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ORIGINAL PAPER

Toxicity and potential utility of ivermectin and moxidectin as xenointoxicants against the common bed bug, *Cimex lectularius* L.

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Abstract The recent resurgence of the common bed bug *Cimex lectularius* L. throughout western industrialized nations has been facilitated in part by the insect becoming pesticide-resistant. Novel control strategies, including xenointoxication, should be considered to combat *C. lectularius*. Ivermectin, a U.S. Food and Drug Administration (FDA)-approved treatment for several human parasites, and the antiparasitic drug moxidectin, currently being explored in human clinical trials, were evaluated for efficacy against *C. lectularius*. Results showed that *C. lectularius* fed on ivermectin or moxidectin blood concentrations of >25 ng/mL and had significantly higher mortality (50–100 %) than controls (0–6 %) by day 13. Bed bugs that survived a blood meal containing >2.5 ng/mL of ivermectin suffered long-term sequelae including reduced fecundity, feeding difficulty, and incomplete ecdysis. Some insects that survived a blood meal containing ≤75 ng/mL moxidectin were able to feed and reproduce.

Keywords Ivermectin · Moxidectin · *Cimex lectularius* · Bed bug · Mortality · Treatment · Xenointoxication

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Introduction

The common bed bug, *Cimex lectularius* L., is an obligate hematophagous temporary ectoparasitic arthropod that feeds preferentially on humans. *C. lectularius* is arguably one of the most important human parasites in the United States today, despite not being a definitive vector of disease causing pathogens. The common bed bug, hereafter referred to as "bed bug," is associated with significant social stigma and numerous mental health problems, including high levels of anxiety, social isolation, insomnia, stress, depression, nightmares, and suicidal ideation (Goddard and de Shazo 2012; Rieder et al. 2012). The U.S. Environmental Protection Agency (EPA) and the U.S. Centers for Disease Control and Prevention (CDC) consider *C. lectularius* to be of "significant public health importance" (CDC and EPA joint statement 2010).

The bed bug resurgence is partly due to increased resistance to commonly used pesticides (Romero et al. 2007; Adelman et al. 2011; Anderson and Cowles 2012). The management of bed bug infestations usually involves an integrated pest management (IPM) strategy utilizing a combination of appropriate premise preparation, pesticide applications, and/or heat treatment(s) (Bennett et al. 2016). The management of bed bugs can become financially burdensome and typically require multiple visits by a pest management professional (PMP).

Health-care facilities are increasingly reporting bed bug infestations (Leininger-Hogan 2011; Bandyopadhyay et al. 2015; Totten et al. 2015). In 2012, 33 % of PMPs in Canada reported responding to bed bug infestations in Canadian hospitals, a 50 % increase from 2011 (Williams 2013). Additionally, the number of PMP visits to nursing homes in Canada increased from 25 % in 2011 to 46 % in 2012 (Williams 2013). The National Electronic Injury Surveillance System-All Injury Program (NEISS-AIP)

reported an increase of U.S. emergency department patient visits related to bed bugs, from 2156 in 2007 to 15,945 in 2010, an approximately sevenfold increase (Langley et al. 2014). At one U.S. academic hospital emergency department (ED), a bed bug was found on or associated with a patient once every 3.8 days (Totten et al. 2015). This resulted in a treatment room being removed from service for "decontamination" approximately 18 h per event. Estimated direct cost for an ED room decommissioning due to bed bugs was $29,574 annually (Totten et al. 2015).

Health-care providers increasingly are being asked to diagnose and treat patients who claim they have experienced bed bug-feeding activity. The clinical diagnosis of cimicosis, the bed bug-feeding lesion, is difficult; feeding lesions are usually non-specific pruritic dermal reactions that rarely present concurrently with systemic allergic symptoms, and some patients do not develop skin reactions at all (Goddard and de Shazo 2009). However, if an identified insect is collected in proximity to a patient with dermal reaction consistent with possible cimicosis, the diagnosis of bed bug-feeding activity may be made. Other conditions, such as delusory parasitosis, allergic reactions, and depression, can mimic cimicosis, and these need to be considered in the differential diagnosis.

A problem for health-care providers is the absence of evidence-based recommendations on the symptomatic management of cimicosis and lack of prescription drugs to help manage bed bug infestations (Goddard and de Shazo 2009). Furthermore, there may be reasons a patient with bed bugs, especially in socioeconomically disadvantaged urban or rural communities, is unable to follow advice from health-care providers to engage the services of a PMP. These may include prohibitive financial burden, fear (especially in difficult landlord-tenant relationships), logistics, mental health and substance abuse, and homelessness. Additionally, health-care providers' lack of familiarity with bed bug management is a continuing problem. Physicians may not be familiar with the concept of referring a patient with bed bugs to a PMP, an entity that does not provide medical care, may not provide feedback to the referring physician, and which exists outside the formal health-care system.

