Parasitol Res (2013) 112:S47–S56 DOI 10.1007/s00436-013-3280-z

Monitoring Field Susceptibility to Imidacloprid in the Cat Flea: A World-First Initiative Twelve Years on

Steven Kopp[1] (✉), Byron Blagburn[2], Glen Coleman[1], Wendell Davis[3], Ian Denholm[4], Chris Field[5] Joe Hostetler[3], Norbert Mencke[6], Robert Rees[7], Michael Rust[8], Iris Schroeder[6], Kathrin Tetzner[6], Martin Williamson[4]

[1] University of Queensland, Gatton, QLD, Australia
[2] Auburn University, Auburn, AL, USA
[3] Bayer Health Care, Shawnee Mission, KS, USA
[4] Rothamsted Research, Hertfordshire, United Kingdom
[5] Bayer plc Animal Health Division, Newbury, United Kingdom
[6] Bayer Animal Health GmbH, 51368 Leverkusen, Germany
[7] Bayer Health Care, Brisbane, QLD, Australia
[8] University of California, Riverside, Riverside, CA, USA

Corresponding author:
Steven Kopp
✉ E-mail: s.kopp@uq.edu.au

Abstract

In 2001, an international surveillance initiative was established, utilising a validated larval development inhibition assay to track the susceptibility of cat flea isolates to imidacloprid. In 2009, an Australian node was incorporated into the programme, joining laboratories in the United States and Europe. Field isolates of *Ctenocephalides felis* eggs were submitted to participating laboratories and, where egg quantity and quality was sufficient, were placed in the imidacloprid discriminating dose bioassay for evaluation. Between 2002 and 2012, a total of 2,307 cat flea isolates were received across all sites; 1,685 submissions (73%) were suitable for placement into the bioassay. In the Northern Hemisphere, isolate submission rate was influenced by season, with highest numbers submitted

between June and October. In Australia, pets with flea infestations could be sourced year-round, and submission rate was largely influenced by programme factors and not climate. A total of 1,367 valid assays were performed between 2002 and 2012 (assay validity data was not recorded in 2001); adult flea emergence 5% or greater at 3 ppm imidacloprid was observed in 38 of these assays (2.8%). For these isolates that reached the threshold for further investigation, re-conduct of the assay using either a repeat challenge dose of 3 ppm of imidacloprid or a dose response probit analysis confirmed their susceptibility to imidacloprid. From 2009 to 2012, the Australian node performed valid assays on 97 field isolates from a total of 136 submissions, with no adult emergence observed at the 3-ppm imidacloprid discriminating dose. In addition to reviewing the data generated by this twelve-year initiative, this paper discusses lessons learned from the coordination and evolution of a complex project across geographically dispersed laboratories on three continents.

Introduction

The cat flea, *Ctenocephalides felis felis*, is widely recognised as the most common ectoparasite infesting dogs and cats worldwide (Bond et al. 2007; Rust 2005; Slapeta et al. 2011). Aside from being a prominent cause of dermatitis and anaemia in these companion animals (reviewed by Dryden 2009), the cat flea is also a recognised vector for several disease agents, including *Bartonella* and *Rickettsia* spp. (Bitam et al. 2010), feline calicivirus (Mencke et al. 2009) and feline leukaemia virus (Vobis et al. 2003, 2005). The subcutaneous filarial nematode *Dipetalonema reconditum* and the flea tapeworm *Dipylidium caninum* are also dependent upon the cat flea as an intermediate host (reviewed by Mehlhorn 2012). The fact that *C. felis* vectors at least three zoonotic pathogens, *Bartonella henselae*, *Rickettsia felis* (Bitam et al. 2010) and *Yersinia pestis* (Mehlhorn 2012), shines a spotlight on this parasite in the public health arena (Richter et al. 2002; Oteo et al. 2006; Mosbacher et al. 2010; Williams et al. 2011). Given the close association between owners and their pets, it can be argued that flea control is important not only from a veterinary standpoint, but also as a protective measure for humans.

