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Discussion paper

VICH Anthelmintic Guidelines Task Force

1. Introduction

In 2013, FDA-CVM introduced a draft Concept Paper (CP) outlining topics from the current VICH anthelmintic guidelines (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; 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*) that should be considered for revision and also new topics that should be considered for addition. At the 29th meeting of the VICH Steering Committee (SC), 11-14 November 2013, the SC decided to create a Task Force (TF) with experts who would review the science regarding the issues raised in the draft CP. The mandate of the TF was to:

- Consider the topics outlined in the draft CP through in-depth discussions regarding the current state of scientific knowledge and the current experience gained from the evaluation of anthelmintic animal drugs. This should result in a comprehensive discussion document elucidating the various scientific opinions and arguments.
- Recommend to the VICH SC as to whether an Expert Working Group (EWG) to revise the VICH Anthelmintic Guidelines should be convened after there has been a full discussion by the TF of the issues and the related science. The points to be addressed must be defined.

Participating members of the TF include representatives from each of the following industry and regulatory groups: Japanese Ministry of Agriculture, Forestry, and Fisheries (JMAFF), Japan Veterinary Pharmaceutical Association (JVPA), European Medicines Agency-Committee for Medicinal Products for Veterinary Use (EMA-CVMP), International Federation for Animal Health-Europe (IFAH-EU), Veterinary Drugs Directorate (VDD) from Canada, Animal Health Institute (AHI), and U.S. Food and Drug Administration-Center for Veterinary Medicine (FDA-CVM).

This discussion paper was drafted using the topics in the CP, and outlines the pertinent points of discussion to consider. The TF states for each topic as to whether the topic is recommended for revision, or if there is currently insufficient agreement to warrant further discussion. There are several topics that have been recommended for revision, and therefore the TF recommends an EWG be convened to complete the revisions as specified in this discussion paper.

2. Discussion by topic

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 in dose determination, dose confirmation, field studies, and persistent effectiveness studies; but in certain circumstances there may be conditions acceptable for the use of arithmetic means.

The CP notes that recent published literature advocates the use of arithmetic means in effectiveness estimation based on fecal egg counts and worm counts[1,2,3], and proposes that an EWG examine additional distributional assumptions, methods of analysis, and estimation of means. There were varying opinions on this topic from the TF which warrant further discussion.

For purposes of this discussion, we should clarify the use of the term "geometric means." Alexander (2012) defines geometric mean as "obtained by multiplying together all the data values and then taking the nth root." Using this definition, the geometric mean is zero whenever any of the individual values are zero. Therefore, a modification is often used to accommodate zero values. Also known as the "Williams mean", it is defined as "add 1 to all the data values, then take the geometric mean, then subtract 1 again." Generally, in the TF's experience, the term "geometric mean" is used even when a modification is required to accommodate zero values, and the most common modification includes the addition of 1 to all values prior to log transformation.

Argument for leaving Section 4.2 of the GL unchanged
The skewed distribution of parasite populations makes the use of a log transformation a valid and even necessary approach to reduce the impact of outlier values. This consideration is of importance to avoid overestimation (in case of an outlier value in the control group) or underestimation (in case of an outlier value in the treated group) of the percent effectiveness. Several authors have discussed the use of the geometric vs arithmetic means in effectiveness calculations, but some suggest that "choice of measure is subordinated to the study's objectives", i.e. study dependent (Alexander 2012). This approach is in line with the current guidance in the anthelmintic GL, i.e. use geometric means given the generally skewed parasite population, and use arithmetic mean if deemed appropriate.

Differences between diagnostic methodology and the use of fecal egg counts vs. worm counts at necropsy should be taken into account for this discussion. The Dobson paper

[1] 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.
[2] Alexander, N. Review: analysis of parasite and other skewed counts. Tropical Medicine and International Health, Vol 17, No. 6 (2012): 684-693.
[3] McKenna, P.B. What do anthelmintics efficacy figures really signify? New Zealand Veterinary Journal, 46 (1998): 82-83.

referenced in the CP[1] estimated effectiveness with fecal egg counts, instead of the worm counts that are currently used in laboratory dose determination and dose confirmation studies. Therefore, what may be applicable for fecal egg counts may not automatically apply to post-mortem worm counts.

The CP proposes evaluation of other statistical methods (aside from simple comparison of geometric or arithmetic means) such as 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.

However, concerns with this approach lie in its impracticality for the desired purpose. Industry notes that the majority of people who run anthelmintic studies for animal health companies and who have attempted to use the zero-inflated Poisson (ZIP) distribution have had difficulty getting it to work as intended

Additionally, there are concerns from some TF members that this change is being suggested simply due to the rising concern over developing antiparasitic resistance. Some TF members assert that considering concerns about resistance is not relevant for pivotal studies (pre-registration or pre-approval) as resistance is not yet an issue prior to approval. A prediction regarding resistance development in the field cannot be obtained from the pivotal, pre-approval studies, regardless of the method used to calculate the effectiveness. There is general agreement that anthelmintic resistance is a serious problem that needs to be addressed; however, some believe it should be addressed by recommendations on the use of anthelmintics in the field after registration and not by a different calculation of the effectiveness in pivotal registration studies.

Argument for having an EWG re-evaluate Section 4.2 of the GL
The assumption that the skewed distribution of worm burdens in naturally occurring anthelmintic infections should be normalized before analysis (i.e. comparison of geometric means) may potentially ignore outliers of biologic importance. Outliers may be important if there is a subpopulation with true reduced effectiveness in the general parasite population and should not be disregarded. It is important to consider the biological relevance of a particular parasite distribution in the evaluation. A more in-depth discussion on how to decide when to use the geometric mean and how to calculate the geometric mean is appropriate, including a discussion of how to choose an appropriate constant (added to each count prior to log transformation) if the geometric mean is appropriately used.

As noted in the argument against changing the current GL, one author (Alexander 2012) suggests that the measure of location (method of conveying the average) should be chosen based on the purpose of the study. However, the full context of the quotation is as follows: "the choice of measure is subordinated to the study's objectives, with purely statistical concerns – for example achieving a normal distribution – being secondary." This latter phrase suggests that the stated goal of geometric means in anthelmintic studies, to normalize a skewed distribution, may not always be appropriate. Alexander further notes that logarithmic transformation is not applicable in the presence of zeros, and further discusses weaknesses when a value such as 1 is added before taking the logarithm. He argues that, "if zeros are present, then other techniques, in particular generalized linear models (GLMs)…, are likely to be preferable to normal-theory methods

on transformed data." So while Alexander clearly argues that choice of measure should be based on the study objectives, he also argues standard comparison of log-transformed means (the geometric mean as typically applied to these regulatory effectiveness studies) is not necessarily preferable when analyzing skewed count data.

Additionally, the assumption that worm count data have a skewed distribution may not be correct in all situations. In laboratory studies where animals are artificially infected with the same number of parasites, and appropriately randomized, the worm burden distribution before treatment and in the control group may be expected to be less skewed compared to naturally infected animals[4]. However, the reference listed here is applicable to chickens, but may not be readily extrapolated to other species. In fact, current experience of TF members reviewing data from dose confirmation studies suggests that, unlike the experience with induced ectoparasite infestations, induced infections of helminths still may produce a skewed distribution of infection. The reason for the differences in the variation between induced ectoparasite studies and endoparasite studies are not entirely certain, and may be related to both the infection dose used, and the biology of the parasite. However, not all induced infections can be assumed to be as skewed as in natural infections.

In addition to the Dobson paper that evaluates the use of geometric or arithmetic means in field studies, the CP also references a paper (McKenna[5]) that describes several trials on endoparasites in sheep comparing worm counts. These trials show large discrepancies between the efficacies based on geometric and arithmetic means. FDA-CVM also provided the task force with examples from actual worm count data highlighting similar discrepancies. In these situations, geometric mean based effectiveness estimates do not describe the proportion of parasites being killed and, thus, might overestimate the degree of effectiveness. Effectiveness estimates based on geometric means also tend to mask treatment failures. This point highlights that the choice of the method for calculating the mean should take both biological as well as statistical information into consideration. Additionally, Dobson[6] used Monte Carlo simulation of fecal egg counts to show that effectiveness estimates using geometric means tend to be biased, while the arithmetic means provide estimates very close to the true effectiveness. This was true although the fecal egg count data distributions were skewed. The information from Dobson et al (2009) is relevant to the discussion because field studies may also be conducted as part of demonstration of effectiveness for registration or approval of a product. In field studies, fecal egg counts are currently most commonly used as an estimate for effectiveness. The current GL recommends geometric means for the field studies, which may be subject to the weaknesses described above.

