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EXPERIMENTAL STUDY

Environmental ivermectin concentrations spiked in cattle dung
(T1: 1000 ng/g and T2: 500 ng/g)

Two controls
(C: dung and CS: dung + ethanol)

9 days of exposure

EFFECTS ON *Culex pipiens*

- Survival
- Adult emergence

ACCUMULATION IN ALL DEVELOPMENTAL STAGES

Ivermectin bioaccumulation and transfer through developmental stages in *Culex pipiens* (Diptera: Culicidae)

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Abstract

Ivermectin (IVM), one of the most widely used antiparasitics in livestock, could enters into the aquatic environment because the treated animal metabolizes only a small percentage of what is administered and the rest is eliminated through the feces, largely as a parent drug, imposing a risk to aquatic organisms. The aims of this study were to (1) assess the effect of IVM spiked in cattle dung on the survival and emergence of *Culex pipiens* (Diptera: Culicidae), and to (2) evaluate the accumulation of this drug in the different developmental stages of this taxon. Larvae were exposed to two IVM concentrations (T1: 1000 ng g^{-1} and T2: 500 ng g^{-1}) for 9 days. At days 3, 6 and 9 survival and adult emergence were recorded and samples of larvae, pupae, pupal exuviae and adults were taken to analyze the IVM accumulation. At these concentrations, a reduction in survival and adult emergence of *C. pipiens* was recorded. In addition, the IVM accumulation was observed in all samples analyzed, decreasing it throughout the development of this taxon (larvae>pupae>adults). Although a large proportion of the drug was lost during the metamorphosis, being mainly eliminated through pupal exuviae during molting, this process is not enough to eliminate it completely. Thus, part of the drug was transferred to the adult stage and remains available to the aquatic and terrestrial food webs. These results show that IVM represents a risk to aquatic invertebrates and their predators, which deserves further studies, especially in the context of their bioaccumulation and biomagnification through the aquatic and terrestrial trophic webs.

Keywords: Antiparasitic; Bioaccumulation; Dipteran emergence; Aquatic-terrestrial linkages; Cattle dung

1. Introduction

Ivermectin (IVM) is one of the most widely used antiparasitics worldwide. Since its development in 1981, this drug has been noted for its remarkable broad-spectrum activity, safety profile, efficacy at low concentrations, and easy administration (Lanusse et al., 2018). Ivermectin is administered as endo and ecto parasiticide to livestock, such as cattle, pigs, sheep and horses (Õmura, 2008). Regardless of the route of administration (e.g. oral, injectable, pour-on, bolus) the dose is largely excreted (up to 80-90%), unaltered, in the dung of the treated animal (Alvinerie et al., 1999; Halley et al., 1989). Ivermectin may reach aquatic ecosystems by excretion in the riverine zone or directly into water bodies (Kövecses and Marcogliese, 2005). It has a high affinity for sediment and organic matter (Krogh et al., 2008) as well as a low desorption potential and leaching (Halley et al., 1989; Krogh et al., 2008), which determines its rapid transference and permanence in the environment (Brinke et al., 2010; Liebig et al., 2010; Mesa et al., 2017). It has been observed that high concentrations remain in feces for up to six months after injection (Suárez et al., 2003), imposing a risk for aquatic ecosystems (Davies et al., 1998; Liebig et al., 2010). Thus, it has been considered a substance of high concern and of high priority for further environmental monitoring and risk assessment (Boxall, 2018; Mesa et al., 2020).

Livestock farming is a traditional activity in many wetlands around the world, however, the massive entry of cattle into these ecosystems is relatively recent (Magnano et al., 2013). In Argentina, important changes in wetlands have been observed in recent decades. The intensification of agriculture, particularly the soybean cropping, has forced the relocation of livestock to more marginal grazing lands such as floodplain wetlands (Mesa et al., 2020; Quintana, 2014). This is the case of the Parana River, the second largest watershed in South America after that of the Amazon River. It was due to the availability of a forage rich environment that both the density and numbers of cattle in these floodplain systems have raised over 100% just in only one

decade (Quintana et al., 2014). Further, local ranchers have implemented the systematic and frequent injection of cattle with IVM as a measure for controlling antiparasitic diseases. This practice, in the absence of a strict veterinarian prescription, followed by contact of cattle with wetlands immediately after injection, has raised concerns about the accumulation and effect of this drug in these floodplain environments (Mesa et al., 2020). Knowledge of concentration of IVM in wetlands subjected to cattle use is scarce. Some field works have reported values of 194.5, 1.24 and 17 ng g^{-1} in manure, water and sediments, respectively (Boxall et al., 2006; Mesa et al., 2020).