Approaches to cat flea control have evolved considerably over time. Arguably the most significant change in the last twenty years has been a shift away from strategies that were underpinned by separate and often very frequent chemical treatment of host and environment, towards reliance upon monthly application of treatment to host only (Rust 2005). This has been achieved thanks to the advent of several highly effective insecticide classes that maintain a high degree of residual adulticidal efficacy on the host and, in many cases, possess activity against off-host life stages in the host's environment (Dryden 2009). These newer actives, namely imidacloprid, fipronil, selamectin, dinotefuran and spinosad, have facilitated owner compliance by providing convenience. It must be acknowledged, however, that the desirable persistence of contemporary actives on the host is also a factor that renders potential vulnerability to the development of resistance. While no definitive evidence of high-level *C. felis* field resistance to the neonicotinoid, avermectin, spinosyn or phenylpyrazole classes has been published to date, Payne and colleagues (2001) have previously highlighted a flea isolate that showed reduced susceptibility to topical fipronil from 20 days post treatment. Dryden and colleagues (2005) observed a decline in topical imidacloprid efficacy on cats challenged with infestation 30 days post treatment. A study by Franc and Yao (2007) reported similar findings. It is not entirely clear whether the effective shortening of residual activity described in these reports reflects an absolute reduction in efficacy, or a reduced speed of kill (Kramer and Mencke 2001). It is worth noting that imidacloprid has been demonstrated to have a rapid direct contact effect upon fleas, as opposed to actives such as fipronil and selamectin,

which primarily require ingestion by fleas for pulicidal activity (Mehlhorn et al. 1999, 2001). Such differences in mode of action exert a profound effect upon speed of kill (Everett et al. 2000; Mehlhorn et al. 2001) and cautious interpretation is therefore required when comparing different actives under *in vivo* conditions. What is also unclear is how often suspect flea isolates, rarely reported in the literature, actually occur in the field, and whether such isolates are truly representative of the systematic development of resistance, or merely a reflection of the biological variability that might be expected in different flea populations (Kramer and Mencke 2001).

Given the extensive global use of modern monthly adulticides against *C. felis*, a handful of suspect isolate responses identified in the literature should not be cause for undue alarm. It is, however, worth noting the well-documented historical development of high-level field resistance to predecessor insecticide classes utilised in flea control, including the synthetic pyrethroids, carbamates and organophosphates (Rust and Dryden 1997). Driven largely by the recognised need to make more judicious use of newer insecticides has been the adoption of an integrated flea control (IFC) approach, which was borne out of the integrated pest management (IPM) concept first pioneered in the agricultural sector (Kogan 1998). The IFC approach advocates client education around flea biology, and reinforcement of adulticidal therapy by concurrent administration of insect growth regulators (IGRs) and employment of mechanical control practices such as regular vacuuming of the environment and use of flea traps (Dryden and Broce 2002).

A central tenant of integrated pest management is 'monitoring' (Abrol and Shankar 2012). While this component of IPM traditionally refers to monitoring of pest levels in the environment as a means to inform strategic control practices, it stands to reason that this rationale should extend to surveillance around the development of insecticide resistance in pest populations. To this end, an intensive *C. felis* imidacloprid susceptibility monitoring initiative was established in 1999, comprising an international group of collaborating laboratories. The basis of the monitoring undertaken by this group is a validated imidacloprid larval development inhibition assay (Rust et al. 2002), for which a diagnostic dose (DD) has been determined to facilitate applicability to high-throughput field isolate screening (Rust et al. 2005). While the concept of utilising laboratory bioassays for monitoring parasiticide responses is not novel, the geographic scope, high degree of co-ordination and longevity of this monitoring initiative make it unique, particularly within the field of veterinary parasitology. With the programme entering its twelfth year of formal operations, it is timely to review the data generated by this work and to reflect upon lessons learned from the coordination and evolution of a complex project across geographically dispersed laboratories on three continents.

Materials and methods

Field isolate sampling

Field isolates of flea eggs collected from veterinary clinics were submitted to participating parasitology laboratories in the United States (Auburn, Riverside, Kansas), Germany (Monheim), the United Kingdom (London) and Australia (Brisbane). Upon enrolment into the programme, participating clinics were supplied with a collection kit, comprising equipment and receptacles for flea egg collection, a detailed protocol and a questionnaire requesting information from the owner regarding their pet's signalment, flea preventative treatment history and pertinent details of the animal's environment, including the presence of other pets in the household (Blagburn et al. 2006; Rust et al. 2005).