Further, the GL provides no guidance on how to choose an appropriate constant when estimating effectiveness with geometric means. When zero data are present, it is necessary to add a constant to each data point because the log(zero) is undefined. The choice of constant may have an impact on the results. Several authors have advocated the use of a more thought-out constant, for example, 50% of the detection limit of the

[4] Ferdushy T. et al.: population dynamics of *Ascaridia galli* following single infection in young chickens. Parasitology. 2013, 140 (9):1078-84.
[5] McKenna, P.B. What do anthelmintics efficacy figures really signify? New Zealand Veterinary Journal, 46 (1998): 82-83.
[6] 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.

diagnostic methodology. For example, in case of a McMaster with sensitivity of 50 eggs per gram (epg), the constant should be 25. Regulatory experience suggests that the vast majority of analyses default to using a constant of 1, which may not be appropriate for anthelmintic studies.

Although arithmetic means are mentioned in the guidelines, the geometric mean is generally used as the default method of calculating effectiveness for applications submitted to the EU, Japan, and the United States (US). This decision is usually made independent of a consideration for the distribution of the parasite count data. However, in other countries currently with observer status on the VICH Steering Committee (e.g. Australia, New Zealand, and Canada), arithmetic means are either used exclusively or are at least taken into consideration in those cases where the arithmetic mean is markedly different from the geometric mean. The fact that observer countries already recommend deviations from the current guidelines lends additional credence to the conclusion that further discussion is warranted to promote global harmonization.

The proposal for discussion of this issue is not focused on estimating effectiveness using the most conservative approach available or using pre-approval effectiveness standards as a primary way to minimize resistance development; rather, it stems from a desire to establish an approach that produces effectiveness estimates that are as close as possible to the true effectiveness.

Summary

Based on the comments from all task force members, the TF will further consider whether the following points should be addressed by an EWG:

- A discussion of how and when to use geometric mean and/or arithmetic mean (or other methods of estimating the mean) for different classes of studies (e.g. induced vs. natural infections), including a consideration for both statistical and biological issues.
- A discussion of how to select the appropriate statistical analysis depending on whether the arithmetic or geometric mean is used.
- A more in-depth discussion on how to calculate the geometric mean, including a discussion of how to choose an appropriate constant (added to each count prior to log transformation) if the geometric mean is appropriately used.

Conclusion: This topic remains open for discussion, and warrants further discussion. Due to the significant differences in opinion on this topic between TF members, it appears that perhaps the current GL do not currently sufficiently address all parties' concerns.

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)."

The CP proposes several topics related to adequacy of infection in control animals to be considered for revision in the VICH guidelines, both general and species specific.

Generally, the CP states concerns with the current definitions of an adequate infection in control animals. Within species-specific guidelines, minimum numbers of worms to be considered in individual animals as representative of "adequately infected" are given for some species. The broader questions up for discussion are: should adequate infection be determined by group means or in individual animals; are the numbers used to define adequate infections in individual animals justified; and are there additional numbers that should be added to the guidelines?

The following section of the current VICH GL7 (Section 4.5) suggests that adequacy of infection may not necessarily be defined only by strict worm numbers at necropsy: "One possible statistical method involves the use of calculating the lower 95 % confidence limit of the control group geometric mean burden. If this value is greater than 10 % of the control group, geometric mean burden, then the infection can be said to be adequate. In the case where some of the animals in the control group are not infected (counts=0), then the geometric means should be replaced by the median and the 95 % confidence limit will be based on the control group median burden." Statistical justifications of adequacy of infection have recognized utility; however, strict reliance on a minimum number is most commonly utilized by sponsors. The EWG should consider discussing and clarifying the use of the statistical methods of justifying adequacy of infection. The majority of the TF agrees that this point should be clarified in Section 4.5 of GL7.

Additionally, the GL should clarify how to deal with the statistical difficulties of designing experiments and analyzing data in host-parasite combinations with a low worm burden. For some worm species a higher number of adequately infected animals may be needed because of high variation between experiments (i.e. for some worm species for which we require only a low number of worms, e.g. cestodes). For example, the standard deviation of the statistical distribution with a mean number of 2 worms per infected animal is expected to be higher than the mean. If only low worm counts are available, the number of animals would need to be increased in order to get a meaningful statistical evaluation. In addition to the statistical difficulties of designing experiments and analyzing data in host-parasite combinations with a low worm burden, it should be noted that a situation in which there is a combination of such low natural worm burden and a low prevalence, may pose a special challenge with regard to design and analysis.

Conclusion: The TF recommends revision of GL7 Section A 4.5 to clarify how to use statistical justification in determining adequacy of infection and also in how to evaluate data in host-parasite combinations with a low worm burden.

Other specific comments are discussed below.

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

The CP notes, "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." The CP further outlines each of the listed situations, and justification for use of specific numbers.

Several concerns were raised in considering adding minimum worm counts for additional species of worms. For example, there is support for the concept that the minimum number of parasites per animal or group mean should be driven by a proper statistical analysis defined prior to conduct of the study (as described in Section 4.5 of GL7), and this approach would be preferred over a definition of a discrete minimum number.

However, regulatory authorities generally agree that the definition of minimums for certain parasite species would aid in consistency and international harmonization. Additionally, minimum worm numbers should only be recommended if models used for certain parasite species are validated and well-established.

Further discussion on each topic is reviewed below.

Cestodes in Canines and Felines

Although some TF members supported addition of minimum cestode counts to the species specific GL for canines and felines, many TF members did not provide further specific feedback on this topic.

Adult Heartworms in Felines

Although some TF members supported addition of minimum adult heartworm counts to the species specific GL for felines, many TF members did not provide further specific feedback on this topic.

Dirofilaria immitis *microfiliariae in dogs*

Specific scientific information was reviewed regarding a minimum number of *D. immitis* microfilariae (MF) in dogs. Thus far, there is no correlation between the number of adult heartworms (females specifically) and the numbers of circulating

MF. The suggestion to use 300 MF/mL from the CP is specific to moxidectin from published information regarding that specific NADA.

Bowman and Atkins[7] illustrate in Figure 5 of their publication that MF counts can be quite variable from low to very high in naturally infected heartworm positive dogs. Additionally, numbers of MF <300 can still induce an adverse reaction when treated with a macrocyclic lactone. Bowman and Mannella[8] reported that 1 dog only had 156 MF/mL at the time of treatment (with ivermectin) and developed listlessness after treatment that lasted 48 hours.

However, in laboratory dose confirmation studies the goal of defining the concentration of microfilaria in each control dog that constitutes an adequate infection is not to establish the lowest concentration of microfilaria that could potentially create an adverse reaction. Rather, the concentration of microfilaria in an individual control animal should be sufficient to allow confidence in the infection model. That is, the control dogs should be adequately infected such that one can conclude that zero or low numbers in the treatment group are due solely to the drug effect, and not that the treated dogs did not have a robust infection. Animals in the field may have well over 300 mF/mL[9]; therefore, the use of >300 mF/mL to define adequacy of infection for a "treatment of microfilaria" indication is somewhat conservative. Although the purpose of the TF is not to make an ultimate decision on the number to be included in the GL, it is clear that there are varying interpretations of what might be considered an adequate infection. In the interest of consistency and harmonization, it may be beneficial for the EWG to further discuss how to determine an adequate infection.

Addition of Angiostrongylus vasorum *(GL 19: Canine) and* Aelurostrongylus abstrusus *(GL 20: Feline)*

Regulatory members of the task force noted the absence of direction regarding establishing an adequate infection of lungworm species in dogs and cats, and believe the GL would benefit from including these species.

Conclusion: These topics remain open for discussion, and would benefit from further scientific review by an EWG.

