or abbreviated new animal drug application (ANADA) for the prevention of heartworm disease in dogs.

⁸ Russell WMS, Burch, RL (1959). *The Principles of Humane Experimental Technique*. London: Methuen & Co. Special edition published by Universities Federation for Animal Welfare, 1992. Available online at: http://altweb.jhsph.edu/pubs/books/humane_exp/het-toc. (Accessed November 16, 2022)

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2. Criteria to Demonstrate Effectiveness

In addition to having an adequate infection in the negative control group and a statistically significant difference in *D. immitis* worm count in the investigational new animal drug group compared to the negative control group, investigational new animal drugs should demonstrate at least 99.5 percent effectiveness and no more than one dog in the investigational new animal drug group should have one or more worms identified at necropsy. The geometric group means estimated from the statistical analysis of log-transformed worm counts should be used. Study design features (e.g., blocks, cohorts) should be included in the statistical model for the analysis.

Percent effectiveness = $(M_c - M_t) / M_c \times 100$

Where:

M_c = geometric mean back-transformed from the Least Squares mean of the control group, and

M_t = geometric mean back-transformed from the Least Squares mean of the investigational new animal drug group.

G. Pilot Study Considerations

D. immitis isolates used in pilot laboratory dose confirmation studies should not be used in the pivotal laboratory dose confirmation studies.

H. Pharmacokinetic Evaluation

FDA recommends the collection of pharmacokinetic data during the dose confirmation studies to verify dose exposure.

IV. Field Effectiveness Study

The field effectiveness study should be a multi-site study conducted with investigators in various geographic regions of the continental United States with endemic heartworm disease. FDA's recommendations for specific study design elements are intended to increase the confidence that exposure to infective *D. immitis* larvae, as well as a variety of isolates, occurred during the evaluation period for the investigational new animal drug and that the evaluation of effectiveness is assessed appropriately.

A. Study Duration

Because of the length of the heartworm life cycle and the delay in the ability of diagnostic tests (heartworm antigen and microfilaria tests) to identify infection with adult heartworms after exposure to *D. immitis* larvae, the effectiveness of the investigational new animal drug is ultimately evaluated for only a portion of the total study duration (the effectiveness evaluation window).⁹

⁹ The field safety of the investigational new animal drug is evaluated for the entire study.

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FDA recommends that for products intended for monthly administration, the effectiveness evaluation window be a minimum of 6 months following the first dose. For products with a duration of effectiveness greater than 6 months, the effectiveness evaluation window should be the entire duration of effectiveness intended from one dose administration (for example, 12 months for a product to be administered every 12 months). The study should continue for an additional 240 days after the end of the effectiveness evaluation window to allow for the development and detection of adult heartworms and collection of safety data (for example, a total study duration of at least 420 days for a product intended for monthly administration and at least 605 days for a product intended for yearly administration). Longer study durations may be appropriate to address potential safety concerns identified during product development. Dogs should remain on the same drug product (either investigational new animal drug or active control) throughout the study.

B. Enrollment

The timing of enrollment should result in the investigational new animal drug being evaluated during the time of year that maximizes the likelihood of exposure to mosquitos infected with *D. immitis*. In the Northern Hemisphere, peak transmission is considered to occur in July and August;¹⁰ therefore, unless the peak transmission season for a specific geographic location differs, the effectiveness evaluation window for studies conducted in the continental United States should include July and August. For example, in the case of an oral product intended for monthly administration, for all dogs to be treated with the investigational new animal drug, or active control, during the effectiveness evaluation window of July and August, the enrollment period for a field effectiveness study should be such that first dose administration occurs between April 1 and June 30.

C. Location of Study Sites

To evaluate the effectiveness of the investigational new animal drug in highly endemic portions of the United States, the majority (> 50 percent) of evaluable cases should be from the southeastern United States,¹¹ eastern/central Oklahoma, eastern/central Texas, or the Mississippi Delta Region.¹² No more than 40 percent of evaluable cases should be provided by one site.

D. Assessment of Effectiveness

Effectiveness and detection of pre-existing infection should be determined by commercial adult heartworm antigen testing and microfilaria detection testing by a concentration method (e.g., modified Knotts). FDA recommends that heartworm tests (antigen and

¹⁰ American Heartworm Society (AHS). Current Canine Guidelines for the Prevention, Diagnosis, and Management of Heartworm (*Dirofilaria immitis*) Infection in Dogs. Available online at https://d3ft8sckhngim2.cloudfront.net/images/pdf/AHS_Canine_Guidelines_11_13_20.pdf?1605556516. (Accessed November 16, 2022)

¹¹ See [footnote 5](#) on page 2.

¹² See [footnote 6](#) on page 2.

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microfilaria detection testing) should be performed at baseline (prior to Day 0, the day of the first dose), at appropriate intervals to detect preexisting infection, and on the predetermined pivotal effectiveness study day based on the characteristics of the investigational new animal drug.

For an individual dog to be considered a success, the individual dog should not test heartworm antigen- or microfilaria-positive up to and including 240 days after first dose administration, at any subsequent interim timepoints, and on the predetermined pivotal effectiveness day. For an investigational new animal drug to be considered effective, the lower bound of the 95 percent two-sided confidence interval for the percent success for all evaluable cases in the investigational new animal drug group should be no lower than 98.0 percent. The confidence interval should be computed using exact methods for binomial proportions.¹³

E. Active Control Group

To minimize bias in data collection, the study should include an active control group that is administered an FDA-approved product¹⁴ for the prevention of heartworm disease in dogs.¹⁵ The ratio of cases allocated to the investigational new animal drug group to the active control group should be 1:1 or greater. If possible, the active control should contain the same active ingredient and route of administration as the investigational new animal drug. The active control group is not used in the statistical evaluation of effectiveness.

F. Number of Evaluable Cases

The field effectiveness study should be large enough to provide independent substantiation of evidence and inferential value to the target population. The number of evaluable cases administered the investigational new animal drug should be at least 300 dogs. This number of minimum evaluable cases increases the confidence that exposure to *D. immitis*-infected mosquitos occurred and that dogs were exposed to a variety of *D. immitis* isolates during the study. In addition, sponsors should consider the proportion of likely treatment successes and the corresponding lower bound of the 95 percent confidence interval when considering the target sample size.

G. Pharmacokinetic Evaluation

FDA recommends the collection of pharmacokinetic data during the field effectiveness study to verify dose exposure.

¹³ Clopper, C. J. and Pearson, E. S. (1934) "The use of confidence or fiducial limits illustrated in the case of the Binomial," *Biometrika*, 26, 404 -413.

¹⁴ See [footnote7](#) on page 3.

¹⁵ To avoid the unnecessary risk of developing heartworm disease in client-owned dogs, the study should not include a negative control group.