Strategies for evaluating ectoparasiticides in companion animals

by

Cameron James Sutherland

B.S., Oklahoma State University, 2017
D.V.M., Kansas State University, 2021

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Diagnostic Medicine/Pathobiology
College of Veterinary Medicine

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2025

Abstract

Ectoparasites including fleas, flies, lice, mites, and ticks are targets of past, present, and future veterinary concern. Controlling ectoparasites is critical to mitigate irritation, potential allergies, and transmission of vector-borne diseases. This dissertation focuses on the process evaluating ectoparasiticides before and during market release with an emphasis on the isoxazoline drug class. Chapter 2 focuses on an *in vitro* strategy for ectoparasite rearing and potential use for parasiticide testing. The proposed model utilizes materials that are accessible for small-scale research groups such as universities, industries, and contract research organizations. This model is a 3D-printed artificial flea feeding system that showed success rearing multiple generations of *Ctenocephalides felis* fleas. The ability to rear fleas using this model would make *in vitro* ectoparasiticide testing more accessible to researchers regardless of budget or scale. Chapter 3 focuses on the evaluation of fluralaner oral and topical canine formulations in an off-label use-case by administering it to horses, an animal with no published pharmacokinetic data from the isoxazoline drug class. Ectoparasite control in horses is of great veterinary interest as they can be responsible for pruritus, discomfort, and/or vector-borne disease transmission. This study demonstrated that fluralaner reached a higher plasma concentration when administered orally but had a longer half-life when administered topically. This provides a starting point for finding an appropriate dose and route for developing a highly effective, long-lasting ectoparasite product for horses. Chapter 4 focuses on a post-approval marketing field study administering an oral formulation of lotilaner to animals in homes experiencing flea infestations. This study showed the ability to eliminate environmental flea infestations by focusing treatment efforts on the animals living within the house rather than broad, premises sprays. The contents of this

dissertation endeavor to elucidate novel strategies for ectoparasiticide testing while also highlighting the importance of data generated from field studies.

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Approved by:

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Ectoparasites including fleas, flies, lice, mites, and ticks are targets of past, present, and future veterinary concern. Controlling ectoparasites is critical to mitigate irritation, potential allergies, and transmission of vector-borne diseases. This dissertation focuses on the process evaluating ectoparasiticides before and during market release with an emphasis on the isoxazoline drug class. Chapter 2 focuses on an *in vitro* strategy for ectoparasite rearing and potential use for parasiticide testing. The proposed model utilizes materials that are accessible for small-scale research groups such as universities, industries, and contract research organizations. This model is a 3D-printed artificial flea feeding system that showed success rearing multiple generations of *Ctenocephalides felis* fleas. The ability to rear fleas using this model would make *in vitro* ectoparasiticide testing more accessible to researchers regardless of budget or scale. Chapter 3 focuses on the evaluation of fluralaner oral and topical canine formulations in an off-label use-case by administering it to horses, an animal with no published pharmacokinetic data from the isoxazoline drug class. Ectoparasite control in horses is of great veterinary interest as they can be responsible for pruritus, discomfort, and/or vector-borne disease transmission. This study demonstrated that fluralaner reached a higher plasma concentration when administered orally but had a longer half-life when administered topically. This provides a starting point for finding an appropriate dose and route for developing a highly effective, long-lasting ectoparasite product for horses. Chapter 4 focuses on a post-approval marketing field study administering an oral formulation of lotilaner to animals in homes experiencing flea infestations. This study showed the ability to eliminate environmental flea infestations by focusing treatment efforts on the animals living within the house rather than broad, premises sprays. The contents of this

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Dedication

To Cheyenne, my childhood dog that showed me the importance of the human-animal bond leading me to become a veterinarian

To my current pets, Checkers, Puka, Kona, and Rivia – thank you for being daily reminders for why I pursued veterinary medicine

Chapter 1 - Ectoparasiticide Testing Literature Review

History of Parasiticides

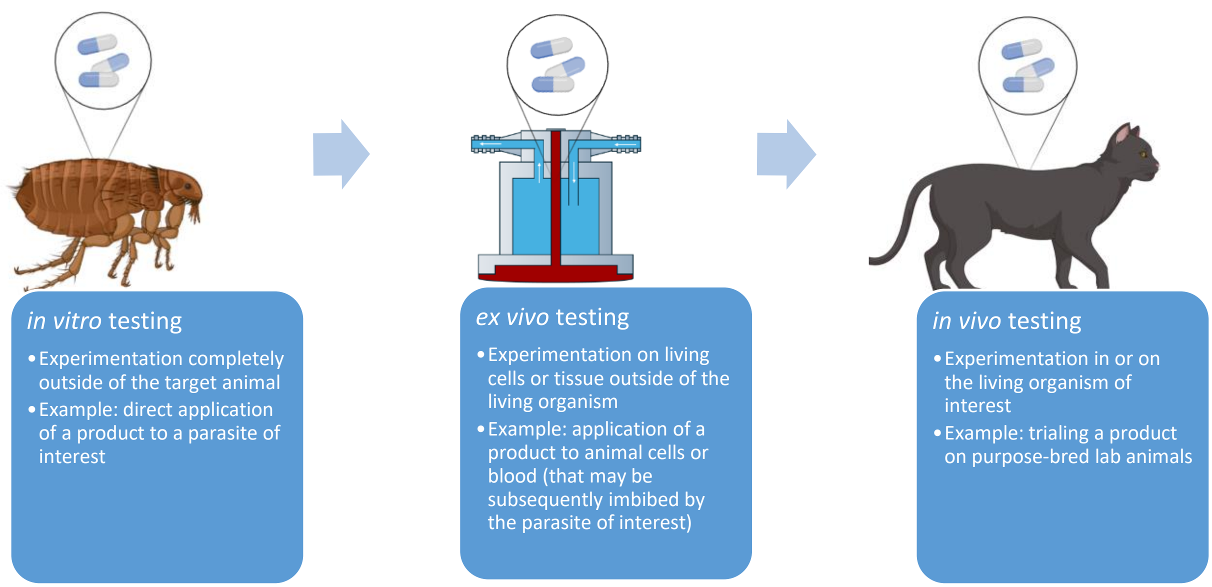
Parasitism refers to the symbiotic relationship between an organism, the parasite, that resides within or on a host that suffers from this relationship. Parasites have demonstrated a history of evolving with their respective hosts through a series of adaptations and reciprocal adaptations (Buckingham & Ashby, 2022). In the wake of this, humans have mitigated parasites through the development of parasiticides to be applied to themselves, their animals, their living spaces, and their crops. This dates to the Sumerians of Southern Mesopotamia who fumigated with juniper powder in locations where locusts laid eggs to protect their crops (Nazari 2023). There are multiple accounts of early parasiticide usage in animals where there is not a singular event denoted as the "first usage of parasiticides in animals." Early accounts of parasite control involve the use of natural substances. This was until the World War II era when synthetic insecticide use began with parasiticides such as Benzene Hydrochloride (BHC) and Dichlorodiphenyltrichloroethane (DDT). DDT, in particular, was made available for agricultural use in 1945. These parasiticides were followed by other drug classes including organophosphates, carbamates, neonicotinoids and more which led to the modern era of parasite control (SPEX CertiPrep Group, 2016). The objectives of parasite control in the present day include parasiticidal, repellency, and mitigation of vector-borne disease transmission. In the present day, pharmaceutical companies routinely perform the process of parasiticide development beginning with an idea (target compound, target receptor, target species, and/or target host) and ending with the product being sold commercially for use on or in animals. This is a multi-step process involving conceptualization of an idea based on veterinary need, identification of a novel compound to meet this need, screening novel compounds against target parasites, development of a suitable formulation and dosage for target animal delivery, assessment of safety and efficacy in the target animal, seeking regulatory approval, and eventually marketing a new drug. This dissertation will focus on testing conducted under the assumption that a novel molecule of interest has already been identified through this drug screening process.

Testing Parasiticides

Parasiticides, amongst other drugs, undergo numerous rounds of testing before administration to the host species of interest. The testing progression leading to trialing a drug in a host species is typically *in vitro* testing followed by *ex vivo* testing followed by *in vivo* testing (Figure 1.1).

In vitro testing consists of experimentation outside of a living organism. In ectoparasiticide testing, this includes direct application of a parasiticide to an arthropod or exposure of an arthropod to a parasiticide via filter paper or other substrate. *Ex vivo* testing consists of experimentation on living tissues or cells outside of a living organism. This would include application of an ectoparasiticide to blood or a tissue culture that arthropods are subsequently introduced to. Researchers will sometimes use *in vitro* and *ex vivo* terminology interchangeably depending on the nature of the experiment (ex: blood testing may be considered *in vitro* or *ex vivo* experimentation). Exposure to treated hair may fall under *in vitro* experimentation if the hair shaft is dead, but *ex vivo* experimentation if the live hair shaft is incorporated into the testing. *In vivo* testing consists of experimentation in/on the living organism of interest. In ectoparasiticide studies, this is typically performed by applying the parasiticide of interest to purpose-bred laboratory animals to evaluate product efficacy against a specific arthropod species. The *in vivo* testing is typically performed last as this step is the most time and resource intensive. In addition, it is the responsibility of the investigators to pursue the "5 Rs" of animal research – Replacement, Reduction, Refinement, Reuse, and Rehabilitation (Kumar 2012). *In vitro* and *ex vivo* testing allows for Replacement and Reduction of animals in research.

Figure 1.1: Progression of product testing from *in vitro* to *in vivo* (created with BioRender.com)



In vitro Testing of Parasiticides

In vitro testing, which translates to "in glass" due to the historical context of experiments taking place in a test tube or other glassware, refers to experimentation in a controlled environment completely outside of a living organism. This often means that *in vitro* experimentation is taking place outside of a parasite's natural environment (on or within a host), and results must be interpreted with caution. In the context of parasiticide testing, however, there are advantages to *in vitro* experimentation. *In vitro* parasiticide testing generates results that are species-specific to the parasite without the influence of the host's physiology or immune system. In addition, the *in vitro* environment is vastly simplified compared to a host organism which permits the control of multiple variables including temperature, humidity, substrate, and compound dose. This results in improved convenience for the tester and rapid generation of massive quantities of data (Marchiondo et al. 2019). The strategies described above reduce the use of animals in research and provide important information before proceeding to *in vivo* testing.

An example of *in vitro* testing in the context of ectoparasites is a contact assay which is either the direct application of an experimental compound directly onto the parasite of interest or the immersion of the parasite into an ectoparasiticide solution. This is commonly performed in fleas (most commonly *Ctenocephalides felis*) and ticks early in drug discovery for small animal ectoparasiticide products. In one study, larval, nymphal, and adult *Rhipicephalus sanguineus* ticks were immersed in fluralaner of varying concentrations to determine the lethal concentrations of fluralaner (Williams et al., 2015). The fluralaner concentrations with a 90% killing effect (LC_{50}) were 2.4 µg/mL, 2.6 µg/mL, and 1973 µg/mL for larvae, nymphs, and adults, respectively (Williams et al., 2015). This study concluded that all life stages of *Rhipicephalus sanguineus* show *in vitro* susceptibility to fluralaner and warrants further investigation. This type of pilot study informs the investigator of several things, including behavior of the ectoparasite in response to the compound and potentially the adulticidal or ovicidal dose. A similar contact assay for *in vitro* testing evaluated the efficacy of varying concentrations of selamectin, fipronil, or imidacloprid against *Ctenocephalides felis* flea larvae (McTier et al., 2003). This study observed a ≥ 93.5% reduction in live larvae 24 hours after exposure to selamectin, but neither the fipronil nor imidacloprid groups reached a 90% reduction in live larvae (McTier et al., 2003). Contact assays such as those described above provide researchers with pilot data on ectoparasiticide efficacy without incorporating any live animals into the study.

Another example of *in vitro* testing in the context of ectoparasites is the impregnation of filter paper sheets with an experimental compound. In one study, *Haemaphysalis longicornis* adult, nymphal, and larval ticks were placed between two pieces of filter paper impregnated with either an imidacloprid/permethrin formulation or a fipronil/(S)-methoprene formulation at different concentrations (Hagimori et al., 2005). Tick behavior was monitored for five minutes via video recording for all life stages, products, and concentrations. A greater avoidance-behavior was observed in the imidacloprid/permethrin group compared to the fipronil/(S)-methoprene group, while both products showed the potential for repellency effects. This sort of experimentation potentially simulates the behavior of ticks towards treated animal hair as the use of dead hair shafts would fall under *in vitro* experimentation (Hagimori et al., 2005). A similar study exposed *Rhipicephalus microplus* larvae to rectangular filter papers containing DDT, dieldrin, coumaphos, or xylene solutions (Stone and Haydock, 1962). This study was used to assess *Rhipicephalus microplus* susceptibility to the ectoparasiticides described. Although this type of *in vitro* experimentation is beneficial for evaluating the effects of environmental exposure of an ectoparasite to compound, it is limited in that the filter paper does not perfectly simulate a treated environment which may include hair, carpet, hard floor, or other surfacing.

In addition, this sort of experimentation completely removes the ectoparasite in question from its natural environment and as a result, the ectoparasite should be considered a dying organism even in the absence of an insecticide or acaracide. This may potentially lead to false-positive results during testing and necessitates the use of a control group. Furthermore, it is unclear whether the organism imbibes the compound during application or if it is the direct contact of the compound itself that elicits the effect (or components of both). Because of these limitations, *in vitro* studies on their own are not sufficient to provide enough data for registration with a federal agency such as the Food and Drug Administration (FDA).

Ex vivo Testing of Parasiticides

Ex vivo, meaning "out of the living/outside the normal living organism," testing utilizes components of a living organism that have been isolated from the original biological environment. This includes experimentation on cells, including blood, and tissue with minimal change to the environment compared to the original biological location. *Ex vivo* experimentation allows for the control of more variables compared to a complete organism (Marchiondo et al. 2019). These variables are numerous and may include temperature, humidity, source of living tissue, and direct drug dosage. In the context of ectoparasiticide testing, *ex vivo* testing may also provide useful toxicity information without concern for animal health. In a study by De Marchi et al. in 2025, the cytotoxic effects of fipronil and imidacloprid were examined on human epithelial cell cultures. Cells were exposed to imidacloprid at concentrations of 62.5 µg/mL, 125 µg/mL, and 250 µg/mL and fipronil at concentrations of 1.25 µg/mL, 2.5 µg/mL, and 5.0 µg/mL. Both the imidacloprid- and fipronil-exposed cells experienced the production of reactive oxygen species and lipid peroxidation in addition to decreased viability of human keratinocyte cells consistent with concentration-dependent cytotoxic effects (De Marchi et al., 2025). However, there are inevitably differences in *ex vivo* environments compared to the natural environment of a complete organism.

An example of *ex vivo* experimentation is the usage of an artificial membrane to feed fleas, ticks, or other arthropods. This sort of experimentation is considered *ex vivo* as the ectoparasites are exposed to blood which is considered living tissue outside of the organism. An example of this is found in Williams et al. from 2014 where *Ctenocephalides felis* fleas were fed on an artificial membrane imbibing blood spiked with fluralaner at sub-insecticidal concentrations between 0.09 ng/mL and 50.0 ng/mL. Measured parameters in this study included assessing flea survival, egg hatchability, oviposition, pupation, and flea emergence. Fleas exhibited a cessation of oviposition in fluralaner blood concentrations as low as 25.0 ng/mL and a larvicidal effect at fluralaner blood concentrations as low as 6.25 ng/mL (Williams et al., 2014). This *ex vivo* study exhibited the ability of fluralaner to control egg laying, larval development, and flea reproduction at sub-insecticidal concentrations. In the case of ectoparasiticide research, *ex vivo* testing is designed to simulate the ectoparasite ingesting a compound present in the blood of a treated animal without the involvement of a live animal. For some products and species, this may provide a simulation of how an ectoparasite is expected to be exposed to a compound in a live organism. Regardless, this type of experimentation, on its own or in combination with *in vitro* testing, is not sufficient for approval of a product under the Food and Drug Administration (FDA) but still provides useful pilot data during product development.

In vivo Testing of Parasiticides

In vivo testing is the administration of a compound in or on the animal in a formulation that is consistent for its use in a real-world setting, although not all compounds make it to a final marketed product. This is different from *ex vivo* testing in that it takes place using an entire living organism rather than living tissue that has been isolated from a living organism. The animal is treated, most commonly orally, topically, or parenterally, and the target parasite of the product is permitted to feed/ingest on the blood, flesh, or host secretions of the animal host

(Marchiondo et al. 2019). In the context of ectoparasiticide testing, *in vivo* experimentation evaluates how the parasiticide performs within the target animal and can include pharmacokinetic parameters that are used to describe absorption, distribution, metabolism, and excretion of a drug. This is demonstrated in Kilp et al. from 2014 where dogs were treated with oral fluralaner at 12.5 mg/kg, 25 mg/kg, or 50 mg/kg. In order to assess pharmacokinetic parameters such as clearance and volume of distribution, these dogs were compared to dogs from a separate study that were treated with intravenous fluralaner at a dose of 12.5 mg/kg as this type of comparison allows the investigators to assess relative oral bioavailability. This study concluded that fluralaner was readily absorbed following oral administration and has a long elimination half-life (Kilp et al., 2014). Furthermore, applying a drug to the target animal is also an opportunity to observe adverse events associated with drug application, if any.

