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Comparative effects of the parasiticide ivermectin on survival and reproduction of adult sepsid flies☆

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ABSTRACT

Ivermectin is a veterinary pharmaceutical widely applied against parasites of livestock. Being effective against pests, it is also known to have lethal and sublethal effects on non-target organisms. While considerable research demonstrates the impact of ivermectin residues in livestock dung on the development and survival of dung feeding insect larvae, surprisingly little is known about its fitness effects on adults. We tested the impact of ivermectin on the survival of adult sepsid dung fly species (Diptera: Sepsidae) in the laboratory, using an ecologically relevant and realistic range of 69–1978 μg ivermectin/kg wet dung, and compared the sensitivities of larvae and adults in a phylogenetic framework. For one representative, relatively insensitive species, *Sepsis punctum*, we further investigated effects of ivermectin on female fecundity and male fertility. Moreover, we tested whether females can differentiate between ivermectin-spiked and non-contaminated dung in the wild. Adult sepsid flies exposed to ivermectin suffered increased mortality, whereby closely related species varied strongly in their sensitivity. Adult susceptibility to the drug correlated with larval susceptibility, showing a phylogenetic signal and demonstrating systemic variation in ivermectin sensitivity. Exposure of *S. punctum* females to even low concentrations of ivermectin lowered the number of eggs laid, while treatment of males reduced egg-to-adult offspring survival, presumably via impairment of sperm quality or quantity. The fitness impact was amplified when both parents were exposed. Lastly, sepsid flies did not discriminate against ivermectin-spiked dung in the field. Treatment of livestock with avermectins may thus have even more far-reaching sublethal ecological consequences than currently assumed via effects on adult dung-feeding insects.

1. Introduction

Over the past decades, research has shown that the use of agrochemicals, in particular pesticides, can have lethal as well as sublethal effects on beneficial, non-target arthropods including pollinators, detritivores, and natural enemies (Desneux et al., 2007; Henry et al., 2012). Insecticides have been shown to impact on feeding and oviposition behaviour, physiology, development, longevity, fecundity and sex ratios (Desneux et al., 2007). These effects ultimately modify the abundance and species composition, impeding important ecosystem functions (Zhang et al., 2007; Lu et al., 2012; Floate et al., 2016). Although less widely recognized, a class of substances with potentially severe and increasing ecotoxicological impact are human and veterinary pharmaceuticals, which can affect arthropod diversity in aquatic as well as terrestrial habitats (Fent et al., 2006; Schmitt and Römbke, 2008). Given the drastic and ongoing decline in insect diversity and abundance (Hallmann et al., 2017), understanding effects of pharmaceuticals and their residues on insects is of utmost necessity.

In this study we investigate the impact of ivermectin, a chemical compound commonly used in veterinary and human pharmaceuticals applied against parasitic nematodes and arthropods such as ticks and lice (Campbell et al., 1983; Õmura, 2008). Treated mammals cannot metabolize ivermectin completely. Therefore, ivermectin residues are regularly present in livestock faeces where they have been shown to

☆ **All authors** conceived and designed the study, and all contributed to the statistical analysis and the writing of the manuscript. **SC, JD, TK & NvK** largely performed the experiments. **JB, MAS & PTR** took care of the fly cultures for extended periods of time.

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Table 1
Origin of the species used, LC50 values, and statistical results from the Cox mixed-effects model with random error.

	Population origin	LC50 larvae	LC50 adult	Single-species models		
		[µg ivermectin/kg wet dung]	[µg ivermectin/kg wet dung]	Hazard ratio	SE [ln(HR)]	*P*
Sepsis cynipsea	Zurich, CH	0.36	644.62	1.002032	2.62e − 4	< **0.01**
S. duplicata	Zurich, CH	0.09	2342.55	1.001502	2.57e − 4	< **0.01**
S. flavimana	Zurich, CH	0.05	2915.50	1.001171	2.55e − 4	< **0.01**
S. fulgens	Zurich, CH	5.68	9961.35	1.000399	4.21e − 4	0.340
S. lateralis	Tenerife, E	0.80	608.53	1.001865	2.81e − 4	< **0.01**
S. neocynipsea NA	Montana, USA	0.23	1603.52	1.000758	2.11e − 4	< **0.01**
S. neocynipsea EU	Sörenberg, CH	1.57	1203.71	1.001679	3.09e − 4	< **0.01**
S. orthocnemis	Zurich, CH	1.09	4196.52	1.000583	2.11e − 4	< **0.01**
S. punctum NA	Ottawa, CAN	1.66	1801.68	1.000572	2.81e − 4	< **0.05**
S. punctum EU	Zurich, CH	4.24	2985.37	1.001136	2.70e − 4	< **0.01**
S. thoracica	Zurich, CH	0.64	794.55	1.001645	3.29e − 4	< **0.01**

have pronounced effects on many non-target, often beneficial organisms that dwell in and feed on dung, thereby breaking it down (Campbell et al., 1983; Herd et al., 1996; Floate, 1998; Gonzalez-Tokman et al., 2017a). Binding to γ-aminobutyric acid and glutamic acid receptors, ivermectin augments the membrane permeability for chloride ions, thus interfering with the organisms' nervous and muscular systems, particularly during moult (Schaeffer and Turner, 1989). Recent experiments investigating the performance of multiple non-target organisms showed that ivermectin sensitivity is a synapomorphy of all ecdysozoa (moulting invertebrates, comprising nematodes and arthropods) (Puniamoorthy et al., 2014).

