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The Efficacy of a Spot-On Pesticide Against Ectoparasites Affecting Poultry in Mississippi

Kevin Steven Maschek

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The efficacy of a spot-on pesticide against ectoparasites affecting poultry in Mississippi

By

Kevin Steven Maschek

A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Poultry Production Medicine
in the College of Veterinary Medicine

Mississippi State, Mississippi

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2015

The efficacy of a spot-on pesticide against ectoparasites affecting poultry in Mississippi

By

Kevin Steven Maschek

Approved:

Michael J. Mael
(Major Professor)

Danny L. Magee
(Committee Member)

Alejandro Banda
(Committee Member)

Sue Ann Hubbard
(Committee Member)

R. Hartford Bailey
(Graduate Coordinator)

Kent H. Hoblet
Dean
College of Veterinary Medicine

Name: Kevin Steven Maschek

Date of Degree: May 8, 2015

Institution: Mississippi State University

Major Field: Poultry Production Medicine

Major Professor: Michael J. Mauer

Title of Study: The efficacy of a spot-on pesticide against ectoparasites affecting poultry in Mississippi

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Candidate for Degree of Master of Science

Ectoparasites are a common occurrence in backyard and commercial chickens and present a severe health challenge in certain circumstances. The accurate identification of the parasite is important in properly treating and preventing re-infestation. The feeding behavior of the pest species and management factors of the chicken affect outcomes of different types of treatment strategies. Most commercial products come in dusts, fogs, mists and sprays. The novel use of a topical spot-on pesticide can provide efficient adulticidal activity against stick tight fleas (*Echidnophaga gallinacea*) and bed bugs (*Cimex lectularius*) associated with poultry in Mississippi.

DEDICATION

To all the itchy chickens in the world, may you one day be completely free of fleas, bed bugs and other despicable pests.

ACKNOWLEDGEMENTS

I would like to take the time to thank all for their support in this project. Dr. Kelli Holloway Jones for providing her time, energy and enthusiasm for acquiring materials (i.e. chickens and test product) and being an inspiration. Dr. Sue Ann Hubbard for her willingness to be my second major professor and amazing ability to get things done. Dr. Michael Mael (third major professor) for cracking the whip to help me meet all the necessary deadlines. You are all my best professors and friends. Thank you to the pharmaceutical companies, private chicken owners and commercial poultry industry for donating materials needed for this project to be successful. Drs. Banda and Magee for their time and effort in reviewing, critiquing the work and mentorship. A very special thanks to Dr. Jerome Goddard for allowing the inclusion of his manuscripts to complete the work of this project.

Behind every great masters student is a graduate committee praying they graduate.

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CHAPTER I
LITERATURE REVIEW AND INTRODUCTION

Pesticide Classes Used In Poultry Production

There are six main classes of pesticides that can be utilized for pest management in the poultry industry: organophosphates, carbamates, pyrethroids, neonicotinoids, botanicals, and inorganics (Hoelscher, 2015; Pickworth & Morishita, 2015; Scott, Alefantis, Kaufman, & Rutz, 2000; Williams, 2010). Most of these pesticides can be used either on live animals or as premise treatments.

Organophosphates

Organophosphates (OPs) are derived from phosphoric acids and at certain concentrations can be toxic to vertebrates and invertebrates resulting in serious illness or even death (Friend, 1999; Cynthia M. Kahn & Line, 2010; Ware, 2000). They have largely replaced the banned organochlorines and represent a major cause of poisoning in animals (Cynthia M. Kahn & Line, 2010). The mode of action of OPs is to inhibit cholinesterase at the neuromuscular junction by irreversible binding (Bajgar, 2004; Ware, 2000). The result is accumulation of acetylcholine causing rapid twitching of voluntary muscles and eventual paralysis (Friend, 1999). The most common OPs used in poultry are chlorpyrifos, tetrachlorvinphos, and dichlorvos. Routes of application of OP's includes direct application to chickens for the treatment of lice, mites, and ticks. Premise

treatments with residual sprays are used for the control of flies, gnats, litter beetles, mosquitoes, spiders, and wasps. An *in vitro* study found organophosphates to be effective against the northern fowl mite in isolates from Virginia (Hall, Townsend, & Turner, 1978).

Carbamates

Similar to organophosphates in action, carbamates inhibit cholinesterase at the neuromuscular junction. However, their binding is spontaneously reversible conceivably making them less toxic to vertebrates (Cynthia M. Kahn & Line, 2010). The action of carbamates is directed toward the central nervous system of insects. Carbaryl (Sevin) was the first successful carbamate introduced in the mid-1950s (Ware, 2000). There are some formulations that are labeled for the treatment of chicken mites, lice, ticks and beetles. It can be misted, fogged or sprayed and applied to cages, birds, roosts or buildings. When applied in the laboratory to treat northern fowl mites, carbamate had acceptable efficacy (Hall et al., 1978).

Pyrethroids

Pyrethroids affect both the peripheral and central nervous system of the insect. They stimulate nerve cells to produce repetitive discharges and eventually cause paralysis. Such effects are caused by their action on the sodium channel, through which sodium ions are permitted to enter the axon to cause excitation (Ware, 2000). Cyfluthrin, permethrin, and gamma cyhalothrin are commonly advertised pyrethroids for use in poultry. They are used for application to buildings, cracks and crevices, and spot treatment for a variety of pest species.