However, although *in vivo* testing in ectoparasiticide research involves the direct application of the compound to the target animal, one animal or group of animals may not be 100% representative of every animal for whom the compound is intended. A guideline review article from Marchiondo et al. from 2007 points out potential factors in field studies that laboratory testing may fail to replicate including differences in breed, behavior, husbandry, environment, infestation pressure, and parasite strain. In the animal pharmaceutical space, this necessitates the pharmacovigilance teams of sponsors/drug companies to receive and report adverse events observed following the release of a product. Adverse events received by a sponsor's pharmacovigilance team are documented in the "Post Approval Experience" of the product label which is explained in greater detail later in this dissertation.

Furthermore, utilization of a live organism presents complex differences in genetics, anatomy, and physiology even amongst animals of the same species. In this case of application, the goal is to evaluate the ability of the drug to achieve the desired outcome (parasiticidal, ovicidal, larvicidal) while documenting and mitigating applicable adverse events. *In vivo* experimentation is critical for providing the robust data required by agencies in the regulatory approval process, especially for FDA-approved products.

Regulatory Approval Process

Once a novel compound has demonstrated efficacy with a potential formulation, pharmaceutical entities will seek regulatory approval from a United States federal agency. The agencies include the FDA (Food and Drug Administration), EPA (Environmental Protection Agency), and USDA (United States Department of Agriculture). The regulatory agency that is contacted depends on the nature of the compound in question. The FDA is the regulatory agency for compounds that meet the definition of a drug. “Drugs” are defined as “articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals” and “articles (other than food) intended to affect the structure or any function of the body of man or other animals” per 21 CFR. § 201.3(a). The EPA is the regulatory agency for compounds that meet the definition of a pesticide. A “pesticide” is “any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest; or intended for use as a plant regulator, defoliant or desiccant” as defined in the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). The USDA is the regulatory agency for compounds that meet the definition of a biological product. “Biological products” are defined as “vaccines and other products intended for use in animals, work primarily through stimulation of the immune system to prevent, treat, or diagnose diseases in animals” (9 CFR § 101.2). The regulatory agencies require different submission materials from organizations seeking commercial approval of a new product. In addition, the preparation and manufacturing processes differ by the nature of the product (drug vs. pesticide vs. biologic). The differences in preparation and manufacturing are significant to the regulatory process because there are standards for these steps established by the respective regulatory agency.

Food and Drug Administration (FDA) Regulatory Process

Within the Food and Drug Administration (FDA), the Center for Veterinary Medicine (CVM) approves and regulates animal drugs. For the approval of a "drug" within the FDA, the agency requires the submission of a New Animal Drug Application (NADA) to the Office of New Animal Drug Evaluation (ONADE) within the CVM. The NADA includes five major technical sections and three minor technical sections. The five major technical sections include: 1) Target Animal Safety, 2) Effectiveness, 3) Human Food Safety, 4) Chemistry, Manufacturing, and Controls (CMC), and 5) Environmental Impact. The three minor technical sections include: 1) All Further Information, 2) Labeling, and 3) Freedom of Information Summary. The FDA provides numerous (hundreds) of documents in the form of a Guidance For Industry (GFI) which provides drug companies/sponsors with the CVM's current opinions on a topic. These GFIs are not legally enforceable; however, following the recommendations within the GFIs increase the likelihood of the CVM's approval of an NADA. The approval of an NADA is a statement from the CVM that a drug is safe and effective when administered according to the target animal according to label directions.

Target Animal Safety

This technical section is intended to assess the safety of a product when used in the animal for which the product will be administered to. This technical section includes the Target Animal Safety (TAS) studies conducted by the drug company/sponsor usually on healthy, laboratory, purpose-bred animals in compliance with Good Laboratory Practice (GLP) and under the direction of the CVM's GFI #185 (also known as VICH GL 43). The goals of the Target Animal Safety studies are to identify potential adverse events as well as to establish a margin of safety (MOS) for the drug. The most significant Target Animal Safety study is the pivotal margin of safety study. This study is performed by administering the drug at higher doses than the product label. This most commonly occurs by administering the drug at 0x (negative control), 1x (highest dose on the product label), then two multiples above the labeled dose, usually 3x and 5x the labeled dose. In addition, per GFI #185, all groups in the margin of safety study are recommended to be treated at three times the proposed dosing duration up to a maximum of 90 days depending on related pharmacologic and toxicologic literature. The purpose of administering the product in this manner is to anticipate and predict adverse events that occur when the product is administered in a clinical environment. Administering the product at a higher-than-labeled dose and duration simulates the usage of the product in the diverse, real-world, target animal populations including those that may be more sensitive to the drug. During the MOS study, the variables collected depend on the drug in question, but almost always include physical examinations/observations, clinical pathology tests, necropsy findings, and histopathologic examinations.

Additional studies are incorporated into the Target Animal Safety major technical section depending on the characteristics of the drug and administration. These studies may include Injection Site Safety Studies, Administration Site Safety Studies for Dermally Applied Topical Products, Reproductive Safety Studies, and Mammary Gland Safety Studies. Injection Site Safety studies elucidate location of administration, duration, vehicle, and maximum volume of injection. These studies also consider the safety consequences of administering an intravascular product by an extravascular route and vice versa. Safety data observations for injection site safety studies include changes in behavior/movement, appearance/inflammation/other changes at injection site, creatine kinase/aspartame transaminase levels, gross pathologic/histologic changes in lesions at injection sites. If inflammation at the injection site has not resolved by the end of the injection site safety study, then the time required for clinical resolution is observed. In addition, Target Animal Safety data is collected during field efficacy studies as these studies commonly recruit a more diverse population of animals compared to the field studies.

Effectiveness

This technical section is intended to demonstrate substantial effectiveness of the drug in question. "Substantial Evidence" is explicitly defined in 21 CFR 514.4 but may be summarized as well-controlled studies that demonstrate the proposed effect of the new animal drug under proposed label conditions. Specifically, the Effectiveness technical section includes the dosage characterization study as well as pilot or pivotal effectiveness studies.

The intent of the dosage characterization study is to determine the minimum efficacious dose of a product that will be used as a starting dose in pivotal efficacy studies. This dose is determined by the drug company/sponsor without requiring the approval of the CVM, but the CVM reserves the right to comment on dosage concerns.

Per the Animal Drug Availability Act (ADAA) of 1996, there are multiple types of pivotal efficacy studies that are sufficient to demonstrate substantial evidence (fda.gov). However, the most common type of pivotal efficacy study is a field study in compliance with Good Clinical Practice (GCP) where all animals selected have the disease or condition for which the proposed drug will be labeled for. Good Clinical Practice, described in CVM GFI #85, sets standards for the accuracy, integrity, and correctness of the data which are all factors that are important to demonstrate substantial evidence. The CVM provides multiple guidance documents for efficacy studies which differ based on drug class, route of administration, or target animal.

Human Food Safety

This technical section is intended to ensure that food products made from animals treated by the New Animal Drug are safe for human consumption. There is potential for drug residue accumulation in food products when a food-producing animal is treated with a drug. The goal of studies pertaining to Human Food Safety is to ensure that food animal products consumed by humans from treated animals are not harmed. There are four components of the Human Food Safety Section including: toxicology, residue chemistry, microbial food safety, and the analytical method used to detect the residue in the tissues. It should be noted that this dissertation focuses on testing ectoparasiticides specifically in companion animals. In New Animal Drug Applications intended for use in companion animals, the Human Food Safety section is omitted.

Chemistry, Manufacturing, and Controls (CMC)

This technical section is included in the NADA in order to demonstrate the drug's strength, quality, and purity. In other words, this section explains the sponsor's protocol for making the drug. This plan includes a description of the Active Pharmaceutical Ingredient (API) and excipients (inactive components of the drug), the source of the API and excipients, the manufacturing process, the location of the manufacturing sites, packing information, and stability conditions that determine shelf-life. This is also the section that includes *in vitro* tests performed by the drug company/sponsor to elucidate batch consistency and lack of impurities. Specifically, Per CVM Guidance for Industry (GFI) # 169, the recommended information to be provided by the drug company/sponsor in the CMC technical section includes: Nomenclature/structure/drug properties, manufacturing, characterization, control of drug substance, reference standards, container closure system, and stability (fda.gov).

Drug monographs published by the United States Pharmcopeia (USP) describe tests to determine physiochemical properties including solubility and the analytical methods to determine potency. The USP is a non-federal organization, but these monographs have become the “gold standard” for quality and safety tests. Therefore, the CVM requires that testing follows the standards from the USP drug monographs if one is available. CVM GFI #234 is a guidance for industry that provides a series of questions focusing on pharmaceutical attributes that determine the quality of New Animal Drugs termed the Question-based Review (QbR). The QbR asks specific questions to inform the CVM on the manufacturing, characterization, control, reference standards, container closure system, and stability of the Drug Substance (API) and Drug Product (API plus excipients). The QbR also includes the sterile process validation process for injectable and some non-injectable drugs (such as those for ophthalmic use). This process streamlines the CVMs review process for the Chemistry, Manufacturing, and Controls technical section.

Environmental Impact

Per the National Environmental Policy Act (NEPA), the CVM is obligated to consider the environmental impact of a New Animal Drug thus necessitating the inclusion of the Environmental Impact Technical Section. For companion animal products, the drug company/sponsor must either provide an environmental assessment or a claim for categorical exclusion (Cat X). A categorical exclusion is bestowed to drugs that fall within a legally defined category (under 21 CFR § 25.30) that is unlikely to affect the environment. Categorical exclusions are most common in companion animal drugs which are administered on an individual patient basis. Drugs that are administered to herds or flocks are more likely to enter the environment due to volume of administration, thus less commonly receiving a categorical exclusion.

For drugs that do not meet the criteria for a categorical exclusion, an Environmental Assessment (EA) is performed by the drug company/sponsor which describes the quantity of a drug that is expected to enter the environment and subsequent consequences associated with potential environmental contamination. The CVM evaluates the EA provided by the drug company/sponsor and considers harvesting practices for the drug to determine whether there will be an environmental impact. This is followed by the CVM writing a “Finding of No Significant Impact” (AKA “FONSI”) in the event of no environmental impact or an Environmental Impact Statement (EIS) in the event of significant environmental impact.

Minor Technical Sections

The minor technical sections of a product under the jurisdiction of FDA regulation include All Further Information, Labeling, and the Freedom of Information Summary (FOI).

“All Further Information” (AFI) includes all information about the drug that was not previously included in the submission for the safety, effectiveness, or human food safety technical sections. This may include information from previously published scientific literature, foreign experience (information on the drug if it is already approved in a country other than the United States), and other supportive studies performed after the submission of the major technical sections. This section is an opportunity for the sponsor to provide supportive information including pilot studies, proof-of-concept studies, or any study not included in a major technical section that supports the approval of a drug. This section is typically submitted towards the end of the application window to maximize the time to accumulate supportive literature.

A product "label" addresses several components. The "label" includes information on the immediate container (plastic bottle in this case), package insert (adhered to the immediate container), outer packaging (cardboard carton housing the immediate container), shipping label (label for box that houses several units of outer packaging), and the client information sheet (optionally written for pet owners to manage expectations with this product) (21 CFR § 201). The product label itself requires several components per the CVM. Any product requiring a veterinary prescription must include the statement: "Caution: Federal law restricts this drug to use by or on the order of a licensed veterinarian". The label must also include the product name, dosage form, route of administration, dosage, dose sizes, and proportion of active ingredient(s). The label also includes the pharmacology/mechanism of action of the product. The label also addresses user safety warnings including the disclaimer that the product is not intended for human use and to keep out of the reach of children. This section also addresses safety concerns such as contacting a physician immediately in the event of inadvertent ingestion and keeping the product out of reach of all animals to prevent accidental ingestion and/or overdose. This section is also where the sponsor addresses disease states or other drugs with which the product should not be combined and whether the product has been tested in pregnant or nursing animals. The product label also includes known adverse events that were observed during the lab and field trials of the product. Included in the label is the effectiveness section which details what parasites the product is used to treat/control/prevent when used according to the label dose and directions. This also includes the results of the well-controlled lab study and well-controlled field study demonstrating treatment/control/prevention of the parasite(s) of concern. The safety section addresses animal safety pertaining to clinical use of the drug (as opposed to the warnings section which addresses handling and accidental ingestion in humans/children/other animals) which includes results from the margin of safety study where the product was administered to the target animal at 1x, 3x, and 5x the label dose to anticipate potential adverse events. There are some drug classes that require additional studies pertaining to known adverse events based on genetics such as the MDR1 gene mutation increasing the incidence of toxicity associated with the usage of macrocyclic lactones. The final component of the product label is storage information.

The final minor technical section addresses the Freedom of Information (FOI) Summary. This is a component that publicly discloses information that the FDA used to evaluate a NADA (new animal drug application), ANADA (abbreviated new animal drug application [generic]), or CNADA (conditional approval) for regulatory approval (21 CFR § 20). The FDA is obligated by Title 21 of the Code of Federal Regulations (federal law) to disclose this information following the approval or conditional approval of an application. This section includes the rationale for the CVMs decision of approval for a drug to be made available to the public. For all applications, the FOI includes the information that the drug company submitted to the FDA to indicate that a drug is safe and effective for intended use. All FOI Summaries are available electronically at animaldrugsatfda.fda.gov or upon a Freedom of Information Act (FOIA) request.

Compliance Post-Licensure

Following the release of a product, a sponsor is required to maintain CGMP standards in manufacturing facilities that are inspected by the CVM to avoid the production of adulterated drugs. Furthermore, a sponsor is required to submit a drug experience report to the CVM one year after the launch of a product which includes sales and Adverse Event reports. The information in the experience report is taken into consideration for any potential changes to the label under a "Post Approval Experience" section. This section is designed to address any adverse events reported by veterinarians to the pharmacovigilance team after the launch of a product. The information in this section is critical as the population of animals receiving the product after launch is far broader and more diverse than those involved in Target Animal Safety and Efficacy studies. During the time after launch, any adverse events that meet the criteria of being a "serious" event per 21 CFR must be reported to the FDA within 15 days of the sponsor learning of the adverse event. Failure to comply with these regulations may result in a warning letter and an injunction. Therefore, it is imperative for the sponsor to remain in communication with the FDA before, during, and after a drug's release onto the market.

Post-Approval Marketing

Adverse events are reported to the FDA as they are experienced in real-world settings. In addition, post-approval marketing studies may be conducted in order to demonstrate safety and efficacy. The FDA requires safety and efficacy studies before a drug is made available on the market, but post-approval marketing studies can demonstrate the degree of safety and efficacy by addressing more diverse patient populations. In the case of ectoparasiticides, post-approval marketing studies often occur in patient populations and environments with higher ectoparasite densities in comparison to the pivotal efficacy studies. These sorts of studies permit investigators to evaluate the efficacy and safety of a product over time.

Off-Label Efficacy

In addition to providing information on a larger and more genetically diverse ectoparasite population, post-approval marketing studies may inform investigators and veterinarians on product capabilities that are not stated on the label. This may include efficacy against a parasite not on the product label, mitigation of vector-borne disease transmission, and amelioration of dermatologic conditions associated with ectoparasite infestations such as Flea Allergy Dermatitis (FAD).

One example of post-approval marketing studies focuses on the off-label administration of a drug in the labeled host species for a parasite that is not on the product label. In one study, ten dogs with clinical signs associated with generalized demodicosis and positive skin scrapings for Demodex spp. were treated with lotilaner at a minimum dosage of 20 mg/kg (same minimum dosage as the product label for flea and tick control) on Days 0, 28, and 56 of the study (Snyder et al., 2017). By Day 56 of the study, there was a >99.9% ($P < 0.0001$) reduction in *Demodex* mites across all treated dogs. By Day 84 of the study, no live mites were detected and showed improved clinical signs associated with demodicosis (Snyder et al., 2017). This study is one of many associated with the usage of a member of the isoxazoline drug class (such as lotilaner) against mite infestations. As a result, the isoxazoline drug class has become the modern-day gold standard for the control of multiple mite species (Beugnet et al., 2016, Six et al., 2016, Djuric et al., 2019, Chiummo et al., 2020, Moog et al., 2021, Becskei et al., 2023) despite the lack of mites on the product label of any isoxazoline drugs.

Furthermore, mitigation of vector-borne disease is critical in association with ectoparasite control even if it is not explicitly stated on the product label. In a study from 2016 by Honsberger et al., dogs were treated with oral sarolaner to prevent the transmission of *Borrelia burgdorferi* (the causative agent of Lyme borreliosis) from wild caught *Ixodes scapularis* ticks. The study was separated into three groups: eight dogs given a placebo on Days 0 and 7, eight dogs treated with 2 mg/kg of oral sarolaner on Day 0, and eight dogs treated with 2 mg/kg of oral sarolaner on Day 7. All dogs were infested with 25 female and 25 male wild caught *Ixodes scapularis* ticks that had a *Borrelia burgdorferi* prevalence of 57% determined by PCR. All ticks were counted and removed on Day 33, and blood samples were collected from dogs on Days 27, 49, 63, 77, 91, and 104. These blood samples were tested for the presence of *Borrelia burgdorferi* and *Anaplasma phagocytophilum* antibodies using SNAP® 4Dx® Plus Tests and quantitatively assayed for *Borrelia burgdorferi* antibodies using an unspecified ELISA test. In addition, skin biopsies were collected on Day 104 from all dogs of all groups to assess the presence of *Borrelia burgdorferi* by bacterial culture and PCR. Live tick counts were performed 1, 2, and 5 days after infestation demonstrating an 86.3%, 100%, and 100% reduction respectively in the group treated 21 days prior to infestation and 90.9%, 97.1%, and 100% reduction respectively in the group treated 28 days prior to infestation (Hornsberger et al., 2016). In both sarolaner treated groups, the geometric mean live tick counts were significantly lower than those the tick counts of the placebo group across all days ($P < 0.0001$). Positive transmission of *Borrelia burgdorferi* in the placebo-treated group was confirmed by positive antibody (6 of 8 dogs), PCR (7 of 8 dogs), and/or culture (7 of 8 dogs). In both sarolaner-treated groups, prevention of transmission of *B. burgdorferi* was demonstrated by negative antibody, PCR, and culture results (Honsberger et al., 2016). This study was performed to demonstrate off-label efficacy of an isoxazoline-based product for prevention of transmission of *B. burgdorferi*. However, this evidence was sufficient for the sponsor to submit this data in Supplemental New Animal Drug Application 141-452 which led to the addition of a label indication for the prevention of *Borrelia burgdorferi* infections as a result of killing *Ixodes scapularis* ticks. Demonstrating off-label efficacy for mitigating the transmission of vector-borne disease aids researchers in making recommendations to veterinarians based on current evidence. There are several other publications demonstrating the ability of ectoparasiticides to mitigate the transmission of vector-borne disease including Fourie et al., 2013, Beugnet et al., 2014, Taenzler et al., 2015, Geurden et al., 2017, and

Chiummo et al., 2023. The Honsberger et al. study from 2016 is an example of a label claim that emerged due to a post-approval marketing study that presented sufficient data to the FDA.

