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Aquatic toxicity of ivermectin in cattle dung assessed using microcosms

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ABSTRACT

Ivermectin (IVM) is a parasiticide widely used for livestock. It is a semisynthetic derivative of avermectin, a macrocyclic lactone produced by *Streptomyces avermitilis*. This drug is only partly metabolized by livestock; considerable amounts of parent drug are excreted mostly via feces. To simulate exposure of aquatic invertebrates and macrophytes to direct excretion of cattle dung into surface waters, a microcosm experiment with IVM spiked in cattle dung was conducted. The objectives of this study were to characterize accumulation of IVM in water, sediment + dung, roots of the floating fern *Salvinia* and the zooplankton *Ceriodaphnia dubia*, the amphipod *Hyalella* and the apple snail *Pomacea*; to determine the effect of this drug spiked in cattle dung on life-history traits of these invertebrates; and to evaluate the influence of IVM on aquatic nutrient cycling. Dung was spiked with IVM to attain concentrations of 1150, 458, 50 and 22 μg kg^{-1}dung fresh weight, approximating those found in cattle dung at days 3, 7, 16 and 29 following subcutaneous injection. Concentrations found in dung during the first week of excretion were lethally toxic to *Ceriodaphnia dubia* and *Hyalella*, whereas no mortality was observed in *Pomacea*. Concentrations of IVM in roots, sediment + dung and *Pomacea* increased significantly from the lowest to the highest treatment level. The effect of this drug on decomposition and release of nutrients from dung would have negative consequences for nutrient cycling in water. Increasing concentrations in sediment + dung with days of the experiment suggested that toxic concentrations would persist for an extended period in the water–sediment system. IVM represents an ecological risk for aquatic ecosystems, underscoring the need for livestock management strategies to limit its entry into water bodies.

1. Introduction

Ivermectin (IVM) has been widely used as veterinary parasiticide for more than two decades (Shoop and Soll, 2002; Õmura, 2008). It is a semisynthetic derivative of avermectin, a macrocyclic lactone produced by *Streptomyces avermitilis* (Campbell et al., 1983). It acts by interfering with glutamate-gated or γ-aminobutyric acid related chloride channels in synapse membranes (Campbell et al., 1983; Duce and Scott, 1985; Cully et al., 1994). Being highly effective against a variety of nematodes and arthropods (insects, ticks and mites), IVM is administered as endo and ecto parasiticide to livestock, such as cattle, pigs, sheep and horses (Strong and Brown, 1987; Shoop et al., 1995; Õmura, 2008). Generally, IVM is only partly metabolized by livestock. As a consequence, considerable amounts of parent drug are excreted, mostly via feces (Halley et al., 1989; Hennessy and Alvinerie, 2002). The high percentage of elimination of the drug via feces causes several environmental problems (Liebig et al., 2010). One of the most important is related to the persistence of IVM in the environment (Kövecses and Marcogliese, 2005). The parasiticide is very persistent in cattle dung with 10–60% of the initially measured IVM concentration still present after 180 days in a field study in Argentina (Suarez et al., 2003). The persistence of this drug in dung could pose a risk for a wide variety of terrestrial insects colonizing and consuming dung pats (Wardhaugh and Beckmann, 1996; Iglesias et al., 2006), potentially limiting the rate of return of nutrients in dung to the soil (Strong and James, 1993; Petney, 1997).

IVM has also been identified as a risk for aquatic ecosystems (Davies et al., 1998), and has been considered of high priority for further environmental monitoring and risk assessment (Boxall et al., 2003). This drug sorbs strongly to soil and has a low potential for leaching (Boxall et al., 2003). As a result, erosion of particulate matter containing IVM and direct excretion by treated pasture animals into water bodies represent the most important routes of IVM entry into the freshwater

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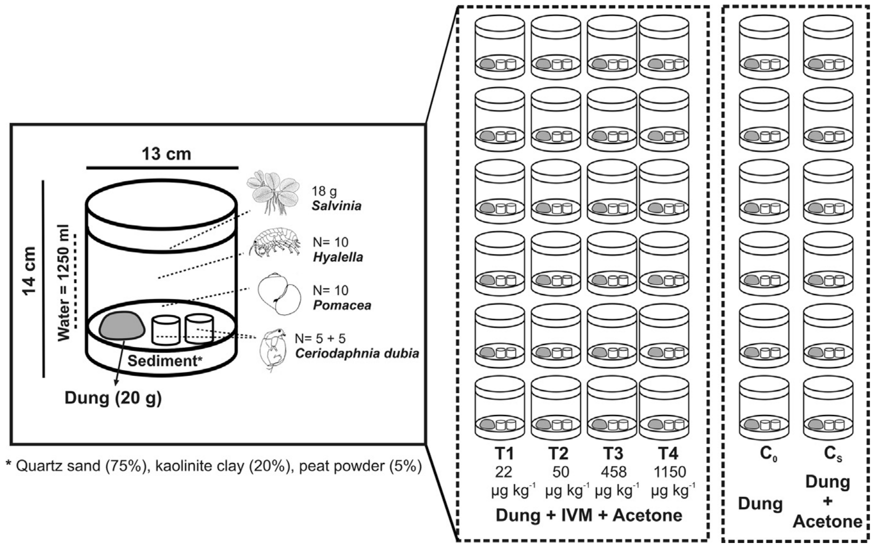

Fig. 1. Experimental design of the water-sediment test system. The four treatments (T1-T4) varied in the concentrations of ivermectin (IVM) in the added dung. IVM-free controls contained either dung alone (C_0) or dung + solvent (C_s).

environment (Kövecses and Marcogliese, 2005).