Flea eggs were collected from patients identified to be carrying a flea burden according to the supplied protocol (Rust et al. 2005). Briefly, the animal was placed into an environment conducive to collection of flea eggs, typically a cage with mesh or grated floor, and provided with *ad libitum* water

and access to food and litter trays as required. An appropriate collection medium, such as newspaper, was placed underneath the cage. Following a period of between 4 h and 24 h, the animal was gently but thoroughly combed in order to remove flea eggs from the pelage. Eggs and debris collected underneath the cage were then transferred to a glass collection tube by use of a supplied sieve and funnel. Where overnight shipping was required, tubes were packed into an appropriately insulated container; for shorter same-day shipping and where climatic conditions were not extreme, tubes were bubble-wrapped for protection and shipped in an envelope.

Diagnostic dose bioassays

Upon receipt of a field submission, the responsible laboratory assessed the submitted material for flea egg quantity using a dissecting microscope. Where flea egg quantity was sufficient to permit conduct of a diagnostic dose (DD) bioassay, this was performed in accordance with the protocol outlined by Rust and colleagues (2005). Batches of 20 eggs were placed into glass Petri dishes containing either larval rearing media pre-treated to achieve a concentration of 3 ppm imidacloprid, or untreated media as a control. Up to six treated and three untreated replicates were prepared for each isolate where egg quantity permitted. As a further quality control measure, 20 eggs representative of in-house *C. felis* reference strains available at each site were placed on untreated media and incubated in parallel with the test isolate, in order to monitor conditions affecting hatchability. All dishes were incubated at 26 ± 2 °C and 80–85% relative humidity. Dishes were inspected after 12 d incubation in order to quantify larvae and pupae, and again at 28 d to quantify emerged adults.

Debris testing

Where no egg hatch was observed in untreated replicates, and sufficient debris was present in the submitted material, a debris test was performed to screen for the presence of inhibitory residues. As outlined by Rust and colleagues (2011), this procedure entailed incubation of 20 control strain eggs with the debris (26 ± 2 °C and 80–85% relative humidity). Failure of any control eggs to hatch and develop to adults after 28 d incubation was considered to indicate the presence of an inhibitory residue in the submission.

Repeat diagnostic dose bioassays

If 5% or greater adult emergence was observed at the 3 ppm DD imidacloprid concentration, emerged adult fleas in the untreated control dishes were retained and placed on a cat in order to propagate the isolate. Where any participating laboratories did not have access to cats for propagation, pupae were promptly shipped to the nearest laboratory with this capacity. Following propagation, flea eggs were collected from the cat as previously described and a repeat 3 ppm imidacloprid DD bioassay was undertaken on three replicates of 20 eggs. At least one untreated control replicate was also setup in parallel. Emerged adult fleas were quantified in all dishes after 28 d incubation.

Full-dose bioassays and probit analysis

Field submissions with 5% or greater adult emergence in both preliminary and repeat DD bioassay testing were designated as isolates of interest. These isolates were further propagated on cats, and eggs were collected for use in full-dose response bioassays, conduct of which has been previously described (Rust et al. 2011). Briefly, larval rearing medium was treated to achieve a gradient of nine imidacloprid concentrations (0.005, 0.01, 0.05, 0.1, 0.3, 0.5, 1, 1.5 and 3 ppm). Twenty flea eggs were added to each dish, however a slight modification for the full-dose bioassays was that eggs were not directly added to the media, but were instead carefully glued (UHU adhesive, Saunders Co., ME, USA) to the lid of each dish. This strategy sought to minimise impacts of cannibalism on egg hatch and larval development, given the higher degree of granularity sought from this level of testing. As for the DD bioassay, untreated media was used to

Table 1 Field submission, DD bioassay and survivorship data across participating sites

Laboratory	Total submissions (2002–2012)	Valid DD bioassays	≥5 % survivorship 3 ppm imidacloprid	Repeat survivorship 3 ppm imidacloprid
Auburn	581	349	12	0
Kansas*	132	71	4	0
Monheim^	491	313	12	0
Riverside	618	300	10	0
London*	349	237	0	0
Brisbane§	136	97	0	0
Total	2307	1367	38	0

* Participated 2002–2006; § Participated 2009 onwards;
^ Samples from country of laboratory except Monheim with samples from across Europe including Germany, France & UK

monitor control mortality. Dishes were incubated at 26 ± 2 °C and 80–85% relative humidity, with larvae and pupae counting at 12 d and quantification of emerged adults at 28 d. Dose response probit lines were generated using the POLO software package (Version 1.0, LeOra Software, Menlo Park, CA, USA), permitting calculation of dose response slope, LC_{50} and LC_{95} values.