To add, post-approval marketing studies can be used to demonstrate the ability of a product to ameliorate secondary disease conditions. An example of this is in a field study from Briand et al. in 2019 where cats experiencing pruritus or skin lesions consistent with flea infestation were treated with a formulation of fluralaner. In this study, pruritus was assessed using a PVAS (Pruritus Visual Analog Scale) on Days 0, 15, 28, 56, and 84 while skin lesions were assessed using a SCORFAD (Scoring Feline Allergic Dermatitis) scale on Days 0, 28, 56, and 84. All cats were treated with fluralaner on Day 0 at a minimum dose of 40 mg/kg. All PVAS assessments post-treatment showed a significant decrease from the pre-treatment PVAS scores from Day 0 ($P < 0.002$) with a reduction of 92% by Day 84. A similar trend was observed with post-treatment SCORFAD scores where there was a significant reduction from Day 0 ($P < 0.002$) with a reduction of 87% by Day 84 (Briand et al., 2019). This study concluded that treatment with fluralaner resulted in a significant reduction of flea bite allergic dermatitis. This is an example of a post-approval marketing study informing researchers and veterinarians on the ability of a product to ameliorate a disease state that may not be on the product label.

Beyond conditions and vector-borne disease, there are post-approval marketing studies that demonstrate a head-to-head comparison between products. These studies may evaluate different parameters between products such as speed of kill or duration of efficacy. A study by Reif et al. published in 2024 assessed the comparative speed of kill of isoxazoline-based drugs including sarolaner (combined with moxidectin and pyrantel), afoxolaner, and lotilaner. Dogs were separated into four groups of eight: one group for each treatment and one negative control. All dogs were infested with 50 adult *Amblyomma americanum* ticks on Days -7, -2, 21, and 28 with tick counts performed on Days -5 and then 4-, 8-, 12-, 24-, 48-, and 72-hours following treatment and future infestations. All treatment group dogs were treated on Day -2, and efficacy was assessed based on the geometric mean of live tick counts. Between all treatment groups, only the lotilaner treated group showed a significantly reduced *A. americanum* infestation by 12 hours (43.3%). At 24 hours, lotilaner efficacy (97.4%) was significantly higher than the sarolaner and afoxolaner treatment group efficacy of 13.6% and 14.9%, respectively. Twenty-four hours after reinfestation on Day 28, lotilaner demonstrated a 92.3% efficacy which was significantly higher than the 4.9% efficacy of sarolaner ($P < 0.001$) and the 0.0% efficacy of afoxolaner (Reif et al., 2024). This study concluded that the lotilaner formulation demonstrated a faster onset of acaricidal activity against *Amblyomma americanum* in comparison to the sarolaner and afoxolaner formulations. In addition, amongst the treatment groups, only the lotilaner maintained the rapid speed of tick kill throughout the dosing period. Vector-borne disease transmission from ticks may occur within 24 hours after attachment which means comparative speed of kill data is valuable to veterinarians in areas endemic to tick-borne disease. This is one of many studies (Varloud and Fourie, 2015, Varloud and Hodgkins, 2015, Becskei et al., 2016, Six et al., 2016, Dryden et al., 2020, Reif et al., 2023) comparing different ectoparasiticide products available on the market. Comparison post-approval marketing studies aid researchers in making informed recommendations to veterinarians based on treatment and prevention goals of a patient or client.

Natural Infestations

Post-approval marketing studies also provide data on natural ectoparasite infestations which may be a better reflection of parasites in nature as opposed to lab colonies. In addition, animals with natural infestations may have lower or higher ectoparasite burdens compared to controlled lab or field studies. Presumably, natural infestations are presumably more genetically diverse than a lab-reared colony of a given ectoparasite which may result in difference in drug susceptibility. In a study from Ouédraogo et al. in 2025, laboratory-reared *Anopheles gambiae/Aedes aegypti* mosquitoes were compared to wild-derived *Anopheles coluzzii/Aedes aegypti* mosquitoes when exposed to ivermectin. After exposure in the larval stage, the following parameters were measured: larval survival, pupation dynamics, teneral emergence rates, and eggs developed and laid by adult females. The ivermectin concentrations to reach a 50% larval mortality were 3.65 ng/mL in the wild-derived *Anopheles* larvae and 1.86 ng/mL in the laboratory-reared *Anopheles* larvae. This was supported in the *Aedes aegypti* mosquitoes as well where the wild-derived larvae reached a 50% mortality at 15.60 ng/mL of ivermectin compared to the concentration of 2.56 ng/mL in laboratory-reared larvae (Ouédraogo et al., 2025). This study demonstrated that wild-derived strains (natural infestations) may exhibit differences in sensitivity compared to a laboratory-reared strain when exposed to an ectoparasite. Therefore, post-approval marketing studies in the field may provide a better example of real-world efficacy of an ectoparasiticide.

A chapter of this dissertation addresses the ability of a drug from the isoxazoline drug class to eliminate home flea infestations using only animal treatments in West-Central Florida without premises treatment. This study was modeled from a line of studies pioneered by Dr. Michael Dryden from Kansas State University, the first of which was a comparison study between topical imidacloprid and oral lufenuron/topical pyrethrin spray (Dryden et al., 1999). Since the first study, numerous subsequent studies (Dryden et al., 2013, 2015, 2016, 2017, 2018, 2019, 2020) have been performed in the same region of Florida investigating more than twenty compounds commonly used for flea control. This set of studies has provided veterinarians with insights on the duration of treatment necessary to eliminate a home flea infestation as well as the current efficacy of a given product which may differ from when the product was initially labeled. These studies also highlight product efficacy against modern-day strains of a parasite experienced in natural infestations.

Post-Approval Marketing Conclusions

Overall, post-approval marketing studies inform investigators on product capabilities outside of the formal process of adding a claim to a label. This allows researchers to make better recommendations to veterinarians and animal owners based on current evidence.

Environmental Protection Agency (EPA) Regulatory Process

The Environmental Protection Agency (EPA) was founded in 1970 following public concern over pollution. The EPA's responsibilities include research on pollutants, monitoring the environment, minimizing negative industry impact on the environment, enforcing air and water quality standards, and to financially/technically assist states and industries looking to expand pollution control programs (epa.gov). Within the EPA, there are multiple offices with different responsibilities including the Pesticides Office. The Pesticides Office is responsible for regulating pesticide residues in or on food, monitoring pesticide levels on food or in the environment, reviewing pesticide formulations for efficacy and adverse effects, assessing compliance with pesticide labels, and researching impacts of pesticides on human/wildlife/environmental health. EPA regulation within the animal parasiticide space sometimes results in jurisdictional disagreements between regulatory agencies. The EPA was not initially founded with companion animal parasiticides in mind but remains the regulatory body for topically applied, topically acting ectoparasiticides. As such, there are situations where proposed pharmaceutical products meet the criteria to fall under the regulatory authority of multiple agencies. In the ectoparasiticide space, this is most observed between the EPA and the

FDA as there are situations where a product may be classified as a “pesticide” (EPA regulation) and as a “drug” (FDA regulation). To resolve these conflicts, there are several Memoranda of Understanding (MOU) between the agencies that outline the sharing of information and the delineation of responsibilities over certain products (MOU 225-24-023).

It is important to consider which regulatory body governs a certain product as there are fundamental differences in regulation and use. For example, off-label usage of EPA-registered products is illegal and prohibited under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. §136-136y [1996]). However, veterinarians are permitted to prescribe FDA-registered products off label due to the Animal Medicinal Drug Use Clarification Act (AMDUCA) in situations that the veterinarian deems medically appropriate (Animal Medicinal Drug Use Clarification Act, 1994). This dramatically changes the manner in which veterinarians prescribe ectoparasiticides.

From a product-development perspective, “Target animal” in the case of veterinary use describes the “pest” or parasite that the pesticide is used to mitigate, not the animal that the product is applied to. Thus, “Target Animal Safety,” a requirement for FDA registration of products, is not policed in the same manner within the EPA.

Global Harmonization

Global harmonization is the practice of drug companies/sponsors performing studies that meet the requirements of both domestic and foreign regulatory agencies for drug approval to streamline the process of marketing a drug on a global scale. This practice can shorten the timeline for approval in multiple countries and reduce the possibility of performing redundant studies for approval of the same product in multiple countries. There are multiple organizations that exist between the United States and foreign countries to facilitate this process. A trilateral cooperation exists between the United States, Europe, and Japan called the International Cooperation of Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (VICH).

The World Organization for Animal Health (WOAH) Guidelines

The World Organization for Animal Health (WOAH), also known as the Office International des Epizooties (OIE) is considered the global authority on animal health. There are different regulatory bodies that govern the usage and safety of ectoparasiticides in countries other than the United States. In other words, identical products are subjected to vastly different registration processes depending on the country where they will be used and sold. WOAH was founded in response to rinderpest, a deadly viral disease of cattle that was negatively impacting the cattle populations of multiple countries. The eradication of rinderpest required a coordinated effort from multiple countries, and WOAH became the organization to facilitate this coordination. WOAH now resides as the global reference for animal health standards that are enforced under the World Trade Organization's (WTO) SPS agreement (World Trade Organization, 1994). Specifically, WOAH provides animal health codes that establish an international standard for the trading of animal products between countries.

World Association for the Advancement of Parasitology (WAAVP)

The World Association for the Advancement of Veterinary Parasitology (WAAVP), while not an official governing body, has set standards for study design within the parasitology community. The WAAVP provides guidelines established by researchers from an international collaborative effort. These resources provide guidelines based on parasite of interest or host species of interest. An example of ectoparasite guidelines is provided in Marchiondo et al., 2013. These guidelines provide a blueprint for evaluating the efficacy of ectoparasiticides for treating/preventing/controlling flea and/or tick infestations of dogs or cats. For evaluating efficacy, these guidelines describe *in vitro* techniques used in preclinical phases of product development, graduating to *ex vivo* testing with whole blood, and finally *in vivo* testing on experimentally infested laboratory animals. Publications such as the one provided offer a baseline to multinational sponsors that would seek regulatory approval in multiple countries. Therefore, these guidelines highlight the importance of multiple kinds of testing (*in vivo/ex vivo/in vivo*) even on the international scale.

Isoxazolines

Isoxazolines are a drug class commonly used in modern, effective, companion animal ectoparasiticide product formulations. Since the introduction of NexGard® (afoxolaner) in 2013, isoxazolines have rapidly become the gold standard drug class for effective companion animal flea and tick control. Isoxazolines are structurally characterized by a 5-membered heterocycle and were initially discovered by researchers from Nissan by chemically altering the structure of diamides. Isoxazolines are allosteric modulators of GABA-gated chloride channels as well glutamate-gated chloride channels to a lesser extent (Gonçalves et al., 2021).

Gamma-aminobutyric acid (GABA) receptors are present on the chloride channels of insects and other arthropods where GABA acts as an inhibitory neurotransmitter. GABA binds to the receptor of GABA-gated chloride channels resulting in hyperpolarization of the membrane to produce an inhibitory post-synaptic action potential (Gonçalves et al., 2021). Isoxazolines bind to the GABA receptor to prohibit the influx to chloride ions which results in a spastic-paralysis of the insect and subsequent death.

There are four overlapping but distinct sites on the GABA receptor that are known for pesticidal activity (Gonçalves et al., 2021). Organochlorines, another pesticide drug class, act on one of these sites known as the NCA-IA site. However, isoxazolines act on the NCA-II site which decreases the possibility of cross-resistance with an existing pesticide drug class. The isoxazolines also demonstrate a high margin of safety as isoxazolines have a much higher degree of selectivity for GABA receptors in insects compared to mammals. In a study by Rufener et al., from 2017, lotilaner, an isoxazoline was assayed to assess the affinity of the compound to tick and crustacean GABA-gated chloride channels. This study also assessed the affinity of lotilaner to dog $GABA_A$ where no activity was observed (Rufener et al., 2017). Despite the data from this study, the FDA has issued a communication alerting veterinarians and pet owners of neurologic adverse events associated with the isoxazoline drug class. When used judiciously under the direction of a licensed veterinarian, the isoxazoline drug class has become the modern-day gold standard for flea and tick control in companion animals.

Afoxolaner

The first U.S. formulation of afoxolaner was approved by the FDA in 2013. Today, afoxolaner is used in canine formulations of NexGard® (afoxolaner) and NexGard® PLUS (afoxolaner, moxidectin, and pyrantel). Esafoxolaner, the active enantiomer of afoxolaner, is an active ingredient in NexGard® COMBO (Esafoxolaner, eprinomectin, and praziquantel), a feline preventive formulation. Label indications vary based on product, but all formulations containing afoxolaner or esafoxolaner are labeled for control of *Ctenocephalides felis* (cat fleas) and certain species of ticks.

Afoxolaner was first described in Shoop et al. in 2014. This study began using an *ex vivo* approach to determine the blood concentration of afoxolaner that would be necessary to kill

Ctenocephalides felis fleas. Titration studies were performed using a membrane flea feeding system. Afoxolaner was first diluted in dimethyl sulfoxide (DMSO) This method utilized dilutions of 0.32, 0.16, 0.08, 0.04, 0.02, and 0.01 µg/ml of afoxolaner in citrated bovine blood performed in triplicate. A control of 1% DMSO and 99% citrated bovine blood was included as well. One hundred *Ctenocephalides felis* fleas per replicate was permitted to feed on citrated bovine blood that had been spiked with afoxolaner, and assessment of live and dead fleas were made at 24, 48, and 72 hours. This resulted in an efficacy of >95% against fleas in the 0.16 µg/ml dilution at 24 hours, 0.08 µg/ml at 48 hours, and 0.02 µg/ml at 72 hours (Shoop et al., 2014). This was followed by an *in vivo* study, where dogs (beagles) were administered afoxolaner orally at a dosage of 2.5 mg/kg, and different groups were challenged with fleas (*Ctenocephalides felis*) every 7 days and ticks (*Dermacentor variabilis*) every 7 days in comparison to a vehicle control to assess efficacy. This resulted in 100% efficacy against fleas for up to 28 days and >98% efficacy against ticks for up to 30 days (Shoop et al., 2014). This strategy illustrated the approval of afoxolaner in the stair-step design of utilizing *in vitro* or *ex vivo* pilot studies before performing *in vivo* efficacy studies.

Fluralaner

The first U.S. formulation of fluralaner was approved by the FDA in 2014. Today, fluralaner is used in canine formulations of Bravecto® Chews for Dogs, Bravecto® (fluralaner topical solution) for Dogs, Bravecto® 1-Month, and Bravecto® Quantum (fluralaner extended-release injectable suspension). Fluralaner is also used in feline formulations of Bravecto® for Cats (fluralaner topical solution) and Bravecto® Plus for Cats (fluralaner and moxidectin topical solution). These products are marketed by Merck Animal Health for control of different species of fleas and ticks for different durations depending on the product.

Fluralaner was first described in Williams et al. in 2014. This began with an *ex vivo* experiment that fed fluralaner-spiked blood to *Ctenocephalides felis* fleas in a membrane system. This study used fluralaner concentrations between 0.09 and 50.0 ng/mL in the blood over 10 days where an ovicidal effect was observed at 25.0 ng/mL (Williams et al., 2014). This was followed by an *in vivo* experiment where dogs were infested with 100 *Ctenocephalides felis* on day -28 and day -21 pre-treatment to simulate a home flea infestation. Dogs were infested with fleas again on days 22, 50, and 78 post-treatment to simulate the introduction of a new infestation. Flea control efficacy across every time point for 12 weeks was greater than 99% (Williams et al., 2014). This again follows the progressive design of moving from *in vitro/ex vivo* studies before performing *in vivo* efficacy studies.

Lotilaner

A formulation of lotilaner was first approved in the U.S. by the FDA in 2018. Lotilaner is an isoxazoline present in multiple companion animal flea, tick, and combination products including Credelio®, Credelio® CAT, and Credelio Quattro™ marketed by Elanco Animal Health (Greenfield, IN, USA). The label indications vary based on the product, but all lotilaner formulations include adult *Ctenocephalides felis* fleas.

