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Ivermectin alters reproductive success, body condition and sexual trait expression in dung beetles

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HIGHLIGHTS

- Dung beetles exposed to the veterinary drug ivermectin suffered lethal and sublethal effects.
- Ivermectin affected beetle emergence, body size, physiological condition and sexual traits.
- Contaminants can alter the population size and the evolutionary trajectories of wild organisms.

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ABSTRACT

Ivermectin is a very common parasiticide used in livestock. It is excreted in the dung and has negative effects on survival and reproduction of dung-degrading organisms, including dung beetles. Here we exposed the dung beetle *Euoniticellus intermedius* to different concentrations of ivermectin in the food and evaluated reproductive success and the expression of traits associated with survival and reproduction under laboratory conditions. It is the first time the effects of ivermectin were evaluated on offspring physiological condition and the expression of a secondary sexual trait. We also registered the number of emerged beetles, sex ratio and body size of emerged adult beetles. Besides reducing the number of emerged beetles and body size, as found in the same and other insects, ivermectin at high doses reduced muscle mass while at intermediate doses it increased lipid mass. Ivermectin changed offspring sex ratio and at high doses increased the size of male horn, which is an important trait defining the male mating success. Our results highlight the importance of regulating parasiticide usage in livestock in order to maintain ecosystem services provided by dung beetles and confirm that contaminants impose new environmental conditions that not only impact on wild animal survival, but also on evolutionary processes such as sexual selection.

1. Introduction

Environmental contamination by pesticides and residues of medical and veterinary products has strong demographic effects on populations of non-target organisms. Such effects can be lethal and immediately causing population reduction, or sub-lethal, imposing risks on long term viability of local populations. Sub-lethal effects of pollutants can be reflected in individual development, morphology, physiology or behavior and ultimately will impact on individual fitness and population persistence (Desneux et al., 2007).

Among the biological traits affected by contamination, those involved in sexual selection can be particularly relevant because they define individual mating and reproductive success, and some potential consequences are the reduced availability of attractive

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mates (Arellano-Aguilar and Macías-García, 2008) or the incorrect interpretation of sexual signals (Cole, 2013). Sexual trait expression is particularly sensitive to phenotypic variation due to environmental stress (Warren et al., 2013). For example, male wasps *Trichogramma brassicae* exposed to organophosphorous insecticides become less responsive to female pheromones, whereas females exposed to the insecticide are more attractive for males (Delpuech et al., 1998).

Ivermectin is one the most widely used anthelmintic and insecticide products in livestock, mainly against nematodes and ticks, that is particularly dangerous for non-target insects during larval stages (Lumaret et al., 2012). Besides causing death in some species, ivermectin causes developmental stress that is reflected in wing asymmetry in *Scatophaga stercoraria* dung flies (Strong and James, 1993 but see Floate and Coghlin, 2010 for lack of effect on asymmetry) and affects development, body size and growth rate in sepsid flies (Blanckenhorn et al., 2013). Given the negative effect on dung decomposing fauna, ivermectin may prevent dung pats from being naturally degraded in pastures (Madsen et al., 1990; Römbke et al., 2010; but see Tixier et al., 2016 for apparent lack of effect of ivermectin on dung degradation).

Dung beetles are among the most important insects threatened by ivermectin. These insects play fundamental role in natural and productive ecosystems, as some species bury dung from the soil surface to feed and provide their offspring, facilitating its decomposition, fertilizing the soil and reducing the proliferation of noxious coprophagous fauna (Nichols et al., 2008). Moreover, dung beetles contribute to reduce greenhouse gas emissions from cattle farming (Slade et al., 2016). Residual ivermectin in dung can affect beetles as it can inhibit or reduce breeding (Wardhaugh et al., 2001; Martínez et al. in press), delay development and sexual maturation (Lumaret et al., 1993; Wardhaugh et al., 1993, 2001; O'Hea et al., 2010), alter sensor and locomotor functions and reduce foraging success (Verdú et al., 2015). In *Onthophagus landolti* beetles, ivermectin reduces survival and fecundity and their function of dung removal, and increases the developmental time of offspring under laboratory conditions (Pérez-Cogollo et al., 2015). The effect of ivermectin can be particularly dangerous in species that are attracted to dung of treated animals, such as the dung beetle *Euoniticellus intermedius* (Reiche) that showed in some cases preference for contaminated over control dung, although such discrimination between dung types was probably attributable to some unknown side effect of the treatment rather than being a direct response to the drug itself (Holter et al., 1993). Despite *E. intermedius* not being severely affected by some agrochemical products such as some herbicides (González-Tokman et al., 2017), ivermectin delays or prevents adult emergence and has negative effects on survival and fecundity in this species (Krüger and Scholtz, 1997; Cruz-Rosales et al., 2012). Moreover, low doses of ivermectin alter the morphology of the ovary and stop vitellogenesis, causing oocyte resorption and thus decreasing fecundity (Martínez et al. in press).