Aquatic invertebrates are likely to be exposed to IVM by consumption of particulate matter of sediment and by bioaccumulation of dissolved IVM (Mesa et al., 2017). However, only a few studies have addressed IVM exposure of aquatic assemblages via sediment (Allen et al., 2007; Davies et al., 1998; Egeler et al., 2010; Slootweg et al., 2010; Thain et al., 1997) or feces (López et al., in press; Mesa et al., 2017; Schweitzer et al., 2010). These laboratory experiments have documented IVM accumulation in sediment, macrophytes and invertebrates such as *Pomacea* sp. and *Lumbriculus variegatus,* as well as lethal effects on *Ceriodaphnia dubia* and *Hyalella* sp. and behavioral response of tadpoles (López et al., in press; Mesa et al., 2017; Schweitzer et al., 2010). Mesa et al. (2020) reported accumulation of IVM in aquatic assemblages in wetlands subjected to different cattle use and frequency of injection of this drug. Accumulation of IVM in aquatic organisms is alarming because it would be biomagnified through food webs, with consequences for several ecosystem functions (Mesa et al., 2020).

Insects, such as Diptera, are among the target organisms of IVM (Õmura, 2008; Strong and Brown, 1987). Therefore, effects of the parasiticide on non-target insect species in the environment are to be expected (Schweitzer et al., 2010). In this sense, it has been observed that short-term exposures (5-60 min) to an IVM solution produced

paralysis, high levels of larval mortality, mobilization of substances stored in the fat body and reduction of the number of eggs laid in the adult stage of *Culex quinquefasciatus* (Alves et al., 2004, 2011; Freitas et al., 1996). Similar effects have been observed in *Aedes aegypti* larvae (Rosa et al., 2011). Other authors have evaluated the toxicity of IVM on *Chironomus riparius* larvae in a water-sediment test system over a long exposure period (>28 days) (Egeler et al., 2010; Schweitzer et al., 2010). These authors reported significant effects on larval survival and growth, adult emergence and development rate on this chironomid species.

Food webs are linked across ecosystem and habitat boundaries through movements of animals; in particular, insects that emerge from freshwaters can be important vectors of materials to the terrestrial ecosystem (Baxter et al., 2005; Polis et al., 1997). Due to their ubiquity and quality as prey, some insects provide a critical resource for consumers in multiple habitats, but they can also spatially propagate contaminants and their effects across ecosystem boundaries (Walters et al., 2008). The transport of contaminants via animal movement is of increasing environmental concern (Sullivan and Rodewald, 2012), but little is known about the effect of IVM on the metamorphosis, the physiological transition from preimaginal stages to adult of aquatic insects, its translocation to adults and its consequences for terrestrial ecosystem.

Culex pipiens (Diptera: Culicidae) is a mosquito species with a wide distribution characterized by immature stages (larvae and pupae) that develop in aquatic environments until adult emergence. These organisms are generalists in their habitat requirements and use different types of breeding sites, both natural and artificial (Berón et al., 2016). Due to their characteristics, wetlands present a great abundance and diversity of breeding habitat for these organisms (Dale and Knight, 2008). This taxon constitutes a trophic resource, both for aquatic organisms in their immature stages (e.g. Odonata nymphs, coleopterans, amphibians and fish) and for terrestrial organisms in

their adult stage (e.g. arachnids, amphibians, birds and reptiles) (Bay, 1974; Marti et al., 2006; Shaalan and Canyon, 2009).

To examine the potential toxicity and bioaccumulation of IVM through developmental stages in *C. pipiens* and its transfer to the terrestrial ecosystem, an experimental design was conducted with dung spiked with this drug. In this sense, the objectives of this study were to (1) assess the effect of IVM spiked in cattle dung on the survival and emergence of *C. pipiens* and to (2) evaluate its accumulation in the different developmental stages (larvae, pupae and adults) of this taxon. We hypothesize that IVM decreases the survival and adult emergence of *C. pipiens* and that it accumulates in preimaginal stages and can be transferred to the adult stage.

2. Materials and methods

2.1 Experimental design

Preliminary assays were conducted to evaluate IVM toxicity levels to *C. pipiens* and to determine the experimental conditions to be used in the definitive assay. The methodology was adapted from international protocols for determining the susceptibility or resistance of mosquito larvae to insecticides (WHO, 1981) and from previous studies that evaluated toxicity of IVM spiked in cattle dung (López et al., in press; Mesa et al., 2017). Different experimental conditions were tested and those that ensured a mortality <25% in the controls towards the end of the assay according to protocols (WHO, 1981), and the highest amount of biomass of the different developmental stages of *C. pipiens* were selected in order to determine the bioaccumulation of IVM.