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

[7] Bowman DD, Atkins CE. Heartworm biology, treatment, and control. Vet Clin North Am Small Anim Pract 2009; 39:1127–1158.
[8] Bowman DD and Mannella C: Macrocyclic lactones and Dirofilaria immitis microfilariae. Topics in Companion Animal Medicine, 2011. 26 (4):160-172.
[9] Bowman DD, Johnson RC, Ulrich ME, Neumann N, Lok JB, Zhang Y, Knight DH. Effects of Long-Term Administration of Ivermectin and Milbemycin Oxime on Circulating Microfilariae and Parasite Antigenemia in Dogs with Patent Heartworm Infections. In: Soll MD, Coleman MW, ed. Proceedings of the Heartworm Symposium '92. Austin, Texas: American Heartworm Society, 1992; 151-158.

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.

Determination of the Definition of Adequate Infection a priori

The CP recommended 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). 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. All TF members agreed that adequate infection should be defined for a particular study during the protocol stage, prior to the conduct of the study. However, the VICH GL should still allow flexibility in cases where the control group comes very close to the pre-defined adequacy of infection, but does not meet the exact definition.

Conclusion: In light of the above agreement, the following sections of the GLs should be considered for revision:

1. GL7, Section A. 4.5 Adequacy of Infection. Specifically, consider the following statement for revision: "in the development of study protocols the adequacy of infection should be addressed..". It may be more appropriate to change "addressed" to "defined", although the EWG should ultimately decide the most appropriate revision.

2. GL12, GL13, GL14 and GL15, Section A. 4.3 Adequacy of Infection. Specifically, consider the following statement for revision: "Concerning minimum adequate number of helminths, the decision will be made when the final report is submitted based on statistical and historical data, literature review, or expert testimony." As suggested in the GL7, and in this discussion paper, adequacy of infection should be determined *a priori*. However, it may be valuable to allow some flexibility in the scientific interpretation of the data once the data has been collected. An EWG should evaluate this section for revision.

Evaluation of Adequate Infections: Individual vs. Mean worm counts

The CP recommended that removing the word "mean" be considered from the following statement in Section A. 4.3 in GL12, GL13, GL14, and GL15, "Generally the minimal mean number of nematodes recommended as adequate is 100." The concern here is the potential for studies to be accepted in which the control group consists of a few animals with very high parasite numbers along with animals with very low or even no parasites, possibly decreasing the confidence in the model. However, TF members argue that other aspects of GL7 appropriately address this concern, avoiding the potential issue. Specifically, GL7, Section A. 4.5, states, "The level of infection, *and its distribution*, among control animals should be adequate to permit the appropriate standards of effectiveness to be met with acceptable statistical and biological certitude/confidence." The GL already suggests that in addition to an overall level of infection, the distribution of infection throughout the control group should be adequate. Also, the same section of GL7 states, "whatever statistical method may be recommended, adequate infections are still required in (a minimum of) 6 control animals as outlined in the relevant species-specific guidelines," further requiring an emphasis on adequate infections in individual animals within the control group.

Conclusion: There is not currently sufficient agreement within the TF to warrant revision of this section of the respective GL.

Consideration of increased worm counts for certain nematode species

The CP proposes that the GL should clarify 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.

Many TF members assert that there is no scientific justification for the use of 1000 as a minimum mean worm count for certain species of nematodes (e.g. *Cooperia* species in cattle). However, the proposal in the CP did not specify that 1000 should be used as a minimum mean worm count. Rather, the CP notes that natural infections in cattle, based on FDA-CVM's regulatory experience, often include numbers well over 1000. Therefore, for this species a group mean of 100 may not represent an "adequate" natural infection.

EMA notes that with recent review of dose confirmation studies with induced infections, they agree that *Cooperia* infections often involve well over 1000 worms in both individual animals and when considering group means. In animals experimentally infected with 14,000 to 30,000 L3 larvae of *Cooperia*, individual worm counts at necropsy ranged from 20 to 21,580 in 32 control animals. The arithmetic mean in the control group was 5725 worms and the geometric mean 3111.9 worms. Only two animals had worm counts below or equal to 100. Three animals had a worm count between 100 and 1000. Out of 32, 27 had a worm count above 1000. Therefore, EMA agrees that a group mean of 100 may not

represent an adequate induced infection as it is far below the mean achieved with the recommended number of infective larvae. These examples are consistent with FDA-CVM's regulatory experience. In FDA-CVM's experience, worm counts in the low thousands are common for both induced and natural infections of *Cooperia* in cattle.

According to Yazwinski TA *et al.* 1999[10], the *Cooperia* prevalence in lactating dairy cows were 90, 60, 50 and 30% of the animals in the control group for *Cooperia* spp females, *C. punctata* male, *C. oncophora* males and *C. surnabada* males, respectively. The worm count of *Cooperia* spp adult females ranged from 0 to 5956. It has also been noted that levels of infections may be higher in beef calves than in cows depending upon the season (Couvillion et al, 1996[11]).

Conclusion: This topic remains open for discussion, and should be considered further by an EWG

Defining a minimum number of other nematode species in bovines

GL12 mentions several species for which the mean worm count in the control group would expected to be lower than 100, including *Bunostomum* spp., *Oesophagostomum* spp., *Trichuris* spp., and *Dictyocaulus* spp. Additionally, the GL remain silent on what is considered an adequate infection of L4 and hypobiotic L4. Although not discussed in the CP, some TF members indicated a need for clarification of appropriate numbers for these species.

Conclusion: This topic remains open for discussion, and would benefit from further scientific review.

Defining a minimum number of cestodes in equines

The CP noted that GL15 does not currently define a minimum number of worms for an adequate infection for cestodes in equines. Considering the biology of the parasite, in that smaller numbers of the parasite are pathogenic, and natural infections typically include less than 100 cestodes, the recommendation of 100 minimum worms as noted for nematodes is not appropriate. For example, one Italian study[12] reported that at the end of the grazing season, when the heaviest parasite burdens are expected, the majority of 31 horses sampled at slaughter with *A. perfoliata* infections had worm burdens ranging from 34-91. These numbers are consistent with those reported in other studies. Pavone et. al. considered worm burdens of 20-100 representative of a moderate level of

[10] T.A. Yazwinski et al.: Dose confirmation of moxidectin pour-on against natural nematode infections in lactating dairy cows. Veterinary Parasitology 86 (1999) 223–228
[11] Couvillion C.E. et al. Epidemiological study of nematode infections in a grazing beef cow-calf herd in Mississippi. Veterinary Parasitology, Vol. 64, Issue 3, (1996) 207-218.
[12] Pavone S, Veronesi F, Genchi C, Fioretti DP, Brianti E, Mandara MT. Pathological changes caused by Anoplocephala perfoliata in the mucosa/submucosa and in the enteric nervous system of equine ileocecal junction. 2011. *Vet. Parasit*. 176 (1); 43-52.

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infection, and these burdens were associated with gastrointestinal pathology. The highest worm count noted was 146, making a requirement for individual worm counts of over 100 highly impractical for this cestode species.

The TF agrees that defining a minimum number of cestodes to be considered an adequate infection in equines would be useful.

One TF member proposed that a useful addition to GL15 would be to list species of parasites within the genus that are considered pathogenic.

Conclusion: The TF recommends an EWG consider the addition of worm counts of equine cestodes sufficient to represent an adequate infection to GL15. The addition of a list of pathogenic species remains open for further scientific review and discussion.

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]

The CP states, "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."

Many TF members agreed that this topic should be included for further discussion; however, they presented concerns. Weakening of biological confidence or validity is usually detected by statistical evaluation of the data distribution, which is already addressed (e.g. GL7 section 4.5). Defining a maximum number of animals as a general guidance to cope with concerns over specific models, might also not be the preferred approach; a case-by-case approach might be more valuable.

Furthermore, the number of control animals needed to achieve the required number of adequately infected animals also depends on the definition or criteria of "adequate infection". If the requirements for an adequate infection are altered, consequently, the number of control animals will also have to be adapted as higher worm burdens cannot necessarily be achieved by simply increasing the

infection dose. Otherwise, depending on the parasite and host species of concern, the proposed alterations in the definition of adequate infection in individual control animals may result in an increased likelihood of overexposure or even failure of an infection. EMA noted a specific concern when an adequate model for a parasite species is not available, for example, *Ascaris suum* in swine. Setting a maximum number of animals could lead to situations where it is not possible to perform a study with sufficient statistical power.