Xenointoxication, the concept of "host intoxication," is a novel approach for bed bug control (McNeil 2012 and Sheele et al. 2013). This is when a drug with limited toxicity to humans at therapeutic doses (e.g., ivermectin or moxidectin) becomes systemically absorbed and becomes toxic or lethal to a blood-feeding ectoparasite (McNeil 2012). Ivermectin has been used as a xenointoxicant in the management of two other important human ectoparasites, human scabies, *Sarcoptes scabiei* var. *hominis* and the human head louse *Pediculus humanus capitis* (Currie and McCarthy 2010; Feldmeier 2014). Ivermectin also has a long history as a xenointoxicant in livestock and in deer for tick management (Davey et al. 2001; Lancaster 1982; Miller 1999; Pound 1996). Previous reports have demonstrated bed bug morbidity and mortality in an artificial feeding system using ivermectin in a mouse model and in humans taking a single 0.2-mg/kg dose of ivermectin (Sheele et al. 2013). Additional studies using fourth instar nymphs fed on mice that had consumed ivermectin-laced food for 5 days prior to test (>5 parts per million (ppm) or 5000 ng/mL) caused 100 % paralysis in the insects (Ostlind et al. 2001). In addition, it was shown that low doses of ivermectin caused delayed nymphal molting.

Ivermectin, discovered by Satoshi Ōmura and William C. Campbell (who won the 2015 Nobel Prize in Physiology or Medicine for this work), was derived from a compound produced by the bacterium, *Streptomces avermitilis*. Approved for medical and veterinary use in 1981, ivermectin is now one of the most ubiquitous antiparasitic drugs with activity against numerous invertebrate parasites. More than 570 million doses of ivermectin have been given to humans during the last 20 years in mass drug administrations for the control of onchocerciasis (Geary 2005; Ōmura 2008; González Canga et al. 2008; Thylefors et al. 2008).

The FDA-approved dose of ivermectin in humans is 0.15–0.2 mg/kg. In two studies, fasting healthy human adult volunteers were given a single dose of 0.165 mg/kg of ivermectin. Blood plasma levels peaked at 46.6 (range 16.4–101.1) and 30.6 (range 13.9–68.4) ng/mL ~4-hour post ingestion (Merck package inset). Additional reviews on the human pharmacology, pharmacokinetics, and the toxicity of ivermectin have been published (Edwards et al. 1988; Baraka et al. 1996; Guzzo et al. 2002; Geary 2005; Ōmura 2008; González Canga et al. 2008; Thylefors et al. 2008).

Moxidectin, derived from nemadectin, a fermentation product of the Actinobacteria *Streptomyces cyanogriseus*, was discovered in Australian soils during the late 1980s. It is a macrocyclic lactone, structurally aligned within the milbemycin family (Prichard et al. 2012). It is currently used in veterinary medicine for the control of several parasitic organisms (Prichard et al. 2012). Moxidectin's mode of action is similar to ivermectin, binding to glutamate-gated chloride channels of invertebrates causing paralysis (Ōmura 2008; Prichard et al. 2012). Humans lack glutamate-gated chloride channels which limits human toxicity (Cotreau et al. 2003). A principal advantage of oral moxidectin over oral ivermectin for human use is that the serum half-life of moxidectin is ~20–35 days compared to ~18–22 h with ivermectin (Cotreau et al. 2003; Korth-Bradley et al. 2011, 2012). Moxidectin is not FDA-approved for use in humans. However, there have been six human clinical trials reported on the federal website clinicaltrials.gov (clinicaltrials.gov 2015b). Recently, the Global Health Investment Fund (GHIF) committed $10 million to complete the dossier for the registration of moxidectin for the treatment of onchocerciasis (WHO press release 2015). Reviews on moxidectin's pharmacology and pharmacokinetics in humans have been published (Cotreau et al. 2003; Korth-Bradley et al. 2011, 2012).

Ivermectin has already been found to be toxic to *C. lectularius* (Ostlind et al. 2001 and Sheele et al. 2013). This study tested select doses of ivermectin against *C. lectularius*, with an extended period of post feeding observation (up to 91 days), to evaluate viability and fecundity of treated insects. Additionally, this was the first study to assess moxidectin toxicity against *C. lectularius*.

Materials and methods

Insects were from the Ridge strain of bed bugs first collected from an infested apartment in New Haven, Connecticut (2009), and maintained in the laboratory. Insects for these particular tests were carefully selected for health and low stress, only young adults, and healthy nymphs. Specimens were laboratory-raised under constant monitoring in established natural refuges following natural daylight cycles at a constant temperature of ~24 °C and 40–50 % RH. Populations were never handled except for shipment. Field-collected insects were not considered, because of unknown history and condition. Laboratory-raised specimens were shipped by Dr. Ridge overnight from The Connecticut Agricultural Experiment Station (CAES) to the laboratory of Dr. Sheele before each feeding experiment. All feedings took place in the laboratory of Dr. Sheele. Unless otherwise indicated, the number and life stages of specimens were not held constant. This mimicked unknown readings of a natural population. Live specimens were held in either 50-mL conical test tubes or 2-mL microcentrifuge tubes. Each test tube contained one piece of cardboard for the insects to cling to, and the open mouth was covered with a small piece of sheer bridal veil secured in place, through which the insects fed. This was natural behavior for these insect, because they were reared to feed through sheer fabric at CAES.