2.2. Test organisms

Each experimental unit consisted of a plastic vessel containing 1 L of dechlorinated water and 25 larvae of third and fourth instar of *C. pipiens*. The test organisms were taken from our own stock cultures. Larvae were selected and placed in the experimental units for acclimation without feeding 24 h before the beginning of the experiment. Laboratory conditions were kept constant during the acclimation and experimental periods and were 27 ± 1 °C with a photoperiod of 12 h light, 12 h dark.

2.3. Spiking dung with IVM

Fresh cattle dung used in the experiments was collected near wetlands of the Middle Paraná River system before the injection of cattle with IVM in order to ensure minimum concentration of this drug. Dung was homogenized and kept refrigerated until the initiation of the experiment. Commercial formulation of IVM (Ivomec®, IVM 1%) was used to prepare stock solutions diluted in ethanol as solvent (dilution factor = 1/1000). One gram of cattle dung was spiked with different concentrations of IVM. The applied nominal IVM concentrations were 1000 (T1) and 500 ng g^{-1} (T2) dung fresh weight, corresponding approximately to those found in cattle dung at days 3 and 7 in studies conducted in Argentina following subcutaneous injection (Lifschitz et al., 2000; Suárez et al., 2003). The IVM solution was added on the surface of dung to obtain the aforementioned nominal concentrations. Special care was taken to allow the complete absorption of the solution into the dung. An identical methodology was used in Mesa et al. (2017) and confirmed analytically by high recovery rates compared with the nominal concentrations. Samples were left for 90 min to allow evaporation of the ethanol.

2.4. Experimental procedures

Nine test vessels (plastic glasses, 10 cm diameter, 17 cm height, 1 L volume, covered with a 1 mm mesh) were prepared for control (C), for the solvent control (CS) and for each treatment (T1 and T2) (total = 36 test vessels). Individuals of larvae, pupae and adults, and pupal exuviae were taken from cultures for IVM measurements before the initiation of the experiment. The exposure started when one gram of fresh cattle dung with the different nominal concentrations of IVM was placed into each vessel. To simulate direct dung deposition by cattle into water bodies, fresh dung was introduced into each vessel from the surface of the water, falling through the water column, and remaining on the bottom until the end of the experiment. Samples of dung, *C. pipiens* individuals and pupal exuviae were taken from three replicates of the controls and treatments on days 3, 6 and 9 (end of the experiment). Survival and number of emerged adults were recorded at each of these days. Larvae, pupae, pupal exuviae and adults collected on each day were separately pooled, washed with distilled water and preserved at -20 ºC for analysis of IVM accumulation.

Conductivity, pH, dissolved oxygen and water temperature (all measured with a Hach® meter), were determined at the beginning of the experiment (day 0) and at days 3, 6 and 9.

2.5. Analysis of IVM concentrations

Samples of dung collected in the field and used in this trial were analyzed for IVM to evaluate the presence of the drug. Samples of dung, larvae, pupae, pupal exuviae and adults of *C. pipiens* of each treatment and days of the experiment were analyzed to determine IVM concentrations. The extraction from samples and analysis of IVM were carried out following Lifschitz et al. (1999). Concentrations were determined by high-pressure liquid chromatography (HPLC) with a Shimadzu 10 A fluorescence detector (Lifschitz et al., 1999; Mesa et al., 2017). The extraction of IVM from spiked