Aquatic invertebrates are thus more likely to be exposed to IVM by consumption of particulate matter than by bioaccumulation of dissolved IVM. However, only a few studies have addressed IVM exposure of aquatic benthic invertebrates via sediment (Thain et al., 1997; Davies et al., 1998; Allen et al., 2007; Egeler et al., 2010), and only one study has addressed exposure via feces (Schweitzer et al., 2010). In addition, the effect of this drug on decomposition of dung in aquatic systems, and the consequences for nutrient cycling, are completely unknown.

Land use in the Middle Paraná River floodplain in Argentina has changed significantly in recent decades, as the expansion of upland soybean production has forced the relocation of cattle to marginal floodplain sites, increasing the stocking density significantly (PROSAP, 2009; Quintana et al., 2014). Injection of cattle with IVM has been a practical and accessible tool for parasite control in this region. The massive administrations of IVM to cattle and direct contact with seasonally inundated wetlands following injection have raised concerns about the risk of ecotoxicity of the active drug excreted in the floodplain environments.

To examine the potential toxicity of IVM in cattle dung to freshwater invertebrates, a four-species water–sediment microcosm experiment was performed to expose representative invertebrates to cattle dung spiked with IVM. The objectives of this study were to (1) characterize accumulation of IVM in water, sediment + dung, roots of *Salvinia* and planktonic *Ceriodaphnia dubia*, pleustonic *Hyalella* and benthic *Pomacea*; (2) determine the effect of this drug spiked in cattle dung on life-history traits (survival, growth, and reproduction) of these invertebrates; and (3) evaluate the influence of IVM on nutrient cycling in water. We hypothesized that IVM would accumulate in sediments, plants, and invertebrates at concentrations that would be toxic to at least some species, and that it would reduce the rate of natural nutrient regeneration from the dung.

2. Materials and methods

2.1. Test organisms

Each microcosm consisted of a vessel containing water, sediment, a small floating aquatic fern (*Salvinia* sp.) and three invertebrates – the zooplanktonic microcrustacean *Ceriodaphnia dubia* (Crustacea: Branchiopoda), the amphipod *Hyalella* sp. (Crustacea: Amphipoda), and the apple snail *Pomacea* sp. (Mollusca: Gastropoda). These invertebrates were selected as representative of planktonic, pleustonic and benthic taxa of floodplain water bodies along the Middle Paraná River, respectively. *Salvinia* sp. was included as a widely distributed macrophyte in wetlands of this floodplain system. All these taxa were taken from our own stock cultures. Water temperatures averaged from 25 ± 1 °C in the cultures. *C. dubia* were fed with *Chlorella* sp. ad libitum, *Hyalella* sp. with Tetramin® fish food, and snails with romaine lettuce every day before the initiation of the experiments.

2.2. Spiking dung with IVM

Fresh cattle dung used in the experiments was collected near wetlands of the Middle Paraná River system where cattle congregate to sleep. Dung collection was done 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.

IVM (CAS-No. 70288-86-7; 94% IVM B1a, 2.8% IVM B1b) was obtained from Sigma-Aldrich GmbH (Taufkirchen, Germany; lot no. 051K1374). Stock solutions and dilutions in cattle dung were prepared with acetone as solvent. Twenty grams of cattle dung was spiked with different concentrations of IVM. The applied nominal IVM concentrations were 1150 (T4), 458 (T3), 50 (T2) and 22 $\mu g\ kg^{-1}$(T1) dung fresh weight, corresponding approximately to those found in cattle dung at days 3, 7, 16 and 29 in studies conducted in Argentina following subcutaneous injection (Lifschitz et al., 2000; Suarez et al., 2003). The IVM solution was added on the surface of dung to obtain the afore mentioned nominal concentrations. Special care was taken to allow the complete absorption of the solution into the dung. Samples were left for 90 min to allow evaporation of the acetone.

2.3. Microcosm set-up

Six test vessels (glass flasks, 13 cm diameter, 14 cm height, 1.45 L volume) were prepared for each treatment (T1, T2, T3 and T4), six for the control (C_0) and six for the solvent control (C_s) (total = 36 test vessels) (Fig. 1). The artificial sediment consisted of kaolinite clay (20%), quartz sand (75%) and peat powder (5%). Individuals of *C. dubia* were placed inside two small flasks (6 cm height, 2 cm diameter, 30 ml volume, covered with a 50 µm mesh), to avoid the loss of these invertebrates and to enable the rapid visual inspection of the individuals in each vessel (Fig. 1). The experiment was carried out under a light regime of 12 h light/12 h dark at constant environmental conditions (continuously gently aerated water, water temperature 25 ± 1 °C), without food addition. During the course of the experiment, evaporated water was replaced by non-chlorinated water. Before the initiation of the experiment, ten *C. dubia* (five in each small flask), ten *Hyalella*, and ten *Pomacea* were introduced in each test vessel (Fig. 1).