C. lectularius specimens were fed once on different concentrations of ivermectin- and moxidectin-treated blood using an artificial feeding system modeled after (Chin-Heady et al. 2013). Blood containing Hanks balanced salt solution (HBSS) alone, ivermectin + HBSS, or moxidectin + HBSS was placed in a cap. The cap was heated to ~37 °C/98.6 °F (normal human body temperature), using a hot plate or water bath. Each test tube, holding the bed bugs, was covered with parafilm to prevent drowning. To facilitate feeding, each test tube was put into the warm blood. Insects then fed through the fabric and parafilm to repletion. Specimens that did not feed were removed from the experiment and destroyed. Those that exhibited evidence of feeding, including partial feeding, were placed in clean test tubes and observed. Partially fed bed bugs were not separated from fully engorged insects, mimicking conditions that might occur in a real-world infestation.

For all ivermectin experiments, a 1.0 % suspension of ivermectin (Noromectin w/v Multi Injection Solution for Injection, Norbrook® Laboratories (GB) Ltd.) was diluted in HBSS. The first three moxidectin feeding tests used QUEST® Equine Oral Gel (moxidectin 20 mg/mL (2.0 % *w*/*v*), Pfizer Animal Health 0.4 oz (11.6 g)) diluted in HBSS. For ease of additional dilutions, remaining moxidectin treatments utilized Cydectin® Injectable Moxidectin (Boehringer Ingelheim Vetmedica) containing propylene glycol (50–75 %), ethanol (20 %), and 1 % moxidectin diluted in HBSS. It was not possible to use human blood so insects were fed on animal blood (Barbarin et al. 2013). The first four feeding tests used defibrillated rabbit blood, and the last three used defibrillated sheep's blood. *C. lectularius* naturally host switch; and a difference in host blood in these experiments should not have significantly affected the insects (Usinger 1966). Blood was obtained from Hemostat laboratories, Dixon, CA and stored at 1–2 °C. Since long-term storage of blood is toxic for *C. lectularius*, blood was stored for no longer than 3–4 weeks before discarding (Bell and Schaefer 1966).

There were five feeding tests using nymphs and adults. Test six used only adults to study the effects of ivermectin and moxidectin on fecundity. In each experiment, the total volume of HBSS (+/− ivermectin or moxidectin) remained constant, but the percent volume of HBSS in blood samples varied.

Test one: a total of 50 microliters (μL) of HBSS was used as the control; then, concentrations of ivermectin or moxidectin diluted in HBSS to a final volume of 50 μL were added to 4950 μL of defibrillated rabbit blood. The final concentrations of ivermectin or moxidectin in the blood samples were 0 (control), 25, 50, 75, or 100 ng/mL.

Test two: a total of 2.5 μL of HBSS was used as the control; then, concentrations of ivermectin or moxidectin diluted with HBSS to a final volume of 2.5 μL were added to 4997.5 μL of defibrillated rabbit blood. The final concentrations of ivermectin or moxidectin in the blood samples were 0 (control) and 5 ng/mL.

Test three: a total of 100 μL of HBSS was used as the control; then, concentrations of ivermectin or moxidectin diluted in HBSS to a final volume of 100 μL were added to 4900 μL of defibrillated rabbit blood. The final concentration of ivermectin in the blood samples were 0 (control), 25, 50, 75, or 100 ng/mL. The final concentration of moxidectin in the blood samples were 0 (control), 25, 50, 75, 100, 125, or 150 ng/mL.

Test four: a total of 100 μL of HBSS was used as the control; then, concentrations of ivermectin or moxidectin diluted in HBSS to a final volume of 100 μL were added to 990 μL of defibrillated rabbit blood. The final concentrations of ivermectin or moxidectin in the blood samples were 0 (control), 2.5, 25, 50, 75, or 100 ng/mL. Engorged

insects from this experiment were sent to the laboratory of Dr. Ridge and observed for 81 days to look for drug-induced long-term morbidity and mortality, which included nymphal molt failure, adult fecundity, behavior change, and feeding disorder.

Test five: a total of 2 μL of HBSS was used as the control; then, concentrations of ivermectin or moxidectin diluted in HBSS to a final volume of 2 μL were added to 998 μL of defibrillated sheep blood. The final concentrations of ivermectin or moxidectin in the blood samples were 0 (control), 0.25, 2.5, 25, 50, 75, or 100 ng/mL. Fed insects from this experiment were sent to the laboratory of Dr. Ridge and offered to feed on a human host on days 97 and 112.

Test six: a total of 2 μL of HBSS was used as the control; then, concentrations of ivermectin or moxidectin diluted in HBSS to a final volume of 2 μL were added to 998 μL of defibrillated sheep blood. The final concentrations of ivermectin was 0 (control), 0.25, 2.5 ng/mL and moxidectin 2.5 ng/mL in the blood samples. Fed insects from this experiment were sent to the laboratory of Dr. Ridge and offered to feed on a human host on days 92 and 107.

A comparison of proportions analysis was used to determine statistical significance of results. Mortality rates for every dose were compared with mortality rates for the controls during each period of observation. In all the experiments, "mortality" was defined as an insect that was minimally responsive to stimulation or unable to cling to a substrate when gently shaken.