An example of the regulatory process for FDA approval of a lotilaner formulation can be found in the Freedom of Information Summary for NADA 141-494 (Credelio™). This document describes the studies utilized in determining the dose of the product (Dosage Characterization), the studies used to determine the efficacy of the product (Field Effectiveness Study), and the target animal safety (Margin of Safety Study). In a pharmacokinetic study published by Toutain et al. in 2017, lotilaner blood concentrations were measured using LC-MS/MS in dogs treated orally or intravenously with lotilaner. This study found that lotilaner had an oral bioavailability of 80% and a terminal half-life of 30.7 days under fed conditions (Toutain et al., 2017). In a separate study published by Cavalleri et al. from 2017, dogs were infested with *Ctenocephalides felis* fleas in a laboratory study and treated with oral lotilaner at a minimum dose of 20 mg/kg. At eight hours post-treatment, there was a 99.6% flea count reduction (Cavalleri et al., 2017). This study concluded that lotilaner can alleviate flea irritation associated with existing infestations. The Freedom of Information Summary also includes the FDA's conclusions on the safety and efficacy of the product specifying which ectoparasites that the product is used to treat and control. The data provided in the studies described above led to the release of Credelio® (lotilaner) onto the market.

Sarolaner

Sarolaner is another isoxazoline with the first formulation approved in the U.S. by the FDA in 2016 with the release of Simparica®. Sarolaner is present in multiple companion animal ectoparasiticide products including Simparica®, Simparica Trio®, and Revolution® Plus.

Similar to lotilaner, the Freedom of Information Summary for NADA 141-452 (Simparica®) addresses studies used to generate data that led to the FDA's approval of the drug. In a dose determination study published by McTier et al. in 2016, dogs were infested with *Dermacentor reticulatus* ticks, *Rhipicephalus sanguineus* ticks, and *Ctenocephalides felis* fleas prior to oral treatment with sarolaner at 1.25 mg/kg, 2.5 mg/kg, and 5.0 mg/kg. The lowest dose of sarolaner (1.25 mg/kg) provided 100% efficacy against fleas through Day 35 and diminishing to 98.4% efficacy by Day 56. This same dose resulted in 99.7-100% control of both tick species through Day 28. A different group of dogs was infested with *Amblyomma maculatum* ticks and treated orally with sarolaner at 1.0 mg/kg, 2.0 mg/kg, and 4.0 mg/kg. This study found that there was no significant improvement in efficacy between the 4.0 mg/kg and the 2.0 mg/kg dose ($P >$

0.05) at any timepoint. However, the 1.0 mg/kg dose showed a decline below 90% efficacy by Day 28 (McTier et al., 2016). Therefore, 2.0 mg/kg was selected as the dose of sarolaner for at least one month of flea and tick control. This dosage characterization study in addition to the pivotal Efficacy Studies and the Margin of Safety Studies contributed to the technical sections submitted to the FDA leading to the approval of a sarolaner formulation on the market.

Off-Label Usage of the Isoxazoline Drug Class

The Animal Medicinal Drug Use Clarification Act of 1994 (AMDUCA) permits the usage of drugs outside of the indications on the product label under the direction of a veterinarian. This "Extra label use" is defined as "Actual use or intended use of a drug in an animal in a manner that is not in accordance with the approved labeling. This includes, but is not limited to, use in species not listed in the labeling, use for indications (disease and other conditions) not listed in the labeling, use at dosage levels, frequencies, or routes of administration other than those stated in the labeling, and deviation from labeled withdrawal time based on these different uses" (21 CFR § 530.3(a)). Per 21 CFR, veterinarians are permitted by the FDA to use prescription drugs off-label within the confines of a Veterinarian-Client-Patient Relationship (VCPR).

The research projects within this dissertation focus on both the off-label and on-label use of the isooxazoline drug class found in Chapter 3 and Chapter 4, respectively. As the isoxazoline drug class is the modern-day gold standard for flea and tick control in dogs and cats, it is worth investigating the use of this drug class in other species experiencing ectoparasite infestations. Options for testing strategies (*in vitro*/*ex vivo/in vivo*) are imperative to facilitate the use of an effective drug class such as isoxazolines in existing approved species as well as novel species.

Chapter 2 - Utilization of a 3D-Printed Apparatus for the Rearing of Cat Fleas (*Ctenocephalides felis*)

Abstract

Ctenocephalides felis, also known as the "cat flea," is an ectoparasite of veterinary and public health importance. It is common to rear cat fleas on live cats for research purposes, but this can be expensive to sustain and presents an opportunity for animal replacement. Commercial artificial flea systems available for research purposes may be cost-prohibitive or inappropriately scaled for certain flea studies. The implementation of a 3D-printed artificial feeding system presents a cost effective, adaptable, and sustainable solution for the future of insecticide studies. For this study, existing 3D models used for mosquito feeding were adapted in Autodesk Inventor© (Autodesk; San Francisco, CA, USA) to house and feed fleas. A total of six units were printed on a Formlabs® Form 3B+ printer with each unit consisting of a blood/water chamber, flea chamber, and egg chamber. Two strains of fleas were tested on these membranes, one which had been reared on cats (WILD), and a second that had been reared on a commercial artificial membrane (MEM). Fleas were monitored for evidence of feeding, egg production, and frass production. First attempts of flea feeding the WILD fleas on the membrane showed signs of feeding with positive Hemoccult® tests and microscopic visualization of blood within the flea, but no egg production. The MEM strain of fleas readily fed and began laying eggs approximately 24 hours post-feeding. Across a 41-day study period, 2493 eggs were collected from the Mem strain for an average of 0.48 eggs/flea/day. These eggs were viable, and the subsequent generations successfully fed on both the artificial membrane as well as cats. The 3D-printed artificial flea feeding system presents a modular and scalable option for flea rearing and *in vitro* research capable of rearing multiple generations of fleas.

Introduction

It is common practice to rear cat fleas (*Ctenocephalides felis*) on purpose-bred laboratory cats for research purposes and flea colony/strain maintenance. Although this method historically yields high flea numbers, this strategy is expensive to maintain and presents an opportunity for animal replacement in research. There are existing models used in commercial businesses for rearing cat fleas that are based on an artificial membrane originally by Wade and Georgi at Cornell University (Wade and Georgi, 1988). This initial design was a water-jacketed, glass bell, adapted from those used for mosquito feeding, that used a Parafilm membrane for the fleas to feed from (Cosgrove et al., 1994). This initial design was scaled up for mass flea rearing to a self-contained chamber, named the "artificial dog", with twenty-five 5-cm flea cages that could raise up to 12,000 fleas at one time (Georgi and Georgi, 1991). These original designs continue to be used in laboratories and contract research organizations for a variety of purposes, including flea rearing and pathogen transmission (Napoli et al., 2014). The most recent offer to sell an "artificial dog" to the authors was ~$20,000 (Herrin, Personal Communication). This initial design was further scaled for high-throughput drug screening by creating a contained device that had 104, 2.5-cm flea cages, named "Greyhound" for its ability simultaneously test many drug compounds (Zakson-Aiken et al., 2001). While these apparatuses are excellent at rearing fleas, studying pathogen transmission, and testing novel compounds, the large scale and monetary cost may not be appropriate for all laboratories.

Additive manufacturing (AM), known colloquially as 3D printing, is the practice of generating three-dimensional objects from computer-aided design (CAD) software (Gao et al., 2015). Today, 3D printers are available on the market at a price that is suitable for personal use as opposed to exclusively commercial use resulting in large scale innovation in numerous scientific avenues. 3D printing is already being utilized within scientific research for arthropod rearing and vector transmission models (Harrison and Etheridge, 2025, Snyder et al., 2022, Witmer et al., 2018). Witmer et al. published an open-source, 3D-printed membrane feeder for mosquitoes that is a new iteration on the historic water-jacketed glass bell (Witmer et al., 2018). In that study, not only did they show successful feeding and egg production, but also the transmission of *Plasmodium falciparum* across the membrane at the same rate as the commercial feeding apparatuses. This study aims to build upon that work to create a sustainable artificial *ex vivo* flea-rearing system using components produced from additive manufacturing. Success of this system is predicated on the ability for the system to produce flea eggs to be reared through the entire flea life cycle while maintaining the ability for subsequent generations to be reared on cats for *in vivo* applications.

Methods

Design and Flea Colony Maintenance

Artificial feeder design

The original design for the 3D-printed apparatus was derived from an open-source mosquito-feeding apparatus that was created as a transmission model and has been adapted for flea-rearing applications (Witmer et al., 2018). The original mosquito feeder was re-created in Autodesk Inventor© (Autodesk; San Francisco, CA, USA) and adjustments were made including increasing the diameter of the apparatus to suit flea rearing, creating a small chamber to house the fleas, moving the blood chamber to the center of the unit to allow for more heat exchange with the water, and bolstering the water inlet/outlet stems to allow for greater shearing force and durability (Figure 2.1, 2.2). All 3D-printed components were printed on a Form 3B+: An

Advanced Desktop 3D Printer Designed for Healthcare using Clear Resin V4.1 (Formlabs; Sommerville, MA, USA). The final flea feeding apparatus consists of three sections: 1) a 3D-printed blood/water chamber, 2) a 3D-printed flea containment chamber, and 3) a glass petri dish positioned beneath the flea containment chamber to catch eggs (Figure 2.3).

Figure 2.1: Cross-sectional depiction of the blood/water chamber of the artificial flea feeding apparatus. Water is indicated in blue with white arrows indicating the directional flow of the water. Defibrinated bovine blood is indicated in dark and lies central to the circulating water to maximize thermal contact.

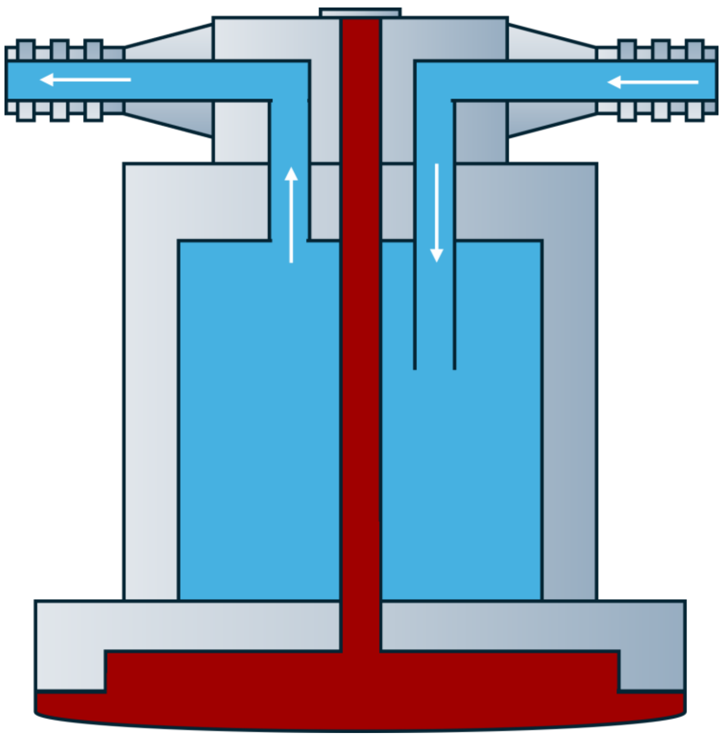
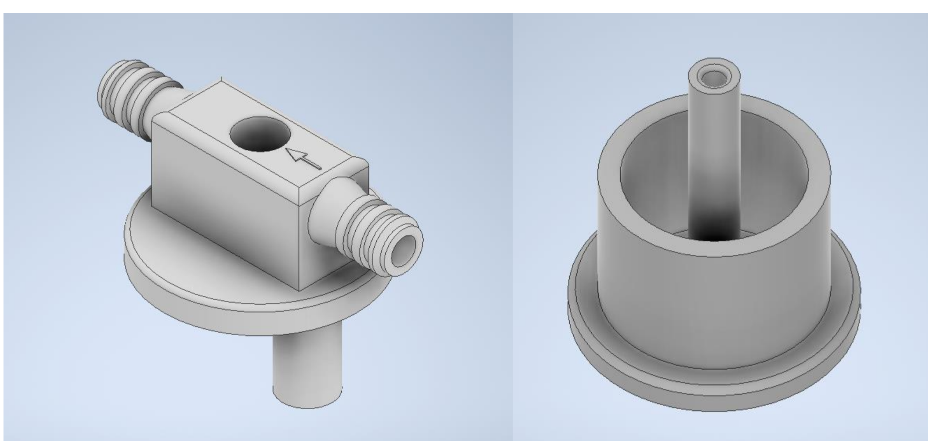


Figure 2.2: 3D rendering of the two separate prints that make up the blood/water chamber



Fleas

Two *Ctenocephalides felis* flea strains were used for this project. The first (WILD) is a strain of fleas that has been reared on cats in a laboratory setting since the early 1990s. This flea colony is a wildtype flea collected from animals with new genetics added in Summer 2024. The fleas for this study were raised under the approved IACUC #4983 at Kansas State University. The second *C. felis* strain of fleas were purchased from EctoServices (Henderson, NC). These fleas are raised on an artificial feeding system ("artificial dog") at EctoServices. All fleas were kept in an incubator maintained at 24°C and 80% relative humidity until ready for use. Approximately fifty fleas were added to a flea chamber in a given cohort. Experiments 1 and 2 attempted to feed the WILD and MEM strains, respectively on an artificial membrane. Experiment 3 took the 2nd generation progeny (n=50) from fleas that were successfully reared on the artificial membrane were put back onto cats to confirm their ability to successfully feed *in vivo*. Experiment 4 attempted to co-feed 25 female, second generation, MEM strain ticks with 25 WILD strain male ticks on an artificial membrane.

Artificial feeder conditions

The two components of the blood/water chamber were sanded and bonded using an N-[3-(Trimethyoxysilyl) propyl]-1,2-ethanediamine (1760-24-3) – based adhesive (Clear Gorilla Glue®) (Figure 2.2). Parafilm® was cut into squares measuring 4 by 4 inches and was pre-stretched using a plastic square of the same dimensions. The Parafilm® was stretched over the bottom of the blood water chamber and affixed to the base of chamber using a rubber band. Lastly, painter's tape was stretched around the outside of the perimeter of the lip of the blood/water chamber to tightly adhere the Parafilm® to the chamber and mitigate potential leaks from tears. In each flea chamber, a pipe cleaner was added as a substrate, and a Parafilm®

membrane was stretched over the bottom of the blood/water chamber to act as the membrane component which the fleas feed through. The blood/water chamber was affixed to the flea chamber using an adhesive foam ring. Approximately 1.5 mL of defibrinated bovine blood (Innovative Research; Novi, MI, USA) was added to the blood/water chamber where the blood sat atop the Parafilm® membrane while maintaining thermal contact from the circulating water. Additional blood was added to the blood/water chamber approximately every five days. The flea chamber consisted of a shallow plastic cylinder with an open top and 500 µM mesh bottom. The assembled apparatus was placed in a petri dish and elevated with cut tongue depressors to allow space for eggs to fall through the mesh of the flea chamber and into the petri dish. The assembled feeding apparatus was connected to a TC120-ST5 Heated Circulating Water Bath (Grant Instruments; Royston, UK) via flexible PVC tubing. The water pump was filled with distilled water to minimize calcium buildup throughout the circuit, and the temperature of the water pump was set to 40°C. An infrared thermometer was used to ensure that the artificial membrane surface resided between a temperature of 37°C and 40°C. The temperature-controlled water was pumped through the circuit at a rate of 16 liters/minute.

Figure 2.3: Complete assembly of the artificial flea feeding apparatus

Data collection

Parameters collected during establishment of the artificially-fed flea colony included the presence/absence of digested blood in fleas, production of flea feces, and number of eggs produced. In the absence of frass, fleas were squished onto a Hemoccult® blood test to indicate blood within the flea. Eggs were collected from the petri dishes daily by gently agitating the flea chamber, and eggs were placed in larval growth media and kept in an incubator at 24°C and 80% relative humidity. The larvae that emerged from eggs were permitted to pupate and moved to glass mason jars until adult fleas emerged (Dryden, 1989). Adult fleas that emerged from these pupae were added to the artificial flea feeding system to maintain egg production and preserve the life cycle. Days where no eggs were collected due to no egg production or lack of personnel availability were omitted from the analysis.

Results

Final Assembly

The final assembly of the 3D-printed blood/water was completed in two prints – a top component with stems to establish directionality of water flow and a bottom component to house the water with a central cylinder to house the blood (Figure 2.1, 2.2). Two versions of the flea chamber were made, one was a cylinder with a radius of 0.75 inches and height of 2.0 inches with an open top and bottom that were covered by mesh, and the second was altered to a height of 0.5 inches and the top was not covered in mesh. Both versions were adhered directly to the blood/water chamber, with the first having a mesh barrier between the fleas and Parafilm® membrane, while the second had no barriers between flea and membrane. Fleas in the deeper flea chamber were not successful in feeding despite having substrate to reach the membrane. Thus, the final, successful feeding apparatus utilized the flea chamber with 0.5-inch depth. The apparatus was able to hold blood for approximately 5 days before requiring a change in the membrane. The temperature of the blood across the membrane was able to be held consistently at 37°C. At any given time during this period, between one and four units were connected to the circuit.