and experimental samples was performed following the technique described by Lifschitz et al. (1999) and adapted by Mesa et al. (2017) for these matrices. Samples were weighed, homogenized, and combined with the internal standard compound. Briefly, 0.5 g of dung was combined with abamectin (Sigma-Aldrich) as the internal standard (10 ng). One milliliter of acetonitrile was added, and the mixture was shaken (Multi Tube Vortexer, VWR Scientific Products, USA) for 20 min. After mixing the dung and *C. pipiens* samples were sonicated for 10 min (Transsonic 570/H, Lab Line Instruments Inc., USA). The solvent-sample mixture was centrifuged at 2000 g for 15 min. The supernatant was manually transferred into a tube and the procedure repeated once. The pooled supernatants were then placed on an Aspec XL autosampler (Gilson, Villiers Le Bell, France) for the automatic solid-phase extraction process. The methanol elution was collected and concentrated to dryness under a stream of nitrogen. The derivatization of IVM was done with 100 µl of a solution of N-methylimidazole (Sigma Chemical, St. Louis, MO) in acetonitrile (1:1) and 150 µl of trifluoroacetic anhydride (Sigma Chemical) solution in acetonitrile (1:2). After completion of the reaction (< 30 s), an aliquot (100 µl) of this solution was injected directly into the HPLC system. IVM concentrations were determined by HPLC using a Shimadzu 10 A HPLC system with autosampler (Shimadzu Corporation, Kyoto, Japan). HPLC analysis was undertaken using a reverse phase C_{18} column (Kromasil, Eka Chemicals, Bohus, Sweden, 5 µm, 4.6 mm × 250 mm) and an acetic acid 0.2% in water/methanol/acetonitrile (1.6/60/38.4) mobile phase at a flow rate of 1.5 mL min^{-1} at 30 °C. IVM was detected with a fluorescence detector (Shimadzu, RF-10 Spectrofluorometric detector, Kyoto, Japan), reading at 365 nm (excitation) and 475 nm (emission wavelength).

2.6. Data analysis

Survival and adult emergence were calculated in percent as the ratio of the number of survivors in the different development stages (larvae, pupae and adults) and

the number of adults relative to the initial number of larvae, respectively. Mean accumulation of IVM in larvae, pupae, adults and pupal exuviae of *C. pipiens* was calculated as the product of tissue sample weight (in grams) and measured concentration of IVM in the tissue. In addition, the Bioaccumulation Factor (BAF) of IVM in each treatment for each developmental stage and pupal exuviae was calculated as the ratio between the IVM concentration in the tissue sample and the nominal IVM concentration added to each treatment in dung.

Survival and adult emergence between control and solvent control were compared using Student t-test. If no significant differences between control and solvent control were detected, treatments were compared with the control, using one way ANOVA followed by Dunnett test if any significant differences were found ($p < 0.05$). Mean IVM accumulation in larvae, pupae, pupal exuviae and adults among treatments and between days and developmental stages/pupal exuviae in each treatment was compared using the Mann-Whitney *U* test. Data normality was tested with the Shapiro-Wilk normality test, and homogeneity of variance was assessed with Barlett's test. All tests were performed at the 5% level of significance using the R Studio Software (version 2022.02.1+461).

3. Results

3.1. Physicochemical variables

Water temperature, pH, conductivity, and dissolved oxygen remained relatively constant throughout the study across all treatments, with values of 27.7 ± 1 °C, 6.9 ± 0.3, 681 ± 61 μS cm^{-1}, and 6.6 ± 0.8 mg L^{-1} respectively.

3.2. Effects of IVM on *C. pipiens*

Adult emergence and survival of *C. pipiens* throughout the experiment in controls (C and CS) and treatments with IVM (T1 and T2) are shown in Fig. 1. No significant differences were observed between C and CS for both variables (Student t-test, $p > 0.05$). Also, there were no significant differences between T1 and T2 for both variables (ANOVA, $p > 0.05$).

Regarding adult emergence, at day 3 of the experiment no significant differences were observed between treatments with IVM and control (ANOVA, $p > 0.05$). There were significant differences between treatments and control on days 6 and 9 (ANOVA, $p < 0.01$, Fig. 1A), with lower emergence in treatments, observing an effect of the drug on this variable, delaying or preventing it.

Respecting survival, no significant differences were observed between treatments with IVM and control at day 3 (ANOVA, $p > 0.05$). From day 6 of the experiment significant differences were observed between treatments and control (ANOVA, $p < 0.01$, Fig. 1B). In both controls, survival did not differ between days (ANOVA, $p > 0.05$). In contrast, in both treatments, survival decreased over time, with significant differences between day 3 and 9 (ANOVA, $p < 0.05$). Towards the end of the experiment, IVM showed a clear effect on survival, being less than 40% in both treatments.

