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Concept Paper for FDA-CVM's Proposal to Re-Open the VICH Anthelmintic Guidelines for Revisions

VICH Steering Committee Meeting
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1. Introduction

The VICH anthelmintic guidelines were recommended for consultation at Step 7 of the VICH process at various time points in November 1999 (VICH GL7, Effectiveness of Anthelmintics: General Recommendations; VICH GL12, Effectiveness of Anthelmintics: Specific Recommendations for Bovine; VICH GL13, Effectiveness of Anthelmintics: Specific Recommendations for Ovine; VICH GL14, Effectiveness of Anthelmintics: Specific Recommendations for Caprine) or June 2001 (VICH GL15, Effectiveness of Anthelmintics: Specific Recommendations for Equine; VICH GL16, Effectiveness of Anthelmintics: Specific Recommendations for Porcine; VICH GL19, Effectiveness of Anthelmintics: Specific Recommendations for Canine; VICH GL20, Effectiveness of Anthelmintics: Specific Recommendations for Feline; VICH GL21, Effectiveness of Anthelmintics: Specific Recommendations for Poultry–Gallus gallus) by the VICH Steering Committee.

In the years since these finalized documents have been in effect, areas of incomplete information within the VICH documents and/or new scientific knowledge not included in the VICH documents have been identified. Revision of the guidelines would make them more informative and help with consistency across sponsors and regulatory authorities.

Since the time the guidelines were written, more scientific knowledge of the development of antiparasitic resistance in gastrointestinal (GI) nematodes, specifically in cattle, small ruminants, and equines, has come to light. There is a need to address this growing worldwide problem with proactive revision of the effectiveness evaluation for certain species of target animals and parasite species in the appropriate VICH guidelines. There are also growing concerns about potential anthelmintic resistance developing in canine heartworm disease.

Achieving consensus through VICH on how to incorporate the current knowledge of veterinary parasitology into these guidelines would help both sponsors and regulatory agencies to advance development of new effective and safe antiparasitic products and control resistance to these important drugs.

FDA-CVM first presented a concept paper on these topics to the Steering Committee in November 2012. This updated concept paper was created after receiving feedback. This updated concept paper outlines in further detail the issues FDA-CVM would like the Steering Committee to consider when voting to re-open the anthelmintic guidelines for revision, with the intent that these concepts would be discussed by the Expert Working Group if one is formed.

2. Problem

Japan, the European Union (EU), and the United States (US) have developed an Effectiveness of Anthelmintics General Recommendations Guideline (VICH GL7) and eight species specific VICH guidelines as stated above. There are areas in the guidelines that are silent or not informative and/or specific enough on a number of issues related to study design, methodology, and the basis of study conclusions. Additionally, there has been much discussion and increasing awareness of

the emerging global problem of antiparasitic resistance. Many veterinary and parasitological professional organizations, such as the World Association for the Advancement of Veterinary Parasitology (WAAVP), The American Association of Veterinary Parasitologists (AAVP), American Association of Bovine Practitioners (AABP), The American Consortium for Small Ruminant Parasite Control (ACSRPC), and the American Association of Equine Practitioners (AAEP) have featured this topic as part of their agendas for annual meetings. The recommendation for the use of standardized methods to detect and mitigate parasite resistance is critical to the preservation of the effectiveness of anthelmintic drugs in cattle, small ruminants, and equines across the world.

Revision of the existing guidelines will unify the global veterinary community's understanding of the basic principles upon which effectiveness determinations are based.

3. Impact on Public Health, Animal Health, and Animal Welfare

3.1 Animal Welfare: Revised harmonized anthelmintic guidelines will provide additional information on certain aspects of study design that, if followed, could minimize the number of studies that need to be conducted, thereby reducing the number of animals that need to be used in the demonstration of effectiveness of antiparasitic drug products.

3.2 Animal Health: Revised harmonized anthelmintic guidelines will also enable member countries to recommend comparable methods for evaluating effectiveness and enable the use of data by multiple regulatory authorities. This may decrease the regulatory burden for drug sponsors and encourage development of new drug products to ensure successful parasite control.

In a global environment, the development of antiparasitic resistance within one country can affect the effectiveness of products in surrounding countries due to increases in international animal movement. Ultimately, protecting the effectiveness of existing anthelmintic products and development of new effective antiparasitic drugs is critical for animal health and well being through minimizing the damaging effects of parasitic infections. Therefore, revisions to the effectiveness criteria should be discussed.