Results

The results of tests 1, 3, 4, and 5 are reported in Tables 1, 2, 3, and 4. Data indicate bed bugs fed on 25–100 ng/mL ivermectin or moxidectin-laced blood experienced high levels of mortality. Within 60 min after feeding, some insects showed lethargy, lack of aggregation behavior, and a desire to disperse (Fig. 1). This was in marked contrast to alert, active controls, which is normal healthy behavior.

Test two had four fed bed bugs in the HBSS control group, three in the 5-ng/mL ivermectin group, and four in the 5-ng/mL moxidectin group. Post feeding observations after 36 days showed two dead bed bugs in the control group, none in the ivermectin group, and one dead insect in the moxidectin group. Day 29 showed eggs in the ivermectin group and newly hatched 1st instar nymphs in the moxidectin group. Observations on day 36 showed eggs laid by ivermectin-treated females failed to hatch (Fig. 2) (Fig. 3).

Test six used lower doses of ivermectin or moxidectin on adult males and females to test against fecundity. Observations on day 16 after the feeding showed:

- HBSS control—four alive adult females plus eggs;
- 2.5 ng/mL moxidectin—three live adults, one dead adult, and eggs;
- 0.25 ng/mL ivermectin—three live adults, one dead adult, and eggs;
- 2.5 ng/mL ivermectin—one dead male and three dead females, no 1st instar nymphs or eggs.

Observations on day 76 before being shipped to Dr. Ridge for additional observation:

- HBSS control—one dead female, three alive females, 26 eggs, and 1st instar nymphs;
- 2.5 ng/mL moxidectin—three dead adults, one lethargic adult, one 1st instar nymph, and 17 unhatched eggs;
- 0.25 ng/mL ivermectin—four dead adults, five unhatched eggs, and 19 hatched 1st instar nymphs;
- 2.5 ng/mL ivermectin—one dead male and three dead females, no 1st instar nymphs or eggs.

Bed bugs offered a human blood meal on day 92 and observations were made within 24 h of the feeding:

- HBSS control—one dead female, three alive females, three dead 1st stage instar nymphs, eight live 1st stage instar nymphs, and all surviving bed bugs took a human blood meal;
- 2.5 ng/mL moxidectin—one dead male, three alive adult females, one live 1st stage instar, 17 unhatched eggs, and all living bed bugs took a human blood meal;
- 0.25 ng/mL ivermectin—one dead male and three live adult females, 19 1st stage instar nymphs, five unhatched eggs, and all live bed bugs took a blood meal;
- 2.5 ng/mL ivermectin—one dead male and three dead females, no 1st instar nymphs or eggs.

Bed bugs offered a second human blood meal on day 107 and observations were made within 24 h of the feeding:

- HBSS control—one dead female, three alive females, six dead 1st stage instar nymphs, 23 live 1st stage instar nymphs, five live 2nd stage instar nymphs, three dead 2nd stage instar nymphs, and all surviving bed bugs took another human blood meal;
- 2.5 ng/mL moxidectin—four dead adults, one live 1st stage instar which fed but did not molt after the day 92 feeding;
- 0.25 ng/mL ivermectin—four dead adults, 14 live 2nd stage instar nymphs, five dead 2nd stage instar nymphs,

Table 1 Test 1: percentage mortality with different concentrations of ivermectin or moxidectin and days observed

Drug and dose	Day 5	Day 6	Day 10	Day 13	Day 20	Day 24	Day 34	Day 36	Day 41	Day 48
0 ng control	0 %	0 %	0 %	6 %[ab]	6 %	0 %	0 %	5 %[c]	0 %	0 %
	$n=15$	$n=15$	$n=15$	$n=16$	$n=16$	$n=15$	$n=16$	$n=22$	$n=33$	$n=29$
25 ng ivermectin	92 %	92 %	100 %	100 %	100 %	92 %	92 %	100 %	100 %	100 %
	$n=12$	$n=12$	$n=12$	$n=12$	$n=12$	$n=12$	$n=12$	$n=12$	$n=12$	$n=12$
	$p<0.001$									$p<0.001$
50 ng ivermectin	89 %	100 %	100 %	100 %	100 %	78 %	89 %	89 %	89 %	89 %
	$n=9$	$n=9$	$n=9$	$n=9$	$n=9$	$n=9$	$n=9$	$n=9$	$n=9$ (1 nymph alive)	$n=9$ (1 1st instar nymph)
	$p<0.001$									$p<0.001$
75 ng ivermectin	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %
	$n=2$	$n=2$	$n=2$	$n=2$	$n=2$	$n=2$	$n=2$	$n=2$	$n=2$	$n=2$
	$p<0.001$									$p<0.001$
100 ng ivermectin	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %
	$n=16$	$n=16$	$n=16$	$n=16$	$n=16$	$n=16$	$n=16$	$n=16$	$n=16$	$n=16$
	$p<0.001$									$p<0.001$
25 ng moxidectin	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %
	$n=6$	$n=6$	$n=6$	$n=6$	$n=6$	$n=6$	$n=6$	$n=6$	$n=6$	$n=6$
	$p<0.001$									$p<0.001$
50 ng moxidectin	83 %	83 %	67 %	67 %	67 %[#]	67 %	67 %	67 %	67 %	25 %
	$n=6$	$n=6$	$n=6$	$n=6$	$n=6$ (2 adults alive)	$n=6$	$n=6$	$n=6$	$n=6$[@]	$n=16$
	$p<0.001$								$p<0.001$	(10 1st instar nymphs)
75 ng moxidectin	100 %	100 %	100 %	100 %	100 %	100 %	100 %	67 %	67 %	67 %
	$n=6$	$n=6$	$n=6$	$n=6$	$n=6$	$n=6$	$n=6$	$n=6$	$n=6$	$n=6$
	$p<0.001$									$p<0.001$
100 ng moxidectin	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %
	$n=5$	$n=5$	$n=5$	$n=5$	$n=5$	$n=5$	$n=5$	$n=5$	$n=5$	$n=5$
	$p<0.001$									$p<0.001$