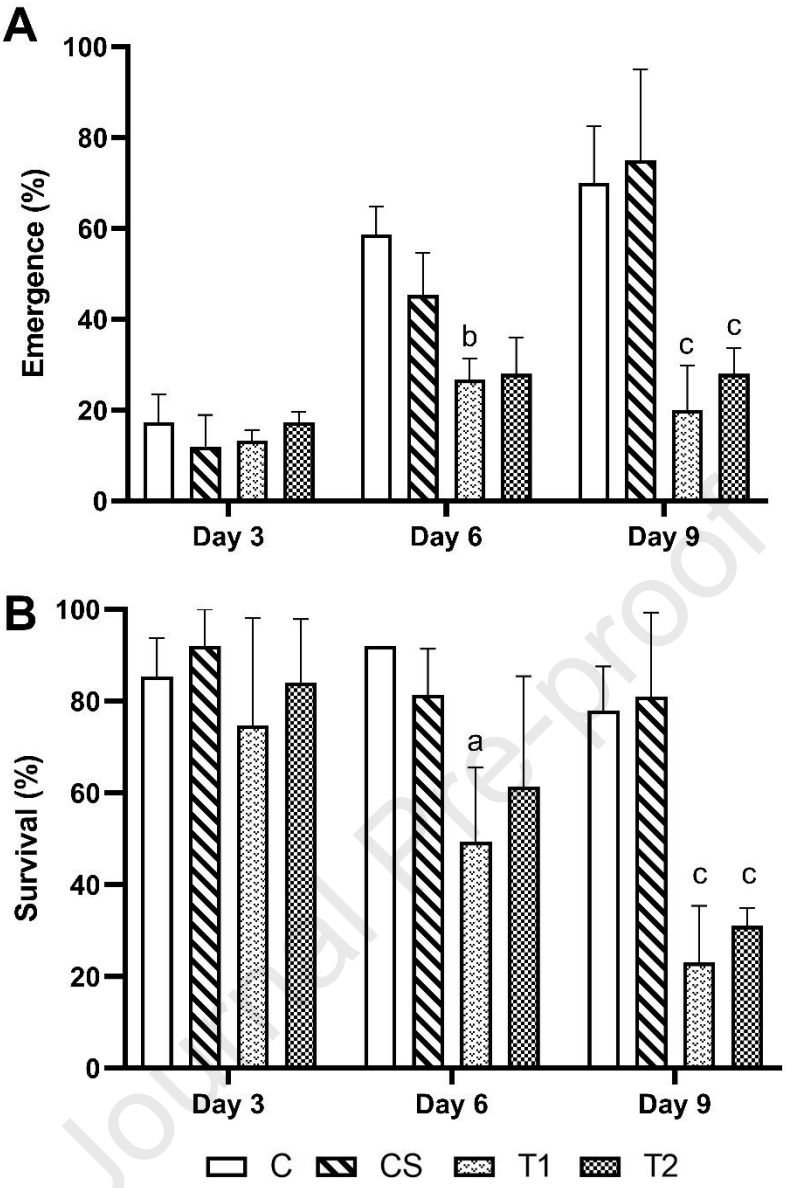


Fig. 1. Mean adult emergence (A) and survival (B) for each control and treatment at days 3, 6 and 9 of the experiment. Results of ANOVA test between treatments and control are shown. a = $p < 0.05$; b = $p < 0.01$; c = $p < 0.001$.

3.3. Accumulation of IVM in *C. pipiens*

For both treatment levels, measured IVM concentrations in dung samples were in good agreement with nominal concentrations and remained relatively constant throughout the experiment (Table 1). The mean recovery of IVM from the different biological matrices was between 76 and 85 %. The limit of quantification of IVM was

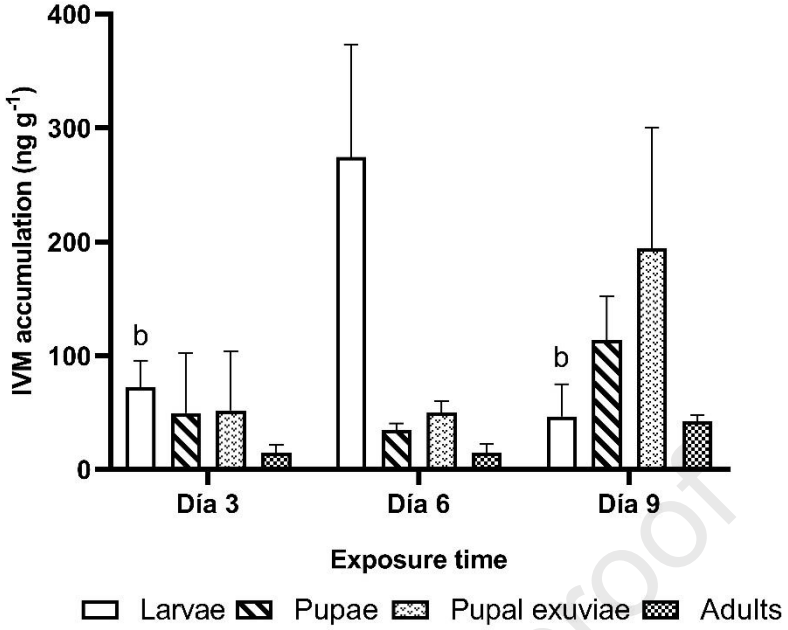
285 0.5 ng g^{-1} and the limit of detection was 0.2 ng g^{-1}. The drug was not detected in the
286 control samples.