Results

Field isolate sampling

While the programme was formally initiated in 2001, and 240 field isolates were submitted in this first year of operation, bioassay data was formally recorded and archived from 2002 onwards. Between January 2002 and December 2012, a total of 2,307 field isolates were received across all sites. This was represented by 1,516 isolates obtained from cats (approx. 66%) and 770 isolates from dogs (approx. 33%); a host species was not recorded for 21 submissions. An overview of submissions received across investigator sites is provided in Table 1. Submissions in North America totalled 1,331, 840 were received across Europe and 136 were received in Australia. Egg numbers in each submission ranged from zero to greater than 5,000, although only 2% of submissions contained more than 1,000 eggs. There was an average of 208 eggs per submission across the programme. In the Northern Hemisphere, which represents all sites outside of Australia, field isolate submission rate tended to peak between June and December, while submissions in the Southern Hemisphere (Australia) were highest between August and October.

Diagnostic dose bioassays

Of the 2,307 field isolates received between 2002 and 2012, 1,685 (73%) were suitable for placement into the imidacloprid DD bioassay. The greatest factor rendering isolates unusable was insufficient flea egg numbers. Of the 1,685 bioassays performed, 1,367 (81%) were valid as determined by hatching of at least one egg in control (untreated) media. The dorsal hind tibia of all emerging adults was examined and all specimens were consistent with *C. felis* and not the closely related species *Ctenocephalides canis*.

Discriminating dose bioassays conducted between 2002 and 2012 exposed 95,729 individual *C. felis* eggs to the 3 ppm imidacloprid diagnostic dose. A total of 453 eggs (0.47%), across 78 isolates, developed to adulthood. With respect to individual field submissions, flea emergence 5% or greater at 3 ppm imidacloprid was observed in 38 of the 1,367 tested in valid DD bioassays (2.8%) (Table 1). No clear trends emerged with respect to either percentage egg emergence, which ranged yearly

from <0.1–1.6%, or percentage field isolate survivorship at 5% or above, which ranged yearly from 0.6–9.8%. Of the 38 field isolates with 5% or greater emergence at 3 ppm imidacloprid, 26 were sourced in North America and 12 from Europe. No emergence at 5% or greater was observed in any field isolates tested in the Southern Hemisphere (Australia). With respect to invalid DD bioassays, 67% of those for which debris could be tested showed nil control strain egg hatch suggesting the presence of an inhibitory residue.

Repeat diagnostic dose bioassays and full-dose bioassays

Propagation of isolates reaching or exceeding the 5% survivorship threshold in preliminary testing could not be achieved in many cases. Factors impeding propagation included submissions containing only enough eggs to run a single control replicate, resulting in few emerged adults, isolates for which control replicate hatchability was poor, isolates for which adults emerged from control media with poor fitness, and isolates that would not establish a fertile infestation even when apparently fit adult fleas were placed onto the pelage of a cat. In all cases where adequate propagation could be achieved, repeat diagnostic dose testing failed to identify any isolates that reached or exceeded the 5% survivorship threshold a second time, implying that survival was a chance effect rather than indicating a systematic shift in susceptibility.

While no isolates were deemed to require full-dose gradient profiling on the basis of reaching or exceeding the survivorship threshold in two consecutive DD bioassays, some isolates were selected to undergo full-dose testing on the basis of moderate to high emergence of adult fleas (generally above 10%) in preliminary DD testing. Full-dose gradient profiling of these isolates is outlined in Table 2. None of these isolates had observed slope, LC_{50} or LC_{95} characteristics that were appreciably different from the susceptible reference strains UCR, AUB and KS1.

Discussion

The imidacloprid susceptibility monitoring initiative reviewed in this paper represents the most ambitious and comprehensive surveillance project for any parasiticide in the veterinary sector. Over a period of twelve years, more than 1,300 individual cat flea isolates were screened with valid imidacloprid discriminating dose bioassays across three different continents. It is notable that just 0.47% of all eggs assayed over this period developed to adult fleas in the presence of 3 ppm imidacloprid, and just 2.8% of field isolates exceeded the diagnostic dose threshold. Crucially, none of these isolates reached or exceeded the 5% survival threshold on repeat testing, hence they could not be characterised as possessing field resistance to imidacloprid. As commented an earlier review of this initiative (Rust et al. 2011), it is likely that initial survivorship in the DD bioassay that cannot be replicated represents a chance finding, and not the systematic development of resistance in that population.