Any increase in populations over time was because of successful hatching by 1st instar nymphs

n = the number of bed bugs that were recorded

[a] molts present

[b] eggs present

[c] live hatched 1st instar nymphs

12 only partially fed, and two did not feed, of which one repeatedly groomed its extended stylus which could not penetrate skin and the other ran in random directions;

- 2.5 ng/mL ivermectin—one dead male and three dead females, no 1st instar nymphs or eggs.

Observations of tests 5 and 6 showed that some insects that were offered a second post treatment blood (97–112 days after the original ivermectin or moxidectin blood meal) fed slowly to partial repletion, some were disinclined to feed, some ran in random directions and did not feed, and others tried to feed but could not because the maxillary-mandibular stylets did not penetrate the feeding surface. The stylets either buckled and emerged as a loop between the labrum and maxillary lobe at the base of the rostrum (beak) or if elongated, could not be controlled by the insect. Insects that exhibited stylet failure, repeatedly groomed the rostrum, and became increasingly distressed. Those insects that did feed, defecated at site of feeding, which was abnormal behavior. Normal behavior of *C. lectularius* post feeding is to usually defecate away from the host to avoid detection. Lastly, it was observed that some nymphs either did not molt or had an incomplete molt.

Discussion

Results show that bed bugs suffer significant long-term morbidity and mortality after feeding on blood meals containing

Table 2 Test 3: percentage mortality of bed bugs fed on different doses of ivermectin or moxidectin on sequential days. Hatching 1st instar nymphs, added to control group population

Drug and dose	Day 5	Day 7	Day 8	Day 13
0 ng control	4 %	0 %	0 %	0 %
	$n=17$	$n=17$	$n=22$	$n=23$
25 ng ivermectin	25 %	65 %	75 %	95 %
	$n=20$	$n=20$	$n=20$	$n=20$
	$p=0.0815$	$p<0.001$		$p<0.001$
50 ng ivermectin	100 %	100 %	89 %	100 %
	$n=9$	$n=9$	$n=9$	$n=9$
	$p<0.001$			$p<0.001$
75 ng ivermectin	100 %	100 %	100 %	100 %
	$n=16$	$n=16$	$n=16$	$n=16$
	$p<0.001$			$p<0.001$
100 ng ivermectin	97 %	100 %	94 %	100 %
	$n=34$	$n=34$	$n=34$	$n=34$
	$p<0.001$			$p<0.001$
25 ng moxidectin	80 %	80 %	80 %	100 %
	$n=5$	$n=5$	$n=5$	$n=5$
	$p<0.001$			$p<0.001$
50 ng moxidectin	82 %	73 %	68 %	82 %
	$n=22$	$n=22$	$n=22$	$n=22$
	$p<0.001$			$p<0.001$
75 ng moxidectin	100 %	100 %	100 %	100 %
	$n=16$	$n=16$	$n=16$	$n=16$
	$p<0.001$			$p<0.001$
100 ng moxidectin	91 %	91 %	86 %	82 %
	$n=22$	$n=22$	$n=22$	$n=22$
	$p<0.001$			$p<0.001$
125 ng moxidectin	98 %	100 %	100 %	100 %
	$n=43$	$n=43$	$n=43$	$n=43$
	$p<0.001$			$p<0.001$
150 ng moxidectin	100 %	100 %	98 %	98 %
	$n=43$	$n=43$	$n=43$	$n=43$
	$p<0.001$			$p<0.001$

n = the number of bed bugs that fed.

the drugs ivermectin or moxidectin at concentrations seen in humans taking those drugs. Long-term observations of bed bugs that survived a blood meal of ivermectin at 2.5–100 ng/mL showed they could not reproduce. Observed eggs laid by females that had fed on concentrations of 2.5 ng/mL ivermectin or higher lacked yolk, and embryos did not develop (Fig. 2). However, at 0.25 ng/mL ivermectin, approximately, a 200-fold lower concentration than equivalent peak plasma levels observed in humans taking a single prescribed dose of 0.2 mg/kg ivermectin, there was lower mortality, insects mated, and viable eggs were laid resulting in live 1st instar nymphs. It is unclear whether these nymphs would mature to adulthood. First instar nymphs hatched from eggs laid by females exposed to 2.5 ng/mL moxidectin successfully took blood meals and molted, indicating short-term viability. The toxic effects of moxidectin did not appear to be as long lived, because surviving bed bugs were able to feed and reproduce.