287 Table 1: Concentrations of IVM (SD) in dung, larvae, pupae, pupal exuviae and adults of *Culex pipiens* on
288 days 3, 6 and 9 of the experiment. ND = not detected; the quantification limit was 0.5 ng g^{-1}.

		Day 3		Day 6		Day 9	
	C	**T1 (1000 ng g^{-1})**	**T2 (500 ng g^{-1})**	**T1 (1000 ng g^{-1})**	**T2 (500 ng g^{-1})**	**T1 (1000 ng g^{-1})**	**T2 (500 ng g^{-1})**
Dung (ng g^{-1})	ND	1046.25 (167.09)	686.75 (211.92)	873.95 (79.41)	715.2	807.8	407.66 (66.11)
Larvae (ng g^{-1})	ND	67.07 (27.68)	77.57 (22.48)	278.4 (120.63)	270.7 (120.5)	26.4	66.7
Pupae (ng g^{-1})	ND	86.6	12.5	38.8	30.6	123.9 (60.1)	103.7 (18.53)
Pupal exuviae (ng g^{-1})	ND	88.8	15.2	47.9 (16.69)	51.8 (5.23)	252.5 (48.79)	78.9
Adults (ng g^{-1})	ND	19.8	9.9	18.25 (6.72)	7.8	46.2	38.6

289

290 IVM concentrations have been detected in all developmental stages and pupal
291 exuviae of this taxon in each day and for both treatments (Table 1). There were no
292 significant differences in the quantitative IVM accumulation between treatments for
293 each item analyzed (Mann-Whitney *U* test, $p > 0.05$). Thus, IVM accumulation was
294 compared between days for each developmental stage with the pooled treatments (Fig.
295 2).



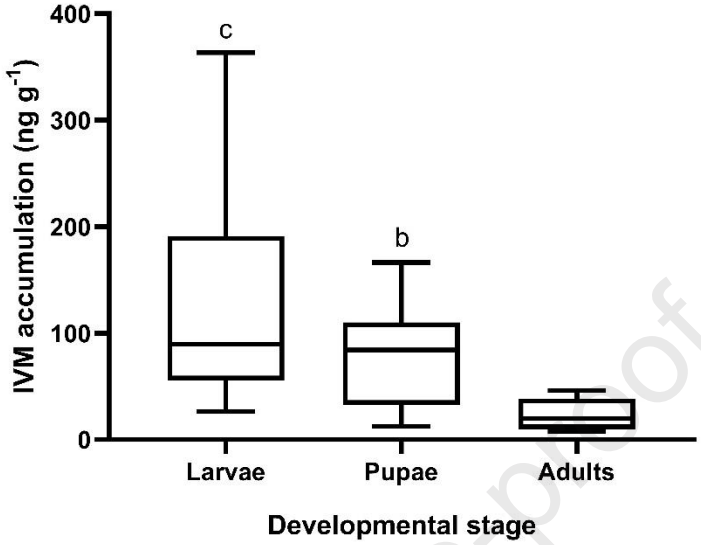
296

297 Fig. 2. Mean accumulation of IVM in larvae, pupae, pupal exuviae and adults of *Culex pipiens* on days 3,
298 6 and 9 of the experiment. Results of Mann-Whitney *U* test between days in the larval stage are shown. b
299 = $p < 0.01$.

300 The IVM accumulation in larvae increased from day 3 to 6 and declined at day 9
301 with significant differences between days (Mann-Whitney *U* test, $p < 0.01$, Fig. 2). In
302 relation to pupal stage, the IVM accumulation on days 3 and 6 was similar and
303 increased until day 9 but no significant differences were found between days (Mann-
304 Whitney *U* test, $p > 0.05$). As shown in Fig. 2 the concentration of IVM in pupal exuviae
305 and adults exhibited the same trend, on days 3 and 6 the accumulation was similar and
306 increased until day 9 but no significant differences were found between days (Mann-
307 Whitney *U* test, $p > 0.05$).

308 Averaging the accumulation values between both treatments and days of
309 exposure showed that IVM accumulation decreased throughout the development of *C.*
310 *pipiens* (Fig. 3). The accumulation of IVM in pupae corresponds to 58% of that
311 accumulated in larvae. In adults, the percentage of accumulation with respect to larvae
312 drops to 17% (Mann-Whitney *U* test, $p < 0.001$). This value was also significant

313 between adults and pupae, with a difference in accumulation of 40% (Mann-Whitney *U*
314 test, $p < 0.01$).