Neonicotinoids

Developed within the last three decades, the neonicotinoids are the newest major class on insecticide with good potency. They have a low affinity for vertebrate nicotinic receptors making them less toxic to vertebrates (Tomizawa, 2005). They act on the central nervous system of insects, causing irreversible blockage of postsynaptic nicotinic acetylcholine receptors. Imidacloprid is the active ingredient in many poultry products used as baits for the control of flies (Ware, 2000).

Botanicals

This class is of interest for poultry producers looking to move away from traditional pesticides and use more natural compounds. Pyrethrum is the most notable of the botanicals. It is derived from the chrysanthemum plant. It stimulates nerve cells to produce repetitive discharges, leading eventually to paralysis of the insect. It is used commonly as a mist for the knockdown of adult flies in caged layer facilities.

Inorganics

Inorganics is the termed used for pesticides that lack a carbon at the molecular level. Sulfur is the oldest of these insecticides and is used in a dust or spray formulation for the treatment of mites and lice. It may be applied for area treatment or directly to birds.

Important ectoparasites in commercial chickens

There are several types of arthropods that affect commercial chickens including mites, lice, ticks, and bed bugs. The degree and type of infection is influenced by the production method, housing type, feed and water equipment, manure disposal and

environmental quality in the house (Axtell & Arends, 1990; Hinkle, 2013). The following will cover the most economically important and relevant species occurring in today's modern production units.

Mites and Lice

The two mite species of economic importance to commercial poultry are the northern fowl mite and the red chicken mite (Wang, Wang, Xu, Liang, & Pan, 2010).

The northern fowl mite, *Ornithonyssus slyvarium*, is ubiquitous (Ruff, 1999) and thrives in modern high-density layer operations and breeder flocks (Axtell, 1999). They are found on more than 72 bird species providing a natural reservoir host and a source of reinfestation to chickens (Hinkle, 2013). The life cycle of a mite can be completed in as few as 4-5 days allowing mite populations to explode quickly and spread rapidly through flocks (Axtell & Arends, 1990; Hinkle, 2013). All life stages occur on the chicken; they feed on the blood of chickens and can cause anemia, scabbing and pruritus in severe infestations. Rooster libido can be affected by pruritus and irritation to the vents, thus, interfering with mating and resulting in lower egg fertility (Hinkle, 2013). Nuisance to workers can occur when handling birds or when mites are dislodged during heavy infestations (Abdigoudarzi, Mirafzali, & Belgheiszadeh, 2014; Nordenfors & Hoglund, 2000).

The poultry red mite, *Dermanyssus gallinae*, differs from the northern fowl mite in how they live and feed. Life stages of the poultry mite are completed off of the host. They feed at night from the host and during the day they hide in cracks and crevices in the environment. Although infestations in U.S. caged layer operations is rare, they are occasionally found on breeder farms (Hinkle, 2013; Cynthia M. Kahn & Line, 2010;

Nordenfors & Hoglund, 2000). Egg production and fertility are impacted by mite infestations as well as feed conversion efficiency (Hinkle, 2013; Cynthia M. Kahn & Line, 2010).

Poultry lice belong to the order Mallophaga, which is known as the chewing lice, biting lice or bird lice. They are wingless insects that feed on feathers, feather debris, skin scales, and occasionally blood of irritated areas (Cynthia M. Kahn & Line, 2010). Lice infestation can be very damaging to young birds resulting in poor growth and feed conversion ratio (Hinkle, 2013). The female louse can lay up to 300 eggs in its lifespan and deposits them on the feather shafts of its host (Pickworth & Morishita, 2015).

Bed bugs

Like the poultry red mite, the bed bug (*Cimex lectularius*) is a hematophagus bug that hides during the day in cracks and crevices of the poultry roost and house. They move to feed on birds at night and are rarely observed by people until populations have reached enumerable concentrations (Hinkle, 2013). Concerns are that bed bugs may move to public or private facilities from poultry houses or be carried on the clothes of infested poultry facility workers. Bed bug infestations of broiler breeder farms have been implicated in 10% egg production loss and anemia (Cater, Magee, & Edwards, 2011).

Ticks and Fleas

The sticktight flea, *Echidnophaga gallinacea*, is an ectoparasite of both birds and mammals. Female fleas can be found attached to the skin of the head of the host in clusters of up to 100 fleas (Hinkle, 2013; Ruff, 1999). Eggs from these sessile females are expelled into the environment where they develop into pupae. In order to break the

lifecycle of the flea, additional cleaning and treatment of the host environment is important to destroy flea eggs and larvae (Hinkle, 2013).

The two major classes of ticks are hard ticks and soft ticks. The most important tick in poultry is the soft-bodied tick, *Argas persicus*. Similar to the red mite and bed bugs, *Argas persicus* spends most of its life in cracks and crevices, only to feed on its hosts at night (Hinkle, 2013). Repeated feeding by nymphal instars is required for growth, so blood loss, emaciation and fatal anemia may occur in poultry (Hinkle, 2013). Ticks serve as vectors for disease such as spirochetes, piroplasmosis, rickettsial and bacterial diseases (Cynthia M. Kahn & Line, 2010).