Results are congruent with those observed in mosquitoes where ivermectin had been shown to be more toxic than moxidectin (Butters et al. 2012; Foy et al. 2011). There are currently several clinical trials underway to determine if ivermectin can reduce malaria transmission by adversely affecting *Anopheles* sp. mosquitoes (Foy et al. 2011 and clinicaltrials.gov 2015a). The toxicity levels we observed in bed bugs is similar to reported ivermectin toxicity in deer where serum ivermectin levels of ≥15 ng/mL provided >90 % control of female *Ixodes scapularis* ticks, subsequent oviposition, and larval eclosion (Rand et al. 2000).

At sub-lethal doses of ivermectin or moxidectin, insects exhibited lethargy, a lack of aggregation behavior, and a desire to disperse. *C. lectularius* naturally does "not evenly distribute over its environment" but is thigmotatic (Usinger 1966). This is a fundamental behavioral drive for full-body contact with others insects and substrates. By seeking a crevice, insects find physical protection and are able to conserve fluids and energy reserves while remaining quiescent. Insects that loose thigmotaxis disperse; yet, they may also be fleeing a perceived threat or responding to illness. If exposure to ivermectin or moxidectin causes this loss in behavior, then the isolation may render the insects susceptible to desiccation, unwanted mobility, and subsequent depletion of stored reserves. *C. lectularius* is also xerophilic, tolerating one-third water loss, high compared to other insects (Benoit et al. 2007). Isolated individuals have difficulties surviving for long periods of starvation, because of a high surface area to volume and lack of protective contact with other insects. Bed bugs cannot absorb water from the air and so depend on blood meals for hydration. Though there was a small population, numbers were not enough to protect against dehydration with clustering behavior, and so weaker individuals may have died. Although ivermectin or moxidectin may not cause immediate mortality, behavior changes caused by these drugs may result in subsequent mortality downstream.

In order to do serious harm to an established *C. lectularius* population, a drug may not need to have immediate knockdown. Populations of bed bugs might be eliminated through attrition when fecundity or ability to feed is adversely effected by ivermectin doses >2.5 ng/mL. Ivermectin caused higher *C. lectularius* mortality in adults than nymphs and delayed or prevented nymphal molting (Sheele et al. 2013). If nymphs cannot molt or feed as can be seen with partial stylus paralysis at 0.25-ng/mL levels, then development is halted.

Using healthy, low-stressed specimens, ivermectin and moxidectin doses of 25–100 ng/mL were lethal to most bed bugs. Surviving insects were often paralyzed and unable to

Table 3 Test 4: percentage mortality of bed bugs fed different doses of ivermectin or moxidectin on different days

Drug and dose	Day 6 mortality	Day 81 post feeding observations of living bed bugs
0 ng control	0 % $n = 27$	21 eggs, 7 nymphs fed to repletion, 1 adult fed to repletion, 6 exuviae
2.5 ng ivermectin	50 % $n = 12$ $p < 0.001$	4 surviving nymphs with arrested molts, cannot feed (not probing), 1 alive male, cannot feed (not probing)
25 ng ivermectin	60 % $n = 10$ $p < 0.001$	1 nymph alive, cannot feed (not probing). Rest of population dead
50 ng ivermectin	70 % $n = 10$ $p < 0.001$	All dead
75 ng ivermectin	84 % $n = 19$ $p < 0.001$	All dead
100 ng ivermectin	100 % $n = 13$ $p < 0.001$	1 live nymph that cannot feed (not probing). Rest of population dead
2.5 ng moxidectin	26 % $n = 23$ $p = 0.0052$	18 eggs (incl. one lacking yolk), 22 live nymphs (incl. 16 1st instars fed to repletion), and 7 exuvae
25 ng moxidectin	88 % $n = 17$ $p < 0.001$	2 hatched eggs, 5 nymphs (incl. one 1st instar) fed to repletion, 3 live adults able to fed to repletion
50 ng moxidectin	75 % $n = 8$ $p < 0.001$	1 nymph and 1 adult fed to repletion
75 ng moxidectin	100 % $n = 8$ $p < 0.001$	2 live nymphs, failed to molt, readily fed to repletion, 1 adult alive and readily fed to repletion
100 ng moxidectin	83 % $n = 12$ $p < 0.001$	All dead

n = the number of bed bugs that fed

feed. Post-treatment live insects survived on reserves, but eventually died. It might be speculated that bed bugs fed on blood containing 2.5–100 ng/mL ivermectin, and to a lesser extent, higher doses of moxidectin, in a real-world infestation, may lead to greater knockdown than in laboratory-raised specimens. This is because "wild" insects live under greater stress in building environments compared to laboratory specimens and are weaker (Ridge unpublished observations).

The resurgent global bed bug pandemic that started during the mid-late 1990s, at least in part, was due to adaptive resistance by *C. lectularius* to commonly used pesticides. These include the pyrethrins and pyrethroids. They act by preventing closure of the voltage-gated sodium channels in the axonal membranes, the pyrroles (e.g., Chlorfenapyr), by uncoupling mitochondrial oxidative phosphorylation (EPA website). Other pesticides, such as neonicotinoids, act at the nicotinic receptors of the nervous system, and insect growth regulators, mimic juvenile growth hormones preventing development (EPA website). None of these pesticides kill *C. lectularius* by the same proposed mode of action as ivermectin or moxidectin, suggesting that pesticide-resistant insects may be susceptible to these two drugs.