Conclusion: The TF did not agree that defining a maximum number of animals in the control group (or fixing a percentage) would be a universally acceptable method of establishing confidence in the study design. However, it is clear that revision of GL7 Section A. 4.5, as described in (ii.) Adequacy of infection/Number of Helminths in Six Individual Control Animals above could potentially also address this concern. Therefore, the TF recommends the EWG consider revision of Section A. 4.5 of VICH GL7 in order to clarify the method of defining an adequate infection, but does not recommend defining a maximum number or fixed percentage in control group when considering adequacy of infection.

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

The CP states, "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."

The TF members all generally agreed that there should be guidance on how to determine a representative number of animals in which adequacy of infection will be determined if the statistical unit is a pen.

Conclusion: The TF recommends revision of GL16 and GL21, Section A 4.3, to provide guidance on how to determine a representative number of animals in which adequacy of infection will be determined if the statistical unit is a pen.

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%. In the CP, FDA-CVM proposed that 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. FDA-CVM acknowledged that further investigation is needed to determine the width of the confidence bound (e.g. 80, 90, or 95%) and its use in the approval process, e.g., used for informational purposes only or as part of the assessment of effectiveness. Almost all TF members expressed some reservation with this proposal, citing concerns that use of confidence bounds may increase the numbers of animals required in a given study to demonstrate effectiveness, or use of additional animals in further studies. The primary concern is that when the data contain a high variability, establishing acceptable confidence bounds for a given drug in studies with low numbers of animals would be effectively applying a more rigorous standard. EMA noted that retrospective analyses on historical study data may determine if the benefit obtained by this additional information outweighs the expected higher sample size in studies. JMAFF expressed concern that the overall effectiveness in a whole population may not be demonstrated when using pooled data from different geographic regions due to the increased variability, especially if there are regional differences regarding parasite susceptibility to an anthelmintic.

All TF members (including FDA-CVM) oppose the use of more animals than currently required for anthelmintic studies, especially the terminal dose confirmation studies which are required for worm counts. However, this proposal was not intended to require larger numbers of animals in dose confirmation studies. FDA-CVM proposed that the width of the confidence bound and its use should be further evaluated by an EWG.

Additional concerns were also cited. IFAH-Europe noted the differences in effectiveness calculation versus effectiveness estimation. As currently described in the guidelines, the evaluation of effectiveness in pivotal studies is an effectiveness calculation, not effectiveness estimation. IFAH-Europe is of the opinion that combining estimations of the lower confidence bound with an effectiveness calculation is not the correct mathematical procedure, as one is a statistic and the other is an arithmetic procedure. However, even if the lower confidence bounds would be used to evaluate the impact of intra-group variability on the difference between treated groups and the control group, certain members do not see a benefit compared to the current procedures where the significance of difference between the treated and control group (p-value) is calculated by the application of a statistical test. In contrast, the lower confidence interval provides less reliable information compared to the p-value. Others disagree with this assertion, quoting the Guideline

on statistical principles for veterinary clinical trials (EMA/CVMP/EWP/81976/2010 section 7.6.2), "P-values should always be accompanied by estimations of confidence intervals for the effect size to allow the discussion of the results' clinical relevance." Other authors have also suggested that estimates of treatment effectiveness should always be reported together with confidence intervals[13].

FDA-CVM does not specifically propose that the criteria for evaluation of effectiveness should include bounds on the confidence interval, but suggests that additional information, such as the lower confidence bound on this estimate, may be helpful. For example, two studies may have the same point estimates of percent effectiveness, but the confidence bound from the more variable study will be wider. The Guideline on statistical principles for veterinary clinical trials (EMA/CVMP/EWP/81976/2010 section 7.6.2) states that "to assess the precision of point estimates of treatment effects, these should be accompanied by confidence intervals, whenever possible".

In the current process for evaluating effectiveness, the statistical test is for the difference between mean counts in each group; the p-value for this test is based on the difference between means. There is no direct relationship between the estimated percent effectiveness and the difference between means. Therefore, the confidence bound on percent effectiveness provides additional information that is different from the inferential value from the statistical test. Even in the (unlikely) event that 2 tests have the same p-value, the lower confidence bounds may still be different.

IFAH-Europe acknowledged that there is an ongoing discussion on evaluating anthelmintic resistance by fecal egg counts (in live animals), and on the added benefit of the confidence intervals to evaluate the true anthelmintic resistance status using this parameter. However, there is no clear benefit to this approach when evaluating effectiveness by worm counts, especially considering the above arguments.

FDA-CVM agreed that many of the concerns cited by the task force members are valid, suggesting it may be difficult to come to a consensus on this topic at this time. FDA-CVM requested that in addition to the original proposal (evaluation of confidence intervals as an additional tool), the task force consider defining the conditions under which the 90% standard may not be sufficient. In Section 5, GL7 states the following: "there are regional differences where the epizootiology of certain parasitic infections may recommend higher minimal effectiveness, especially when the aim for drug effectiveness is focused specifically on preventing pasture contamination. These will be covered in the individual host species guidances." The individual species guidances for sheep (GL13), cattle (GL12), and goats (GL14) do not provide any additional information; the canine guidance (GL19) states that for some parasites with public health or animal welfare/clinical implications, e.g. *E. granulosus*

[13] Schall, R., Luus, H.G., Comparative evaluation of ectoparasiticide efficacy: Non-inferiority and superiority assessment. Vet. Parasitol. (2011)

and *D. immitis*, respectfully, higher effectiveness standards (i.e. up to 100%) may be appropriate. The task force should consider whether the statement regarding prevention of pasture contamination is outdated and whether the guidelines should more clearly define the conditions under which an effectiveness of greater than 90% may be appropriate for grazing species.

The strategy of parasite control that includes preventing pasture contamination appears to contradict refugia-based strategies that are generally accepted as an appropriate management tool to slow the development of antiparasitic resistance. Experts have suggested that for refugia-based strategies (targeted treatment of high-shedding animals) to be most effective, they need to be used with drugs that have high effectiveness.[14,15] Specifically, FDA-CVM recommends we remove the phrase "prevention of pasture contamination" from this part of the GL.

Finally, there may be other valid conditions under which higher effectiveness standards may be appropriate for anthelmintics in grazing species, including parasites with greater clinical importance, drugs at greater risk for resistance development (e.g. drugs with persistence where the rate of resistance development has been related to the initial effectiveness of the drug); and certain combinations of anthelmintics. Combination antiparasitic drugs for which the spectrum of action of the drug is similar or identical (combinations in which the indications for use, or drug claim, are highly overlapping), are generally created in order to either slow the development of anthelmintic resistance or to address a current resistance problem. Such combinations are theoretically ideal when the overall effectiveness of the combination is very close to 100%.[16] FDA-CVM recommends that an EWG consider this topic for revision to the species specific guidelines for grazing species (GL12, GL13, GL14, and GL15).

EMA noted a concern that use of combination anthelmintics in the absence of an adequate level of refugia of unselected parasites has the potential to select for antiparasitic resistance to several actives simultaneously (Leathwick and Besier 2014). EMA noted a concern that although the use of combination anthelmintics appear to slow the development of resistance because they afford the highest possible kill of nematodes their use does not eliminate the significant risk for resistance posed by dosing strategies that allow livestock to graze low contamination pastures after treatment. The practice of allowing livestock to graze low contamination pastures after treatment readily selects for resistant populations

[14] FDA's Public Meeting on Antiparasitic Drug Use and Resistance in Ruminants and Equines. March 2012. See slide presentations for Kaplan (starting on slide 16) and Leathwick (starting on slide 7) for more details found here: Link to CVM website
[15] Leathwick DM, Besier RB. The management of anthelmintic resistance in grazing ruminants in Australasia-Strategies and experiences. 2014. *Vet. parasit*. 204; 44-54.
[16] FDA's Public Meeting on Antiparasitic Drug Use and Resistance in Ruminants and Equines. March 2012. See slide presentations on combinations for Prichard (Combinations, Good and Bad, When and How to use Combinations) and Leathwick (Combination Anthelmintics and the Role of Worms in Refugia) for more details found here: Link to CVM website

(Geary et al 2012)[17]. FDA-CVM reiterated the potential usefulness of combinations "as part of an integrated package of resistance management strategies," and that the choice of active ingredients in a combination should be made carefully (avoiding a shared mechanism for resistance, unfavorable pharmacokinetic (PK) or pharmacodynamics (PD) interaction between actives or excipients, etc.)[17]

IFAH-EU expressed concern regarding possible additions to the GL regarding higher effectiveness. Specifically, situations where higher effectiveness may be appropriate could also change over time, making it difficult to provide further detailed guidance than what is currently in GL7. FDA-CVM acknowledged that while it may be difficult to specify the exact parameters for each of the above examples, updating the current GL to keep up with current science is valuable to all parties. A simple removal of the outdated "prevention of pasture contamination" example and addition of some or all of the examples cited would provide valuable information to all users of the GL.