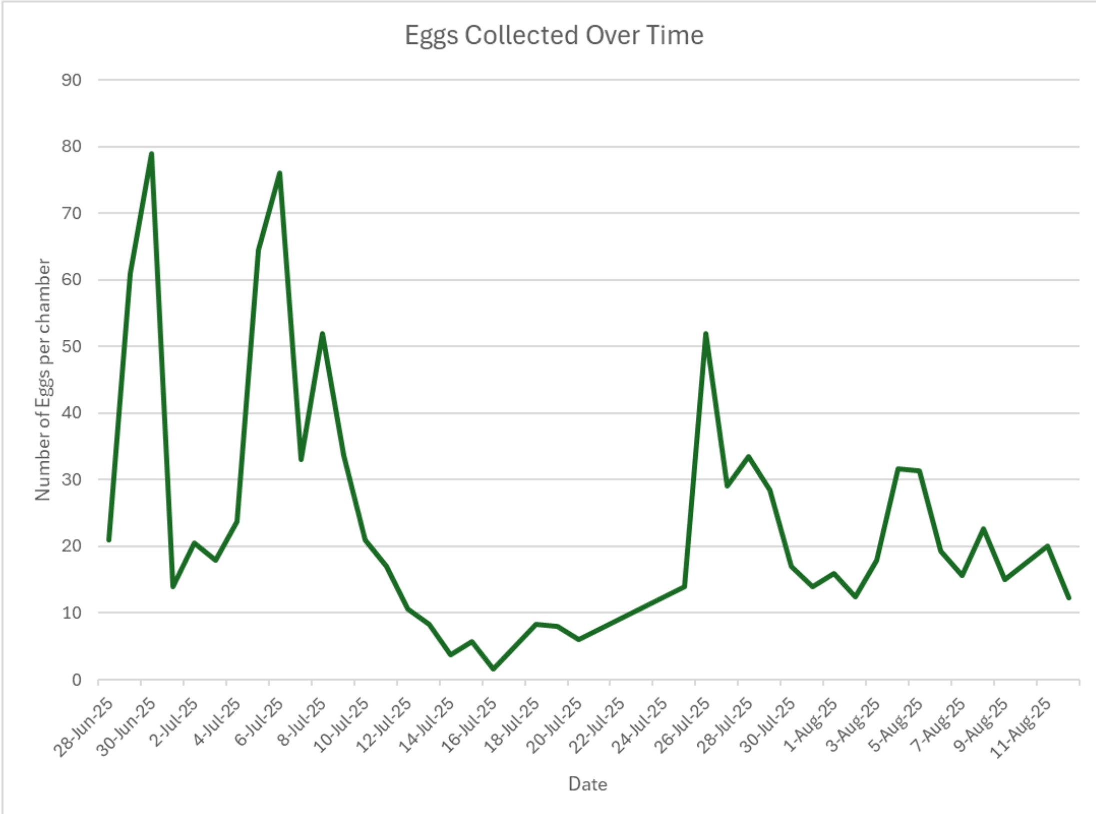
Experiments 1&2: Artificially Fed *Ctenocephalides felis* fleas reared on 3D-Printed Apparatus

The first round of flea feeding using the WILD strain (cat adapted) resulted in sporadic feeding, evidenced by frass and positive Hemoccult® tests, but only a single, non-viable egg was produced.

The second round of flea rearing using the MEM strain of fleas purchased from EctoServices. These fleas readily fed on the membrane and began producing eggs as soon as 24 hours after feeding was initiated. Egg counts from the artificial flea feeder were measured over 41-days, and a total of 2493 viable flea eggs were collected from the artificial flea feeding apparatus during this study period. The trend of eggs collected each day is depicted in Figure 2.4. Given that 50 fleas were added to each feeder, this gives an average of 0.48 eggs/flea/day.

Eggs collected from the initial fleas purchased from EctoServices were reared into adults and added to the artificial flea feeder system. The first generation was also able to produce viable eggs, although these were not enumerated. This was repeated for one more generation for a total of two generations produced after the initial purchased fleas.

Figure 2.4: Eggs collected per chamber over the course of the study



Experiment 3: Artificially fed *Ctenocephalides felis* fleas reared on live cats

Following the 41-day study period, 50 fleas produced from the 2nd generation of artificially fed fleas were placed on live cats (*Felis catus*), and egg counts were assessed over a 5-day period. A total of 821 eggs were produced resulting in an average of 3.28 eggs/flea/day.

Experiment 4: Co-feeding strains

After feeding for 24 hours, there was frass and eggs in the petri dishes. Female fleas also had microscopic evidence of feeding.

Discussion

Under experimental circumstances, the 3D-printed artificial flea feeding system successfully reared fleas through at least two complete generations. Parameters that resulted in successful egg production included shortening the chamber to 0.5 deep, utilizing a pipe cleaner substrate, and stretched Parafilm® membrane. The fleas in the deeper, 2.0-inch, chamber spent more time towards the bottom regardless of substrate, dog hair, cat hair, or pipe cleaners. We theorized that the fleas might not be close enough to get signal to feed, which prompted the shortening of the flea chamber. At 0.5 inches, the pipe cleaners worked well as a substrate, removing the need for dog hair or cat hair, which might, unknowingly, have insecticides or environmental bacteria which may disrupt specific studies. There are relatively few publications describing changes to this system to optimize flea feeding and egg production. One such study looked at the addition of dog hair into the flea chamber to stimulate feeding and found that the fleas housed with dog hair were less likely to die (2.4%) compared to those housed without dog hair (61.2%) (Pullen and Meola, 1995). In comparison, there is significant literature on the optimization of artificial feeding systems for ticks, which historically were difficult to rear on membrane as well (Romano et al., 2018, Bonnet and Liu, 2012, González et al., 2021). These optimization changes include utilizing a silicone/cellulose membrane (with and without mammal hair embedded), addition of mammal hair to the tick chamber, and the addition of attractants to encourage feeding. These changes have led to the successful establishment of artificial feeding systems for at least 20 tick species although there is significant variation between research groups and exact models (Rochilin et al., 2024).

Initially, the fleas used in the 3D-printed artificial flea feeding apparatus were the WILD *C. felis* reared on live cats. This strain resembles the wildtype strain of *Ctenocephalides felis* and had been maintained by Kansas State University for more than 20 years. This strain of fleas appears to be adapted to a cat-rearing model, thus, the fleas' inability to feed on the membrane was somewhat expected. This is a concern, as the ability to take a wildtype strain of fleas of interest and study them more closely in a laboratory setting is of interest. The WILD strain in this study is not a true, field-collected wildtype strain, but a continuous line of fleas reared on cats, and thus may be even more selected for specifically cat feeding than even field-collected wildtype strains. This potentially presents limits the options of flea strains that may be selected for studies utilizing the artificial model for future studies. Future attempts to utilize the artificial feeding system should collect fleas from a variety of animals to attempt to establish colonies.

The MEM strain readily fed on the artificial membrane and consistently produced eggs across the multiple generations. The average egg production of 0.48 eggs/female/day was lower than that when the second generation of MEM fleas was applied to cats, 3.28 eggs/female/day. This drop in production (14% of output compared to on-animal) is consistent with the literature. In fact, even the initial description of flea feeding on artificial membranes described the membrane-fed fleas only had 13-19% the reproductive output of fleas fed on cats (Wade and Georgi, 1988). Similar decreases in feeding and egg production have been described in ticks, where only 76% of ticks attached on membrane to feed compared to 100% on-animal, and only 41% of egg clusters hatched compared to 76 – 81% on-animal (Sebastian et al., 2023). While feeding and egg production may be diminished, this flea feeding apparatus is a viable option for the replacement of animals with its ability to easily scale to add more fleas or chambers to sustain a colony.

The second generation of MEM fleas successfully infested cats and produced viable eggs, which is key for maintaining a colony to be used for *in vivo* studies at a later date. The egg production of these fleas was 3.28 eggs/flea/day, which is similar to the regular colony maintenance of the WILD strain of 2.236 eggs/flea/day (Data unpublished).

A key for any ectoparasite colony is the ability to add new genetics into the colony to maintain vigor and more closely reflect wildtype organisms. In this study, the MEM fleas were co-fed with the WILD fleas and were able to successfully reproduce. While the WILD strain alone was not able to feed, this does at least present a viable option for continually updating a flea strain with new genetics.

During the experiments conducted in this study, fleas added to chambers were added at random and were not sexed. This means that when fifty fleas were placed in a chamber, the ratio of females to males was not known. This system could potentially produce more eggs/flea/day with a higher female to male ratio. Female fleas can lay up to sixty eggs/day, and a male flea can mate multiple times (Bourne et al., 2018). Therefore, it stands to reason that reason that the presence of more females in a given chamber presents the opportunity for higher egg production per chamber.

Conclusion

Overall, the 3D-printed apparatus successfully reared *Ctenocephalides felis* fleas through two complete generations, although it appears that there is some level of feeding adaptation needed to transition fleas fed on-animal to the membrane. In addition, artificially fed fleas were able to produce eggs when reared on cats. This shows promise for future research applications as flea colonies can be maintained *ex vivo* while maintaining the ability to be used in *in vivo* studies. More research is needed to optimize the flea feeding apparatus to increase feeding and reproduction success as well as determine methods for adapting fleas from animals to the membrane.

Chapter 3 - Pharmacokinetics of Fluralaner in Horses Following Oral or Topical Administration

Abstract

Horses routinely experience ectoparasite infestations including mites, lice, flies, and ticks. Ectoparasite control in horses is challenging as drugs available to horses on the market and not both long lasting and efficacious. The current study evaluates the pharmacokinetic parameters of fluralaner, a member of the isoxazoline drug class, when administered orally and topically to horses (*Equus caballus*). Eight horses were included in the study where four horses received an oral formulation of fluralaner while four horses received a topical formulation of fluralaner. Blood specimens were collected at pre-treatment and then at 1, 2, 4, 8, 12, 24, 48, and 72 hours and at 7, 14, 21, 28, and 35 days post-treatment. Plasma was separated after collection, and plasma fluralaner concentration was calculated using Ultra-Performance Liquid Chromatography with Electrospray ionization. The orally treated group had an average C_{max} ± STD of 363.5 ± 191.55 ng/ml and t_{max} ± STD of 10.5 ± 9.43 hours while the topically treated group resulted in an average C_{max} ± STD of 29.8 ± 13.1 ng/ml and t_{max} ± STD of 294 ± 160.85 hours. The C_{max} for orally treated horses was significantly higher than those treated topically (t=3.476, P = 0.0132) at a significantly earlier t_{max} (t = 3.519, P = 0.0125). This resulted in a significantly higher AUC of the horses treated orally compared to topically, AUC_{Oral}=28,718.2 and $AUC_{topical}$= 8,665.7, respectively (t = 5.9088, P = 0.0010). The horses administered oral fluralaner reached significantly higher plasma concentrations than the horses that received topical fluralaner, although the half-life of fluralaner in the orally treated group was shorter. More work is needed to determine appropriate drug concentrations of oral and topical fluralaner for the creation of a product for safe and effective ectoparasite control.

Introduction

Ectoparasites including mites, lice, flies, and ticks are a ubiquitous problem of horses around the world. These ectoparasites can cause skin irritation, pruritus, and have the ability to transmit a variety of pathogens which can negatively impact the host's quality of life. A variety of tick species are documented parasites of horse and pose the added concern of transmitting vector-borne pathogens such as *Babesia caballi* and *Theileria equi*, the causative agents of Equine Piroplasmosis (Duell et al., 2014; Rocafort-Ferrer et al., 2022). Although Equine Piroplasmosis is endemic in tropical climates, this is a disease that the United States is trying to mitigate establishment within the country's borders.

Currently, equine ectoparasite control consists of products including pyrethroids such as permethrin and organophosphates such as coumaphos – products with a re-application timeline as short as daily. Therefore, ectoparasite control in horses is challenging as there is not an efficacious, long-lasting drug on the market. Fluralaner is a drug of the isoxazoline class that selectively antagonizes GABA-gated chloride channels of arthropods leading to spastic paralysis and death (Gassel et al., 2014). This novel drug has shown prolonged plasma concentrations in dogs and cats following topical administration with mean $T_{1/2}$ of 21 ± 3 days and 12 ± 4 days respectively when administered at the labeled dose (Kilp et al., 2016). Currently, there are no published data on the pharmacokinetic parameters of fluralaner in horses. However, this drug has been administered to multiple species including dogs, cats, poultry, and bare-nosed wombats either orally or topically while reaching an efficacious concentration with a relatively wide safety margin (Kilp et al., 2016, Takano et al., 2023, Sari et al., 2022).

Furthermore, in a previous study in dogs, fluralaner was found to be at a plasma concentration of 17.7 ng/ml 84 days after oral administration of a dose of 25 mg/kg (Kilp et al., 2014). A different study in dogs evaluating dose confirmation for fluralaner showed a 99.0% effectiveness against Dermacentor variabilis (American Dog Tick) 86 days after drug administration (Bravecto® Freedom of Information Summary) at an oral dose of 25 mg/kg. As Dermacentor variabilis is a tick that also affects horses, it can be extrapolated that a plasma concentration of at least 17.7 ng/ml should be an initial target following equine administration of fluralaner. This would provide information on adjustments needed to be made to dosage or formulation to achieve tick control in horses.

Although the anatomy of the integumentary system is similar between mammalian species, there is a variance in thickness of the epidermis and dermis which may impact topical drug delivery (Song et al., 2023). There is a previous PK study repurposing a transdermal flunixin solution labeled for bovine use that was administered topically in horses where the drug reached anti-inflammatory concentrations in the horse (Knych et al., 2021). This shows potential for repurposing topically administered drugs labeled for other mammalian species to equine use.

The current study aims to evaluate pharmacokinetic parameters of fluralaner in horses following topical administration to determine the viability for future ectoparasite control. Based on pharmacokinetic studies performed in other species, it is hypothesized that fluralaner will reach and maintain the targeted plasma concentration of 17.7 ng/ml over the 35-day study.

Methods

Study Horses

All animal handling and sample collection were approved by the Kansas State University IACUC #4884. All horses were evaluated by an equine-focused veterinarian, received a Complete Blood Count (CBC) and serum chemistry, and underwent a 48-hour quarantine prior to treatment. A total of 8 horses were included in this study. Relevant medical information for each horse is provided in Table 3.1.

#	Breed	Age (y) / Sex	Weight	BCS	Medical condition	CBC/Chemistry performed on intake
1	Quarter Horse Dream	21 YO Mare	513.6 kg	4/9	Historic melanomas and poor body condition	Mild leukocytosis and mature neutrophilia consistent with a stress leukogram
2	Percheron Slim	20 YO Gelding	656.8 kg	5/9	Poor doer in winter	Mild neutrophilia consistent with a stress leukogram.
3	Percheron Shorty	21 YO Gelding	522.7 kg	4/9	Poor doer in winter	Hyperproteinemia, hyperglobulinemia.
4	Quarter Horse Pie	23 YO Mare	454.5 kg	4/9	Infertility	Mild leukocytosis and mature neutrophilia consistent with a stress leukogram.
5	Quarter Horse Six	23 YO Mare	554.5 kg	3/9	Poor body condition	No significant abnormalities
6	Quarter Horse Sister	25 YO Mare	395.5 kg	2/9	Poor body condition	Mild hypoalbuminemia
7	Quarter Horse Barbara	22 YO Mare	377.3 kg	2/9	Poor body condition	Leukocytosis, mature neutrophilia, monocytosis, hyperfibrinogenemia consistent with inflammation and/or infection.
8	Quarter Horse Jackie	20 YO Mare	454.5 kg	4/9	Infertility. Reactive granulation tissue mass on left hind fetlock region. Severe Lymphangitis of both hind limbs from hock distal.	Leukocytosis, mature neutrophilia, mild anemia, hyperfibrinogenemia, consistent with inflammation and/or infection. Mild hypoalbuminemia.

Table 3.1: Medical information for study horses

Treatments

All horses were fasted for 12 hours prior to treatment but allowed access to hay immediately after dosing . Four horses received topical treatments of Bravecto® Topical Solution (Elanco Animal Health; Greenfield, IN, USA) as close as possible to 10 mg/kg using a 5 ml syringe to draw up and dispense the liquid. Four horses received oral treatments using a combination of Bravecto® Chews (Elanco Animal Health; Greenfield, IN, USA) to achieve a dose as close as possible to 10 mg/kg. Oral Bravecto® was only administered as whole chews and no halved chews were used to avoid inaccurate dosing from nonuniform drug distribution within the chew. For the oral dose, a nasogastric tube was placed for each horse. The chews for each horse were crushed into approximately 0.5 L of water and pumped into the stomach via the nasogastric tube. The bucket was rinsed with approximately 17 L of water which was also pumped into the horse post-treatment.

Table 3.2: Horse dosing information

Horse	**Route**	**Dose in mg (mg/kg)**	**Volume of Bravecto® Topical Solution (280 mg/ml)**	**Number and type of Bravecto® Chews**
Horse 1 Dream	Topical	5152 mg (10.03)	18.4 ml	NA
Horse 2 Slim	Topical	6580 mg (10.02)	23.5 ml	NA
Horse 3 Shorty	Topical	5236 mg (10.02)	18.7 ml	NA
Horse 4 Pie	Topical	4564 mg (10.04)	16.3 ml	NA
Horse 5 Six	Oral	5600 mg (10.10)	NA	Four 1400 mg chews
Horse 6 Sister	Oral	4200 mg (10.62)	NA	Three 1400 mg chews

Horse 7 Barbara	Oral	3800 mg (10.07)	NA	Two 1400 mg chews Two 500 mg chews
Horse 8 Jackie	Oral	4700 mg (10.34)	NA	Three 1400 mg chews One 500 mg chew

Blood Collection

Blood specimens were collected from the jugular vein in BD Vacutainer™ Plastic Blood Collection Tubes with K2 EDTA: Hemogard™ Closuretubes (Fisher Scientific) pre-treatment and at 1, 2, 4, 8, 12, 24, 48 and 72 hours and at 7, 14, 21, 28 and 35 days post-treatment. Whole blood samples were centrifuged at 3000 RPM for 10 minutes, and plasma was aliquoted into 2 ml cryogenic storage tubes and stored at -80°C until pharmacokinetic analysis.