Not all insects in these experiments fed, and these were destroyed. This was consistent with normal bed bug-feeding behavior. Nymphs and adults during the immediate post molt period are disinclined to feed, females often skip a blood meal to avoid mating, and some individuals can be over stimulated, seeking a blood meal in the wrong direction (Ridge unpublished research.) Additionally, insects do not necessarily feed to repletion but take smaller blood meals. All these factors play into determining the efficacy rates of a drug. These initial experiments showed even partially fed insects suffer harm when exposed to higher dose concentrations of ivermectin or moxidectin.

Table 4 Test 5: percentage mortality for bed bugs fed on different doses of ivermectin or moxidectin on different days

Drug and dose	Day 4 mortality	Day 31 mortality	Day 91 mortality
0 ng control	0 % $n=23$	0 % A great deal of exuviae, no eggs, no 1st instar nymphs	13 %. No 1st instar nymphs or eggs
0.25 ng ivermectin	0 % $n=16$	6 % ($p=0.499$) Exuviae, eggs, and 1st instar nymphs present	25 % ($p=0.34$) Some treated bed bugs lethargic. Excuviae, eggs, and 1st instar present. Some1st instars had partial stylus paralysis
2.5 ng ivermectin	0 % $n=6$	33 % ($p=0.248$) Exuviae present	17 % ($p=0.80$) Nymphs lethargic, excuviae present
25 ng ivermectin	50 % $n=6$ $p<0.001$	50 % ($p=0.05$) All nymphs with incomplete molts, no eggs, no exuviae	83 % ($p=0.0008$) One live very lethargic insect
50 ng ivermectin	94 % $n=16$ $p<0.001$	88 % ($p<0.0001$) Live insects able to cling to substrate, but do not respond to touch. No eggs, no exuviae	88 % ($p<0.0001$) with the two live bed bugs lethargic to touch
75 ng ivermectin	100 % $n=15$ $p<0.001$	80 % ($p<0.0001$) Two bed bugs fell off substrate with agitation, but climbed back. One live nymph able to cling to substrate; lethargic to touch	93 % ($p<0.0001$) One live nymph lethargic to touch
100 ng ivermectin	92 % $n=26$ $p<0.001$	84 % ($p<0.0001$) Only nymphs survived; no molts, no eggs	96 % ($p<0.0001$) One live nymph
0.25 ng moxidectin	11 % $n=18$ $p=0.1073$	6 % ($p=0.4634$) Two live 1st instar nymphs, 2 eggs, and exuviae	39 % ($p=0.0575$) Population healthy with 1st instar nymphs
2.5 ng moxidectin	63 % $n=8$ $p<0.001$	38 % ($p=0.137$) One live adult female, no eggs	38 % ($p=0.137$) Live bed bugs, lethargic to touch; no eggs, no excuviae
25 ng moxidectin	50 % $n=6$ $p<0.001$	17 % ($p=0.820$) there were three eggs noted but no 1st stage instar nymphs	50 % ($p=0.05$) Eggs and 1st stage instars present
50 ng moxidectin	81 % n = 21 $p<0.001$	33 % ($p=0.112$) some nymphs with incomplete molts, exuviae present	48 % ($p=0.013$) One surviving bed bug is lethargic, no eggs or 1st stage nymphs present, but excuviae was present
75 ng moxidectin	70 % $n=10$ $p<0.001$	20 % ($p=0.611$) with one nymph having an incomplete molt, exuviae present	50 % ($p=0.025$) Surviving bed bugs lethargic, no eggs or 1st stage nymphs
100 ng moxidectin	0 % $n=4$	100 % ($p=0.0003$)	100 % ($p=0.0003$)

n = the number of bed bugs that fed with observations

C. lectularius life cycle is such that eggs take approximately 7–10 days to develop. After hatching, first instar nymphs rest for a period before seeking a blood meal. Bed bug nymphs need a blood meal before eclosion to the next instar. They feed approximately once a week depending on temperature and humidity. There are five instars before eclosion to adult. Adult females feed every 6–12 days, and adult males feed every 10–14 days. By understanding the biology of the *C. lectularius* and the pharmacokinetics of ivermectin, it may be possible to design a drug regimen to maximize harm to the insect through a temporal approach. Based on the known pharmacokinetics of ivermectin, a daily dose of 0.2 mg/kg of ivermectin taken by healthy adult humans would result in steady-state blood levels of ivermectin >25 ng/mL. In addition, it had been reported that the pharmacodynamic effects of ivermectin appear to exceed the drug's pharmacokinetics, because antiparasitic effects persisted for days after a single dose of the drug (Sylla et al. 2010 and Foy et al. 2011). There have been multiple animal studies involving the administering of ivermectin daily for more than three consecutive days; however, there have been few reports on humans (Hauber et al. 2005; Diazgranados-Sanchez et al. 2008;

Fig. 1 Cimex lectularius specimens exhibiting post-treatment stress by not aggregating after being fed ivermectin or moxidectin-treated blood (25–100 ng/mL) (left) compared to normal aggregation behavior after being fed untreated blood (right)