3.3 Impact on Public Health: The revised harmonized anthelmintic guidelines will help to minimize parasites in our companion animals and will help to control zoonotic parasites that are a threat to human health. Control of parasites in food animals is vital to protect and ensure a safe and nutritious food supply.

4. Anticipated Benefit

The benefits that will be obtained through the revision of the current harmonized VICH anthelmintic guidelines are in keeping with the stated VICH objectives to:

- Establish and implement harmonized regulatory requirements for veterinary medicinal products in the VICH Regions, which meet high quality, safety, and efficacy standards while minimizing the use of test animals and the costs of product development, and ensuring consistent interpretation of data requirements between sponsors and across different regulatory agencies.

Considerations for addressing the development of antiparasitic resistance will:

- Bring about a constructive dialogue between regulatory authorities and industry to provide technical guidance enabling response to the significant emerging global issue of antiparasitic

resistance that impacts regulatory requirements within the VICH regions.

- Ensure that the newly approved anthelmintic drugs withstand or help minimize the biological pressure of resistance development.

5. Discussion

A. PROPOSED TOPICS FOR REVISION OF EXISTING GUIDELINES

i. Use of geometric means [Section A 4.2, GL7]

VICH GL7, Section 4.2 Geometric versus arithmetic means, states … "Differences in effectiveness may be seen whether geometric or arithmetic means are used. However, in the context of harmonization, recommendations are needed for one method of calculating the means…The use of arithmetic means to evaluate effectiveness has been considered to be a more stringent criterion reflected in a more conservative estimation of therapeutic activity of the product and may be acceptable in certain circumstances only." Further, the guideline recommends that generally, geometric means should be used in the estimation of percent effectiveness but in certain circumstances there may be conditions acceptable for the use of arithmetic means.

Parasitic nematode infections in both companion and food animals are recognized to have a skewed distribution, meaning that most of the nematodes are found in a small percentage of the animal population (generally 20 to 30% of hosts harbor most of the parasites)[1,2]. Because of the skewed distribution and in accordance with the guideline, data have been log-transformed for analysis and to obtain the geometric mean estimates. An additional effect of using the log transformation (geometric mean) is that impact of extreme values on the estimate of the mean is mitigated and a lower mean estimate is obtained compared to the arithmetic mean. On the other hand, using non-transformed values, the arithmetic mean is an unweighted estimate of the parasite burden and may be considered reflective of total parasite burden. In contrast to GL7, recent published literature advocates the use of arithmetic means in effectiveness estimation.[3,4,5]

Therefore, the difference in effectiveness estimates using these mean types is dependent on the distribution of observed values. As the observed values become more uniform or, if the control group has an adequate infection and post-treatment counts are substantially reduced, the estimate of effectiveness becomes more similar whether the arithmetic or geometric mean is used.

In proposing to reopen the guidelines, it is suggested that additional distributional assumptions, methods of analysis, and estimation of means be examined. An alternative is

[1] Grenfell, B.T., et al. Modelling patterns of parasite aggregation in natural populations: trichostrongylid nematode–ruminant interactions as a case study. Parasitology, 111 (1995): S135–S151.
[2] Galvani, A.P. Immunity, antigenic heterogeneity, and aggregation of helminth parasites. Journal of Parasitology, 89 (2003): 232-241.
[3] Dobson, R.J., et al. Geometric means provide a biased efficacy result when conducting a faecal egg count reduction test (FECRT). Veterinary Parasitology, 161 (2009): 162-167.
[4] Alexander, N. Review: analysis of parasite and other skewed counts. Tropical Medicine and International Health, Vol 17, No. 6 (2012): 684-693.
[5] McKenna, P.B. What do anthelmintics efficacy figures really signify? New Zealand Veterinary Journal, 46 (1998): 82-83.

the zero-inflated Poisson (ZIP) distribution, which jointly models the probability of observing zero counts with the Poisson distribution of counts when observed. This distribution allows for frequent zero-valued observations combined with skewed positive counts and may be appropriate for data with excessive zeros, such as those occurring in successfully treated animals.

In evaluating the different methods, one should consider not only the magnitude of the estimated percent effectiveness but the benefits and risks of each alternative and how each addresses the concern for development of antiparasitic resistance. Prior to beginning the procedure, one needs to define the criteria to be used in evaluating the benefits and risk of each alternative. The proposed statistical test for each alternative should be determined based on the stated assumptions.

ii. Adequacy of infection/Number of Helminths in Six Individual Control Animals [Section A 4.5, GL7]

VICH GL7, Section 4.5 Adequacy of Infection states, "…Because of the inherent differences in the helminths, a universal definition of adequacy of infection should not be formulated. However, protocols should address adequacy of infection and appropriate standards of effectiveness should be met with acceptable statistical and biological certitude/confidence. Adequate infections are still recommended in (a minimum of six control animals)."