Integrative Pest Management Systems

The objectives of Integrative Pest Management Systems are to monitor pest populations, use appropriate techniques to control pest populations, allow producers to decide if pesticide application is necessary, and to avoid the unnecessary or unprofitable pesticide applications (Rutz, 2000). Multiple control measures should be implemented as no one product or technique can satisfactorily control pests. The following methods for Integrative Pest Management Systems is extracted from Rutz, 2000 and Williams, 2010:

Cultural/Physical Control

Cultural control is used primarily in fly control where manure management is the key to controlling fly populations. Reducing moisture of manure can help prevent and discourage flies from breeding. Maintaining equipment such as leaking water lines and ventilation fans can help reduce the accumulation of moisture in manure. Sticky traps or live bug traps can also be instituted to help control fly populations.

Biological control

The most notable example of biological control in pest management is the use of parasitic wasps to control fly populations. Proper use of these methods can suppress house fly populations. The macrochelid mite lives in manure and has been known to eat up to 20 fly eggs daily. They can provide substantial reduction in fly populations but large mite populations are needed. More information regarding biological control of flies can be found in Rutz, 2010. There are several potential control methods for use against the northern fowl mite; predatory mites, *Bacillus thuringiensis* exotoxin, and plant derived chemicals that act to repel or deter mites (Chauve, 1998). To the author's knowledge, biological control of poultry ectoparasites is not widely used by the industry.

Chemical control

Chemical control using approved pesticides is a common strategy of pest control in poultry production. Treatments may not be 100% effective for several reasons such as missed birds during application, sprayer malfunctions preventing penetration to the skin where mites live, and chemical resistance (Hinkle, 2013). Pyrethroids are a commonly used pesticide for the control of mites, lice, beetles, bed bugs, and ticks. They come in various sprays, aerosols, and dusts formulations. Direct application of pesticide to the vent of chickens is required for northern fowl mite control (Hinkle, 2013; Rutz, 2000).

Types of pesticide applications used in poultry

There are numerous types of pesticide products labeled for use in poultry. How they may be applied can vary by the product, with some products having multiple formulations.

There are a number of chemical control measures that can be implemented in poultry housing to control pests such as residual sprays, foggers and mists, baits and traps, resin strips, and fly ribbons.

Residual Sprays

Residual sprays are an effective means of controlling pest populations such as flies, mites, and bed bugs. At least two sprays 5-6 weeks apart should be performed in a poultry house to treat life stages of ectoparasites not affected during the initial application. The application of product to surfaces in which the pests will be in contact is important. These areas include poultry house framework, ceiling, walls, trusses, nest boxes, slats, electrical light cords, and attics.

Foggers and Misters

Portable fogger and mister machines are convenient, efficient, and labor saving in caged layer operations. They can provide a rapid knockdown of ectoparasites and flies but have a poor residual. The droplet size delivered is important and should be less than 30 microns for use against house flies.

Baits and Traps

Baits work well to control fly populations. Application of these bait systems could be helpful in the control of bed bugs. The drawback to these baits is possible ingestion by chickens or accidental mixing in with feed. They can be used in layer operations outside of cages. The common pesticides used in baits are dichlorvos, trichlorfon, and tetrachlorvinphos. Mite traps treated with pesticide are a less commonly used control method reported in the literature. According to (Chirico & Tauson, 2002) a 95% and 99%

reduction of red poultry mite was seen in 8 week and 2 week trials respectively in layer housing when traps treated with pesticide were placed strategically in a caged layer operation.

Resin Strips and Fly Ribbons

Resin strips containing a common OP (dichlorvos) may be placed in poultry housing to control flies. Strips may need to be replaced as they lose effectiveness. Fly belts can be placed in strategic locations like ceilings and walls to provide additional control. As strips and belts become aged and dusted they should be monitored and replaced to ensure efficacy (Hoelscher, 2015).

CHAPTER II
LABORATORY ASSAYS WITH A TOPICAL CANINE INSECTICIDE AGAINST
BED BUGS

Background

Bed bugs are small, flat, oval-shaped, wingless insects that feed on the blood of warm-blooded animals such as humans, bats, birds, and pets (Little & West, 2008; Reinhardt & Siva-Jothy, 2007; Ryckman, Bently, & Archbold, 1981; Thomas, Kihiczak, & Schwartz, 2004). They have traditionally been common in the developing world, and especially in areas of extreme poverty and crowding. These blood-sucking parasites had nearly disappeared in developed countries when a dramatic increase and spread of the insects began in the 1980's (Goddard & De Shazo, 2008; M. F. Potter, 2006). Since then, bed bugs have been increasingly reported inside U.S. hotel rooms, dorms, and apartments (Anderson & Leffler, 2008; Cleary & Buchanan, 2004; Gangloff-Kauffmann et al., 2006). Bed bugs feed at night, hiding in crevices during the day. Hiding places include seams in mattresses, crevices in box springs, and spaces under baseboards or loose wallpaper. There are 5 nymphal stages that must be passed before development to adulthood. Once an adult, the bed bug has a life span of 6 to 12 months. At each nymphal stage, bed bugs must take a blood meal in order to complete development and molt to the next stage. The bugs take about 5 to 10 minutes to ingest a full blood meal. Bed bugs can survive long periods without feeding. When their preferred human hosts are absent they

may take a blood meal from any warm-blooded animal, including pets and poultry (C.M. Kahn, Line, & Aiello, 2008; Steelman et al., 2009). Health effects from bed bug bites include pruritic bumps and rashes, bullae, and rarely systemic allergic reactions (Bircher, 2005; Brasch & Schwarz, 2006; Churchill, 1930; Elston & Stockwell, 2000; Goddard & De Shazo, 2008; Hwang, Svoboda, De Jong, Kabasele, & Gogosis, 2005). As far as is known, bed bugs do not transmit human disease agents (Goddard & de Shazo, 2009). This study was initiated to generate some preliminary data as to the effect of a canine spot-on insecticide product (containing 36.08% Permethrin, 4.95% Dinotefuran, and 0.44% Pyriproxyfen) against bed bugs.