Euoniticellus intermedius males develop a cephalic horn whose expression varies depending on larval developmental time and environmental conditions, including the weight of the brood ball where it develops (Reaney and Knell, 2015) and exposure to herbicides (González-Tokman et al., 2013). The size of this horn is a good indicator of physical condition, measured as strength and resistance to forced long runs in the laboratory (Lailvaux et al., 2005), and is correlated with phenoloxidase immune response (Pomfret and Knell, 2006b). In this species, horn length is also a good predictor of mating success, especially in even contests between large males (Pomfret and Knell, 2006a) and, together with body size, are good predictors of individual fitness (Pomfret and Knell, 2006a; b).

In the present study we used *E. intermedius* beetles (Coleoptera: Scarabaeinae) to test the effect of ivermectin on reproductive success, sex ratio, body size, physiological condition and the expression of a sexually-selected trait. This African species invaded Mexico in the 1980's after being introduced to the United States in the 1970's. Now it is one of the most abundant dung beetle species in cattle pastures in tropical Eastern Mexico (Montes de Oca and Halffter, 1998; Flota-Bañuelos et al., 2012). This species shows preference for cattle dung deposited 24 h earlier. Females dig galleries in the soil just below the dung pat, and deposit brood masses that take around 35 days to develop (Cruz-Rosales et al., 2012). As the effects of ivermectin are not always the same in males and females, one potential consequence of ivermectin contamination is a change in sex ratio (Desneux et al., 2007; Garric et al., 2007). Such changes can cause a reduction in mate availability, an increase in competition for mates and consequently in the strength of sexual selection (Kvarnemo and Ahnesjö, 1996).

2. Materials and methods

We collected 50 adult *Euoniticellus intermedius* (Coleoptera: Scarabaeinae) beetles (sex ratio ca. 1:1) in September 2015, in Zapoapan ranch, San Andrés Tuxtla, Veracruz, Mexico (18°27′N, 95°19′W; 166 m a.s.l.) by searching them manually in fresh dung pats from 10:00 to 14:00 h. Beetles were transferred to the laboratory in 20 L plastic buckets, half filled with local soil, and bred in plastic containers ($57 \times 30 \times 37$ cm), with 15 cm of sterile, compact and moist local soil. During the experiment, beetles were fed ad libitum with cattle dung collected fresh from the field in Rancho Palo Alto, Acajete, Veracruz, Mexico (19°35′N, 97°00′W), where parasiticides and herbicides were not applied according to the ranch owner. Dung (moisture content = 83.6 ± 0.9%, pH = 6.40) was homogenized in an industrial mixer (Bathammex, Mexico) and kept in the freezer at −18 °C for at least 48 h before feeding the beetles. The used dung was always from the same homogenate. Moisture content was quantified as the weight loss after desiccation in an oven (Casa Ríos, Mexico) at 60 °C and pH was measured with a potentiometer (Jenco Electronics, Taiwan). Dung was replaced three times per week. Rearing of beetles and trials were carried out at 27.07 ± 0.85 °C, 14 h photoperiod and relative humidity of 70%.

Newly emerged beetles (new generation coming from wild parents, which developed their whole life cycle in untreated dung) were stored in a clean container until individuals were 5–10 days old and then they were separated by sex for 24 h. After that time we randomly formed couples of a male and a female that were placed in 1 L containers, corresponding to the nine different treatments (seven experimental and two controls; see below). Ten couples (families) were used for each treatment. Beetle couples were allowed to breed for 21 days and then they were removed to allow the development and emergence of a new generation in the corresponding treatment. This new generation that developed its whole life cycle in the corresponding treatment, was used for further analyses of reproductive success and body condition.

Experimental treatments consisted on defrosted dung mixed with ivermectin (spiked dung) (Sigma, CAS-Number: 70288-86-7 with a purity of ≥90% ivermectin B1a and ≤5% ivermectin B1b; Batch number: SLBG8734V) diluted in acetone (Sigma; CAS-Number: 1567-89-1; purity > 99.8%; 5.2 mL acetone + ivermectin per kg of fresh dung). The treatment was slowly applied while dung was being mixed and the solvent was allowed to evaporate for at least 8 h at 25 °C before feeding the beetles. As ivermectin is excreted largely unchanged in dung of treated livestock (Canga et al., 2009), spiking ivermectin for testing the effect on fauna is comparable with findings in dung of treated cattle, and therefore it is recommended for laboratory assays with this antiparasitic drug