It is noted that the general guidelines and all the species specific guidelines state that six animals in the control group should be adequately infected. As the determination of helminth infection by worm counts requires animal necropsy, it is not possible to determine whether the animals in treated groups were adequately infected prior to administration of the anthelmintic. We note this is an assumption that is not confirmed based on basic study design. An adequate infection in the treated group is assumed based on confirming an adequate infection in the control group. This assumption is valid if 1) the two groups are randomly assigned, 2) the cure is not spontaneous, and 3) at necropsy the control group has an adequate infection. If these criteria are met, then the assumption that the treated group had an adequate infection is valid. In accordance with this topic, we propose that the following should be discussed when considerations of revising the guidelines are made:

a. Helminth numbers in canines and felines [Section A 4.3, GL19 and GL20]

VICH GL19, Section A 4.3 Adequacy of Infection states, "….With respect to the minimum adequate number of helminths, the decision should be made when the final report is submitted based on historical data, literature review, or expert testimony. Generally the minimal number of nematodes in canines recommended as adequate is in the range of 5 to 20. Higher counts are to be expected with *Ancylostoma caninum* and *Uncinaria stenocephala*."

Section 4.3 Adequacy of Infection from the VICH GL20 is worded similarly to VICH GL19, with the exception that, "Counts higher than the 5 to 20 range are to be expected with *A. tubaeforme*."

In order to protect both veterinary and public health, an adequate infection should be defined for some species to ensure consistent standards. At present, the guidelines remain silent in regard to the adequacy of infection for cestodes, feline heartworm, and

Dirofilaria immitis microfilaria, among other helminths. We believe that it is necessary to discuss updating the guidelines so that the regulatory requirements become standardized in these categories to reduce the use of test animals, reduce the costs of product development, and ensure consistent interpretation of data requirements between sponsors.

Cestodes in Canines and Felines

While the VICH guidelines currently do not address the minimum number of worms for an adequate infection for cestodes, some regulatory authorities have accepted a minimum of two *Dipylidium caninum* and three *Taenia pisiformis* worms as evidence of an adequate infection. Since *D. caninum* has the potential to be zoonotic (e.g. if a child ingests a flea infected with *D. caninum*), a lower level of infection is acceptable compared to *T. pisiformis*.

Adult Heartworms in Felines

Although cats are susceptible to heartworm disease, they tend to be more resistant to infection than dogs[6]. Overall, worm burdens tend to be between one and nine worms in cats; dogs often have twenty or more. The number of worms that can produce severe clinical signs in cats can be as low as one to two[7], whereas in dogs, that number is typically ten or more. Some cats also clear the adult worms spontaneously, such that they have no adult worms but still demonstrate cardiovascular and respiratory pathology consistent with heartworm disease. Additional differences between heartworms in dogs and cats include life span of the parasite (five to seven years in dogs versus two years in cats) and size (smaller in cats than in dogs[8]).

The VICH guidelines remain silent regarding the adequacy of infection for *D. immitis* in cats. The number of infective stages (third stage larvae) recommended for induced *D. immitis* infections is 30 to 100. Studies using numbers at the higher end of the range could reasonably be expected to increase the chances of producing robust infections. Even with higher numbers of larvae inoculated, however, the disease is so highly variable in cats that it can be challenging to obtain five adult heartworms per cat.

Jacobs (1994), states that 70% of cats inoculated with 30 to 100 heartworm larvae will become infected with an *average* of four to five worms. Thus a group size of approximately nine cats is needed to achieve six adequately infected animals[9]. This inoculation success rate is supported by other literature[10,11,12].

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[6] McTier, T., et al. Prevention of experimentally induced heartworm (Dirofilaria immitis) infections in dogs and cats with a single topical application of selamectin. Veterinary Parasitology 91 (2000): 259 – 268.
[7] Jacobs, D., et al. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P) guidelines for evaluating the efficacy of anthelmintics for dogs and cats. Veterinary Parasitology 52 (1994): 179 – 202.
[8] McCall, J., et al. Biology of Experimental Heartworm Infections in Cats. Proceedings, American Heartworm Society Symposium, 1992.
[9] Op. cit., Jacobs.
[10] Op. cit., McCall.
[11] McTier, T., et al. Prevention of Heartworm Infection in Cats by Treatment with Ivermectin at One Month Post-Infection. Proceedings, American Heartworm Society Symposium, 1992.
[12] Atkins, C., et al. Echocardiographic quantification of Dirofilaria immitis in experimentally infected cats. Veterinary Parasitology 158 (2008): 164 – 170.