Pharmacokinetic Analysis

Fluralaner standard was purchased from Cayman Chemical (Ann Arbor, MI, USA). Fluralaner-d4 was purchased from Clinivex® (Scarborough, Ontario, Canada). LC/MS grade methanol and acetonitrile were purchased from Fisher Scientific (Waltham, MA, USA). The fluralaner and fluralaner-d4 stock solutions were prepared in DMSO at 1 mg/mL and stored at -80°C freezer.

The stock solutions were moved out of the – 80°C freezer and allowed to back to room temperature before use. For daily analysis, the fluralaner stock solution was diluted with the negative horse plasma to achieve a fluralaner concentration of 100 µg/mL. Subsequently, the above fluralaner solution was further diluted to get a set of concentrations at 0.25, 0.5, 1, 2.5, 5, 25, 50, 250, and 500 ng/mL in the negative horse plasma.

Fluralaner-d4 was used as an internal standard for fluralaner. The fluralaner-d4 stock solution was diluted in methanol to get concentration at 10 µg/mL and then diluted in 4% phosphoric acid at 50 ng/mL. Quality controls (QC) were prepared daily in the negative horse plasma at 5, 10, 100 ng/mL.

The horse plasma samples, calibrations, blank (negative horse plasma), and QC were extracted and cleaned-up by solid-phase extraction (SPE) using Oasis HLB Prime µElution 96-wells plate (Waters Co, Milford, MA) (Elliot et al., 2024). Briefly, 100 µL of the horse plasma sample, 50 µL of the internal standard solution (fluralaner-d4), and 150 µL of 4% phosphoric acid were added to a 48-well non-tissue culture-treated plate (Corning Incorporated, Corning, NY), respectively. The 48-well plate was shaken gently at 350 rpm for 20 min at room temperature with the IKA MTA 2/4 Shaker (IKA® Works Inc., Wilmington, NC). Next, the 48-wells plate was centrifuged at 2,000 × g for 30 min at 20°C (Thermo Scientific ST16 Benchtop Centrifuge, Waltham, MA). The supernatant was transferred to the wells of the Oasis HLB Prime µElution 96-wells plate. Following a wash with 300 µL of 5% methanol and both the target component and internal standards were eluted with 50 µL 90/10 acetonitrile/methanol. 50 µL of 10 mM ammonium acetate solution was added onto each well of the 96-wells square collection plate (Waters Co, Milford, MA) and then was shaken gently at 300 rpm for 3 min at room temperature with the IKA MTA 2/4 Shaker before the UPLC-MS/MS analysis.

The analysis was carried out by UPLC–ESI–MS/MS instrument, consisted of a Waters Acquity Ultra Performance LC with a Waters column manager and heater/cooler, binary system manager, sample manager coupled to a Waters Xevo TQ-Absolute triple quadrupole mass spectrometer equipped with electrospray ionization (ESI) (Waters Acquity Ultra Performance LC, Xevo TQ-A MS/MS, Waters Co., Milford, MA, USA). An Acquity UPLC C18 column (1.8 µ, 2.1 x 100 mm) was held at 40°C with eluents composed of mobile phase A (10 mM of ammonium acetate) and mobile phase B (acetonitrile). The flow rate was 0.4 mL/min. Following gradient program was used: from 0-0.2 min phase A (30%), at 0.2 min phase A (30%) and at 3.0 min phase A (10%), at 3.10 min phase A (30%) and hold for 4.90 min 0.99. Total run time was 8 min. The triple quadrupole mass spectrometer was Xevo TQ-A MS/MS equipped with an electrospray ionization (ESI) interface. For fluralaner analysis, the ionization mode used was negative ionization (– ESI). The operating parameters for the mass spectrometer were as follows: capillary voltage was -2.80 kV, source and desolvation temperatures were 150°C and 600°C, respectively. The source and desolvation temperatures were 150°C and 600°C, respectively. The cone energy was set at 71V for fluralaner and fluralaner-d4. Nitrogen was used as the desolvation gas and cone gas. Helium was used as the collision gas. The collision gas flow was 0.15 mL/min. Desolvation and cone gas flow were 1000 and 150 L/h, respectively. The data acquisition and quantification were performed using the Waters MassLynx and TargetLynx software 4.1, respectively.

The developed method was validated in reference to the FDA guidelines for the Bioanalytical Method Validation Guidance for Industry (2018). Ions were monitored in the multiple reaction monitoring (MRM) mode with transitions at m/z 554.16 → 494.088 (qualifier), 554.16 → 534.12 (quantifier) for fluralaner, m/z 558.16 → 497.243 (qualifier), 558.16 → 538.12 (quantifier) for fluralaner -d4. For fluralaner quantification, the calibration curve was linear from 0.25 to 500 ng/mL with the correlation coefficient R2 at least 0.99. The lower limit of quantitation (LLOQ) for fluralaner was 0.25 ng/mL. The inter-day precision was 4.69%, 9.28%, and 7.96% at 5, 10 and 100 ng/mL, respectively. The accuracy was 92.58%, 95.60% and 100.33% at 5, 10 and 100 ng/mL, respectively. The validation results of fluralaner spiked in horse plasma is shown in the Supplemental Table 5.1 in Appendix A.

Analysis of the pharmacokinetic data was calculated using PKcalc package from Plotly (Montreal, Quebec, CA).

Results

Four horses were enrolled into the topical treatment group, receiving an average dose of 10.03 mg/kg (Range = 10.02 mg/kg – 10.04 mg/kg) (Table 3.2), and four horses were enrolled into the oral treatment group, receiving an average dose of 10.28 mg/kg (Range = 10.07 mg/kg – 10.28 mg/kg) (Table 3.2).

All horses treated orally with fluralaner at 10 mg/kg had detectable levels of fluralaner in the plasma by 1-hour post-treatment. Plasma concentrations increased over time reaching a mean C_{max} = 363.5 ng/ml (Range = 201.3 – 600.9 ng/ml) by an average T_{max}= 10.5 hours (Range = 2 – 24 hours) (Table 3.3). Two horses, O-1 and O-2, had higher plasma concentrations and once C_{max} was reached there was one defined peak followed by a steady decline over time and both had no detectable fluralaner in their plasma by Day 14. The other two orally-treated horses, O-3 and O-4, had overall lower C_{max} that peaked and plateaued for 24 – 48 hours before decreasing. While these two had detectable fluralaner longer, Day 21 and Day 28 respectively, the later concentrations were < 4 ng/ml after D7. The mean $t_{½}$= 77.55 hours with a major outlier of O-4, whose $t_{½}$= 186.9 hours.

Table 3.3: Pharmacokinetic Parameters

Parameter	Oral 10mg/kg n = 4	Topical 10mg/kg n = 4
C_{max} (ng/ml) ± STD (Range)	363.5 ± 191.55 (201.3 - 600.9)	29.8 ± 13.1 (14.3 - 46.4)
t_{max} (hours) ± STD (Range)	10.5 ± 9.43 (2 - 24)	294 ± 160.85 (168 - 504)
$AUC_{(0\rightarrow t)}$ (hours*ng/ml) ± STD (Range)	28718.2 ± 6626.8 (21607.3 – 37487.6)	8665.7 ± 1467.4 (7031.1 – 9923.9)
$AUC_{(0\rightarrow \infty)}$ (hours*ng/ml) ± STD (Range)	29077.5 ± 6870.8 (21885.1 – 38321.7)	8758.0 ± 1436.8 (7122.7 – 9997.6)
$t_{1/2}$ (hour) ± STD (Range)	71.5 ± 77.55 (24.4 – 186.9)	84.3 ± 18.3 (67.2 – 110.2)

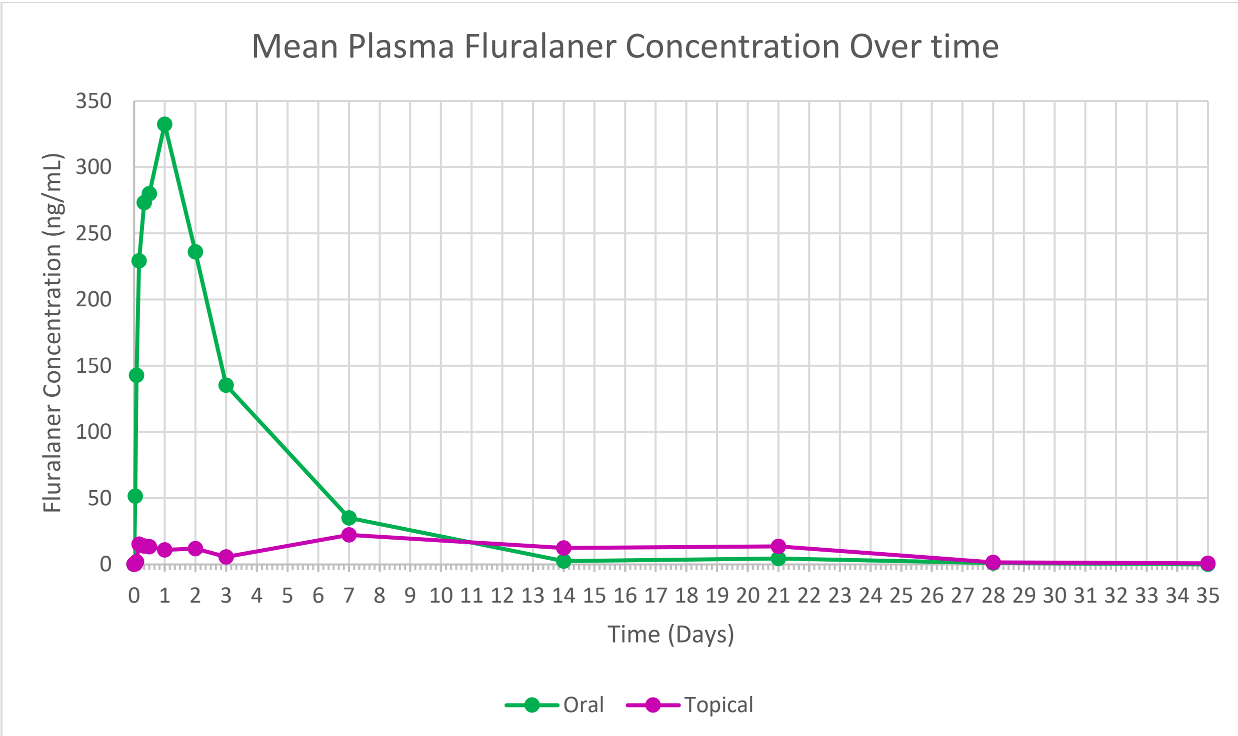
Topical Dose

Three of the four horses treated topically with fluralaner had detectable plasma concentrations within 1 hour post-treatment, while the final horse (T-4) did not have detectable levels until 4 hours. The average C_{max} for the 4 horses treated with topical fluralaner was 29.8 ng/ml at an average T_{max} = 294 hours (12.3 days) (Table 3.3). Three of the horses (T-1, T-2, T-3) had an earlier peak between 4 – 24 hours that plateaued until T_{max} was reached, while the fourth horse (T-4) had a low plasma concentration (< 2 ng/ml) for the first 14 days followed by a single peak at Day 14. The mean t½ for horses treated topically with fluralaner was 84.3 hours.

Comparison

The C_{max} for orally treated horses was significantly higher than those treated topically (t=3.476, P = 0.0132) at a significantly earlier t_{max} (t = 3.519, P = 0.0125). This resulted in a significantly higher AUC between the horses treated orally compared to topically, AUC_{Oral}=28,718.2 and $AUC_{topical}$= 8,665.7, respectively (t = 5.9088, P = 0.0010). There was no significant difference in the t½ between the orally and topically treated horses (t = 0.3213 hours, P = 0.7589).

Figure 3.1: Depiction of fluralaner concentration over time in both the oral and topical study horses



Discussion

Two prior studies of fluralaner use in horses have been documented. The first is a study of 10 horses naturally infested with *Amblyomma cajennense* and *Rhipicephalus microplus* ticks and treated at 25 mg/kg (Romero Núñez et al. 2023). Tick numbers were reduced >94% by day 14 post-treatment, and no ticks were recovered between Day 30 and Day 90. This study does not report on the route of treatment (topical vs. oral) and does not describe if the horses were reinfested with ticks or were in areas where they would be re-exposed to ticks over the study period (Romero Núñez et al. 2023). The second report is a study presented of 6 horses treated orally with fluralaner at 10 mg/kg (Lehman, 2024).

All 8 horses were successfully treated with fluralaner, either orally or topically, and completed the full 35-day study. All horses had a rapid uptake of fluralaner with detectable concentrations in the plasma by 1 hour in 7/8 of the horses, with the final horse having detectable levels by 4 hours.

The orally treated group had a rapid increase in the fluralaner plasma concentrations that resulted predominantly in a single C_{max} peak that decreased over time. Horse O-4 had a longer $t_{½}$ than the other orally treated horses, and that skewed the mean $t_{½}$ for the group from 33 hours (O-4 excluded) to 71.5 hours (O-4 included). This seems to represent the trend slightly better as the product appeared to rapidly peak and then be rapidly cleared. All orally treated horses were negative by Day 35 and had concentrations below expected therapeutic levels (<17 ng/ml) by Day 14. The large variation in C_{max} between the orally treated group may be due to natural physiologic variation between horses or differences in diet. A previous description of oral fluralaner in horses reported a similar C_{max} of 162.1 ng/ml at 0.42 days and a half-life of ~6 days. These are comparable to the results seen in the orally treated horses from this study. In comparison, average C_{max} fluralaner concentrations in dogs treated orally at 12.5mg/kg were 2,144 ng/ml, peaked 1-day post-treatment and had a half-life of 13 days (Kilp et al., 2014). The overall plasma concentrations seen in orally treated horses would likely be effective in controlling active ectoparasite infections in horses, although it would not provide the same extended ectoparasite control of 1 month that is seen with orally administered fluralaner products for dogs (Kilp et al., 2014).

The topically treated group appeared to have a slower absorption of the product with an initial peak happening between 4 – 12 hours in three of the horses followed by the true C_{max} later between 7 – 12 days. This peak and plateau are seen in dogs treated topically with fluralaner, although the drug concentrations are much lower in the horses. Dogs treated topically at 12.5mg/kg had an average C_{max} of 358 ng/mL with a half-life of 17 days, both of which are significantly lower in horses from this study (Kilp et al., 2016). Given that the C_{max} was low in the topical treatments, much higher doses might be needed to achieve effective ectoparasite control. In dogs, doubling the topical dose applied increased the C_{max} by roughly double as well, while maintaining a similar t½, 17 – 21 days (Kilp et al., 2014). Increasing the dose applied may push the plasma concentrations to therapeutic levels, but it is unlikely to solve the shorter half-life issue that would preclude the use of a fluralaner product from being effective for a full month.

Limitations

This pilot study utilized horses that were opportunistically enrolled as they became available. Therefore, there were a variety of breeds, body condition scores, and pre-existing medical conditions that may affect the drug pharmacokinetics. The low body condition score of the horses has an increased likelihood of influencing the results of the study as fluralaner is a highly lipophilic drug; therefore, the volume of distribution of the fluralaner in horses with low fat percentage is likely to be decreased. However, this study did not utilize an intravenous (IV) administration. Therefore, the volume of distribution and the true bioavailability of the fluralaner could not be calculated.

Adverse Events

Despite all 8 horses completing the full duration of the study, three had medical issues following fluralaner treatment. One horse (Topical-2 Slim) experienced colic signs 7.5 hours post-treatment and was treated with xylazine, flunixin meglumine, and mineral oil. Colic signs resolved within 4 hours, and the horse was able to complete the study. One horse (Oral – 2 Barabara) experienced a choke issue and loose stool which was determined to likely be due to poor dentition and parasitism. The horse was treated with xylazine, detomidine, flunixin meglumine, gentamicin, procaine penicillin G, and ivermectin. The choke resolved easily, and the horse was able to complete the study. The third horse (Oral-1 Sister) had loose stool that was determined to be likely due to parasitism. This was treated with ivermectin and the signs resolved within 48 hours. Each of these adverse events was attributed to old age and pre-existing conditions.

Conclusions

This is the first published study evaluating the pharmacokinetic parameters of fluralaner, or any isoxazoline, given both orally and topically in horses. The oral fluralaner reached significantly higher plasma concentrations than the topical route, although it had a shorter half-life. Further studies with healthy horses evaluating a variety of dosages are necessary to fully determining both the safety and likely efficacy of fluralaner against ectoparasites.

