#229

Guidance for Industry

Evaluating the Effectiveness of New Animal Drugs for the Reduction of Pathogenic Shiga Toxin-Producing *E. coli* in Cattle

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Additional copies of this guidance document may be requested from the Policy and Regulations Staff (HFV-6), Center for Veterinary Medicine, Food and Drug Administration, 7519 Standish Place, Rockville, MD 20855, and may be viewed on the Internet at either http://www.fda.gov/AnimalVeterinary/default.htm or http://www.regulations.gov.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Veterinary Medicine
October 2015

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

I. INTRODUCTION

This guidance provides recommendations to industry relating to study design and describes criteria that the Center for Veterinary Medicine (CVM) thinks are the most appropriate for the evaluation of the effectiveness of new animal drugs that are intended to reduce pathogenic Shiga toxin-producing *Escherichia coli* (STEC) in cattle.

Section II discusses general considerations regarding the development of protocols, study conduct, animal welfare, substantial evidence of effectiveness, experimental parameters, nutritional content of experimental diets, and the assessment of drug concentrations in experimental diets.

Section III discusses the studies and analyses CVM recommends for sponsors to substantiate the effectiveness of pathogenic STEC reduction drugs.

The guidance is not a comprehensive source of information on conducting clinical effectiveness studies. Alternative study designs for providing substantial evidence of effectiveness may be acceptable. Sponsors should contact CVM to discuss their development plan prior to initiating any studies. Sponsors and clinical investigators should consult the Code of Federal Regulations (21 CFR Parts 511 and 514) for information on the proper shipment, use, and disposition of investigational new animal drugs, as well as submission of the results of clinical investigations.

This guidance does not address the evaluation of human food safety with respect to microbial food safety and/or concerns related to antimicrobial resistance. CVM encourages sponsors to discuss any related concerns in their project plan with CVM as early as possible in the development process.

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

II. GENERAL CONSIDERATIONS

A. Protocol Development

A protocol should be developed to specifically describe the plan for conducting an effectiveness study. The protocol must include a clear statement of the study objective(s), state the hypothesis, describe the experimental design in detail, and include success, entrance, and exclusion criteria (21 CFR §§ 514.117(b)(2), (4)-(6)). A protocol should be based upon sound scientific principles and procedures. The characteristics of an adequate and well-controlled study are described in 21 CFR § 514.117. Sponsors should follow the format for writing protocols that is recommended in CVM Guidance for Industry No. 85: Good Clinical Practices: VICH GL9, Final Guidance, 05/09/01, section 6 (http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM052417.pdf).

CVM recommends that sponsors submit protocols for review and concurrence before beginning essential studies.[1] CVM's concurrence with a protocol represents a fundamental agreement between CVM and the sponsor that we agree with the design, execution, and analyses proposed in the protocol. CVM concurrence represents a commitment that we will not later alter our perspectives on these issues unless public or animal health concerns appear that we did not recognize at the time of the protocol assessment. Because this concurrence does not extend to any subsequent changes a sponsor may make to the protocol, sponsors may want to seek concurrence on the revised protocol if they make changes. Protocol concurrence does not guarantee that the results of the study will support a particular finding or approval of the new animal drug.[2]

Sponsors may choose to submit either a master or a site-specific protocol. Sponsors should identify which type of protocol they are submitting. A master protocol provides general information on principles that apply to all study sites. Master protocols provide any clinical investigator the details to conduct the entire study, including, but not limited to the following: test and control article specifications, blocking and randomization schemes, a description of the animal model, inclusion/exclusion criteria, variables of interest, statistical analysis, treatment groups, schedule, and success criteria. Additionally, a master protocol may be used as the basis from which more detailed, site-specific protocols are developed and written.

A site-specific protocol should contain information present in a master protocol plus any detailed information pertaining to the study site, including, but not limited to the following: location of the study/studies, personnel involved, diet(s) to be fed, detailed facility diagrams showing pen locations, location of feeders and waters, environmental conditions, and standard operating procedures.

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[1] See Guidance for Industry #215: Target Animal Safety and Effectiveness Protocol Development and Submission, Final Guidance.