315

316 Fig. 3. Mean accumulation of IVM in larvae, pupae and adults of *Culex pipiens*. Results of Mann-Whitney
317 *U* test between larvae, pupae and adults are shown. b = $p < 0.01$; c = $p < 0.001$.

318 The Bioaccumulation Factors (BAFs) for the overall experiment were 0.13±0.13
319 for larvae, 0.09±0.05 for pupae, 0.14±0.11 for pupal exuviae and 0.03±0.01 for adults
320 in the case of T1. In the case of T2 BAFs were 0.28±0.23 for larvae, 0.13±0.10 for
321 pupae, 0.10±0.05 for pupal exuviae and 0.04±0.03 for adults.

322

323 **4. Discussion**

324 The current trial evaluated the effects and accumulation of the parasiticide
325 ivermectin throughout the development of *Culex pipiens* and its potential transfer to the
326 terrestrial ecosystem. To simulate exposure by direct excretion of cattle into surface
327 waters, IVM was applied via spiked cattle dung. Test conditions appeared to be
328 appropriate for this mosquito species: physicochemical conditions were representative
329 of wetlands affected by livestock activities (Gutierrez et al., 2022) and the control
330 organisms showed no signs of impairment. Also, the initial larval density selected for

331 the experiment were in the range of those found in natural wetlands of South America
332 in summer season (Maciá et al., 1996; Silva, 2002).

333 The results showed that IVM concentrations commonly found in dung during the
334 first week of excretion of cattle following injection were highly toxic to *C. pipiens*,
335 producing mortality of larvae and pupae and affecting adult emergence. These results
336 are in agreement with those found by other authors who evaluated the behavior and
337 survival rate of *Culex quinquefasciatus* after exposure to an IVM solution, reporting
338 ataxia, paralysis in larvae and adults and high mortality rates (greater than 70%) (Alves
339 et al., 2004; Consoli et al., 1986; Freitas et al., 1996). The observed effects of IVM on
340 the mosquito larvae is probably correlated with blocking action of this drug on
341 neuromuscular junctions as suggested by Freitas et al. (1996). Ivermectin blocks
342 neurotransmission, interfering with neuromuscular synapses. It acts on glutamate-
343 gated chloride channels, which are common in nematodes, insects and ticks, thereby
344 paralysing pharyngeal and somatic muscles (Õmura 2008). Given that insects are one
345 of the major target groups for IVM (Õmura 2008, Strong and Brown, 1987), the high
346 toxicity of the parasiticide to *C. pipiens* is not surprising.

347 Significant effects on survival and adult emergence were observed from day 6,
348 and in the case of survival, the results of the present study indicate a significant time-
349 dependent trend. This is in agreement with other authors who found that longer
350 exposures and higher concentrations of IVM resulted in increased mortality and several
351 toxicological effects in diverse aquatic organisms (Brinke et al., 2010; Daoud et al.,
352 2018; Domingues et al., 2016; Ezenwaji et al., 2017; Gerhardt, 2009; Lozano et al.,
353 2021; Nunes et al., 2021; Ogueji et al., 2020).

354 In this study, no significant differences were found between the different
355 concentrations of IVM tested for any of the variables evaluated. In fact, even at the
356 lowest IVM concentration (500 ng g^{-1}), corresponding to dose found in dung seven

days after cattle injection, lethal and sublethal effects on *C. pipiens* were observed. Regarding to emergence, the observed effect in treatments with IVM (a reduction of 67% towards the end of the experiment) is likely caused by a reduction in adult survival resulting from the accumulation of direct and indirect effects of IVM mainly on larvae stages during their permanence in aquatic environment, as well as by energy-demanding process of metamorphosis. Emergence is an important ecosystem service that strongly shapes consumer distribution (Kraus et al., 2014a) and the effects we report here would have important ecological consequences for aquatic and terrestrial food webs.

To our knowledge, this is the first study reporting accumulation of IVM in *C. pipiens* and its different developmental stages. This taxon had a high capacity to accumulate IVM through cattle dung, with transfer of the drug through developmental stages. Mosquito larvae are omnivorous and feed mainly on microorganisms, organic matter and biofilm (Clements, 1992). In our experiment, larvae of *C. pipiens* were observed feeding on the cattle dung, so this probably explains how IVM would be mainly incorporated by larvae. Dung enters into aquatic systems as a partially decomposed organic matter, constituting a possible food source for larvae of this taxon (del Rosario et al., 2002). In addition, the decreasing in IVM accumulation in larvae from day 6 to day 9, suggest that a detoxification process is taking place, probably as result of detoxificant enzymes action, as suggested by other authors in relation to the resistance of some mosquito larvae to insecticides (Alves et al., 2011; Georghiou and Pasteur, 1978). The excrement (meconium) of last instar larvae is also proposed as a mechanism of contaminant loss (Kraus et al., 2014b).