Chapter 4 - Assessment of lotilaner (Credelio® CAT) for control of in-home *Ctenocephalides felis* infestations

Sutherland, C., Tomlinson, T., Wilson, G. et al. Assessment of lotilaner (Credelio® CAT) for control of in-home *Ctenocephalides felis* infestations. *Parasites Vectors* 18, 462 (2025). https://doi.org/10.1186/s13071-025-06888-8

Abstract

Flea infestations remain a major issue in veterinary medicine. Highly effective flea control for dogs and cats remains the foundation for eliminating infestations from homes and improving skin conditions associated with flea-feeding. Homes with pet cats were screened by flea-history questionnaire. Qualifying homes were subselected into "high" (≥ 5 fleas on ≥ 1 cat, and ≥ 5 fleas collected in environmental flea traps over a 16–24 h period), "low" (< 5 fleas on all cats, < 5 in traps), and "no" homes (no evidence of fleas on cats or traps). All cats and dogs in a household were treated with a lotilaner oral tablet (Credelio® CAT and Credelio®, respectively) in weeks 0, 4, and 8. On-animal and trap counts were performed for: "high" at weeks 0, 1, 2, 4, 6, 8, and 11–12; "low" at week 0 and at approximately 2-week intervals through week 11–12; and "no" only at week 0. During each visit, one owner completed a pruritus assessment (PVAS) and a veterinary dermatologist assessed dermatologic lesions using the feline allergic dermatitis (SCORFAD) scale. A total of 46 homes met inclusion criteria and completed the study: 19 "high" (35 cats); 17 "low" (27 cats); and 10 "no" (14 cats). By week 1, relative to pretreatment, there was a 99.3% reduction in flea counts on "high" cats, with 31 of 34 cats (91.2%) flea-free. By week 11–12, flea counts across all study cats and traps were zero. Prior to the first treatment, mean PVAS scores were: "high" 6.6; "low" 5.5; and "no" 1.9. By week 1 there was a significant

decrease in mean PVAS score of cats from “high” homes to 2.9 ($P < 0.0001$), and mean week 11–12 scores were 0.5 and 0.8 for “high” and “low” homes, respectively. For SCORFAD, by week 11–12, relative to week 0, there was a significant decline in mean scores of cats from both “high” (8.0 to 1.7) ($P < 0.0001$) and “low” homes (3.3 to 0.9) ($P < 0.0001$). Lotilaner was 100% efficacious in eliminating flea infestations from animals and their homes. The monthly lotilaner treatments of cats and dogs in flea-infested homes resulted in clinical resolution of pruritus and dermatologic lesions.

Introduction

The cat flea, *Ctenocephalides felis*, is a blood-feeding ectoparasite that can infest a wide variety of mammals, including dogs, cats, and humans (Blagburn and Dryden, 2009). Blood loss arising from heavy flea infestation can result in iron-deficiency anemia (Blagburn and Dryden, 2009). Flea allergy dermatitis (FAD), a hypersensitivity response to antigenic material from flea saliva of the cat flea, is a common cause of pruritic dermatitis in dogs and a major contributor to miliary dermatitis in cats (Blagburn and Dryden, 2009, Santoro et al., 2021, and Bond et al., 2007). Although recent studies have suggested an association between flea burden and lesions on the ventral abdomen and cervical regions in cats, there is not a recognized pathognomonic reaction pattern for FAD in cats as there is with the dorsal tail base in dogs (Sampeck et al., 2024, McAuliffe et al., 2022). Such pruritic skin diseases can impair the quality of life of pets, and of their owners, with potential undermining of the human–animal bond (McAuliffe et al., 2022, Linek et al., 2010, Bruet et al., 2022, Noli, 2019). Fleas can also transmit bacterial pathogens (including *Rickettsia felis* and *Bartonella* spp.), and act as intermediate hosts of other parasites (*Dipylidium caninum* and *Acanthocheilonema reconditum*) (Otranto et al., 2013, Abdullah et al., 2019, Minahan et al., 2023).

Historically, cat flea infestations have been challenging to clear from a home due to the continued emergence of adult fleas from the environment and inadequate efficacy of premise sprays. It has now been shown that home infestations can be controlled by treating all pets with a highly effective product that meets success criteria: killing fleas before these can lay eggs (within 24 h of infesting a host) and a speed of flea kill that is then sustained throughout the entire dosing period (Dryden et al., 2013, 2015, 2016, 2017, 2018, 2019, 2020). Fleas emerging from the environment are then killed when finding a treated host, before egg-laying can begin. In addition to eliminating egg-laying, expedient product efficacy is desirable to reduce the allergen load associated with flea burden. These treatments must be continued until the environmental burden of fleas has been eliminated, typically at least 2–3 months (Dryden et al., 2013, 2015, 2016, 2017, 2018, 2019, 2020). Stopping treatment before environmental flea stages are eliminated will result in reinfestation of the animals and subsequently the home. Similarly, without a sustained quick speed of kill throughout the recommended treatment interval, fleas will be able to lay eggs before dying, contaminating the environment with new eggs. Reduced residual speed of kill may be the first sign of a product's inability to control fleas on cats and dogs, which has historically been documented via post-approval assessment of insecticides through in-home, natural infestations (Bruet et al., 2022, Dryden et al., 2013). Thus, in-home evaluation of canine and feline flea control products has become the gold standard for the continual monitoring of product efficacy to confirm a drug class's ability to continue to kill fleas rapidly throughout the entire dosing period (Dryden et al., 2013, 2017, 2019, 2020).

The isoxazoline drug class has demonstrated efficacy at eliminating home cat flea infestations due to a rapid residual speed of kill (Dryden et al., 2015, 2016, 2017, 2018, 2019, 2020). This drug class primarily works to inhibit GABA-gated chloride channels of invertebrates, leading to spastic paralysis and death (Rufener et al., 2017). Lotilaner is an isoxazoline, and the active ingredient in Credelio® CAT (Elanco Animal Health; Greenfield, IN) and Credelio® for dogs and one of the active ingredients in Credelio® Quattro (Elanco Animal Health; Greenfield, IN) for dogs. A previous study showed that home infestations with *C. felis* could be controlled by treating all dogs with lotilaner at monthly intervals for 3 months (Dryden et al., 2015). In a field study, flea counts of 228 cats, each infested with at least five fleas, were reduced by 98.3, 99.9, and 99.9% on days 30, 60, and 90, respectively, following lotilaner treatment on days 0, 30, and 60 (Chappell et al., 2021). The current study was designed to build on that finding by investigating whether three consecutive monthly lotilaner treatments of flea-infested cats could eliminate household flea infestations with resulting reductions in pruritus and resolution of skin lesions.

Methods

Inclusion Criteria

Residential homes in the Tampa area on Florida's Gulf Coast were recruited in May–June of 2022 through veterinary referrals and Facebook advertisements. Each home was evaluated for inclusion in the study on the basis of a screening questionnaire concerning pet-relevant activities, including time spent indoors and history of flea infestations and control measures. To qualify for enrollment, all household cats and dogs had to spend at least 12 hours indoors per day, as reported by the owner, and have no history of flea control measures within 1 month beyond the labeled efficacy duration for the product, and no reported premise treatment within 6 months prior to enrollment. Pet owners had to agree to in-home visits by study staff over the 12-week study. All cats in the home had to be at least 8 weeks old and weigh at least 1 kg, and all dogs at least 8 weeks of age and at least 2 kg. Each enrolled home could have up to ten dogs and cats in total, but no more than five animals were enrolled for data collection. Any dogs or cats not enrolled but residing within the household were treated with the appropriate lotilaner product on the same schedule as any cat that was enrolled. All animal handling and treatment procedures were reviewed and approved by Kansas State University IACUC #4704 and Kansas State University IRB#: 11,060.

Enrolled homes were categorized as “high” homes, “low” homes, and “no” homes according to the number of fleas present on cats and in environmental traps. For “high” homes, selection criteria included at least five fleas on at least one cat and; at least five fleas in total collected on environmental traps over a 16–24 h period. For “low” homes, the number of fleas on a cat or on environmental traps was lower than five but greater than 0. For “no” homes, no fleas were found on any animals or environmental traps. Any home with dogs or cats that were pregnant, nursing, fractious, or had a history of seizures was excluded from the study. All owners, regardless of study group, signed an informed consent form and questionnaire pertaining to the history of the animals and the home.

Flea Population Assessment

The flea population of every cat enrolled in the study for data collection was assessed using a flea combing technique described in a previous study (Dryden et al., 1994). For pathogen testing at a later date, a subset of fleas was removed from cats with ≥ 10 fleas and the remainder were returned to the animal: for cats with 10–14 fleas, 3 were removed; 15–24 fleas, 6 removed; 25–49 fleas, 9 removed; ≥ 50 fleas, 12 fleas removed.

The environmental flea population in each participating home was assessed using validated, intermittent light traps (Dryden and Broce, 1993). Two traps were placed in separate rooms for a 16–24 h period. The trap locations were selected on the basis of owners' observations of where animals spent most of their time and placed in the same location for each subsequent collection. Fleas collected on the adhesive pads of the traps were counted and morphologically identified.

The on-animal flea counts and flea-trap placements were conducted for "high" homes prior to treatment in week 0, and in weeks 1, 2, 4, 6, 8, and 11–12; for "low" homes in weeks 0, 2, 4, 6, 8, and 11–12; and for "no" homes in week 0 only.

Evaluation of Skin Disease

During each collection period, the same owner completed a pruritus visual analog scale (PVAS) on a nonnumeric data capture form for each enrolled cat (Dryden et al., 2018). The PVAS is a validated scale that assesses the owner's impression of pruritus for dogs (Hill et al., 2007, Rybnícek et al., 2009). While not validated for cats, previous publications have used such a scale to assess feline pruritus (Dryden et al., 2018, 2020, Noli et al., 2019). The pet owners were blinded to the number scale (0 – 10) that was subsequently superimposed over the form to numerically assign a pruritus level.

A board-certified veterinary dermatologist assessed dermatologic lesions on every cat in the home using the scoring feline allergic dermatitis (SCORFAD) scale score for ten different anatomical regions in weeks 0, 4, 8, and 11–12 (Steffan et al., 2012). These anatomical regions were assessed for excoriations, miliary dermatitis, eosinophilic plaques, and self-induced alopecia on a 0–4 scale.

Treatments

All cats and dogs in the household were treated with a lotilaner oral tablet, Credelio® CAT and Credelio®, respectively (Elanco Animal Health, Greenfield, IN, USA) at weeks 0 (day 0), 4, and 8 according to product label instructions. All animals were offered food by the owner 30 min before treatment. The dosing for each animal was conducted on a dose-banded system according to the product labels, and animals were weighed on a calibrated scale before each dose administration. This resulted in a minimum lotilaner dosage of 20 mg/kg and 6 mg/kg to dogs and cats, respectively. All animals were treated either directly by or under the direct supervision of study staff following product label directions. All animals that received lotilaner treatment were monitored for adverse events. Any adverse event that occurred was recorded and the relationship to treatment evaluated. No other topical or systemic products were used to address dermatologic lesions, inflammation, or secondary bacterial infections associated with flea feeding.

Data Analysis

The outcomes of interest in this study included flea counts on animals, flea counts on traps, PVAS scores, and SCORFAD assessments. The percentage changes in flea burdens from pretreatment counts in week 0 were calculated on the basis of the geometric means, and the changes in PVAS and SCORFAD between pre- and posttreatment assessments were compared using arithmetic means. Geometric means for flea and trap count data were calculated in R by means of the ln (count + 1) transformation using the “psych” package (Revelle, 2024).

Spearman's correlation test was also performed in R and used to assess the relationship between flea counts and PVAS scores, and PVAS and SCORFAD scores within "high" and "low" homes.

Results

Homes

From 64 homes that were initially visited, 55 met the inclusion criteria, of which 46 were included in the analysis dataset: 19 "high," 17 "low," and 10 "no" homes. Two homes in the "high" category were excluded from the analysis, one because of the introduction of flea-infested kittens too young for treatment and the other because changing home circumstances prevented completion of study procedures. Seven "low" category homes were excluded from the analysis, either because the owners could not adhere to the schedule or because the study cats could not be found. No homes were removed due to the presence of fleas or adverse events. Of the 17 "low" homes, at the enrollment visit 12 had at least 1 cat with 5 or more fleas but fewer than 5 fleas in traps placed in the home. The remaining five "low" homes had fewer than five fleas counted on any cat and collected in traps.

Animals

Across the 46 qualifying homes for data collection, 76 cats were included in the study calculations: 35 cats in the "high" home category, 27 in the "low", and 14 in "no" homes. Including the additional household pets that were not enrolled for data collection, 146 cats and 69 dogs were treated with Credelio® CAT and Credelio®, respectively. The Credelio® CAT dosages averaged 10.5 mg/kg (range 6.2–22.2 mg/kg) across all cats treated, and the Credelio® dosage averaged 29.0 mg/kg (range 20.2–50.8 mg/kg) across all dogs treated. Treatments were well tolerated by dogs and cats, and there were no treatment related adverse events.

Flea Counts

The week 0 geometric mean flea count on study cats in "high" homes was significantly greater than on cats in "low" homes (W = 757; $P < 0.001$) (Table 4.1). By week 1, relative to mean pretreatment counts, there was a 99.3% reduction in fleas counted on "high" home study cats, and 91.2% of these cats (31/34) were flea-free. Between week 0 and week 11–12 there was a complete (100%) and statistically significant ($V_H = 496$, $V_L = 253$; $P < 0.001$) reduction in flea counts in both the "high" and "low" home categories.

Table 4.1: Descriptive Statistics Summary of on-cat flea counts in study cats

	Week of Visit							
	0	1	2	3	4	6	8	11 - 12
High-flea homes								
Geometric mean	12.7*‡	0.1‡	0.2‡	0.2‡	0.2‡	0.0‡	0.0‡	0.0‡
(Standard deviation)	(10.1)	(0.6)	(0.7)	(1.0)	(0.5)	(0.0)	(0.2)	(0.0)
Arithmetic mean	15.2	0.2	0.4	0.3	0.2	0.0	0.0	0.0
95% CI	9.1-16.0	0.0-0.3	0.0-0.5	0.0-0.5	0.0-0.3	0.0-0.0	0.0-0.1	0.0-0.0
Range	5 - 44	0 - 3	0 - 2	0 - 4	0 - 2	0 - 0	0 - 1	0 - 0
% Reduction compared to Week 0[a]	-	99.3	98.2	98.7	98.7	100.0	99.8	100.0
Low-flea homes								
Geometric mean	3.9*†		0.0†		0.0†	0.0†	0.0†	0.0†
(Standard deviation)	6.7		(0.0)		(0.0)	(0.0)	(0.0)	(0.0)
Arithmetic mean	6.4		0.0		0.0	0.0	0.0	0.0
95% CI	1.6-6.7		0.0-0.0		0.0-0.0	0.0-0.0	0.0-0.0	0.0-0.0
Range	0 - 33		0 - 0		0 - 0	0 - 0	0 - 0	0 - 0
% Reduction compared to Week 0[a]	-		100.0		100.0	100.0	100.0	100.0

CI confidence interval

High-flea homes visited on all shown weeks; Low-flea homes not visited on weeks 1 and 3; no-flea homes not included in table

[a]On the basis of geometric means

*Between-group (high–low) difference significant $P < 0.001$; ‡Mean flea-count reduction significant ($P < 0.001$) between week 0 and all other weeks

†Mean flea-count reduction significant ($V = 253$; $P < 0.001$) between week 0 and all other weeks

The week 0 geometric mean flea count in environmental flea traps for "high" homes was significantly different from the count in "low" homes (V = 190; $P < 0.001$) (Table 4.2). By week 1, in "high" homes there was an 82.3% reduction (V = 171; $P < 0.001$) in geometric mean flea-trap counts and by week 3 the reduction was 94.0% (V = 171; $P < 0.001$) (Table 2). Between week 0 and week 11–12 there was a complete (100%) and statistically significant reduction in flea-trap counts in both the "high" (V = 171; $P < 0.001$) and "low" home categories (V = 36; $P = 0.010$).

Table 4.2: Descriptive statistics summary of flea counts from household flea traps

	Week of Visit							
	0	1	2	3	4	6	8	11 - 12
High-flea homes								
Geometric mean	27.4*‡	4.8‡	3.4*‡	1.6‡	0.9*‡	0.2‡	0.1‡	0.0‡
(Standard deviation)	(85.9)	(12.1)	(30.1)	(20.2)	(7.5)	(1.7)	(0.4)	(0.0)
Arithmetic mean	50.5	10.3	14.4	6.6	2.7	0.5	0.2	0.0
95% CI	9.1-91.9	4.4-16.1	8.6-20.2	0.0-17.2	0.0-6.3	0.0-1.3	0.0-0.4	0.0-0.0
Range	7 - 387	0 - 33	0 - 114	0 - 89	0 - 33	0 - 7	0 - 1	0 - 0
% Reduction compared to Week 0[a]	-	82.6	87.6	94.0	96.9	99.2	99.6	100.0
Low-flea homes								
Geometric mean	0.6*†		0.1*†		0.2*†	0.0†	0.4†	0.0†
(Standard deviation)	1.6		(0.7)		(1.4)	(0.0)	(0.3)	(0.0)
Arithmetic mean	0.7		0.3		0.6	0.0	0.1	0.0
95% CI	0.2-1.1		0.0-0.6		0.0-0.7	0.0-0.0	0.0-0.2	0.0-0.0
Range	0 - 3		0 - 1		0 - 1	0 - 0	0 - 1	0 - 0
% Reduction compared to Week 0[a]	-		79.3		62.9	100.0	93.4	100.0

CI confidence interval

[a]On the basis of geometric means

High-flea homes visited on all shown weeks; low-flea homes not visited in weeks 1 and 3; no-flea homes not included in table

*Between-group (high–low) difference significant $P < 0.001$; ‡Mean flea-count reduction significant between week 0 and all other weeks ($P < 0.001$)

†Mean flea-count reduction significant between week 0 and all other weeks ($P = 0.010$)

Pruritus visual analog scale (PVAS)

Prior to the first treatment, the arithmetic mean PVAS score for cats from “high” homes was 6.6, for cats from “low” homes 5.5, and for cats from “no” homes 1.9 (Table 4.3). At this point there was no significant difference between the mean PVAS score of cats from “high” and “low” homes ($W = 593$; $P = 0.088$), but mean scores of cats from both “high” and “low” homes were significantly higher than cats from “no” homes (1.9) ($W_H = 457$, $W_L= 342$; $P < 0.0001$) (Table 3). By week 1, relative to week 0, there was a significant decrease in mean PVAS score of cats from “high” homes ($V = 600.5$; $P < 0.0001$). By week 11–12, the arithmetic mean PVAS score for cats from “high” homes was not significantly different from the score of cats from “low” homes ($W = 291$; $P = 0.067$). The week 11–12 mean PVAS scores for “high” and “low” cats were both significantly lower than the mean PVAS score of “no” home cats (i.e., flea-free cats) reported at the start of the study ($W_H = 59$, $W_L= 90.5$; $P < 0.05$), as well as significantly different from their respective week 0 values ($V_H = 461$, $V_L= 378$; $P < 0.0001$).