It is worth reflecting upon data pertaining to the flea isolate submission process itself, since this can provide insight into seasonal prevalence of *C. felis*. In the Northern Hemisphere, sampling was undertaken year-round, with submission rate largely driven by the availability of fertile flea burdens yielding eggs. Unsurprisingly, viable submissions peaked during the warmer months between June and October, which is likely to represent the most active period for expansion of flea biomass within a household. However, as noted previously (Rust et al. 2011), the programme has consistently received Northern Hemisphere submissions during winter months, albeit at a reduced rate. This finding advocates year-round treatment, particularly since winter flea burdens are likely to provide a strong impetus for a rise in flea numbers as warmer spring temperatures become more conducive to larval development. In Australia, submission rate was highly influenced by programme factors, since many isolates were sourced from patients admitted

Table 2 Lethal doses of imidacloprid in larval rearing medium required to kill larval *Ctenocephalides felis*

Strain/isolate	n	slope ± SE	LD_{50} (95 % CI)	D_{95} (95 % CI)
UCRI*§	505	6.5 ± 1.16	1.25 (1.038–1.427)	2.24 (1.838–3.756)
UCRII*¤	534	2.68 ± 0.292	0.12 (0.039–0.214)	0.48 (0.260–2.957)
AUBI*§	394	5.5 ± 1.06	0.48 (0.395–0.564)	0.97 (0.802–1.383)
AUBII*¤	410	4.64 ± 0.962	0.12 (0.094–0.158)	0.28 (0.212–0.518)
KS1*§	1208	4.3 ± 0.62	0.73 (0.566–0.851)	1.75 (1.405–2.638)
MON*	403	3.44 ± 0.986	0.11 (0.070–0.154)	0.34 (0.239–0.673)
AUSI*§	268	3.8 ± 0.80	0.48 (0.322–0.611)	1.29 (0.577–2.240)
AUSII*	458	3.16 ± 0.528	0.09 (0.059–0.120)	0.30 (0.207–0.608)
USA1§	245	2.91 ± 0.492	0.18 (0.071–0.291)	0.67 (0.415–1.981)
USA2	435	4.56 ± 0.715	0.18 (0.141–0.216)	0.42 (0.341–0.560)
USA3	373	3.50 ± 0.689	0.21 (0.133–0.272)	0.62 (0.473–0.968)
USA4	438	2.81 ± 0.375	0.15 (0.100–0.198)	0.58 (0.419–0.964)

* Denotes susceptible reference strain; § Denotes that this data is as previously presented by Rust et al. 2011;
USA# – field isolates sourced from United States; ¤ UCRII, AUBII and AUSII are the same continuously maintained strains as UCRI, AUBI and AUSI, UCRII, AUBII and AUSII data reflects profiling of these strains from 2009 onwards

to a desexing clinic associated with that laboratory's institution. This clinic was most active between August and October each year, hence submission rate was strongest during this period. The fact that isolate sampling at the Australian node of the programme was largely sustained by submissions during late winter and early spring is testament to the need for year-round use of flea preventatives in this country also, where much of the population resides in subtropical or mildly temperate climatic zones.

The incorporation of an Australian node in 2009 was an important means of expanding the geographic scope of the initiative. There were, however, significant challenges to be overcome in establishing a participating laboratory on this isolated island continent. Aside from the marked geographic separation of the Australian laboratory from those in the USA and Europe, increasing internationalisation of the initiative required significant co-ordination to achieve import and export of biological materials across international borders. Harmonisation of bioassay procedures has been an ongoing priority for the susceptibility monitoring team, a process that necessitates exchange of flea strains between laboratories. A primary goal upon establishment of the Australian node was shipment of a representative *C. felis* field isolate from that country for full-dose response profiling in collaborating international laboratories. The dose response characteristics of this isolate (AUS 1) were outlined in an earlier review of the initiative (Rust et al. 2011); it was determined to be phenotypically similar to the imidacloprid-susceptible reference strains UCR, KS1 and AUB. Profiling of this isolate was one step in a detailed series of laboratory visits, personnel training workshops and teleconferences that began as early as 2007. This comprehensive planning facilitated a relatively seamless entry of the Australian laboratory into the programme in 2009.