[2] Animal Drug User Fee Act Performance Goals and Procedures - http://www.fda.gov/ForIndustry/UserFees/AnimalDrugUserFeeActADUFA/ucm042936.htm.

B. Animal Welfare Considerations

All studies using live animals that are conducted in the United States must conform to the requirements of the Animal Welfare Act (AWA), which is administered by the United States Department of Agriculture (USDA). The USDA has issued policies and regulations on how to comply with the requirements of the AWA. In addition, many research institutions that conduct research studies using live animals are also accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

Farm animals used for biomedical research (such as drug studies) fall under the purview of the AWA and USDA regulations. Two publications: the "Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching," published by the Federation of Animal Science Societies (FASS), and the "Guide for the Care and Use of Laboratory Animals," published by the Institute for Laboratory Animal Research (ILAR), may assist researchers in the implementation of the USDA regulations. These guides provide information on the appropriate handling, housing, care, treatment, and transportation of farm animals for nonagricultural purposes and should be referenced when designing studies to demonstrate the effectiveness of pathogenic STEC reduction drugs in cattle.

C. Substantial Evidence of Effectiveness

Effectiveness must be demonstrated by substantial evidence consisting of one or more adequate and well-controlled studies [21 U.S.C. §§ 360b(d)(1)(E) and (d)(3); 21 CFR § 514.1(b)(8)(ii); 21 CFR § 514.4(a); and 21 CFR § 514.117].

CVM recognizes that non-specific drugs may have a similar effect on pathogenic STECs other than O157:H7, but only the major serotypes of concern to human health would be currently considered for the indication (specifically, O26, O45, O102, O111, O121, and O145). You should first demonstrate the effectiveness of a drug against *E. coli* O157:H7 if you wish to include any or all of these additional serotypes. CVM does not expect the criteria for including non-O157 serotypes to be as rigorous as for *E. coli* O157:H7. Therefore, although a natural infection field study should be conducted for *E. coli* O157:H7, challenge model studies may be acceptable to demonstrate effectiveness for other pathogenic STECs.

CVM recommends a multi-location field study to establish the effectiveness of pathogenic STEC reduction drugs in cattle. A multi-location field study should be conducted at the appropriate number of sites to ensure that the two principle objectives for the demonstration of effectiveness, independent substantiation and inferential value, are met. Power calculations can help determine the number of experimental units per site. You should conduct the study using at least two different investigators for independent substantiation. You should specifically state in the protocol the number of study sites to be used.

D. Experimental Parameters

1. Study Animals

   Study cattle should be representative of those in US commercial production in terms of class and weight prior to shipment to slaughter.

   You should enroll only healthy cattle in pivotal field studies. Eligible groups of cattle may be screened for the presence of *E. coli* O157:H7 to ensure a useful prevalence in the group prior to enrollment, but should reflect prevalences that are observed among naturally-infected animals. Enrolling only *E. coli* O157:H7-positive cattle does not provide adequate inferential value in a field study.

   You should state in the protocol the criteria to be used to exclude animals or groups of animals from enrollment during the study as well as under what circumstances that would cause a site, pen, or animal to be removed after the study has started.

2. Experimental Unit

   The experimental unit should be the level which is evaluated as the primary variable or variable (typically a pen or group). Typically, this is determined by the smallest unit to which the drug is intended to be administered. In studies of pathogenic STEC reduction drugs that are administered to multiple animals in or on feed or in water, the experimental unit is the pen or group and not the individual animal.

3. Experimental Group

   CVM recommends that the experimental groups include the "treated" group(s) receiving the drug at the proposed label dose(s) for the proposed frequency and duration and a "control" group. CVM recommends that you use a placebo concurrent control (referred to as "control" in the remainder of this document) so that the control group cattle receive the same handling and procedures as the treated group cattle. Using a placebo may also help maintain the masking of the study cattle to their experimental group assignment.

E. Randomization

Randomization in a study design with multiple levels of organization usually includes at least two recommended steps. Using the pen as the experimental unit, the first step should be to randomly assign cattle to pens. The second step should be to randomly assign each pen to an experimental treatment group.

Blocking may be used to maintain balance across treatment groups. Blocking is recommended as a means to reduce variation associated with variables other than the experimental treatment within a block so that the overall unexplained variation will be reduced by accounting for variation among the blocks.