Previous research has focused mainly on the effects of ivermectin on insect larvae (Lumaret et al., 2012). This is because larvae of many (but certainly not all) dung insects consume (contaminated) dung, and toxicologists and regulators are typically interested primarily, if not exclusively, in the direct mortality effects of toxic substances. Nevertheless, sublethal effects at lower ivermectin concentrations must and indeed do occur, for instance in terms of prolonged development, suboptimal growth, or stunted body size (Römbke et al., 2009). Such sublethal effects impede individual performance in various ways directly and indirectly, well known effects in community ecology that are relevant from a biological perspective (e.g. TerHorst et al., 2015). Moreover, many dung organisms rely on dung as food not only as larvae but also as adults (Skidmore, 1991), and different life-stages may vary in sensitivity. Consequently, the impact of environmental toxins on biodiversity and ecosystem functioning are likely systematically underestimated when ignoring sublethal effects and focussing exclusively on juvenile life-stages (Lumaret et al., 2012; Gonzalez-Tokman et al., 2017a).

We here used black scavenger (or dung) flies (Diptera: Sepsidae) to study the fitness consequences of ivermectin for adult dung feeding insects, thus extending previous studies that investigated its effects on their juvenile development and mortality (Madsen et al., 1990; Floate, 1998; Iwasa et al., 2005; Blanckenhorn et al., 2013). We were particularly interested in whether feeding on ivermectin-spiked dung influences adult longevity and offspring production at realistic, ecologically relevant concentrations that remain detectable in the field for long time periods after cattle treatment (Liebig et al., 2010). To address this issue, we exposed adult flies of eleven (sub)species to fresh dung spiked with varying ivermectin concentrations and determined their mortality. If ivermectin sensitivity is indeed an evolvable species-specific physiological property as suggested earlier (Puniamoorthy et al., 2014), we expected that juvenile and adult ivermectin sensitivity should covary across the sepsid phylogeny. In one representative but relatively insensitive species, *Sepsis punctum*, we further tested whether ivermectin affects female fecundity and/or male fertility even when offspring are subsequently raised in untreated dung. We also investigated whether males exposed to ivermectin change their mating behaviour, and whether flies can differentiate between contaminated and uncontaminated dung under natural conditions, complementing our previous common garden laboratory research on larvae (Blanckenhorn et al., 2013).

2. Methods

2.1. Ivermectin treatments

In all experiments, we used dung originally collected from grass-fed cattle that had not been recently treated with parasiticides. The dung was subsequently homogenized and frozen at − 80 °C for several weeks. Six ivermectin concentrations were prepared following a semi-logarithmic scale and covering the wide range of concentrations reported as residues in nature because the substance is rather inert (Liebig et al., 2010): $C_{70} \approx 69$; $C_{300} \approx 269$; $C_{700} \approx 692$; $C_{800} \approx 833$; $C_{1000} \approx 1008$; $C_{2000} \approx 1978$ µg ivermectin/kg wet dung. The ivermectin solution was thoroughly mixed into wet dung and kept overnight at room temperature to allow for evaporation of the solvent acetone. A standard acetone treatment was used as control (C_0).

2.2. Comparative adult longevity

We worked with offspring of 11 (sub)species of black scavenger flies of the genus *Sepsis* that were originally caught in the wild on or around cow dung at various places (Table 1). Laboratory cultures were established in 1-l plastic containers using offspring of at least 10 wild-caught gravid females and thereafter kept in replicate groups in the laboratory for multiple generations prior to our experiment. Fly groups were regularly supplied with fresh cow dung, sugar, and water ad libitum using standard methods. Seven species were represented by one population, and we additionally studied two continental populations each of the widespread *Sepsis punctum* (Zurich (CH) and Ottawa (USA)) and *Sepsis neocynipsea* (Zurich (CH) and Montana (USA)). New and Old World populations of both species differ in life history, morphology and mating system and can thus be treated as independent evolutionary lineages (Puniamoorthy et al., 2012; Rohner et al., 2016, 2018).

We experimentally exposed adult flies of all 11 taxa to ivermectin-spiked fresh dung to assess effects on longevity. Immediately after emergence flies were haphazardly removed from laboratory cultures and continuously exposed to either an acetone control (C_0) or one of five different ivermectin concentrations (C_{300}, C_{700}, C_{800}, C_{1000}, C_{2000}). In each replicate, eight adult flies were kept in a 1-litre plastic container with dry sugar and a plastic dish ($20 \times 40 \times 15\,mm^3$) filled with ca. 10 g of spiked dung. Each treatment was replicated five times (i.e. $n = 5 \times 8 = 40$ flies per treatment and taxon). All test containers were placed in a climate chamber set at 24 °C, 60% relative humidity and 14 h light, and over the four subsequent days mortality was assessed every 24 h.

For the statistical analysis of adult mortality we used Cox mixed-