Therefore, it is reasonable to deviate from the general recommendation of a minimum of five worms per cat as an adequate infection. However, one worm does not provide adequate validation of the experimental model because cats can spontaneously clear worms. In this case, it would be unclear if the product was effective in preventing worms or if the cat cleared the infection on its own in the treated group if adequate infection was defined as one worm. Two worms or greater provides for a better chance of enduring infection for the study duration.

D. immitis Microfilaria in Canines

The VICH guidelines currently do not address the minimum number of microfilaria for an adequate infection to evaluate indications for *D. immitis* microfilaria in dogs. Consideration should be made for including *D. immitis* microfilaria in GL19 with a definition of an adequate infection. When establishing an induced infection, it would be helpful to define a minimum number of microfilaria that would correspond to an expected level of adult heartworms that would be considered adequate.

b. Helminth numbers in livestock and equine species [Section A 4.3; GL12, GL13, GL14, GL15]

VICH GL7 broadly states that a universal definition of adequacy of infection should not be formulated due to the diversity of helminths subject to evaluation but that the concept should be addressed during protocol development in order to permit appropriate standards of effectiveness to be met. The livestock-specific guidelines provide only slightly more explicit recommendations. For example, VICH GL12 Section A 4.3 states that the decision of adequacy of infection, "will be made when the final report is submitted based on statistical and historical data, literature review, or expert testimony." These guidelines offer a general recommendation that the minimal mean number of nematodes is 100 in order to be considered adequate infection, although lower counts should be expected for certain helminth genera. VICH GL13 and GL14 contain similar wording.

We recommend that consideration should be made about whether the nematode numbers constituting adequate infection should be determined prior to the conduct of the study in the bovine, ovine, caprine, and equine specific guidelines (GL12, GL13, GL14, and GL15 respectively).

Additionally, we recommend discussing whether the use of the word "mean" in the sentence, "Generally the minimal mean number of nematodes recommended as adequate is 100," should be removed. We would like to discuss whether adequate infections should be evaluated in individual control animals rather than using a mean across a group, when confirming adequate infections in the minimum number of control animals.

Furthermore, we would like to discuss whether these guidelines should clarify that for some nematode species, the minimum number of nematodes recommended as adequate be greater than 100. For example, in cattle, *Cooperia oncophora, C. punctata*, and *C. pectinata* infections frequently include adult worm counts well over 1,000 and a minimum count of 100 may not be representative of field conditions.

A discussion of these recommendations will enable the creation of a more consistent framework in which to design effectiveness anthelmintic studies. Determining adequate infection in control animals in the final study report after the study has been completed lends itself to differences in opinion about the study conclusion between study investigators and regulatory authorities. If the current guidelines remain as they are, these sections will remain ambiguous, which is not consistent with the objective for harmonization among the international regulatory bodies.

Finally, the VICH GL15 does not address the minimum number of worms for an adequate infection for cestodes in equines. Some authorities have accepted a minimum of ten *Anoplocephala perfoliata* worms as evidence of an adequate infection. Discussion is needed to determine a minimum acceptable number for adequacy of infection in this parasite species.

iii. Adequacy of Infection/Number of animals per group [Section A 4.3, GL7]

a. Number of adequately infected animals [Section A 4.3, GL12, GL13, GL14, GL15, GL19 and GL20]

Adequacy of infection defines the level and distribution of infection of a particular parasite in a given host species. In doing so, adequacy of infection supports the model such that the results can be interpreted with statistical and biological confidence. The existing anthelmintic guidelines (general and species specific) state that an adequate infection is required in a minimum of six control group animals. The guidelines do not specify a maximum number of animals per group nor do they define adequacy of infection as a percentage of control animals. When studies include a large number of animals in the control group to achieve six adequately infected animals, the biological confidence or validity of the model is weakened. We recommend discussing whether the guidelines should define a maximum number of control animals prior to conducting the study to ensure the validity of the experimental model and study design, and thus, ensure confidence in the conclusions drawn from the results.

b. Number of adequately infected animals specific to poultry and swine [Section A 4.3, GL16 and GL21]

The recommendation regarding criteria to grant a claim in the existing VICH guidelines for both porcine and poultry (GL16 and GL21, respectively) is that dose confirmation studies should be conducted with a minimum of six adequately infected animals in the control group and six adequately infected animals in the treated group in each study. However, swine and poultry studies are often designed using pens as the experimental unit, and multiple animals are housed together in each pen. Therefore, six animals may not be sufficient to demonstrate adequate infection, most notably in cases involving large numbers of animals in each pen and/or large numbers of pens. For example, in poultry studies, the control group may include more than 250 birds. In this case, a question arises as to whether six adequately infected controls out of 250 animals provide enough information to characterize the level of infection in the flock. Specifically, we would like to discuss whether having six adequately infected control animals is enough to have confidence to determine if the level of infection is enough to warrant treatment, and to be able to detect and interpret significant differences between control and treated groups.