Although only 97 Australian cat flea isolates have undergone valid DD bioassay testing to date, a reflection of the Australian laboratory's nascent participation and the considerably smaller population base in Australia as compared to North America and Europe, it is noteworthy that none of these isolates enabled 5% or greater survivorship at 3 ppm imidacloprid. This uniform field susceptibility is consistent with the dose response profile of AUS 1, which showed remarkable similarity to

imidacloprid-susceptible reference strains maintained in the USA. It should be noted that, although this isolate was grouped with field survivors in a previous review of this work (Rust et al. 2011), this isolate was profiled as an exercise in bioassay harmonisation and not due to survivorship in the 3 ppm imidacloprid DD bioassay.

Australia is a continent of unique isolation and biodiversity, where a rich agricultural heritage has seen widespread use of pesticides, notably persistent organic compounds such as dieldrin and DDT (Kausik 1991). Although use of these compounds has now been banned in that country (Shivaramaiah et al. 2002), background exposure of parasite populations to bioaccumulative compounds creates uncertainty around how such populations might respond over time to novel parasiticides, particularly where non-specific metabolic resistance mechanisms are possible. Superimposed on this is the high degree of treatment compliance seen in Australian pet owners (Schantz 1991). While commendable, strong treatment compliance obviously drives selection for resistance. It is therefore heartening that, in the sixteen years since Hopkins and colleagues (1996) demonstrated high *in-vivo* efficacy of imidacloprid against a representative Australian *C. felis* isolate, there appears to have been no appreciable shift in imidacloprid susceptibility in that country.

In an earlier review, Rust and colleagues (2011) commented on a significant reduction in control hatchability of field isolates over the first seven years of the programme, a trend that has continued through 2012. As outlined previously, it is possible that this reduction is due to receipt of an increased number of samples contaminated by an IGR, reflecting increased market-share of combination adulticide-IGR flea control products. Driven by concerns around IGR contamination of field isolates, routine testing of debris from invalid DD bioassays has now been formally incorporated into the programme. In 2012, this testing revealed nil control strain egg hatch in debris from 67% of invalid bioassays tested, suggesting IGR contamination in these submissions. While scrutinising debris with a control strain hatchability test is a useful preliminary screen for presence of inhibitory residues, it remains a somewhat blunt tool and requires that appreciable debris are present in submitted material. Given that invalid bioassays are a waste of physical and human resources, it would be useful to be able to identify a contaminated submission upon receipt, and to be able to determine the identity of any inhibitory residue present. This is increasingly possible as technology advances, but requires further investigation.

A further challenge that has emerged more recently is the difficulty encountered in propagating isolates that exceed the survivorship threshold in preliminary imidacloprid DD screening. It is not clear whether this is related to any kind of fitness cost associated with reduced susceptibility to imidacloprid, because survivorship in preliminary 3 ppm DD bioassays is not definitive proof of imidacloprid tolerance. As previously discussed, all surviving isolates for which propagation was achieved did not survive subsequent 3 ppm imidacloprid exposure, and isolates for which full-dose response profiling was undertaken were comparable to susceptible reference strains. Fitness cost has been associated with imidacloprid resistance in crop pests (Baker et al. 2007; Liu and Han 2006), however, it is too early to determine whether this might also occur in *C. felis*, particularly since no imidacloprid-tolerant strains have yet been identified. While failed propagation of interesting isolates is a frustration in one sense, there is also opportunity for investigation into why many of these isolates do not readily favour propagation.

The longevity of this imidacloprid susceptibility monitoring initiative is testament to the high degree of cooperation between collaborating laboratories, and the sustained and valued contributions of the many veterinary clinics participating in the programme. Coordinated surveillance projects such as the one described in this paper are a crucial undertaking; it would be foolish to assume that modern flea adulticides are immune from

resistance and even more foolish to expect that the golden age of parasiticide discovery enjoyed over the last 25 years will continue indefinitely. It is noteworthy, however, that in spite of scrutiny across three continents over an extended period, there continues to be an ongoing lack of *C. felis* field isolates showing resistance to imidacloprid. The adoption of integrated flea control practices is an excellent step that favours responsible insecticide use, however, these ideals must be reinforced by comprehensive surveillance using sensitive tools that are likely to detect emerging resistance long before it manifests as treatment failure in the field.

Ethical standards

All aspects of the programme involving use of live animals conform to the 'Guide for the Care and Use of Laboratory Animals', National Research Council, USA. Additionally, all work was approved by institutional animal ethics committees responsible for oversight of animal welfare at each participating site of the collaboration.

Conflict of interest

The study was supported in part by Bayer Animal Health GmbH, Leverkusen, Germany. Employment of the authors by Bayer as indicated in the list of affiliations.