Table 4.3: Descriptive statistics summary of PVAS of study cats scored by cat owners

	Week of visit							
	0	1	2	3	4	6	8	11 - 12
High-flea homes								
Mean (SD)	6.6 (2.5)[a‡]	2.9 (1.7)[‡]	2.3 (2.2)[‡]	1.5 (2.2)[‡]	1.3 (1.6)[‡]	1.2 (1.3)[‡]	0.6 (0.8)[‡]	0.5 (0.9)[∞ ‡]
Median	6.2	2.5	1.8	0.4	0.7	0.4	0.3	0.2
95% CI	5.7-7.5	2.3-3.5	1.6-3.1	0.7-2.3	0.5-1.9	0.7-1.7	0.2-1.0	0.1-0.8
Range	0 – 9.8	0 – 5.2	0 – 9.7	0 – 9.6	0 – 6.5	0 – 3.7	0 – 2.4	0 – 4.9
Low-flea homes								
Mean (SD)	5.5 (2.3)[a†]		2.0 (1.7)[†]		1.7 (1.3)[†]	1.5 (1.5)[†]	0.9 (0.9)[†]	0.8 (0.9)[⁑†]
Median	5.0		2.0		2.2	0.7	0.5	0.5
95% CI	4.6-6.4		1.4-2.7		1.2-2.2	0.9-2.1	0.5-1.2	0.4-1.1
Range	0 – 0.7		0 – 7.6		0 – 4.9	0 – 4.8	0 – 2.6	0 – 2.6
No-flea homes								
Mean (SD)	1.9 (1.4)[b∞ ⁑]							
Median	1.7							
95% CI	1.1-2.7							
Range	0 – 5.1							

PVAS pruritus visual analog scale, *SD* standard deviation, *CI* confidence interval

High-flea homes visited in all shown weeks; Low-flea homes not visited in Weeks 1 and 3; no-flea homes visited only in week 0

[a,b]Mean PVAS scores in week 0 with different letters are significantly different ($P < 0.0001$)

"High" and "low" week 11 – 12 were significantly different to week 0 "no" homes [∞]$P_H < 0.05$; [⁑]$P_L < 0.05$

[‡]Mean PVAS reduction significant between week 0 and all other weeks ($P < 0.001$)

[†]Reduction significant between week 0 and all other weeks ($P < 0.001$

Scoring Feline Allergic Dermatitis (SCORFAD)

Across the duration of the study, a SCORFAD score was assigned to 35 cats from “high” homes, 26 from “low” homes, and 12 from “no” homes. Before the first treatment, the arithmetic mean SCORFAD score of cats from “high” homes was significantly greater than that of cats from “low” homes ($W = 614.5$; $P < 0.05$) and “no” homes ($W = 353.5$; $P < 0.05$), and the mean SCORFAD of cats in “low” homes was significantly higher than those of “no” home cats ($W = 226.5$; $P < 0.05$) (Table 4.4). By week 11–12, relative to week 0, there was a significant decline in mean SCORFAD scores of cats from both “high” ($V = 351$; $P < 0.0001$) and “low” ($V = 134$, $P = 0.0007$) homes. There was no statistically significant difference in week 11–12 scores between cats in “high” and “low” homes ($W = 432$; $P > 0.1$). For cats in each of those categories, the mean week 11–12 SCORFAD score was not significantly different from that the enrollment SCORFAD score of cats in “no” homes ($W_{HN} = 222.5$, $W_{LN} = 177.5$; $P > 0.1$).

Table 4.4: Descriptive statistics summary of veterinary dermatologist SCORFAD scoring of study cats

	Week of study			
	0	4	8	11 - 12
High-flea homes				
Mean (SD)	8.0 (8.1)[a ‡]	4.3 (5.7)[‡]	2.7 (4.5)[‡]	1.7 (3.0)[‡]
Median	5.5	2.0	1.0	1.0
Range	0 – 33	0 – 20	0 – 24	0 – 15
95% CI	5.1-10.8	2.3-6.3	0.8-4.7	0.6-2.9
Low-flea homes				
Mean (SD)	3.3 (2.3)[b †]	1.3 (1.4)[†]	1.0 (1.6)[†]	0.9 (0.5)[†]
Median	3.0	1.0	0.0	0.0
Range	0 – 12	0 – 5.0	0 – 6	0 – 3
95% CI	1.9-4.7	0.7-1.9	0.3-1.7	0.5-1.3
No-flea homes				
Mean (SD)	0.9 (1.4)[c]			
Median	0.0			
Range	0 – 6			
95% CI	0.0-2.1			

SD standard deviation, *CI* confidence interval, *SCORFAD* scoring feline allergic dermatitis

High-flea homes visited in all shown weeks; low-flea homes not visited in weeks 1 and 3; no-flea homes visited only in week 0

[a,b,c]Mean SCORFAD scores in week 0 with different letters are significantly different ($P < 0.05$)

[‡]Mean SCORFAD scores significantly different between week 0 and all other weeks: $P < 0.0001$

[†]Mean SCORFAD scores significantly different between week 0 and all other weeks: $P < 0.0015$

High-flea homes visited on all shown weeks; low-flea homes not visited in weeks 1 and 3

A Spearman's correlation test found no correlation between mean flea counts and PVAS scores prior to the first treatment for either "high" ($r(32) = -0.0265$; $P = 0.882$) or "low" homes ($r(25) = 0.2604$; $P = 0.1896$). There was also no correlation detected between cat flea counts and SCORFAD scores in either the "high" home ($r(32) = -0.0371$; $P = 0.8378$) or "low" home ($r(24) = 0.2277$; $P = 0.2632$) categories. There was a moderate correlation between the week 0 "high" PVAS and SCORFAD scores $r(32) = 0.59$, $P = 0.0002$, but that was not seen for the "low" group ($r(24) = 0.077$; $P = 0.9702$). That correlation seen within the "high" group at week 0 was not found at any other timepoint $r(32) < 0.15$, $P > 0.40$.

Discussion

The mean flea counts found in this study are consistent with findings from previous studies on household cats, and lower than those reported from dogs in similar studies, likely attributable to feline grooming behavior (Dryden et al., 2013, 2015, 2016, 2017, 2018, 2019, 2020). Most cats (> 90%) in "high" and "low" homes were flea-free within 1 week of the first treatment, confirming lotilaner's rapid onset of efficacy. However, fleas were found on treated cats in a few homes past that first week. In week 8, fleas were also found in traps in each of those homes. Thus, the cat infestations would have been due to larvae that hatched and pupae that emerged from the prestudy environmental contamination with flea eggs. The timing of this emergence is based on a variety of factors including temperature, humidity, and pressure-sensory cues that stimulate adult fleas to emerge from the cocoon (Dryden and Broce, 1993). Overall, complete clearance of on-animal infestations and environmental burden of fleas in homes in Florida takes approximately 2–3 months, yet in cooler, damper conditions, adult flea emergence can occur over a period as long as 6–9 months (MW Dryden, personal communication).

Lotilaner has been shown to kill fleas before egg-laying begins, and the subsequent complete elimination of fleas (from cats and traps) indicates that the fleas found on those cats died before adding to the environmental contamination (Paarlberg et al., 2021).

PVAS

Coinciding with the reduction in on-animal and in-trap flea counts, there was a significant reduction in mean owner-reported pruritus scores after only 7 days following the first lotilaner treatment. In one home, a continued emergence of fleas in weeks 2 and 3 coincided with increased PVAS scores. Prior to any treatment, this home had 99 fleas counted in traps, and enrolled cats were scored 9.7 and 9.8 on the PVAS; 1 week post-treatment, trapped flea numbers dropped to 24 and the corresponding PVAS scores declined to 5.2 for both cats. Then, in weeks 2 and 3, flea-trap numbers rose to 68 and 89, respectively, and PVAS scores increased to 9.7 and 9.6 for one cat (respective flea counts of 2 and 0), and 6.2 and 5.3 for the other (flea counts 0 and 3). Trapped flea numbers then dropped to 1 and 0 in weeks 8 and 11–12, respectively, and scores for each cat were 0 on and after week 8. Given that the PVAS scores of cats with "high" and "low" flea burdens were not significantly different, the study findings highlight that a relatively low flea count, or perhaps more importantly, environmental exposure to fleas, can result in clinically evident pruritus in cats. The consistent use of PVAS scores in cats may be a useful way of monitoring changes in a home's environmental flea burden.

This is the first study of which we are aware that included PVAS scoring of cats with no fleas. The validated PVAS score in "normal" dogs is ≤ 1.9, and in this study the mean PVAS score of cats from "no" homes was 1.9 (median 1.7) (Hill et al., 2007, Rybnícek et al., 2009). This may be an appropriate baseline for monitoring the reduction of pruritus over time in cats with allergic dermatologic conditions. A limitation of this interpretation may be that only 14 cats were included in this group, 2 of which had PVAS scores of 5.1 and 4.3. Additionally, the study-end mean and median PVAS scores of cats in the "high" and "low" categories were much lower (respective means 0.5 and 0.8, medians 0.2 and 0.5). It may be that in the presence of fleas at enrollment, owners were sensitized to their cat's pruritus. Then following elimination of the flea challenge, owners were impressed with the drastic reduction in pruritus, so their scoring declined from very high to very low. This might also explain why the final "high" and "low" category mean PVAS scores were significantly lower than the mean scores of the "no" category. Further study is needed to investigate our conclusions in this regard.

SCORFAD

The SCORFAD scores from cats in "high" homes are consistent with those seen in previous publications looking at skin lesions associated with flea feeding (Dryden et al., 2018, 2020, Briand et al., 2019). The significant difference between cats in "high" and "low" homes prior to the first lotilaner treatment may speak to the volume and chronicity of the infestation, especially given initial PVAS scores that were not significantly different. If left untreated for another month, both the flea numbers and lesion severity of cats in "low" homes may have more closely matched the numbers and lesions in "high" homes that had already reached that point. The high group could also have an over-represented number of cats with true flea allergy, resulting in more significant lesions, although we did not test or select for this in the study. Furthermore, the Spearman's correlation noted that there was no correlation between number of fleas on cats and the lesion severity. On the basis of prior experience of the investigators, this is likely due to the fastidious grooming behavior of cats. The grooming of fleas has been documented in laboratory studies, where cats readily remove free-roaming fleas, and cats with flea allergy remove more fleas than cats that are flea-naive (Dryden, 1989, McDonald et al., 1998). Given that cats in our study may have had significantly different initial flea burdens, there was no obvious correlation between flea counts and SCORFAD scores. Veterinarians should not rule out fleas as a cause of dermatologic lesions just because there are few, and possibly no, fleas seen during the exam. Flea control is an important diagnostic tool in working up dermatologic lesions. There was a consistent reduction in lesion severity over time in response to flea treatment, without any additional systemic or dermatologic treatments. Cats treated with lotilaner showed a significant reduction in lesion scores by the time of the first posttreatment assessment in week 4. The SCORFAD scores were less responsive to flea reduction than PVAS scoring because lesions, such as excoriations and self-induced alopecia, take time to fully resolve. In a clinical setting, lesions may improve faster if effective flea control is combined with other therapies such as antipruritic and immunosuppressive therapies. Regardless, the study-end SCORFAD scores of cats from "high" and "low" homes were not significantly different from the "no" home scores taken at enrollment. In the absence of a validated SCORFAD baseline for cats, the average SCORFAD score of 0.9 for the cats from "no" homes served as a comparative value for local cats and the dermatologic lesions seen during normal grooming behaviors. The reduction of the SCORFAD scores of flea-infested cats toward this presumed baseline is consistent with successful resolution of the lesions with only flea treatments and no other medical interventions. In practice, veterinarians have other drugs available to reduce inflammation and treat secondary infections associated with flea-feeding, but rapid and prolonged killing of fleas remains the key to stopping the progression of and reducing these dermatologic lesions.

Limitations

Due to the nature of an in-home study, there are variables that cannot be controlled. Investigators must trust owners in regard to vacuuming and prior environmental and pet treatments. A limitation for "low" category homes is that there is not a standardized cutoff for the number of fleas that can be on an animal or a trap, and therefore may have a larger variation in dermatologic lesions. Furthermore, this investigation does not begin at a standardized time of the home flea infestation, thus, the duration of the infestation is unknown, which can produce variability in the environmental burden of fleas and severity of lesions. Despite this, there is a consistent, repeatable pattern of environmental flea control, pruritus reduction, and lesion resolution seen in this and previous studies using only highly effective flea control (Dryden et al., 2013, 2015, 2016, 2017, 2018, 2019, 2020, Chappell et al., 2021).

Conclusion

Over the 12-week study period, lotilaner treatment of infested cats and dogs was 100% effective at eliminating flea infestations from animals and their homes. This study illustrates the value of systematic flea control with lotilaner for up to 3 months to eliminate fleas from cats and to prevent reinfestation from the home environment. Flea-trap counts may be a more effective means of monitoring flea challenge to household cats than on-animal flea counts. Reductions in dermatologic and owner pruritis scores (PVAS and SCORFAD) demonstrated that the high lotilaner efficacy resulted in clinical resolution of flea-induced allergic dermatitis in cats.

Chapter 5 - Conclusion

Testing and approval of ectoparasiticides is essential within veterinary medicine, and there are multiple tiers of research required to accumulate the data necessary for a market release of an animal parasiticide product. This is typically a progression from *in vitro* testing outside of a live animal model and progressing up to *in vitro* testing in the live animal. Ectoparasiticides are critical within the veterinary medicine space as ectoparasites detractors of the human-animal bond. Ectoparasites are a persistent problem because there are ectoparasites of interest that are spreading geographically including the New World Screwworm (*Cochliomyia hominovorax*, classified as a nationally reportable disease) and the Asian Longhorned Tick (*Haemaphysalis longicornis*). Ectoparasiticides and therefore ectoparasiticide testing must be available in order navigate on and off-label control strategies for mitigating the spread of damage of ectoparasites of interest.

In Chapter 2 of this dissertation, the blueprint has been provided for establishing an artificially reared laboratory colony of *Ctenocephalides felis* fleas. The system utilized in this chapter uses resources that were publicly available on Kansas State University's campus with a minimal cost of materials. This system successfully produced two generations of *Ctenocephalides felis* fleas. A future direction for this project could be to develop a resistance model utilizing the 3D-printed artificial flea rearing apparatus. Currently, there is no document resistance to the isoxazoline drug class. However, many drugs in the isoxazoline drug class are demonstrating 100% efficacy for eliminating flea infestations, for example. Therefore, the isoxazoline drug class is placing a high selection pressure on fleas and other ectoparasites which increases the likelihood of resistance development. Creating a resistance model on the artificial system could inform the investigators on the flea resistance mechanism to the isoxazoline drug class. This information would be useful to researchers when considering the future of flea control and ectoparasiticide products.

In Chapter 3, fluralaner, a member of the isoxazoline drug class, was administered to horses, a species with no previously published pharmacokinetic data related to this drug class. Understanding the pharmacokinetics of fluralaner in horses provides useful data for future equine ectoparasiticide research. It should be noted that the oral dose reached a much higher C_{max} than the topical dose. In the event of a drug sponsor considering an equine formulation of fluralaner, this study potentially informs the sponsor on expected outcomes based on the route of administration.

In Chapter 4, it was established that treating every pet with an efficacious flea control with high residual speed of kill was effective for eliminating on-animal and environmental flea infestations. The isoxazoline drug class expressed a profound ability to reduce flea counts on animals, flea counts in the environment, owner perception of pruritus, and dermatologic scores. Post-approval marketing studies are useful to assess the efficacy of a drug over time after release. In addition, post-approval marketing studies assess a product in a situation outside of the confines of the studies required for initial FDA approval.

This dissertation covered strategies for evaluating ectoparasiticides beginning with establishing a model for flea rearing and *in vitro* testing in Chapter 2, providing data on the use of isoxazolines in novel species in Chapter 3, and providing an example of a post-approval marketing field study in Chapter 4. Ectoparasites are on the horizon to continue to be a concern of veterinary interest for the clinical lifetime of myself and my colleagues. Strategies such as those demonstrated in this dissertation must be utilized to continue to produce novel ectoparasiticides to the market and demonstrate efficacy of products already on the market.

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Appendix A - Supplemental Table(s)

Table 5.1: Validation results of fluralaner spiked in horse plasma

Fluralaner spiked (ng/mL)	Fluralaner detected (ng/mL)	Accuracy (Recovery %)	Precision (RSD %)
5	4.63	92.58	4.69
10	9.56	95.60	9.28
100	100.33	100.33	7.96