We suggests considering revising both the porcine and poultry guidelines to address the issue of adequacy of infection in the control group when the experimental unit is a pen of animals. We note that there are multiple options for determining adequate infection for these types of studies. Whatever option is chosen, the choice should be determined prior to conducting the study.

iv. Standards of Effectiveness [Section A 5, GL7]

VICH GL7 Section A 5 states that, "A compound should be declared effective only when effectiveness against each parasite declared on the labeling stands at 90% or above, based on calculation of geometric means using pooled data (when appropriate), and there is a statistically significant difference in parasite numbers between control and treated animals." The guidance further advises different effectiveness standards could be used when focusing on preventing pasture contamination (higher standard) or no other effective treatment is available (lower standard).

As stated above, the effectiveness evaluation is based on comparing results from a study to a predetermined effectiveness requirement, e.g., 90%. An additional tool could be the estimation of the lower confidence bound of the effectiveness estimate. The confidence bound would use the count variability in both the treated and control animals and give some indication of the robustness of the effectiveness estimate. Further investigation is needed to determine the width of the confidence bound (80, 90, 95%) and its use in the approval process, e.g., used for informational purposes only or as part of the assessment of effectiveness.

v. Dose Confirmation Studies [Section B 2, GL7]

We would like to discuss whether regulatory authorities would approve an indication in the following situations:

- without at least one study conducted in their country;
- with only one study conducted;
- when both studies are conducted by the same investigator, and/or in the same laboratory, and/or using the same isolate.

If so, what would be the appropriate circumstances to allow for those situations?

In the United States (US), to demonstrate substantial evidence of effectiveness, the studies must demonstrate inferential value and independent substantiation, as described in 21 Code of Federal Regulations (CFR) Section 514.4(b)(3)(i) and the Preamble to the Substantial Evidence of Effectiveness rule (Federal Register/Vol. 64, no. 144, Wednesday, July 28, 1999, page 40747). Studies conducted for FDA-CVM intended to provide substantial evidence of effectiveness shall consist of a sufficient number of studies of sufficient quality and persuasiveness to permit qualified experts to:

- Determine that the parameters measured and the measured responses reliably reflect the effectiveness of the new animal drug and that the finding is not the result of unanticipated, undetected, systematic bias or chance (independent substantiation).

- Determine that the results obtained are likely to be repeatable and that valid inferences can be drawn to the target animal population (inferential value).

Situations in which both dose confirmation studies are conducted using only one isolate (in those situations where only induced studies are appropriate), one foreign location, and/or one investigator may compromise our ability to make appropriate inferential value and independent substantiation conclusions. We would like to discuss including language in the guidelines that is more specific regarding the use of two laboratory studies to support an indication.

vi. Defining the Age of Field Isolates and Laboratory Strains [Section A 2, GL7]

VICH GL7, Section A 2 does not specifically state recommended ages of field isolates or laboratory strains, rather this section only uses the adjective "recent". However, the glossary for this guideline defines a field isolate as less than 10 years old while a laboratory strain is defined as a sub-population of helminths isolated from the field at least 10 years ago. For some strains, using isolates that are 10 years old may not be appropriately representative of the current field situation in light of anthelmintic resistance. We would like to discuss redefining the age of isolates in anthelmintic effectiveness studies for bovine, ovine, caprine, and equine species as something less than ten years at the time the study is conducted. Additionally, we would like to discuss the adjective "representative", as used in the glossary of GL7 to define the term field isolate, and whether it should be defined based on susceptibility to the drug in question, with the goal of selecting strains of median susceptibility. This is especially important for nematodes of cattle, small ruminants, and equines.