Conclusion: The TF has yet to come to consensus agreement on whether to support the proposed revisions. However, there is sufficient discussion provided here for the EWG to consider the topic further.

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

FDA-CVM has been approached by drug sponsors with proposals to deviate from the current VICH guidelines for dose confirmation studies. The following is an excerpt from the current VICH GL7:

"At least two controlled or, when appropriate, critical dose confirmation studies per individual claim are recommended (single or multiple infections). Two studies are the minimum suggested to verify that effectiveness can be achieved against various helminth strains in animals raised in disparate regions and climates and under respective husbandry conditions. At least one of the studies should be conducted in the geographic location where registration is being pursued and both studies should be conducted under conditions that are sufficiently representative of the various conditions under which the product will be authorized. In the event that in certain locations parasites are particularly rare then two trials from outside the location should be acceptable."

Due to an increasing number of proposals deviating from these recommendations, FDA-CVM proposed in the CP 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.

[17] Geary et al. World Association for the Advancement of Veterinary Parasitology Guideline: Anthelmintic combination products targeting nematode infections of ruminants and horses. 2012. *Vet. Parasit*. 190: 306-316.

JMAFF suggested that they do deviate from the GL in that they do not always require two dose confirmation studies, and the dose confirmation study(ies) do not have to be conducted within their country. If two studies are conducted by the same investigator/same laboratory/with the same isolate, this also may be sufficient for approval of an indication in Japan.

However, the EMA stated they generally only deviate in certain circumstances from the current GL. For example, generally both dose confirmation studies for products to be registered in Europe should be conducted in Europe. If certain parasites are not native to Europe, studies could be performed outside Europe. However, reference to these parasites would normally only be made in other sections of the label (e.g. pharmacodynamic properties), but not under "indications."

When considering the investigator, laboratory, and strain, EMA generally considers that two different strains of European origin be used for the two dose confirmation studies. EMA considers it acceptable for the same investigator or the same laboratory to perform the studies with two different strains, provided the studies are GCP compliant. In very rare diseases, where the parasite is known to be difficult to establish and maintain in the laboratory, it would be acceptable to use the same laboratory strain.

For the EMA, only one dose confirmation study may be required for certain generic products[18], minor use products, or new fixed combination products with well-established components. Each of these situations is likely highly dependent on the individual regulations of the country in which the sponsor is seeking approval or registration. Therefore, it would be difficult to add such specific situations to the GL.

In the 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 FDA-CVM's ability to make appropriate inferential value and independent substantiation conclusions.

[18] Provided other field data are available to support the claims and the dose limiting species is known.

EMA further pointed out concerns with the use of naturally infected animals in dose confirmation studies regarding dogs and cats in the EMA. The current GL 7, 19 (canines) and 20 (felines) recommend at least one dose confirmation study be conducted in naturally infected animals for each parasite species claimed. It may be difficult to find naturally infected animals with certain parasite species (e.g. hookworm species) in European countries, in which case many companies use animals in other countries. EMA points out both animal welfare concerns and study validity concerns arising from studies performed in these other countries. Additionally, sponsors have presented concerns to FDA-CVM that some of the remaining countries where these naturally occurring infections may be found are changing the laws regarding the use of these animals for research. In this case, it may not be possible to enroll these animals in terminal dose confirmation studies.

One of the additional concerns EMA presented regarding dose confirmation studies is that selection criteria within the species specific GL may need to be clarified or revised. For example, VICH GL 19 states that approximately six-month-old canines are generally suitable for effectiveness studies, followed by a list of exceptions based on parasite species. One exception is that for *Uncinara stenocephala*, older canines can be used. EMA suggests revision of the defined age in the GL. EMA had some concerns that older animals may show higher self-cure rates, noted in studies where dogs of unknown age were used. FDA-CVM has not necessarily experienced high numbers of "self-cure" due to age with data submitted for approval, but does see cases where the control group did not have an adequate infection as defined by the study protocol and GL definitions of adequacy of infection. Whether the age of animal at enrollment was a factor in those cases in FDA-CVM's experience likely depends on the individual situation.

In Canada, VDD will approve an indication without studies performed in Canada, although sponsors are asked to provide bridging information to show that the pathogenicity and resistance patterns of the organism are similar to what is found in Canada. The bridging report must also demonstrate that rearing practices are the same or similar in the study location, and that climactic conditions are similar to those seen in Canada. Additionally, while not ideal, VDD will approve an indication with evidence from one study only.

Conclusions: The discussion points presented by the TF members demonstrated that, in general, the current guidelines on the number and geographic locations for dose confirmation studies are sufficient for most situations. Unique situations can be addressed on a case-by-case basis in accordance with specific regulatory requirements within the TF member countries.

However, the discussion did highlight two issues that may warrant further consideration by an EWG:

- Naturally infected dogs and cats are becoming increasingly difficult to obtain for dose confirmation studies within the registration country; an EWG should consider exploring alternative study designs and approaches. One of the approaches to consider could include adding flexibility in the GL to the requirement for at least one dose confirmation study in naturally infected dogs or cats.

- It may be appropriate to revisit the defined ages of study animals in GL 19 (e.g. *U. stenocephala*).

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

VICH GL7, Section A2, states that "recent field isolates generally are preferred to develop induced infections, although in some cases laboratory strains should be used." The glossary defines a field isolate as a collection of a sub-population of helminths (considered representative of current parasitic infections in the field) for the conduct of drug effectiveness tests isolated from the field less than 10 years ago. A laboratory strain is defined as a sub-population of helminths isolated from the field at least 10 years ago, characterized, and segregated in the laboratory based on a property that makes it unique for research areas such as resistance to certain antiparasitic compounds.

In the CP, FDA-CVM discussed that, for some strains using field isolates that are up to 10 years old may not be appropriately representative of current parasitic infections in the field, particularly in light of the emerging global problem of anthelmintic resistance. An additional consideration is that an anthelmintic drug approval may occur many years after some studies were conducted. FDA-CVM proposed a discussion about potentially 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, FDA-CVM recommended that an EWG consider whether the adjective "representative", as used in the glossary of GL7 to define the term field isolate, 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.

Most TF members agreed that the revisions to the definitions in the glossary of GL7 should be discussed; however, there were additional comments and concerns presented.

Both EMA and JMAFF recommended that the discussion not be restricted to the guidelines for horses, cattle, and small ruminants; revisions to other target animal species guidelines should be considered as well.

Although EMA agreed that the field isolates and laboratory strains should be representative of strains in the field at the time the study is conducted, obtaining new field isolates may not always be feasible. EMA therefore suggested considering allowing the "refreshing" of existing laboratory strains.

EMA also suggested discussing whether VICH GL 7 should include specific guidance on appropriate methods for detecting resistance patterns in nematodes and trematodes, and the types of data that should be included in an application to address resistance. EMA provided a discussion paper related to this topic to further

this discussion ("CVMP Reflection paper on anthelminthic resistance CVMP/EWP/573536/2013").

One TF member disagreed with any age restrictions on isolates used in a laboratory study as long as the isolates are appropriately defined as susceptible or resistant, depending on the claim being pursued. The TF member asserted that effectiveness relevant to the field should be tested in field studies.

There are scientific and regulatory challenges associated with the identification, characterization (extent of resistance, cross-resistance, correlation of genotype with phenotype, etc), and determination of field representativeness of resistant isolates (e.g. consider variability in mechanisms of resistance). Consequently, FDA-CVM does not currently consider approving indications for treatment and control of resistant strains of parasites. However, FDA-CVM asserts that use of more recent strains than those 10 years old improves confidence that a given strain is representative of current susceptibility patterns in a given area.

Finally, AHI stated that a goal of selecting strains of median susceptibility is one that is unattainable. This point is appropriate given the lack of knowledge of the overall range of susceptibility for any given parasite in a geographic region.

Conclusion: This topic remains open for discussion, and warrants further discussion by an EWG. Due to the significant differences in opinion on this topic between TF members, it appears that the current GL may not currently sufficiently address the concerns of all members and may not address additional concerns raised as a result of the emergence of antiparasitic resistance. The main areas of disagreement are whether the guidelines should provide clarity on the appropriate age of isolates and how field isolates are defined as "representative."

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

Section B4 (GL12, GL13, GL14, and GL15), describes two basic study designs to support persistent effectiveness claims but acknowledges that no standardized protocols have been developed. The guidelines state that persistent effectiveness claims should be supported by a minimum 90% effectiveness. In the CP, FDA-CVM noted that the VICH guidelines do 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. FDA-CVM provided the following example of how persistent effectiveness studies are generally interpreted in the US. If the effectiveness of a drug at 14, 21, 28, and 35 days was 97, 95, 81, and 94%, respectively, the persistent effectiveness claim for the drug would generally be granted for 21 days, not for 35 days, because on Day 28, the effectiveness was less than 90%. FDA-CVM recommended that an EWG discuss revising the VICH guidelines for bovine, ovine, caprine, and equine species to 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.

The regulatory members of the TF supported the proposal to discuss revising the guidelines to clarify that effectiveness should be assessed at different time points after infection in order to define the accurate period of persistent effectiveness. EMA's experience is same as that of FDA-CVM: that persistent effectiveness studies include several time points of evaluation (e.g. by using different time points of challenge per group for 3 or 4 groups slaughtered at the same time, which allows an evaluation of the effectiveness for different intervals from infection to slaughter). VDD added that the intervals would have to be well defined (presumably related to life cycle of the parasite). The evaluation would be particularly important in instances where PK parameters show an inconsistent level of medication throughout the proposed duration.

JMAFF had concerns that these expectations would lead to the need for the use of more animals in research to develop these products. As FDA, EMA, and VDD consider this study design (assessment of effectiveness at regular intervals) commonly used for persistent effectiveness claims, there is no expectation of an increase of the number of animals required for such a study. Products approved in Japan are not necessarily required to demonstrate effectiveness at regular intervals to establish persistent effectiveness, and therefore the EWG should consider sufficient flexibility to any additions to the guidelines to allow for this consideration.

Industry TF members (JVPA and IFAH) did not support the discussion of this topic because they concluded that it could lead to an increase in the number of study animals. However, as it appears the study design described in the CP is fairly standard, at least from the perspective of FDA-CVM and EMA; FDA-CVM does not believe such language would increase the number of study animals used for a persistent effectiveness claim. Rather, it would clarify current standards for the benefit of the drug sponsor developing study protocols.

Finally, AHI commented on FDA-CVM's example and recommended that such examples should be discussed on a case-by-case basis; rather than through a revision to the guidelines. They pointed out that when outliers occur, they may be able to be explained using PK/PD information (if available) or other information. For the example discussed above, such information could potentially be used to justify the persistent effectiveness of the product for a full 35 days. FDA-CVM always evaluates data for a particular product on a case-by-case basis and considers all data available on the product when making regulatory decisions. Addition of language to the anthelmintic GLs could clarify this position to industry members intending to develop protocols for persistent effectiveness.

Conclusion: This topic remains open for discussion, and warrants further discussion by an EWG due to the differences in opinion on this topic between TF members.

viii. Statistical Consideration: Blocking [GL7]

Section A. 6 of the species specific VICH guidelines (GL12, 13, 14, and GL15) states, "Blocking in replicates by weight, sex, age, and/or exposure to parasites may reduce trial variance." The inclusion of this statement in each species specific guideline seems to overemphasize the importance of blocking, as blocking is not universally appropriate. Regulatory experience suggests that sponsors will default to blocking based on this statement, without regard to potential loss in efficiency if blocking is unnecessary. Blocking should only be used when the effect of blocking is expected to reduce residual error and compensate for the corresponding loss of error degrees of freedom.

The guidelines do not provide sufficient direction regarding study design considerations, including how to decide whether blocking should be used for a particular study. FDA-CVM suggests that an EWG should evaluate the best placement for this discussion (General or Species Specific), and also consider adding appropriate direction for when blocking may be appropriate. The guidelines should also state that any design features (such as blocking) should be accounted for in the statistical model.

Conclusion: The TF supports consideration by an EWG to revise the GL to provide more clarification on blocking or other study design considerations, and to state the statistical model should account for any study design restrictions. At minimum, the TF recommends addition of language similar to: "Blocking should only be performed if the effect of blocking will minimize residual error in the study. Blocking is not always the most appropriate method for minimizing variation. If blocking is used, it should be included in the statistical model."

ix. Updating VICH GL16, Effectiveness of Anthelmintics: Specific Recommendations for Porcine

Although not in FDA-CVM's original CP, EMA proposed revision of VICH GL16 Effectiveness of Anthelmintics: Specific Recommendations for Porcine, Section 4.4, Label Claims. The introduction section of GL16 references an outdated publication, the 1986 WAAVP Guidelines for Evaluating the Effectiveness of Anthelmintics in Swine[19]. The current GL states, "For adult claims as a general rule the treatment should not be administered earlier than…65 days for *A. suum*" and "For L4 claims treatments should be given 11 to 15 days for *A. suum*." This treatment timing is not up to date with current scientific knowledge of the *A. suum* life cycle. For adult claims, a shorter time to treatment may be acceptable as the prepatent period is

[19] D. Düwel, D.W. Barth, E.G. Batte, H. Berger, T.B. Stewart, V.J. Theodorides. 1986 World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) Guidelines for evaluating the efficacy of anthelmintics in swine. *Veterinary Parasitology* 21: 69-82.

now recognized to be 42-29 days.[20] For larval claims, the specified 11-15 days may overlap with a natural elimination of larvae that occurs starting 14-21 days post-inoculation, and therefore the treatment timing may also need revision.[2122] [2324] Further, necropsy timing following treatment should be added to GL 16, similar to other species specific GL (e.g. GL 19 and GL 20).

Conclusion: An EWG should consider updating recommendations in GL16 to be consistent with current scientific knowledge and current World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines. Specific items to be addressed remain under discussion.

x. Implementing GLP standards for some or all laboratory dose confirmation studies

EMA proposed another topic in addition to those proposed in FDA-CVM's CP. Currently, VICH GL 7 states under A.1, Good Clinical Practice, "The principles of Good Clinical Practice (GCP) should apply to all clinical studies and sponsors should work within the principles of the GCP recommendations. Non-GCP studies are considered as non-pivotal studies and may be used as supporting data." The GL does not differentiate standards for terminal dose confirmation studies, which are always performed in research animals due to the terminal nature of the studies. EMA proposes that an EWG consider adding that laboratory dose confirmation studies be conducted according to Good Laboratory Practices, as EMA has concerns that the GCP GL are not sufficient for laboratory dose confirmation studies.

Concerns primarily include the source of animals used in dose confirmation studies. As the GCP GL does not address animal source, there could be a variety of sources for these animals. EMA considers that use of laboratory animals would limit the variability that could be expected when animals originate from multiple sources. Further, animals should be of known origin or purpose-bred to ensure both animal welfare and the validity and reproducibility of the studies.

EMA provided the following example of how source of animals could present an ethical problem: Recruiting animals for studies that are conducted under GCP conditions is addressed in VICH GCP GL.9. An informed consent is mandatory to

[20] Hennessy DR, Bauer C, Boray JC, Conder GA, Daugschies A, Johansen M-V, Maddox-Hyttel C, Roepstorff A. 2006. World association for the advancement of veterinary parasitology (WAAVP): Second edition of guidelines for evaluating the efficacy of anthelmintics in swine. *Veterinary Parasitology*. 141: 138-149.
[21] Roepstorff A, Eriksen L, Slotved HC, Nansen P. 1997. Experimental Ascaris suum infection in the pig: worm population kinetics following single inoculations with three doses of infective eggs. *Parasitology*. 115: 443-452.
[22]-Masure D, Wang T, Vlaminck J, Claerhoudt S, Chiers K, et al. (2013) The Intestinal Expulsion of the Roundworm Ascaris suum Is Associated with Eosinophils, Intra-Epithelial T Cells and Decreased Intestinal Transit Time, PLoS *Negl Trop Dis* 7(12).
[23] Miquel N., Roepstorff A., Bailey M, and Eriksen L. (2005): host immune reactions and worm kinetics during the expulsion of *Ascaris suum* in pigs, *Parasite immunology*, 27, 79-88.
[24] Nejsum P, Thamsborg S.M., Petersen H.H., Kringel H., Fredholm M., Roepstorff A.: Population dynamics of Ascaris suum in trickle-infected pigs, J. Parasitol., 95 (2009), 1048–1053

confirm the owner's willingness to allow their animal(s) to participate in a study. However, many owners would be reluctant to provide animals for studies with terminal outcome (i.e. need for post-mortem examination). In such cases, a pharmaceutical company or research organization may purchase the animals themselves e.g. animal of unknown origin, and then give their owner's consent to allow participation in a trial. This approach would technically follow the VICH GCP guideline. However, EMA believes this does not reflect the spirit in which the VICH GCP guideline was written, and would potentially raise ethical and even animal welfare concerns.

EMA proposed that Section A.1, Good Clinical Practice, of the VICH GL 7, be considered for revision as dose confirmation studies conducted under laboratory conditions might also comply with GLP recommendations (see also definitions of terms 2.3 of the OECD Principles on Good Laboratory Practice).

FDA-CVM does not agree that the current animal sourcing situations for dose confirmation studies in companion or food animal species leads to compromised animal welfare or ethical concerns. FDA-CVM agrees that the VICH GCP guideline does not differentiate standards for animal sourcing for terminal dose confirmation studies; however, FDA-CVM asserts that changing the standard should not change the burden of responsibility to properly ensure animal welfare in the studies, regardless of the source. Animal welfare is addressed in Section A.6 (Animal Selection, Allocation and Handling) of the species specific VICH Anthelmintic guidelines. In addition, the fact that a pharmaceutical company purchases animals for a study does not mean that animals are of unknown origin. Situations in which a pharmaceutical company purchases companion animals from owners who are unaware of the potential for that animal's use in a terminal study is very rare (to non-existent) in the US.

FDA-CVM notes that the OECD GLP and FDA GLPs (21 CFR Part 58) have similar language regarding documentation of the animal source (Section 8.2 of OECD GLP; 21 CFR 58.120(a)(4)) as the VICH GCP guideline; none of the standards necessarily limit the use of a variety of sources, as long as the source(s) is appropriate for the study objectives.

- Section 5.2 of the OECD GLPs[25] states that "records of source….should be maintained." And 8.2 of the OECD GLPs states that "the study plan should contain, but not be limited to the following information: … Characterisation of the test system, such as the species, strain, sub-strain, source of supply, number, body weight range, sex, age, and other pertinent information…"

- 21 CFR 58.120(a) states that "each study shall have an approved written protocol that clearly indicates the objectives and all methods for the conduct of the study.

[25] http://ec.europa.eu/health/files/eudralex/vol-7/a/7ag4a_en.pdf

The protocol shall contain, as applicable, the following information…..”(4) The number, body weight range, sex, source of supply, species, strain, substrain, and age of the test system…”

- Section 6.3.9 (Animal selection and identification) of the VICH GCP guideline states that the protocol should specify the “…source, number, identity and type of study animal to be used, such as species, age, gender, breed category, weight, physiological status and prognostic factors.”

FDA-CVM notes that the FDA GLP regulations (21 CFR Part 58) apply to non-clinical laboratory studies, which are defined as follows: “in vivo or in vitro experiments in which test articles are studied prospectively in test systems under laboratory conditions to determine their safety. The term does not include studies utilizing human subjects or clinical studies or field trials in animals…” The FDA GLP regulations are intended for safety rather than effectiveness studies.

IFAH suggested that animal source might be directly addressed in the anthelmintic guidelines, rather than indirectly through a GLP requirement. However, for livestock species “known” source may be more appropriate than “purpose-bred,” as generally this terminology doesn’t apply to cattle and horses. IFAH further noted that type of animal required in a given study (purpose-bred vs. known origin) is likely related to the research objective of that particular study, and even purpose-bred animals can originate from a variety of sources. If there is concern about the source variety having an influence on the study results, this could be addressed with appropriate randomization techniques.

IFAH raised further concerns about requiring dose confirmation studies to conform to GLP standards. GLP requires a certain infrastructure that many investigators and parasitological experts are not able to provide. In contrast, GCP is a very established and practical quality standard that can be applied under many conditions. For example, most universities would not be able to conduct studies under GLP. There may be specific situations where a laboratory dose confirmation study could be conducted under full GLP; however, extension of the scope of GLP to veterinary effectiveness studies may be further complicated by the fact that GLP-surveilling authorities worldwide would have to acquire expertise specific to these types of studies. Currently GLP regulations worldwide largely target animal toxicity and safety studies.

JMAFF does not support discussing the issue in the EWG. JMAFF is concerned the current proposed Anthelmintic Guideline revisions isn’t an appropriate forum to discuss changing the standards (GLP/GCP) for laboratory dose confirmation studies. Only studies concerning toxicity, safety of animals and residue studies are required to be done under the GLP in Japan. JMAFF believes that the effectiveness of the product is confirmed by the clinical studies conducted under the GCP guidelines.

AHI emphasized that effectiveness studies should be performed to GCP standards for the following reasons: The additional costs and logistics of running effectiveness studies, in particular in food animal species, are not appropriate using GLP standards. GLP should be reserved for target animal and PK types of studies. AHI does not support this proposed revision.

Conclusion: The majority of the TF does not support further consideration by an EWG of the requirement for dose confirmation studies to be performed to the GLP standards. However, the EWG could consider whether animal sourcing can or should be addressed in more detail in the canine and feline species-specific Anthelmintic GL.

B. PROPOSED TOPICS FOR ADDITION TO EXISTING GUIDELINES

i. Approach to New Indications [GL19 and GL20]

From the CP: "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."

Generally, most TF members agreed that such revision is supported. However, AHI specifically noted that new indications have been successfully obtained for a number of different anthelmintic products for such rare or relatively uncommon parasites. Examples include *Crenosoma vulpis*, *Thelazia callipaeda*, *Angiostrongylus vasorum*, and microfilariae of *D. immitis*. AHI therefore noted that it is unclear what additional information might be provided to these existing guidelines since drug sponsors have been successful to date in adding label claims for these exemplified parasite species. There will still be some discussion with regulatory agencies on a case-by-case basis depending on the parasite or indication under review.

FDA-CVM noted that in the US, the only approved indication listed above is for microfilariae of *D. immitis*. FDA-CVM believes that such framework could aid sponsors further as they consider pursuing new indications.

IFAH-EU does believe that further guidance can be welcomed and can be considered as beneficial, but is also of the opinion that sufficient flexibility should be retained for all eventualities. VDD suggested that, for species not specifically described in the guidelines, the manufacturer could provide justification for the study design, explaining the reasoning for specific protocol design. A risk: benefit discussion should also be included that is regionally pertinent. It would also be beneficial if the manufacturer could provide references to (regional) specialists (2 to 3) that could be contacted.

JPVA does not consider these new or rare indications for parasites of cats and dogs to be a concern in Japan; however, they support to further consider this topic because the addition of "framework" for the effectiveness evaluation of new indication would shorten the duration from application by drug sponsors to the approval.

JMAFF supports the discussion of this topic by an EWG. They suggest the establishment of criteria for which parasites this framework may be applied (e.g. only those parasites for which eggs and/or worms can be accurately counted in the host species). JMAFF emphasized that the framework should not be excessively strict.

Conclusion: The TF supports addition of general points to consider when approaching new or rare indications. However, there remains significant discussion about the level of detail that can be provided.

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

From the CP: "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."

TF members generally agree that due to increasing concerns regarding animal welfare and animal use in research, it has become difficult to obtain dogs and cats naturally infected with intestinal parasites for use in terminal dose confirmation laboratory studies. Thus, using naturally infected dogs/cats for 1 of the 2 required dose confirmation endoparasite studies as recommended by the guidelines has become difficult for drug sponsors.

On the other hand, the use of induced infections may be difficult for some parasites, e.g. *Toxocara canis*, *Dipilidium caninum*, *Trichuris vulpis*[26]. Results of experimental models may also appear less valid compared to those obtained from naturally-infected animals. Using alternative methods in field trials, if feasible, would then be useful. In accordance with the European draft guideline on regulatory acceptance of 3 R (replacement, reduction, refinement), alternative methods for replacing animal studies is the ultimate goal. Nevertheless, alternative methods currently still need to be evaluated for their suitability and be scientifically validated. At this point in time, the use of endoscopic video capsules does not seem viable to provide inferential value based on published literature[27]. The use of Elisa methods used on fecal samples may be a consideration in the future for certain parasites such as *Trichuris vulpis* in dogs[28]. The use of non-terminal fecal nematode egg counts (pre- versus post-treatment) is a more viable option in induced experimental laboratory infections in purpose bred dogs/cats but their inferential value in client-owned dogs is questionable. IFAH noted that inferential value to the client-owned population can be obtained generally from field studies, which are typically conducted in conjunction with laboratory dose confirmation studies.

The VDD would support exploring other options for studying natural infections in dogs and cats in a non-terminal manner. VDD suggested only using induced infections in specifically bred animals for terminal studies and performing larger field studies/clinical trials that would follow the animals for a longer duration. IFAH agreed that replacement of terminal laboratory studies with field data was a good idea. However, longer duration under field conditions where re-infection cannot be excluded may complicate the determination of effectiveness, especially for parasites with short life cycles.

IFAH-Europe also supports efforts to explore possible alternatives to terminal studies. However, a very broad discussion (e.g. workshop outside VICH) and more data would be needed to further develop a proposal before it could be included into a guideline. Alternative methods, e.g. for faecal egg counts, faecal worm counts or even worm counts by endoscopy would need to be validated and evaluated for their suitability for the different worm species. Historical effectiveness data obtained with

[26] Jacobs DE et al. Vet Parasitol. 1994 Apr; 52(3-4):179-202. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guidelines for evaluating the efficacy of anthelmintics for dogs and cats.
[27] Lee, et al., 2013. Veterinary Parasitology 196, 538–540
[28] IDEXX Laboratories, 2014

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natural infections could be compared with data obtained with experimental infections and with field studies. IFAH-Europe proposed the WAAVP as a suitable forum of experts for scientific discussion to further develop a proposal.

JMAFF does not support the discussion of this topic in an EWG because such discussions may be premature due to the state of the science on the issue (e.g. questions about the performance and reliability of the fecal egg counts). JMAFF suggests that GLs should allow for sponsors to use other methods if they can provide an adequate justification.

Conclusion: All TF members agree that the concerns regarding naturally occurring infections presented in the CP are valid concerns. However, there are varying opinions on how these concerns could be addressed, and whether the VICH GL is the most appropriate place to address these concerns. An EWG should further consider this topic, especially as new diagnostic techniques continue to emerge.

iii. Fecal Egg Count Reduction Tests (FECRT) [GL12, GL13, GL14, and GL15]

From the CP: "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 effectiveness of a drug at the time of approval. Not all drugs have the same effectiveness 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."

IFAH-Europe does not support this proposal. We believe anthelmintic resistance and effectiveness investigation are different topics, and that AR should not be discussed within the framework of effectiveness claims, especially since it is not possible to acquire a claim of efficacy against resistant worms. Furthermore, IFAH-EU would like to highlight there is no scientific consensus at the moment to adequately identify resistance.

The AHI does not believe that revisions to the ruminant/equine referenced guidelines around the use of FECRT provides any inferential value since as frequently stated, fecal egg counts are already performed in treated animals both pre- and post-treatment. The monitoring of anthelmintic resistance should occur post-approval and currently falls under pharmacovigilance practices mandated by the various regulatory agencies.

FDA-CVM does not propose to evaluate or estimate levels of resistance pre-approval. Rather, FDA-CVM intends to establish a baseline against which future monitoring can be compared. FDA-CVM believes this baseline information is critical to end users of the products, and as the data is already collected in current field studies, there is no significant burden to the industry.

The EMA-CVMP strongly recommends monitoring resistance in the EU. Applicants already have to address the resistance of the product in the authorization file (part IV). Nevertheless, the use of FECRT data (using novel techniques) from clinical studies would be additional useful information to monitor the resistance and evaluate the effectiveness of the veterinary product. It could also be a valuable baseline for further evaluation of resistance if not yet prevalent. The provision to this approach is, we agree, the development of the FECRT method.

The VDD supports the use of FECRT as supportive evidence for effectiveness in field trial situations, and would support the inclusion of coproculture with susceptibility testing if appropriate. For certain livestock parasites, coproculture and susceptibility testing are available at least in North America (US - Dr. Ray Kaplan's lab) and could provide useful supportive data.

JMAFF does not support discussing the issue in an EWG because the criteria and methods to evaluate antiparasitic resistance are not internationally standardized at the present time, the relationship between resistance and effectiveness is not clear, and there is no consensus on the proper use of the FECRT.

Conclusion: The TF remains divided as to whether the VICH GL should address FECRT. Further discussion by an EWG is warranted.

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

From the CP: "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."

FDA-CVM further clarified that the reason adding females (and sometimes L4s) to the male counts has been proposed in the past is that for some species and types of studies, sponsors have thought that they may have difficulty getting adequate numbers in the control group animals if only males are counted. Therefore, some sponsors have proposed defining the total worm count for the calculation of effectiveness as the sum of the adult males, adult females (proportioned to the various species by location or based on male proportions), +/- L4's (proportioned in the same way as the females). The use of L4s in the total count has been proposed in some induced nematode infection studies with the justification that if L4s are found at necropsy, they should be considered "inhibited" because the pre-patent period had been exceeded.

There are two primary arguments from some TF members against addition of specific recommendations for parasite counts to the VICH GL. The first argument is that for the assessment of effectiveness, the sex ratio is not likely of significant importance. According to literature the susceptibility of both males and females appears to be almost equal[29,30]. Even if there is a minor variation of the sex ratio, depending on the host's age and nematode intensity, the potential impact on worm counts is considered to be minor.

The other argument against adding parasite counting recommendations is that such information can be addressed in other documents, specifically the WAAVP guidelines that are updated at regular intervals by internationally-recognized parasitologists.

EMA noted that they normally receive dose determination/confirmation studies including experimental infection studies with defined species and strains which provide an accurate calculation of effectiveness based on worm counts for each claimed species. Currently, there is no sex speciation for worm counts. An alternative approach to the use of male adults for the identification of the worm species could be to determine the proportion of worm species by using molecular

[29] T.A. Yazwinski et al.: Dose confirmation of moxidectin pour-on against natural nematode infections in lactating dairy cows. Veterinary Parasitology 86 (1999) 223–228
[30] J.C. Williams et al.: Comparative efficacy of ivermectin pour-on,albendazole, oxfendazole and fenbendazole against Ostertagia ostertagi inhibited larvae, other gastrointestinal nematodes and lungworm of cattle. Veterinary Parasitology 73 (1997) 73-82

biological methods like species-specific PCR. However, it is not known yet whether there are standard methods available. Irrespective of the above, where specification is not possible it appears acceptable and reasonable to accept a more general claim, e.g, *Ostertagia* spp.

Also, in the EMA, L4 larvae are not considered in a study for effectiveness testing against adult worms. In principle, L4 stages are considered as separate claims. A separate L4 claim is normally accepted based on artificial infection studies in the target species with defined prepatent periods.

The VDD agreed that the counting and speciation of parasites be re-evaluated to ensure that accurate and representative counts are being provided. The VDD suggested that reporting could include the female and L4 counts as separate numbers as well as the combined calculations with a reference to the method in which the numbers were achieved (ie direct counting or using a ratio).

JMAFF considers this discussion premature and does not support the discussion of this topic in the EWG because the availability of counting methods to discriminate male, female, and L4 larvae for the parasites and the meaning of the discriminated counts is unclear.

Conclusion: The majority of TF members do not agree that revision of this point would add significant value to the Anthelmintic GL. An EWG could consider revisiting the topic if those members deem necessary.