Location and time of study

This work was performed in the Department of Biochemistry, Molecular Biology, Entomology, and Plant Pathology, Mississippi State University, between November 15, 2013 and November 18, 2013.

Source of bed bugs

For the “contact” experiment, a field strain of bed bugs was used, which was wild-caught on November 1, 2013 from a poultry house, located near Union, MS. For the artificial feeding experiment, the Harold Harlan laboratory (provided by Dr. Jerome Goddard, Mississippi State University) strain was used due to its adapted ability to feed through an artificial membrane.

Methods

For the contact experiment, approximately 3-5 drops of the product were wiped (smeared) around in the bottom of three petri dishes and allowed to air-dry for 30 min.

For a control water was smeared in the bottom of three petri dishes and also allowed to dry 30 min. Ten field-caught bed bugs each were placed in the petri dishes (total of 60 bed bugs in the experiment) and counts were made as to number of bed bugs alive at 5 min, 15 min, and 24 hr.

For the artificial feeding experiment, approximately 25 adult bed bugs (Harold Harlan strain) were placed in a vial with a screen top and allowed to feed through an artificial membrane on fresh chicken blood containing sodium citrate. Sodium citrate was the anti-coagulant; Parafilm M[®] was the membrane. A glass feeding device was used which had a surface feeding area of approximately 7.0 cm². Since not all bed bugs will feed through a membrane, the effort was repeated until 10 specimens fully fed through the membrane without any product. This was the control group. This process was repeated, except this time, 0.1 ml of the product was painted on the outer surface of the parafilm membrane. Counts were made as to the number of bed bugs alive at 15 minutes, 2 hours, and 48 hours.

Results

All control bed bugs remained alive through the entire experiment, allowing for good comparisons with the treatments. In the contact experiment, 60-70% of the bed bugs exposed to Vectra died within 5 minutes of exposure (Figure 2.1). There was 100% mortality at 24 hours against the bed bugs. In the artificial feeding experiment, there was 70% control of bed bugs exposed to a membrane which had been treated with product (Figure 2.2). The 30% surviving bed bugs lived without showing any ill effects for 48 hours (although we cannot guarantee that these three bed bugs ever walked on the treated surface).

Conclusions

The product showed mortality in bed bugs exposed to the product in contact and artificial feeding experiments. Painting the artificial membrane created a situation similar to skin where the product has been applied topically. Seventy percent of bed bugs exposed to the treated membrane were dead within 2 hours, indicating potential usefulness of the product against bed bugs in either pets or poultry provided an acceptable delivery method to the animals is identified.

Table 2.1 Results of the contact experiment over a 24 hour period

	# Alive 5 min	# Alive 15 min	# Alive 24 hr
Water in dish Rep 1	10	10	10
Water in dish Rep 2	10	10	10
Water in dish Rep 3	10	10	10
Vectra in dish Rep 1	3	0	0
Vectra in dish Rep 2	4	2	0
Vectra in dish Rep 3	4	1	0

Table 2.2 Results of the artificial membrane feeding experiment.

	# Alive 15 min	# Alive 2 hr	# Alive 48 hr
Membrane with Vectra	10	3	3
Membrane without Vectra	10	10	10

CHAPTER III
A SPOT-ON CANINE INSECTICIDE APPLIED TO BREEDER HENS TO
DEMONSTRATE EFFICACY AGAINST BED BUGS

Introduction

Bed bug infestation in broiler breeder housing is becoming a common reoccurrence amongst several commercial broiler companies in the United States. Effectively eradicating the infestation has proved futile and controlling the infestation is temporary. Bed bugs have an ability to congregate and hide in cracks and crevices making it easy for bed bugs to escape from pesticides while still residing in the vicinity of the hosts. The authors experience with bed bug infestation in broiler breeder housing revealed that the most preferential sites for bed bugs to inhabit are the attic, nest boxes, egg belt covers, and the spaces created by triply on the ceiling. These areas are suspected to go undertreated during normal application of pesticides with industrial applicator systems (i.e. trailer sprayers). Other areas not easily accessible by humans (and pesticides) may also provide great hiding spaces for bed bugs in a poultry house. Bed bugs are not naturally resistant to any one pesticide, however, reports of pyrethroid resistance are common. They can be very easy to kill *in vitro* with a variety of non-pesticide products i.e. soap solutions, rubbing alcohol and powder desiccants.

Controlling bed bugs in commercial broiler breeder housing is challenging with current application strategies. The types of pesticide formulations used to control bed

bugs include mostly dusts and sprays. A topical spot on pesticide (containing 36.08% Permethrin, 4.95% Dinotefuran, and 0.44% Pyriproxyfen) was investigated for its efficacy against bed bugs when applied to breeder hens.