We propose discussing revising these definitions in light of the emerging global problem of anthelmintic resistance in livestock and equine species. Because an anthelmintic drug approval can take many years, ensuring that field isolates are representative of recent isolates at the time the study is conducted and at the time of approval provides greater assurance that the isolates used in the studies are reasonably representative of susceptible field isolates when the product is approved.

vii. Persistent Effectiveness Studies [Section B 4, GL12, GL13, GL14, and GL15]

The recommendation regarding persistent effectiveness studies in the existing VICH guidelines does not state that the study should demonstrate effectiveness at regular intervals within the persistent effect period. If effectiveness is only demonstrated at the end and is not demonstrated at earlier time points, it cannot be determined if the effectiveness of the product was sustained throughout the persistent effect period. For example, if efficacy of a drug at 14, 21, 28, and 35 days was 97, 95, 81, and 94%, respectively, the persistent effectiveness claim for the drug should be granted for 21 days, not for 35 days, because on Day 28, the efficacy was less than 90%. However, if efficacy was only determined at Day 35, a persistent effect claim would be given for 35 days and this would be in error because the product was not effective over the entire 35 day period. Therefore, we would like to discuss whether the existing VICH guidelines for bovine, ovine, caprine, and equine species should describe the evaluation of effectiveness at regular intervals throughout the entire persistent effect period stated in the indication. The schedule of intervals to demonstrate persistent effect may vary for extended release

products based on the duration of persistent effect period stated in the indication.

viii. Statistical consideration: Blocking [GL7]

VICH guidelines suggest that blocking in replicates by weight, sex, age, and/or exposure to parasites may reduce trial variance. This guidance might be too suggestive that blocking is always effective. Effective blocking will result in the reduction of experimental error if blocks are constructed such that the units within a block resemble each other more than units in different blocks. However, blocking may be inefficient if animals are all similar in weight, age, etc. Blocking should be used carefully in order to be beneficial. Additionally, if blocks are used, they should be included in the model.

B. PROPOSED TOPICS FOR ADDITION TO EXISTING GUIDELINES

i. Approach to New Indications [GL19 and GL20]

Some regulators are receiving requests to consider parasite indications not currently addressed by VICH Guidelines. Because this is not addressed in the VICH Guidelines, there is no harmonization for study design for these species or life stages. For parasites that are incompletely described in the existing VICH guidelines (e.g. *Crenosoma vulpis*), additional discussion to revisit the specific gaps present in the current guidelines may be appropriate.

The existing VICH guidelines are silent on the process for evaluating new parasite indications (e.g. study design). General guidelines for study design (numbers per treatment group, geographic considerations, etc.) are provided in VICH GL7, but there are no specific guidelines for how to consider the effectiveness of parasites not presently outlined in VICH GL19 and GL20.

Although specific recommendations cannot be feasibly formulated for every possible new parasite species, adding a framework for evaluation of new parasite species/indications, which outlines a minimum amount of information that should be provided to evaluate effectiveness, is a worthwhile consideration. For example, the framework may include special exceptions for zoonotic parasites, e.g. allowing sponsors to conduct only an induced infection study.

Having a framework in place ahead of time for the review of new parasites/indications can increase efficiency of review (decreasing time between pre-submission discussions and submission of protocol/data) and potentially reduce the number of requests by the regulatory body for more information or corrections after a study is submitted. Due to the variety of parasites, the obvious caveat is that there may be considerations for new parasites of which we are as yet unaware, so there will still need to be some discussion on a case-by-case basis depending on the parasite/indication under review.

ii. Considerations for replacing terminal worm count studies in canines and felines [GL19 and GL20]

In the past, obtaining naturally infected dogs and cats in the US was relatively easy. Sponsors are now experiencing difficulties in this regard. Drug sponsors are stating that this shift is due to restrictions in Class B dealers (dealers that collected dogs and cats from shelters or owners without necessarily divulging that they will be used in terminal studies)

and not that the parasites are becoming less prevalent in the US. If that is the case, and gastrointestinal nematode and cestode prevalence in dogs and cats is still high, we would like to explore a way to take advantage of the naturally-infected dogs and cats in a non-terminal manner. Therefore, we propose to consider whether the use of fecal egg counts (or some other method, like capsule endoscopy) in companion animals could replace the terminal worm count study and provide evidence of effectiveness. Some questions for consideration for relying on fecal egg counts are:

- What is the prevalence of GI cestodes and nematodes in companion animals?
- Can an adequate infection be determined from the fecal egg count?
- Are fecal egg counts a reliable indicator of worm presence (i.e. how intermittent is the fecal egg shedding?) Is this parasite dependent?
- What would be the primary variable of effectiveness?
- How many animals would such a study require to ensure an adequate evaluation, given these limitations?
- How long would the study need to be?
- Could sponsors enlist shelters in this endeavor, since heavily parasitized animals are more likely to be found there than as client-owned animals whose owners go to a veterinary clinic?

iii. Fecal Egg Count Reduction Tests (FECRT) [GL12, GL13, GL 14, and GL15]