If multiple arrival groups or "lots" of cattle are to be enrolled at a given site, care should be taken to randomize cattle so that the cattle within each lot are distributed in a balanced manner across treatment groups in order to avoid any bias offered by a given lot. As an example, pens can be filled with a certain number of cattle from each lot with the number proportional to the size of each lot and then assigning the individual pens to a treatment group.

The protocol should provide the following information concerning the process of randomization:

- Identification of all steps to be used in the process of randomization;
- A description of the method(s) to be used to generate random assignments;
- A description of the relationship between the experimental unit, the process of randomization, and other organizational levels of the study design; and,
- Identification of any blocking variables, restrictions to randomization, and other variables that are part of the randomization process.

F. Masking

In order to minimize bias, you should mask all personnel responsible for day-to-day management of the animals, including those making and recording observations. You must describe masking procedures in the protocol and final study report (21 CFR § 514.117(b)(7)). Masking is an important design technique for avoiding bias in clinical trials (see CVM Guidance for Industry (GFI) #85: Good Clinical Practices: VICH GL9, Final Guidance, 05/09/01).

G. Personnel Training and Experience

As specified in 21 CFR § 511.1(b)(7)(i), all personnel involved with the investigation must have adequate scientific training and experience with the target animal species and disease models used in the studies.

H. Record Keeping

Good record keeping is a critical component in determining the effectiveness of a drug when it is being assessed. The integrity and accuracy of the data collected are critical to the acceptance of a study as substantial evidence of effectiveness. CVM's recommended standard for record keeping and data management is contained in CVM GFI #85: Good Clinical Practices: VICH GL9, Final Guidance, 05/09/01.

I. Nutritional Content of Diets

You should feed animals nutritionally-adequate diets so that observed responses can be attributed to the drug, rather than a possible nutritional effect. You should formulate diets to meet predominant commercial practices for the species and class of animal being fed, and for the geographic region where you conduct the study. You may use agricultural survey data, e.g., Agrimetrics, Agri-Tech, etc., to support information on predominant commercial practices.

Nutrient recommendations for different food-producing animals, published by the National Research Council (NRC), may serve as a reference for formulating diets. You should specify feed additives, such as antioxidants, pellet binders, copper sulfate, etc., in the diet formulation and ensure that any feed additives do not confound the effects of the drug. You should state in the protocol the standard to which the nutrient levels are being compared (e.g., NRC or literature).

To ensure that experimental animals receive proper nutrient densities in the diet, you should conduct proximate and chemical analyses on a composite sample from the uniform basal diet. You should include a description of the analyses that you intend to conduct in your protocol and specify the number of assay replicates. You should indicate whether the analyses are reported on an as-fed or dry-matter basis.

You should divide each batch equally among all treatment groups. When a batch of feed runs out for one or more treatment groups, you should discontinue feeding that batch of feed to all remaining treatment groups and begin feeding the new batch of feed to all treatment groups at the same time. Feed from the previous batch should be weighed and properly accounted for in the final study report.

J. Drug Assays

The purpose of conducting drug assays is to verify that the drug mixed in or on feed or constituted in water and used in the study is present at the appropriate concentration.

For drugs administered in feed, personnel responsible for mixing feed should be aware of the performance and capabilities of the feed mixer(s) that will be used to prepare experimental diets. Medicated feeds should be mixed according to 21 CFR Part 225 - *Current Good Manufacturing Practice for Medicated Feeds*, to ensure adequate homogeneity. All standard operating procedures used for feed mixing should be consistent with applicable GMP regulations for the manufacture of medicated feeds. The protocol should state that the medicated feed will be mixed at a commercial feed mill and shipped to the study site, or that the medicated feed will be mixed using a validated mixer. The final report should include all relevant records.

1. Assay Limits for Drugs in Feed

Assay limits for approved drugs in feeds are codified in 21 CFR § 558.4. Concentrations of approved drugs in experimental feeds should fall within these assay limits regardless of where and when a feed sample is collected.

For investigational new animal drugs for which assay limits have not been codified, levels should conform to the investigational assay limits derived through the feed assay method transfer studies. If the assay limits are not established before conducting studies, the assay method used to determine drug concentration(s) should be no more variable than the method that will be subjected to the method validation process.