Fig. 3 *Cimex lectularius* nymph that fed on ivermectin laced blood (100 ng/mL). It could not feed on a human host 91-day post treatment. The insect exhibited a failed molt. The old exuviae was not shed and remained attached to the distal abdominal wall, head, and thorax

Currie and McCarthy 2010). Thus, safety of such a dosing strategy is unclear. If ivermectin were to be used against *C. lectularius*, a 20-day prescriptive period might be suggested for humans to intercept the insect's behavior rhythms and biological needs. Alternatively, a 60-mg oral dose of ivermectin (0.713–1.091 mg/kg) given every 3 days for a week has been shown to be safe in humans (Guzzo et al. 2002). Based on the published pharmacokinetics of ivermectin, a 30-mg dose of ivermectin, given in the fed state to enhance absorption, administered to a person with a bed bug infestation, likely followed by a repeated dose 2–3 weeks later, may potentially expose almost all insects in an undisturbed population to a toxic dose of ivermectin. The first three doses of ivermectin might incapacitate most actively feeding insects and either kill or render infertile, all of the adult females. A repeated dose of ivermectin 2–3 weeks later may catch 1st instar nymphs that had hatched after the initial treatment and any other bed bugs that had not fed during the first treatment period. This strategy may be very effective if a treated bed bug population is "closed," meaning no insect movement around a building, and all humans living with an infestation concurrently take the drug. Ivermectin could even be utilized alongside existing IPM treatments. Plans for human clinical trials are being developed to assess the efficacy of ivermectin therapy against bed bug infestations.

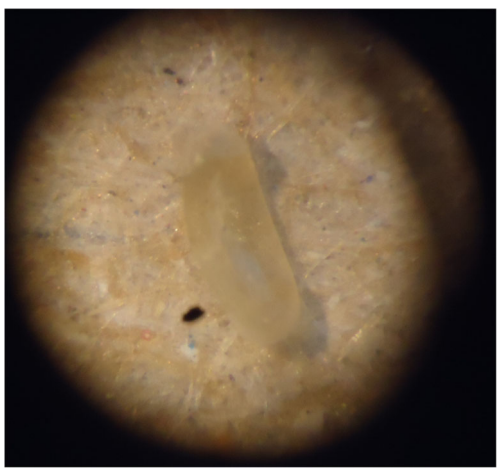

Fig. 2 Non-viable egg laid by female exposed to 2.5 ng/mL ivermectin-laced blood

No previous reports have shown the effects of moxidectin on bed bugs. Bed bugs occasionally appeared to be able to recover from toxic effects of the drug regaining ability to feed and reproduce with higher dose levels than seen with ivermectin (Tables 3 and 4). Moxidectin still may be a possible single-dose alternative to ivermectin for the treatment of *C. lectularius* infestations, because the half-life of oral moxidectin in human plasma is approximately 20–35 days compared to 18–22 h with ivermectin. *C. lectularius* harm was observed at moxidectin levels of 0.25–2.5 ng/mL; however, surviving insects recovered and were able to reproduce. Moxidectin would likely need to be given as part of a coordinated IPM approach rather than a monotherapy.

Oral administrations of 3–36 mg of moxidectin in humans were well tolerated in a single-dose escalation trial, although higher doses of moxidectin did demonstrate increased neurological side effects (Cotreau et al. 2003). A single 18-mg oral dose of moxidectin given with a meal to patients resulted in blood plasma concentration >10 ng/mL for the first 8 days. This then dropped to between 1 and 10 ng/mL during the following 8–50 days (Cotreau et al. 2003; Korth-Bradley et al. 2011, 2012). Based on known pharmacokinetics of moxidectin in humans, and the results herein, it may be assumed that a single oral dose of 18 mg of moxidectin may be lethal to most insects that take a blood meal within the first 7 days of drug ingestion by a patient. Bed bugs that feed after

the first week would likely receive a sub-lethal dose of moxidectin. However, these insects may be exposed to multiple sub-lethal doses of moxidectin over a period of time via repeated feedings, and a cumulative toxic effect may result in reduced fecundity and premature mortality. This was not specifically evaluated in the current study. One concern with low-dose, sub-lethal exposure to moxidectin is that *C. lectularius* populations may become resistant to the drug.

First instar nymphs hatched from eggs laid by females exposed to sub-lethal doses of moxidectin were able to feed, develop, and become fertile adults. The current study used an artificial feeding technique to quantify moxidectin toxicity against bed bugs. Morbidity and mortality to moxidectin has not been evaluated in an animal or human model, but these studies are currently being planned.

Ivermectin or moxidectin shows potential as xenointoxicants against human-feeding bed bugs and may be useful to assist in the control of bed bug infestations. These drugs may find particular utility as adjunctive therapy in severe and hard-to-manage infestations such as in pesticide-resistant populations, in poor building construction with excellent harborage, or where current IPM techniques are failing. For ivermectin or moxidectin to be used as part of a bed bug control strategy, there will need to be increased coordination between the medical community and PMPs as PMPs are unable to prescribe ivermectin (or moxidectin) for human use.

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