Imidacloprid (Advantage®) International Flea Susceptibility Monitoring: Update 2005

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Abstract

An international imidacloprid flea susceptibility monitoring team was established in 1999 to develop and validate a bioassay to effectively monitor and document imidacloprid susceptibility of cat flea (*Ctenocephalides felis*) isolates collected from the field.

During 1999 and 2000 the team developed, standardized, and validated a larval bioassay. An imidacloprid discriminating dose of 3ppm was selected which was approximately 2 times the highest LC_{95} of laboratory flea strains. During the 2001-2004 flea seasons, the team used this standardized bioassay to test a number of flea isolates collected from veterinary clinics within the USA, the United Kingdom ,and Germany. Flea eggs collected from the field were assayed in 5 independent laboratories.

Of the 774 collections, 602 isolates were tested with the assay. Approximately 67% of the isolates were received between August and December during the years sampled. Of those isolates received, 76% were collected from cats while 24 % were collected from dogs. Three isolates collected from the US (Augie, Ginger, and Sunshine) demonstrated adult survivorship in the assay. These isolates were studied further using the full bioassay to determine the LC_{95}s. The LC_{95}s for these isolates ranged from 0.74 to 3.85ppm compared to 0.92-5.55ppm for the baseline isolates collected from US field sites in 2000. None of those isolates had LC_{95}s that were outside the range of the LD_{95}s established with the 2000 baseline field isolates.

These data indicate that the flea strains with adult survivorship had susceptibility to imidacloprid well within the natural spectrum of field isolates ,and provided support for the selection of 3ppm as a single discriminating dose in susceptibility monitoring. None of the field collections evaluated in the 2001–2004 assays were determined to be less susceptible to imidacloprid compared to the 2000 baseline.

Introduction

Imidacloprid, a neonicotinoid insecticide, was introduced to the animal health market in 1996 for the treatment and control of flea infestations (*C. felis)* on cats and dogs (Advantage®). Historically, *C. felis* has developed resistance to a variety of insecticides including pyrethroids, organophosphates, carbamates, and fipronil following repeated exposures[1,2]. In 1999 an international team of veterinarians, parasitologists, and entomologists supported by Bayer Animal Health was formed to develop baseline data regarding the susceptibility of laboratory flea strains to imidacloprid. A larval bioassay was subsequently established with an imidacloprid discriminating dose of 3ppm which was approximately 2 times the highest LC 95 of the laboratory strains. Baseline data were necessary as a comparative standard for evaluation of wild flea strains collected in the field, and to determine any shifts in susceptibility of *C. felis* to imidacloprid [3-6]. Between 2001 and 2004, a total of 774 cat flea field isolates were collected from the United States, United Kingdom, and Germany with 602 of the isolates assayed for imidacloprid susceptibility.

Materials and Methods

Cat flea isolates were submitted from participating veterinary practices. Each practice was provided with a kit and instructions on how to collect and submit eggs to the laboratories. Infested pets were held in cages and cat flea eggs were collected from the cage floor for up to 24 hours. The eggs were then placed in a glass tube for overnight shipping to the laboratory. A questionnaire was completed for each submission which included information on the pet, other pets in the household, and information regarding recent use of insecticides.

The larval assay method developed to monitor large numbers of cat flea isolates collected from the field with the selection of 3ppm as the discriminating dose has been reported[7,8]. A minimum of 40 viable flea eggs were required for each assay. Technical imidacloprid was dissolved at the desired concentrations in acetone, and then mixed with flea rearing media. The acetone was evaporated, and the media was transferred to glass petri dishes. Twenty viable flea eggs were then exposed to the treated media and 20 eggs were exposed to acetone only treated control media. The petri dishes were covered and incubated at 26-28°C, 75-80% RH for 28 days. The petri dishes were examined after 5 days and again at 11-14 days for hatching of larvae and/or pupae development. After 28 days, live adult fleas were counted in both the control and treated dishes. If survivorship occurred in the treated dishes, the adult fleas reared in the control dishes were placed on laboratory cats to expand the populations for further follow-up assays. The LC_{95} values of the surviving isolates were determined with a full dose-response study using flea rearing media containing serial dilutions of imidacloprid ranging, from 0.05 to 30 ppm.

Adult flea hatch was observed at the discriminating dose of 3 ppm imidacloprid from 3 isolates collected from the US designated as " Sunshine" (a multiple cat and dog household) and "Ginger" (a multiple cat household), both from the state of Louisiana; and "Augie" ,an isolate collected from a cat household in California. The isolate designated as "Augie" was evaluated using the full assay at all 5 participating laboratories. The "Sunshine" isolate was assayed with the full assay at Auburn, Riverside, and the Kansas labs ;and "Ginger" was evaluated using the full assay at the Riverside, CA lab. For comparative purposes, the LC_{95} values of Sunshine, Ginger, and Augie were compared to the LC_{95} values of 6 baseline field isolates evaluated at each US lab during 2000.