Acknowledgements

We thank representatives of laboratories previously participating in this initiative; Professor Michael Dryden and Associate Professor Patricia Payne of the Kansas laboratory, and Dr Ross Bond who represented the United Kingdom laboratory. The invaluable contributions of technical staff in each of the participating laboratories, past and present, are also gratefully acknowledged. Lucio Rodriguez (University of California, Riverside, CA, USA), Tracey Land (Auburn University, Auburn, AL, USA), Lyn Knott and Kim Jell (University of Queensland, Gatton, QLD, Australia), Sabrina Collica (Monheim Laboratory, Germany), Chris Booler, Mel Hutchinson and Anna Riddle (Royal Veterinary College, UK), and Vicky Smith (Kansas State University, Manhattan, KS, USA) have all played a vital role in technical aspects of the work.

References

Abrol DP, Shankar U (2012) Integrated pest management: principles and practice. CABI, Cambridge, MA, p 502

Baker MB, Alyokhin A, Porter AH, Ferro DN, Dastur SR, Galal N (2007) Persistence and inheritance of costs of resistance to imidacloprid in Colorado potato beetle. J Econ Entomol 100:1871–1879

Bitam I, Dittmar K, Parola P, Whiting MF, Raoult D (2010) Fleas and flea-borne diseases. Int J Infect Dis 14:e667–676

Blagburn BL, Dryden MW (2009) Biology, treatment, and control of flea and tick infestations. Vet Clin North Am Small Anim Pract 39:1173–1200

Blagburn BL, Dryden MW, Payne P, Rust MK, Jacobs DE, Bond R, Hutchinson MJ, Denholm I, Mehlhorn H, Vaughn M, Mencke N, Schroeder I, Hostetler J, Endrizzi M (2006) New methods and strategies for monitoring susceptibility of fleas to current flea control products. Vet Ther 7:86–98

Bond R, Riddle A, Mottram L, Beugnet F, Stevenson R (2007) Survey of flea infestation in dogs and cats in the United Kingdom during 2005. Vet Rec 160:503–506

Dryden MW (2009) Flea and tick control in the 21st century: challenges and opportunities. Vet Dermatol 20:435–440

Dryden MW, Broce AB (2002) Integrated flea control for the 21st century. Comp Cont Ed Pract Vet 24:36–39

Dryden MW, Smith V, Payne PA, McTier TL (2005) Comparative speed of kill of selamectin, imidacloprid, and fipronil-(*S*)-methoprene spot-on formulations against fleas on cats. Vet Ther 6:228–236

Everett R, Cunningham J, Arther R, Bledsoe DL, Mencke N (2000) Comparative evaluation of the speed of flea kill of imidacloprid and selamectin on dogs. Vet Ther 1:229–234

Franc M, Yao KP (2007) Comparison of the activity of selamectin, imidacloprid and fipronil for the treatment of cats infested experimentally with *Ctenocephalides felis felis* and *Ctenocephalides felis strongylus*. Vet Parasitol 143:131–133

Hopkins TJ, Kerwick C, Gyr P, Woodley I (1996) Efficacy of imidacloprid to remove and prevent *Ctenocephalides felis* infestations on dogs and cats. Aust Vet Pract 26:150–153

Jaga K, Dharmani C (2003) Global surveillance of DDT and DDE levels in human tissues. Int J Occup Med Environ Health 16:7–20

Kausik C (1991) Persistence and metabolism of HCH and DDT in soil under subtropical conditions. Soil Biol Biochem 23:131–134

Kogan M (1998) Integrated pest management: historical perspectives and contemporary developments. Annu Rev Entomol 43:243–270

Kramer F, Mencke N (2001) Flea Biology and Control: The Biology of the Cat Flea, Control and Prevention with Imidacloprid in Small Animals. Springer, Berlin Heidelberg, p 192

Liu Z, Han Z (2006) Fitness costs of laboratory-selected imidacloprid resistance in the brown planthopper, *Nilaparvata lugens*. Pest Manag Sci 62:279–282

Mehlhorn H (2012) Fleas as Underestimated Vectors of Agents of Diseases. In: Mehlhorn H (ed) Arthropods as Vectors of Emerging Diseases. Springer, Berlin Heidelberg, pp 301–328

Mehlhorn H, Hansen O, Mencke N (2001) Comparative study on the effects of three insecticides (fipronil, imidacloprid, selamectin) on developmental stages of the cat flea (*Ctenocephalides felis* Bouché 1835): a light and electron microscopic analysis of in vivo and in vitro experiments. Parasitol Res 87:198–207

Mehlhorn H, Mencke N, Hansen O (1999) Effects of imidacloprid on adult and larval stages of the flea *Ctenocephalides felis* after in vivo and in vitro application: a light- and electron-microscopy study. Parasitol Res 85:625–637

Mencke N, Vobis M, Mehlhorn H, J D'Haese, Rehagen M, Mangold-Gehring S, Truyen U (2009) Transmission of feline calicivirus via the cat flea (*Ctenocephalides felis*). Parasitol Res 105:185–189

Mosbacher M, Elliott SP, Shehab Z, Pinnas JL, Klotz JH, Klotz SA (2010) Cat scratch disease and arthropod vectors: more to it than a scratch? J Am Board Fam Med 23:685–686

Oteo JA, Portillo A, Santibanez S, Blanco JR, Perez-Martinez L, Ibarra V (2006) Cluster of cases of human *Rickettsia felis* infection from Southern Europe (Spain) diagnosed by PCR. J Clin Microbiol 44:2669–2671

Payne PA, Dryden MW, Smith V, Ridley RK (2001) Effect of 0.29 % w/w fipronil spray on adult flea mortality and egg production of three different cat flea, *Ctenocephalides felis* (Bouche), strains infesting cats. Vet Parasitol 102:331–340

Richter J, Fournier PE, Petridou J, Haussinger D, Raoult D (2002) *Rickettsia felis* infection acquired in Europe and documented by polymerase chain reaction. Emerg Infect Dis 8:207–208

Rust MK (2005) Advances in the control of *Ctenocephalides felis* (cat flea) on cats and dogs. Trends Parasitol 21:232–236

Rust MK, Denholm I, Dryden MW, Payne P, Blagburn BL, Jacobs DE, Bond R, Mencke N, Schroeder I, Weston S, Vaughn M, Coleman G, Kopp S (2011) Large-scale monitoring of imidacloprid susceptibility in the cat flea, *Ctenocephalides felis*. Med Vet Entomol 25:1–6

Rust MK, Denholm I, Dryden MW, Payne P, Blagburn BL, Jacobs DE, Mencke N, Schroeder I, Vaughn M, Mehlhorn H, Hinkle NC, Williamson M (2005) Determining a diagnostic dose for imidacloprid susceptibility testing of field-collected isolates of cat fleas (Siphonaptera: Pulicidae). J Med Entomol 42:631–636

Rust MK, Dryden MW (1997) The biology, ecology, and management of the cat flea. Annu Rev Entomol 42:451–473

Rust MK, Waggoner M, Hinkle NC, Mencke N, Hansen O, Vaughn M, Dryden MW, Payne P, Blagburn BL, Jacobs DE, Bach T, Bledsoe D, Hopkins T, Mehlhorn H, Denholm I (2002) Development of a larval bioassay for susceptibility of cat fleas (Siphonaptera: Pulicidae) to imidacloprid. J Med Entomol 39:671–674

Schantz PM (1991) Parasitic zoonoses in perspective. Int J Parasitol 21:161–170

Shivaramaiah HM, Odeh IO, Kennedy IR, Skerritt JH (2002) Mapping the distribution of DDT residues as DDE in the soils of the irrigated regions of northern New South Wales, Australia using ELISA and GIS. J Agric Food Chem 50:5360–5367

Slapeta J, King J, McDonell D, Malik R, Homer D, Hannan P, Emery D (2011) The cat flea (*Ctenocephalides f. felis*) is the dominant flea on domestic dogs and cats in Australian veterinary practices. Vet Parasitol 180:383–388

Vobis M, D'Haese J, Mehlhorn H, Mencke N (2003) Evidence of horizontal transmission of feline leukemia virus by the cat flea (*Ctenocephalides felis*). Parasitol Res 91:467–470

Vobis M, D'Haese J, Mehlhorn H, Mencke N (2005) Experimental quantification of the feline leukaemia virus in the cat flea (*Ctenocephalides felis*) and its faeces. Parasitol Res 97(Suppl 1):S102–S106

Williams M, Izzard L, Graves SR, Stenos J, Kelly JJ (2011) First probable Australian cases of human infection with *Rickettsia felis* (cat-flea typhus). Med J Aust 194:41–43