Materials and Methods

Eight, 33-week old healthy broiler breeder hens were used in three experimental trials. Hens were housed in Sterilite 30 gallon clear plastic isolators (Walmart, Inc) with snap on lids. Four circular cutouts (6 cm in diameter) were created in the sides of the isolators and covered with mosquito screen to allow for air flow to the hens. Play sand from the local hardware store was used as bedding to cover the floor of the isolator to a depth of 1 inch. Adult bed bugs were enumerated and placed into the isolators containing the hens. The hens were treated with 1.6 mL of the product along the featherless areas of the ventrum. The criteria used for determining dead and live bugs included: Dead- no movement of legs after one minute of observation after stimulation with a probe. Live – movement of legs within one minute of observation after stimulation with a probe.

Trial One

One, 33-week old hen was treated with the product and placed into the isolator as described above. Another hen was used as a control and placed into an identical but separate isolator with no application of product. Fifty adult bed bugs were enumerated and placed into each isolator. Feed and water were provided to each hen using containers made for raising poultry. The isolators were placed inside of the poultry house in the scratch area near the cool cell end next to the slats. The hens were rechecked 24 hours later and bed bugs were enumerated for live and dead counts.

Trial Two

The same procedures for trial 1 were also followed for trial 2. However, feed and water were not provided to hens. In this trial 100 bed bugs were enumerated and placed into each isolator with a hen. The hens were rechecked 24 hours later and bed bugs were enumerated for live and dead counts

Trial Three

The same procedures for treatment of hens were followed as previously stated. However, the author's used three hens in the treatment group and one hen in a control group. One hundred adult bed bugs were enumerated and placed into each of the four isolators. Feed and water were withheld and a recheck was performed 48 hours later.

Results and discussion

In trial one, there was zero mortality observed. In trial two, the mortality rate in the treated group was 5% compared to 3% in the control group. The author's noticed the behavior and movement of the bed bugs exposed to a treated hen were slower and more lethargic than the bed bugs in the control group.

In trial three, the three replicate groups had 62%, 64%, and 64% mortality compared to 0% mortality in the control group (n=1). Recovery of bed bugs was 83, 73 and 78 respectively in treated group and 70 in the control.

The authors experienced issues with performing the experiment in trials one and two. Ultimately, solutions for experimental design came from these trials that led to an improved trial three. In trial one, the authors found a breach in one isolator allowing escape of the hen. The bedding was saturated with spilled water which made recovering

bed bugs difficult. Due to poor recovery of bed bugs, the authors decided to retry the experiment and withhold feed and water.

There were several challenges the authors had to overcome when designing the experiment. First, they needed to be able to appropriately house both chicken and bed bugs to ensure exposure of the bed bugs to the product was possible. Second, eliminating factors that may cause mortality to bed bugs causing a false positive was necessary. This proved difficult, in trial 1, when feed and water were spilt by the hen onto the sand causing the loss of bed bugs to drowning and poor recovery.

Adequate exposure time of bed bugs to treated hens occurred up to 48 hours before mortality in bed bugs was seen. There are many reasons this may be the case. In interpreting the results, there is an assumption all bed bugs crawled on the hen at some point during the study. It was not determined how many bugs fed or were in contact and for how long.

The need for a pesticide that has residual and high efficacy is paramount in poultry production. Application of a spot-on pesticide can be done during times of handling for other necessary procedures such as vaccination. It has been suggest by commercial chicken companies affected with bed bugs to hire vaccine crews to apply these types of pesticides. The cost associated with this application is most likely not economical if used on a regular basis.

CHAPTER IV

A FIELD TRIAL AGAINST STICK TIGHT FLEAS, *ECHIDNOPHAGA GALLINACEA*, USING A TOPICAL CANINE INSECTICIDE IN BACKYARD CHICKENS

Abstract

Ectoparasites are a common occurrence in backyard and commercial chickens and present a severe health challenge in certain circumstances. The accurate identification of the parasite is important in properly treating and preventing re-infestation. In this field report, we discovered a novel case of sticktight flea (*Echidnophaga gallinacea*) infestation in a backyard chicken flock. The flock experienced mortality of 78% over the duration of one year. Refractory to several of the most common pesticides labeled for poultry, a canine topical insecticide was used experimentally to demonstrate potential insecticidal efficacy. All affected birds (n=17) were treated topically on two occasions with a spot-on canine insecticide (containing 36.08% Permethrin, 4.95% Dinotefuran, and 0.44% Pyriproxyfen). Physical examination and digital photographs were taken to document the infestation load in each bird over the course of two months. After one year post treatment, the subjects were re-evaluated for the presence of fleas. Treatment efficacy was found to be 100% in all treated birds 2 months post treatment. One year later there were no signs of re-infestation.

Introduction

A small hobby chicken flock located in Laurel, Mississippi was investigated for mite infestation and mortality in chickens. The affected chickens were housed in a pole barn with hog panel fencing constructed around the perimeter and dividing the barn into four equal sized pens. Each pen contained 10-20 chickens at a time. The pen on the south end of the barn had 100% mortality and was the most severely affected. As the investigators moved towards the north end of the barn, it was noted that each pen had a decreasing percentage of sick and dying chickens. The owner noted that there was a seasonality associated with the mite infestation. In addition, there was an active breeding site for wild Martins located five feet away from the south end of the barn

Upon physical examination of the affected chickens, a presumptive diagnosis was made based on the physical characteristics of the parasites and location on the bird that the parasites were stick-tight fleas (*Echidnophaga gallinacea*). A confirmed diagnosis was made by submitting samples to Mississippi State University's Entomology Department. The feeding behavior of the stick-tight flea resembles that of a tick whereby the flea embeds its anterior end into the host's skin and continuously feeds on the host's blood. Female fleas will stay in this position to breed and deposit eggs by expulsion into the environment (Boughton et al., 2008). A study was conducted to determine the efficacy of the topical spot-on canine insecticide against stick tight fleas in chickens (*Gallus gallus domesticus*).