Results and Discussion

A total of 774 cat flea isolates were submitted to the 5 participating laboratories between 2001– 2004, and assays using the 3 ppm imidacloprid discriminating dose were conducted in 602 of these isolates (Table 1).

Flea egg isolates were collected from 582 cats [76 %] and 184 dogs [24%], (Table 2). Approximately 67% of the isolates were received between the months of August and December (Table 3).

Flea treatment history provided for the 3 field isolates with adult survivorship at 3 ppm imidacloprid ("Sunshine", "Ginger", and "Augie") did not indicate previous use of Advantage® in these households.

The LC_{95}S for Sunshine, Ginger, and Augie determined with the full assay ranged from 0.74 to 3.85 ppm (Table 4).

The LC_{95}S of these 3 isolates compared to the LC_{95} values of 6 baseline isolates evaluated at the 3 US labs in 2000 is displayed in Figure 1. None of the 3 isolates had LC_{95}S that were outside of the range of the LC_{95}S established with the initial 2000 baseline field isolates(i.e. 0.92-5.55 ppm).

Table 1: *C. felis* Egg Collections Received (Assayed)

Participating Laboratory	2001 - 2004			
	2001	2002	2003	2004
Auburn Univ.	45 (36)	18 (16)	20 (16)	45 (40)
Kansas State Univ.	39 (25)	16 (11)	26 (16)	40 (34)
Monheim, Germany	37 (23)	27 (26)	25 (25)	51 (41)
Univ. Calif, Riverside	46 (26)	17 (15)	22 (17)	44 (40)
RVC, London, UK	73 (53)	59 (50)	57 (40)	67 (52)
Total	**240 (163)**	**137 (118)**	**150 (114)**	**247 (207)**
		Total Received		**774**
		Total Assayed		**(602)**

Table 2: *C. felis* Eggs – Collections from Cat vs. Dogs

	2001	2002	2003	2004	2005
Cat	166	112	110	194	582
Dog	70	25	38	51	184
Total	**236**	**137**	**148**	**245**	**766**

	Auburn	Kansas	Monheim	UCR	RVC	Total
Cat	87	63	138	69	225	582
Dog	41	57	2	57	27	184
Total	**128**	**120**	**140**	**126**	**252**	**766**

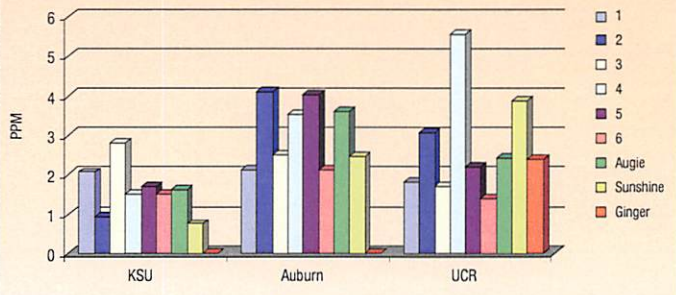
Table 3: *C. felis* Eggs – Collections from Cat vs. Dogs

	2001	2002	2003	2004	Totals
January		4	9	11	24
February		3	6	4	13
March		1	7	4	12
April	4	3	6	6	19
May	6	11	17	8	42
June	10	8	5	32	55
July	24	20	14	30	88
August	33	18	12	47	110
September	49	28	32	60	169
October	64	17	23	15	119
November	28	12	9	14	63
December	13	12	6	14	45
(blank)	5	-	2	-	7
Totals	**236**	**137**	**148**	**245**	**766**

Table 4: Imidacloprid LC_{50} and LC_{95} of Three *C.felis* Isolates with Adult Survivorship at 3ppm

	Augie		Sunshine		Ginger	
	LC_{50}	LC_{95}	LC_{50}	LC_{95}	LC_{50}	LC_{95}
Auburn	1.7	3.6	0.541	2.435	-	-
Riverside	0.824	2.4	0.34	3.85	0.59	2.38
Kansas	0.668	1.61	0.39	0.74	-	-
Monheim	0.238	1.58	-	-	-	-
London	0.415	0.962	-	-	-	-

Figure 1 Comparative Imidacloprid LC_{95} Values



Conclusions

None of the field collections evaluated in the 2001-2004 assays were determined to be less susceptible to imidacloprid compared to the 2000 baseline.

These data indicate that the 3 flea strains with adult survivorship had imidacloprid susceptibility well within the natural spectrum of the field isolates, and provided support for the selection of 3ppm imidacloprid as a single discriminating dose in susceptibility monitoring.

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