The diagnosis of anthelmintic resistance of gastrointestinal nematodes to anthelmintic drugs is of increasing concern for producers, veterinarians, and animal owners of cattle, sheep, goats, and horses. Currently, the most practical and available on-farm test for the evaluation of the efficacy of anthelmintics is the fecal egg count reduction test (FECRT). Although methods for performing the FECRT are currently only standardized for sheep, useful methods are also available for goats, cattle and horses. Presentations made during FDA-CVM's *Antiparasitic Drug Use and Resistance in Ruminants and Equines Public Meeting*, (http://www.regulations.gov/#!docketDetail;dct=FR%252BPR%252BN%252BO%252BSR%252BPS;rpp=25;po=0;D=FDA-2012-N-0102), in March 2012, highlighted the fact that one of the problems in the development of useful guidelines for the diagnosis of resistance within the context of the FECRT is a lack of data on the expected efficacy of a drug at the time of approval. Not all drugs have the same efficacy in susceptible parasite populations at the time of approval and a lack of baseline data of FECRT may lead to the underestimation or overestimation of resistance for some drugs when the FECRT is used in the field after approval. Therefore, we suggest discussion regarding the modifications of the species specific VICH guidelines for cattle, sheep, goats, and horses to allow for the calculation and analysis of FECRT in dose confirmation and/or field study protocols.

These FECRT data could be used as part of the design of appropriate labeling recommendations in order to assist end-users with the evaluation and monitoring of the development of antiparasitic resistance on their farms. We believe that collected FECRT data would not be used as a primary criterion to establish effectiveness, but rather function as supportive information. In horses, the FECRT should not replace the evaluation of egg reappearance periods.

We are aware that additional guidelines pertaining to FECRTs are under development by the WAAVP and recommend that any revisions to the VICH guidelines allow for and encourage

the use of standardized procedures and the use of enhanced diagnostic tests as they become available. Procedures should be recommended to minimize known sources of variability within the test procedures such as use of adequate animal numbers; sampling the same animals pre- and post-treatment; use of appropriate blocking in the study design, appropriate sampling, collection, and storage of fecal samples; and replicated use of a fecal egg count method with adequate sensitivity. Finally, the data should be analyzed using appropriate statistical methods.

The addition of a FECRT calculation to a dose confirmation study may allow for a comparison to the "true efficacy" based on worm counts but would be limited by small animal numbers in each treatment group. We do not anticipate this extra calculation to be burdensome to the sponsor. For dose confirmation studies, fecal egg counts are already performed at the beginning of the study for study inclusion purposes and the addition of post-treatment fecal egg counts is not likely to incur prohibitive study costs.

If the FECRT is calculated within a field study, the calculation could be performed in addition to the standard calculation of efficacy using fecal egg counts from treated and control animals. Because fecal egg counts are already performed in treated animals both pre- and post-treatment, the addition of the FECRT calculation should not be a prohibitive burden to sponsors. We would like to discuss whether the field study design should include a consideration for the use of coproculture (or other diagnostic techniques as they become widely available) at the individual animal level pre- and post-treatment in order to provide a qualitative assessment of the distribution of the parasite species and an improved ability to interpret field study data (particularly FECRT data). Performing coprocultures in this context could assist the sponsor in characterizing the parasite population in the study animals and help explain situations in which the FECRT is lower than expected (for example, if the parasite population in the field study is mixed heavily with parasites for which the test article is not effective). The FECRT results could be interpreted in the context of the dose confirmation study data rather than using a strict % efficacy cut-off. Additionally, for equine field studies, fecal egg counts are taken at regular periods for the determination of egg reappearance periods. FECRTs would not add extra burdens in these cases, as this calculation would use the same data.

iv. Parasite Counting: Speciation of males and females, inclusion of Fourth Stage Larvae (L4) in adult counts [GL12, GL13, and GL14]

VICH guidelines do not address specific recommendations for parasite counts. For dose confirmation studies, worm counts are the pivotal variable for determining effectiveness. However, with certain gastrointestinal nematodes, female parasites within a genus cannot be speciated, leading to situations of possible inaccurate worm counting. The same problem is often encountered when counting the further larval (L4) stages of nematodes. We recommend adding details to the current guidelines that outline how to distribute female worm counts within certain genuses based on the biology of the parasite and the host species.