Materials and Methods

Treatment 1

Seventeen mixed breed chickens were selected for treatment with the product (CEVA Animal Health, Lexena, KS) and leg banded with a unique number for tracking purposes. The investigators applied 1.6 mL of product (by body weight as labeled for canines <10lbs) topically with a 3 mL syringe over four sites of each chicken: under right and left wing, between scapula, and the base of the tail. At day 10 post treatment, chickens were re-evaluated and photographs were taken of treated areas.

Treatment 2

Reapplication of the product was done 21 days after initial treatment. The location of treatment was directed towards the base of the head and neck using the previously described quantity and technique. Rechecks were scheduled at 4 days, 30 days and 365 days after second application.

Results and Discussion

Before the experiment started, the owner was advised not to consume any meat or eggs from treated chickens for 60 days after final treatment due to potential chemical residues. Testing residues in meat, fat and eggs was not performed in this study. Owner did not agree with sacrifice of all birds treated. The chickens were used exclusively for breeding and exhibition.

Mortality in the flock ceased after initial treatment. Historically, the owner reported it was common to pick up one dead chicken a day. Ten days post treatment; flea infestation per bird appeared to be reduced by 50-90% by visual assessment on pre and

post treatment photographs (Figure 4.1). After reapplication of the product around the head and neck there were no fleas present on any treated birds 4 days post application. Follow up visits 28 days and one year after treatment confirmed the complete resolution of the stick-tight flea infestation. It was suspected the martins were a source of stick tight fleas as they would congregate with the chickens to feed. The martin house was removed from premises the day after initial treatment. The owner treated the environment with a natural insecticide product to help control larvae and other life stages of the flea.

Topical pesticide application to chickens as a spot-on formulation is a novel treatment not described previously in the literature. Application of a topical spot-on pesticide to treat an infestation of stick tight fleas was successful with proper application and follow-up treatments and monitoring. The best success of these types of products will come from private veterinary clinicians working on backyard chickens and looking to utilize dog and cat products in their pharmacy.

Failed attempts to treat the stick tight fleas with approved products in this case are multifactorial. Application of dusts (sulfur, permethrin) to chickens may not have allowed localized treatment to the head, especially when birds are allowed to dust themselves with the product. In addition, reapplication with any pesticide is necessary to kill live stages that hatch after treatment which is dependent on owner's follow up.

The product contains 36.8% Permethrin, 0.44% Pyriproxyfen and 4.95% Dinotefuran. It is available by veterinary prescription only. Dinotefuran is part of the neonicotinoid class of insecticides. It acts indirectly to stimulate nicotinic receptors in insects resulting in paralysis and death. It has less affinity for human receptors (meaning

much safer for human handling) than organophosphates do, which are more common in poultry labeled products.

Disadvantages for commercial application would be high labor cost and high cost of the product. Hiring vaccination crews would be the most logical way to apply the medication. However, training on proper application would be required when using new chemicals and products. In addition, a poultry specific label is needed to stay in compliance with the Environmental Protection Agency's regulation on pesticide usage. Until then, it is illegal to use pesticides not in accordance with the EPA approved label.

Advantages of this spot-on product is its unique dispersive characteristics over the skin and its residual efficacy. The investigators consider the efficacy of the active ingredients to be prolonged (i.e. 30 days) in spot-on formulations for chickens as it is for dogs and cats. Further research is needed to determine such claims. From an industry perspective, products that need only be applied once and have extended residuals are ideal from an economic and labor standpoint.

The results of this field trial suggest topical spot-on formulations work well against stick tight fleas in chickens. Most importantly, the martin house that could have provided a source of fleas was removed. Follow up environmental treatments with a natural product may have helped control some stages of the flea lifecycle (i.e. larvae, eggs). The effectiveness of spot on formulations are due to residual activity and unique dispersion across the skin. In labeled poultry products such as dusts and aerosols, it is difficult to quickly and effectively disseminate product across the entire chicken. Vectra 3Ds relatively low toxicity profile makes it a great addition to formulations currently used in poultry.



Figure 4.1 Serial photographs of chickens before treatment and 10 days after.



Figure 4.2 Serial photographs of chickens before treatment and 10 days after.

CHAPTER V

LABORATORY ASSAY WITH VARIOUS INSECTICIDES AGAINST BED BUGS TAKEN FROM A POULTRY HOUSE IN MISSISSIPPI

Abstract

Bed bugs may be severe pests in poultry houses, especially broiler breeder houses, leading to stress, anemia, and lowered egg production in the birds. Insecticides are important tools in managing these pests; however, efficacy data for many commercially available products is lacking. This laboratory study evaluated 8 insecticides (5 traditional residual products and 3 natural or “green” products) against bed bugs collected from a poultry house in Mississippi during August 2014. Invader[®] (propoxur), Ravap[®] (rabon/vapona combination), and Cimexa[®] (silica gel) killed 100% of bed bugs when exposed to ceramic tiles sprayed with the products 24 hours earlier. Interestingly, Ravap[®] is labeled for use inside poultry houses even with birds present. Cimexa[®] is a non-toxic powder which desiccates bugs. Tempo[®] and Suspend[®] (pyrethroids) killed 73% and 93% of bed bugs, respectively, indicating that the poultry house field strain used in this study was likely not pyrethroid resistant. The organophosphate, Durashield[®] (chlorpyrifos), also showed good efficacy against poultry bed bugs, killing 96% of the bugs in this test, but can only be used during clean-out. Alpine dust[®] (diatomaceous earth/dinotefuran) and EcoExempt[®] (phenethyl propionate/soybean oil/clove oil), both

considered natural products, only killed about half of the exposed bed bugs in this study (46% and 45% control, respectively).

Introduction

Bed bugs are small, flat, oval-shaped wingless insects that feed on the blood of warm-blooded animals such as humans, bats, birds, and pets (Little & West, 2008; Reinhardt & Siva-Jothy, 2007; Ryckman et al., 1981; Thomas et al., 2004; Usinger, 1966). They have traditionally been common in the developing world, and especially in areas of extreme poverty and crowding. These blood-sucking parasites have recently increased in both numbers and geographic distribution (Goddard & De Shazo, 2008; M. F. Potter, 2006), and are increasingly being reported inside U.S. hotel rooms, dorms, and apartments (Anderson & Leffler, 2008; Cleary & Buchanan, 2004; Gangloff-Kauffmann et al., 2006; Goddard, 2011). Bed bugs can survive long periods without feeding. When their preferred human hosts are absent they may take a blood meal from any warm-blooded animal, including poultry (C.M. Kahn et al., 2008; Steelman et al., 2009). Poultry houses, and especially broiler breeder houses, may be severely affected, leading to stress, anemia, and lowered egg production in the birds (Cater, Magee, Hubbard, Edwards, & Goddard, 2011; Steelman et al., 2009). This study was initiated to examine the effectiveness of several commercially available insecticides against bed bugs collected from a broiler breeder poultry house.

Methods

Eight commercially-available insecticides were used in this study (Table 5.1). All are appropriately labeled for bed bug control except for Invader[®] (propoxur, a

carbamate), which was included as a standard known to kill 100% of bed bugs (Goddard, 2013). EcoExempt[®], Alpine dust[®], and Cimexa[®] are all considered low-toxicity, natural or green products. The others are all traditional residual insecticides -- Durashield[®] and Ravap[®] are older organophosphates, while Tempo[®] and Suspend[®] are commonly used pyrethroid products. All bed bugs used in this study were field-caught from a poultry house on August 6, 2014, located near Noxapater, MS, and were used within two weeks of capture. Substrates used in this study were 4.5 cm square ceramic tiles (Lowe's Inc.) which were sprayed/dusted exactly as per pesticide label directions. They were then flipped over and the other side treated since bed bugs will invariably crawl to the underside of a substrate. Tiles were allowed to dry for 45 minutes and then placed in standard 100 mm x 15 mm petri dishes (Fisher Scientific Co.). Bed bugs were then placed directly on the tiles in the dishes, 20 per dish, and allowed to remain on the treated substrates for 24 hr, after which they were moved to clean plates with filter paper for another 24 hr before observation. Twenty bed bugs were used per petri dish x 9 treatments (8 treatments and 1 water control), 3 replicates of each treatment yielding a total of 540 bed bugs tested. Bed bugs were examined for mortality only – those with no leg movement were considered dead. Results were recorded and entered into an Excel spreadsheet for analysis.

Results and Discussion

All control bed bugs were alive at the time of observation, allowing for good comparisons with the treatment groups (Figure 5.1). Tempo[®] and Suspend[®] (the pyrethroids) killed 73% and 93% of bed bugs, respectively, indicating that the poultry house field strain used in this study was likely not pyrethroid resistant, a finding similar

to that published earlier (Goddard, 2013). NOTE: many populations of human dwelling-inhabiting bed bugs are highly resistant to pyrethroids (Gordon, Goodman, Potter, & Haynes, 2014; Romero, Potter, Potter, & Haynes, 2007), but none of those found in Mississippi chicken houses have thus far shown any resistance (J. Goddard, unpublished data). Invader[®] killed 100% of bed bugs, which was not unexpected, but so did Ravap[®] and Cimexa[®]. The Ravap[®] result is good news in that the product is labeled for use inside poultry houses even with birds present. Other products like Durashield[®] and Tempo[®] require birds to be absent during treatment. Cimexa[®] is a non-toxic silica gel which dries out the bugs, and has recently been shown to hold great promise in bed bug control (M.F. Potter et al., 2014). The organophosphate, Durashield[®], also showed good efficacy against poultry bed bugs, killing 96% of the bugs in this test, but as mentioned, can only be used during clean-out after the birds are gone. Alpine dust[®] and EcoExempt[®], both considered natural products, only killed about half of the exposed bed bugs in this study (46% and 45% control, respectively).