Note that a permissible analytical variation (PAV) relates to a single application of the assay method. Under the proper mixing conditions using the correct quantity of the drug, a single assay of a feed sample should fall within the assay limits. If a drug assay(s) falls outside of the assay limits, the feed should not be used in the study and the reason for the out-of-assay-limit result(s) should be investigated and discussed in your final study report.

2. Sampling for Drug Assays

You should propose a feed sampling method for the study drug assay in your protocol. The method used should provide for a representative sample for the drug assay.

The most relevant sampling point is where and when the test article is offered to the animals, with the representative composite samples collected when the product is presented to the animals. For feed-based drugs, you may collect samples for the assessment of the test article from the batch prepared at a feed mill.

In addition, you should assay every batch of feed (medicated and control feed) or water for the presence of the last non-study drug used in the feed or water delivery system unless all of the equipment used in the study is dedicated and isolated to avoid cross-contamination. If dedicated equipment is used, you should test for the last run drug at the beginning of the study. Samples for this purpose should be collected from the point that the product is provided to the animals.

K. Combination Approvals

The Animal Drug Availability Act of 1996 (ADAA) amended the Federal Food, Drug, and Cosmetic Act (the act) and changed the requirements for the approval of certain combinations of new animal drugs that have been previously separately approved. There are important criteria to consider before a sponsor submits a combination new animal drug application. For example, under section 512(d)(4) of the act, a sponsor seeking approval of a combination of two or more previously approved new animal drugs may not need to conduct additional effectiveness studies, if each new animal drug in the combination has at least one unique non-overlapping indication. However, among other things, all of the following criteria must be met: the new animal drugs must provide for appropriate concurrent use, must be physically compatible, and not have disparate dosing regimens (21 U.S.C. §§ 360b(d)(4)(C) & (D)). If you intend to submit a combination new animal drug application, we recommend you contact CVM for specific requirements.

III. RECOMMENDED STUDY DESIGNS AND ANALYSES

Drugs that are intended to reduce the exposure risk of consumers to pathogenic STECs are expected to predominantly exert their effect on finishing cattle by reducing the shedding or colonization of the rectal mucosal surface of asymptomatically-affected cattle by pathogenic STECs. Drug intervention in the live animal therefore represents a pre-harvest intervention that should be used in concert with other interventions to reduce the exposure of beef carcasses to fecal contamination by these pathogens.

CVM limits indications for pathogenic STEC reduction drugs to the effect of the drug in live cattle prior to shipment to slaughter, and also considers the duration of the drug's effect to allow for transit time to slaughter. For this reason, CVM will evaluate study designs to support the following suggested indications for the reduction in the prevalence or quantity of pathogenic STECs in feces, with *E. coli* O157:H7 as the principal target:

> "For the reduction in fecal pen prevalence of *Escherichia coli* O157:H7 among feedlot cattle prior to shipment to slaughter"
>
> "For the reduction in fecal pen quantity of *Escherichia coli* O157:H7 among feedlot cattle prior to shipment to slaughter"
>
> "For the reduction in fecal pen prevalence and fecal pen quantity of *Escherichia coli* O157:H7 among feedlot cattle prior to shipment to slaughter"

You should clearly state the intended targeted indication(s) in the protocol.

A. Variables for Evaluation of Effectiveness

Pathogenic STECs contaminate beef carcasses primarily due to exposure to fecal matter during the removal of an animal's hide. The sampling variability and uncontrollable environmental factors commonly encountered in a field study precludes the use of hide samples as evidence of effectiveness. Because the ultimate source of hide contamination is feces and because significant fecal cross-contamination of hides occurs during transit of cattle, fecal samples (fecal grab samples from the rectum) should be collected from live cattle prior to shipment to evaluate the effectiveness of pathogenic STEC reduction drugs.

You should collect fecal samples from all animals at several time points during the study and used to calculate results for groups or pens of cattle. You should collect at least one sample prior to treatment with the drug and at least two post-treatment samples for the pivotal analysis of the below variables.

Because the contamination of beef carcasses by strains of *E. coli* O157:H7 appears to be more closely associated with the contamination of hides during lairage at the slaughter facility than a source feedlot, any approvable product should demonstrate effectiveness for at least the greater of the pre-slaughter withdrawal period or 24 hours. An ideal live animal intervention would be one that demonstrates a period of effectiveness for roughly 9 to 14 days prior to shipment to slaughter in order to provide time for any fecal reduction in *E. coli* O157:H7 to be mirrored by a reduction on the hides of treated cattle.

1. Fecal Pen Prevalence

   You should measure the prevalence of *E. coli* O157:H7 among all animals within a pen using an appropriate and sensitive culture technique at each sampling time point. You should confirm suspect colonies as *E. coli* O157:H7 using confirmatory genetic or immunologic methods.

   For studies that seek a fecal pen prevalence reduction indication, these data represent the primary response variable. For studies that seek a fecal pen quantity reduction indication, these data will be used to evaluate whether the drug may unintentionally increase the fecal pen prevalence of *E. coli* O157:H7 despite any apparent reduction in the concentration of the pathogen.

2. Fecal Pen Quantity

   You should collect and quantify the fecal concentration of *E. coli* O157:H7 from each animal in the field studies at each sampling time point. You should confirm suspect colonies as *E. coli* O157:H7 using confirmatory genetic or immunologic methods in a manner that will allow CVM to assess the concentration of the specific pathogen in feces, expressed in colony-forming units (CFU) per gram of dry feces.

For studies that seek a fecal pen quantity reduction indication, these data represent the primary response variable. For studies that seek a fecal pen prevalence reduction indication, these data will be used to evaluate whether the drug may unintentionally increase the fecal pen quantity of *E. coli* O157:H7 pathogen in feces despite any apparent reduction in the prevalence of the pathogen in feces.

3. Screen for Induced Resistance

If it is possible that the pathogenic STEC reduction drug may engender resistance in *E. coli* O157:H7 to the drug and thus limit its effectiveness over time, you should assess this potential within the field study. CVM recommends that you use a selective medium that has a concentration of drug that will allow you to more sensitively detect a change in susceptibility to the drug among *E. coli* O157:H7.

B. Statistical Methods

The final report should contain:

- Raw data in its original form;
- Raw data in an electronic format (Excel, SAS or text file);
- Program code to read raw data and create calculated variables;
- Statistical programs with documentation; and
- All statistical output (e.g., analysis results).

You should also provide a document identifying the purpose or content of each file and a document that describes variable names, abbreviations, formats, and how variables are used in the analysis.

C. Basis for Study Conclusions

In order to conclude that the drug is effective for a reduction in fecal pen prevalence indication, the study should meet the following success criteria:

1. A statistically significant (α=0.05, two-sided) difference in fecal pen prevalence of *E. coli* O157:H7 between the treated and control group with a lower prevalence in the treated group to establish a post-treatment effectiveness period of at least 24 hours in duration throughout a minimum of two sampling time points within this period; and,

2. No numerically higher pen average fecal concentration of *E. coli* O157:H7 in treated group pens compared to control group pens throughout the above-defined effectiveness period at each site.

In order to conclude that the drug is effective for a reduction in fecal pen quantity indication, the study should meet the following success criteria:

1. A statistically significant (α=0.05, two-sided) reduction in the fecal pen average concentration of *E. coli* O157:H7 in the treated group compared to the control group to establish a post-treatment effectiveness period of at least 24 hours in duration throughout a minimum of two sampling time points within this period; and,

2. No numerically higher fecal pen prevalence of *E. coli* O157:H7 in treated group pens compared to control group pens throughout the above-defined effectiveness period at each site.

For both indications, the effect of the drug treatment should be biologically-meaningful. You should propose and provide information to support the biological relevance of the observed effect, which may include such things as the similarity to seasonal effects (e.g., prevalence of pathogens at winter levels), Hazard Analysis and Critical Control Point (HACCP) interventions, or other similar relevant comparators.

In addition, for the evaluation of effectiveness, there should be no scientifically significant increase in resistance to the drug among *E. coli* O157:H7 isolated at the post-treatment time points in the treated group compared with those isolated prior to treatment.

Other reasonable scientific approaches will be considered.