The existing VICH anthelmintic guidelines do not contain language on how to speciate all recovered worms of certain nematode genera during worm counts for dose confirmation studies, specifically for cattle and small ruminants. The inability to speciate certain female parasites and L4s creates complications for dose confirmation studies in which counting and identifying parasites is a primary variable, leading to situations of possible inaccurate worm

counting. While the VICH guidelines are silent on this aspect of dose confirmation studies, the WAAVP Guidelines for Evaluating the Efficacy of Anthelmintics in Ruminants (1995) state that, "The numbers, species, and stages of nematodes in each aliquot from each animal should be recorded." These guidelines further direct the investigator to remove 100 male nematodes (or as many as possible up to that number) for identification.

Currently, the WAAVP guidelines assume that parasites have a 1:1 sex ratio between males and females, therefore making the counting and speciating of only male parasites acceptable. However, literature suggests that a 1:1 sex ratio may not always be biologically true. The distribution of males and females in a nematode population depends on many factors, such as the mating system (monogamous versus polygamous) and the number of morphotypes within a particular parasite species, as well as the host animal's age and the intensity of the parasite infection within the host[13]. Such differences appear most dramatically in the genus *Teladorsagia*. As such, there are cases where proportioning the male worms of each species in the aliquot sample and multiplying by the total number of worms in that sample to determine the number of worms of each species present would not provide accurate numbers, since one cannot assume a 1:1 sex ratio.

We would like to discuss whether for dose confirmation studies, all worms (male and female) within an aliquot be counted and speciated to provide the most accurate estimate of the total worm burden in the animal. In cases where females are not able to be identified to the species level, we would like to discuss whether methods to determine how to assign species should be decided before the study is conducted based on the most recent scientific literature to ensure the most accurate worm counting. If unspeciated female parasites are not taken into account, is the true efficacy represented by male-only worm counts?

Additionally, in some dose confirmation studies, L4 stages are counted and added to the adult counts after proportioning according to species of males. Given the inability of differentiating L4s to the species level, these numbers may provide inaccurate counts for the primary variable. We would like to discuss what to do with L4 counts when the study is for adult worm efficacy.

6. Recommendations

We recommend that the VICH Steering Committee should consider re-opening the anthelmintic guidelines for possible revision and consideration of the above mentioned topics. Many of the issues identified here are common across the regulatory jurisdictions of the VICH countries. Therefore, we recommend that VICH establish an Expert Working Group (EWG) to elaborate harmonized guidelines utilizing the basic principles underlying the topics outlined above.

[13] Craig, B., et al. Sex ratio and morphological polymorphism in an isolated, endemic Teladorsagia circumcincta population. Journal of Helminthology 84 (2010): 208-215.

7. Timetable and Milestones

Step 1	Establish the EWG through recommendations received by the SC.	3 months
Step 2	The EWG decides which revisions, additions to existing guidelines, and new guidelines should be developed and drafts them. A face-to-face meeting of the EWG will be convened to facilitate successful harmonization on the scientific issues. The EWG submits the guideline to the Secretariat with the signatures of all experts.	24 months
Step 3	The draft revised and new guidelines are submitted to the Steering Committee for approving their release for consultation.	12 months
Step 4	Once adopted by the SC, the draft revised and new guidelines are circulated to all interested parties for consultation, applying an appropriate consultation period (normally 6 months). The regulatory coordinators should inform VICH secretariat when the consultation process in their region is delayed.	12 months
Step 5	The comments received are directed to the EWG for consideration. At this step, the topic leader must be a representative of a regulatory authority. The EWG prepares a revised draft and submits it to the Secretariat with the signature of all experts.	12 months
Step 6	The draft revised and new guidelines are submitted to the SC for approval.	24 months
Step 7	Once approved by the SC, the final Guidelines and a proposed date for their implementation are circulated to the regulatory authorities represented in the SC.	
Step 8	The SC members report to the SC on the implementation of the Guidelines in their respective regions.	
Step 9	Monitoring, maintenance and review of Guidelines	Continuous with formalized review 3 years after implementation

8. Impact Assessment

Industry

1. The guidelines will provide improved clarity of the effectiveness standards for anthelmintic drug products.

2. Unified requirements may lead to a reduction in number of studies needed to obtain global marketing. As a result, the numbers of test animals used should also decrease, promoting animal welfare and the 3R's principles.

3. Most importantly, these guidelines will allow for global consistency in evaluating effectiveness studies.

Regulators

1. Revised guidelines will increase the clarity of the requirements in VICH countries, and therefore there will be less uncertainty and fewer questions expressed by industry.

2. Revised guidelines will lead to a consistent approach in interpretation and assessment by the competent authorities.

3. Revised guidelines will decrease the number of submissions with studies that are inadequate for determining effectiveness of new antiparasitic drugs.