Our results, although a preliminary screening only, indicate that several insecticide tools are available to aid poultry growers and technicians in managing bed bug populations. Although certain insecticides have been demonstrated as effective against bed bugs in a laboratory setting, eliminating or even just managing bed bug infestations in an ongoing poultry operation is extremely difficult. No one product is a magic bullet and successful bed bug control involves an integrated approach using both chemical and non-chemical methodologies.

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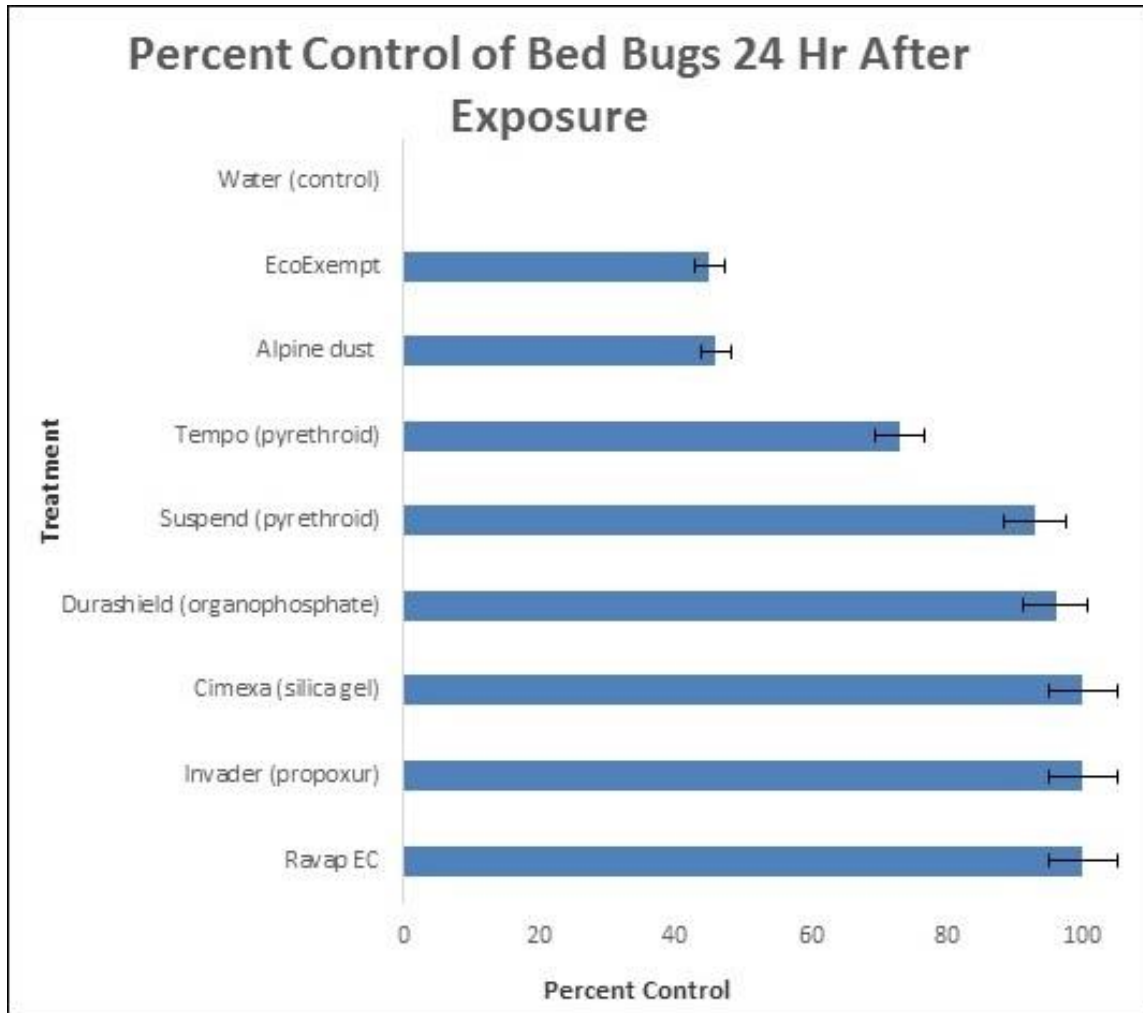


Figure 5.1 Effectiveness of insecticides tested against wild type bed bugs.

Table 5.1 Insecticides used in the study.

Product	% Active ingredients	How applied
Alpine dust (BASF Corp.)	Dinotefuran 0.25%	0.32 oz per 10 ft ²
Suspend SC (Bayer Corp.)	Deltamethrin 4.75%	1.5 oz per gallon, 1 gallon per 1,000 ft ²
Cimexa (Rockwell Labs)	100% Amorphous silica gel	2 oz per 100 ft ²
Tempo SC Ultra (Bayer Corp.)	B-Cyfluthrin 11.8%	0.54 fl oz per gallon; general surface application, fan spray
Durashield (BASF Corp.)	Chlorpyrifos 20.0%	5 fl oz per gallon, 1 gal per 1,000 ft ²
Invader (FMC Corp.)	Propoxur 1.0%	Per label, "crawling pests, 1 sec spray per spot"
Ravap E.C. (Bayer Corp.)	Rabon 23.0%; Vapona 5.3%	5 fl oz per gallon, 1 gal per 150 ft ²
EcoExempt D (EcoSmart Technologies)	2-Phenethyl propionate 4.5%; Soybean oil 2.0%; Clove oil 1.75%	2 oz per 100 ft ²

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