The pupal stage is a phase where energy saving is prioritized, since it is the stage in which physiological and structural changes of high energy cost will take place, aimed at the formation and emergence of the adult. During this stage, the organisms do not feed, and they also have a double cuticle that isolates them from the outside

(Clements, 1992), so the concentration of IVM found in pupae would come mainly from what is accumulated by the larvae. If we observe the IVM accumulation over days and developmental stages, we can see that at the end of the experiment the accumulation in pupae was higher than in larvae. Metamorphosis changes body chemistry in insects by altering protein and lipid content, metabolizing stored resources, and breaking-down cellular structures. During this process insects lost mass. These changes alter contaminant body burdens (contaminant mass/individual) and concentrations (contaminant mass/insect mass) (Kraus et al., 2014b).

A high concentration of IVM was observed in pupal exuviae, inferring that these organisms would be eliminating a large part of the drug through the molting process. Other authors have observed the elimination of another kind of xenobiotics through the molting process in dipterans (Hare, 1992; Nybom et al., 2016). In general, for organic contaminants most of their loss is accounted for by contaminant burden within exuvia (Kraus et al., 2014b). However, this process is not enough to eliminate the totality of the drug, transferring it to the adult stage and thus entering the terrestrial ecosystem.

Actual contaminant exposure and flux among food webs are determined both by the concentration in insects and by the effects of contaminants on insect survival to emergence (Kraus et al., 2014b). Ivermectin could alter both aquatic subsidies (prey quantity) and exposures (prey quality) mainly for terrestrial predators (e.g. birds, bats, reptiles and arachnids). This drug would have strong effects on adult densities because of the physiological and metabolic stress of metamorphosis following development in a contaminated larval environment. Because this drug is also lost in a large percentage during metamorphosis, its primary impact on adult food webs would be through reduction of prey quantity, in addition to the direct contaminant transfer. In this sense, *C. pipiens* may serve as a vector of IVM to aquatic and terrestrial food webs.

While this study demonstrates that IVM incorporated through cattle dung can accumulate in *Culex pipiens* larvae, remain throughout the development of this taxon and become available through adults to terrestrial food webs, there are some important limitations that present opportunities for additional study in future work. For example, given that for all contaminants cross-system impacts on riparian consumers are mediated by the relationship between bioavailable aquatic contaminant concentration, aquatic insect emergence, and adult aquatic insect contaminant concentrations (Kraus et al., 2014a; Menzie, 1980), we suggest further studies to better understand how the impact of this drug on aquatic insect communities may affect their respective terrestrial consumers. In addition, given that IVM can reach aquatic environments in large amounts through cattle feces (Kövecses and Marcogliese, 2005), remaining for a long time in the environment and being able to accumulate in sediment and several organisms such as plants, fish and invertebrates (Mesa et al., 2017, 2020; Slootweg et al., 2010; Wang et al., 2019), and that a relationship has been found between the environmental concentrations and the number of treated cattle and frequency of IVM injections, further studies, both experimental and field, are suggested to clarify the biomagnification process of this drug through aquatic and terrestrial food webs.

5. Conclusions

The results of this research showed that IVM represents a risk for aquatic insects and it should be considered a contaminant of high concern due to its potential to affect the survival and emergence of these organisms. Bioaccumulation and biomagnification of IVM in food webs, both aquatic and terrestrial, require more study but the observed accumulation of IVM in *C. pipiens* is concerning because aquatic insects constitute a trophic source for several species of the aquatic and terrestrial ecosystems. Furthermore, considering the high potential risk of IVM in floodplain and wetlands

environments (Mesa et al., 2020; Liebig et al., 2010; Schweitzer et al., 2010) due to livestock, we suggest further field studies for the development of livestock management strategies and good practices in the use of antiparasitics to limit the impact on aquatic and terrestrial communities.

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- Ivermectin affected the survival and adult emergence of *Culex pipiens.*
- Accumulation of ivermectin was observed at all stages of *Culex pipiens* development.
- The drug was eliminated in a large proportion during molting through pupal exuviae.
- *C. pipiens* may serve as a vector of this drug to aquatic and terrestrial food webs.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: