# The Impact of Ivermectin Treatment in Cattle on Dung Degradation and Fauna Abundance and Diversity in Tanzania

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## Academic Abstract

Ivermectin, also called the wonder drug has been used over the years to control internal and external parasites in livestock. In humans it has been used for the control of several neglected tropical diseases. With regards to efforts to control malaria, mass drug administration (MDA) of ivermectin in humans and livestock has been considered as a potential tool. This is due to challenges in malaria preventive strategies such as insecticide resistance in mosquitoes, shift in their behaviors and residual transmission. Ivermectin reduces malaria transmission by targeting the mosquito nervous system resulting in their death. Ivermectin and its metabolites could have great impacts on the environment as well as human and health. In temperate settings, impacts of ivermectin in the environment were studied due to intense use of the drug in livestock. There is also a necessity to study effects of ivermectin in the tropics before MDA for malaria vector control. Despite its great potential, previous studies recorded toxicity and sensitivity of the drug to most arthropods, mainly dung organisms because ivermectin is released in dung at high concentrations for the case of livestock. With dung organism activity being affected the dung decomposition process is disrupted, cattle avoid these grazing areas leading to financial losses. In the tropics where there is a high number of malaria cases, there is no information on the impact of the drug on the environment. We placed standardized dung pats from ivermectin-treated and control cattle to determine the effect of ivermectin on dung degradation and dung fauna in Tanzania. For the dung degradation study, at 15, 30 and 45 days post placement, we observed a total of 220 dung pats in the field. We measured termite colonization; wet weight of the entire pat; water content; dry weight of the 10 g subsamples and organic matter from subsamples and the whole pat. For the dung fauna study, we collected fresh dung 3, 10 and 29 days post treatment and put the pats out in the field to be colonized by insects before being transferred to emergence traps. We also did a semi field study where we collected dung pats 1,2,3 and 5 days post treatment to obtain larvae counts. We qualitatively assessed insect larvae activity in the field experiment and observed and counted larvae in the semi field study. We found that termites colonized pats from cattle treated with ivermectin more readily compared to controls ($p < 0.001$). Compared to control pats, the treated pats' wet weight decreased more slowly on day 15 ($p < 0.001$), day 30 ($p < 0.001$), and on day 45 ($p = 0.037$). Percent dry weight increased over time and similarly between the treatments as water content decreased. Organic matter of the 10g sub samples was similar between the treatments. Total organic matter in the whole pats showed significant differences on day 15 ($p < 0.001$), and day 30 ($p = 0.003$), but not on day 45 ($p = 0.291$). Qualitatively, we saw that pats from treated cattle had less insect larvae activity as compared to controls in the field study. In the semi field study, we counted less larvae in the pats from ivermectin-treated cattle than in the control pats ($p<0.001$). Our results indicate that ivermectin and its residues affect dung degradation and dung fauna in tropical

savanna settings, and the environmental safety may be at risk upon mass drug administration in livestock.

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**General Audience Abstract**

Ivermectin is a drug commonly used in livestock and humans to control most parasitic infections. Malaria is a disease transmitted by female *Anopheles* mosquitoes and prevalent in tropical regions mostly the sub-Saharan Africa. There are efforts worldwide to reduce transmission of malaria such as the used of insecticide treated bed nets as well as use of repellents and spraying insecticides indoors. These efforts are challenged by insecticide resistance in mosquitoes, change in mosquito behavior as well as remaining malaria cases after such interventions are applied. With such challenges comes a need to use ivermectin which can kill mosquitoes. Despite its great potential, evidence from temperate regions record that ivermectin affects the environment by decreasing dung insect activity, affecting the developmental process, and causing a delay in dung decomposition. These effects cause cattle to avoid such pasture areas, resulting in an increase in pest pressure and affecting the economy in general. In the tropics where malaria is prevalent and plans are in place to use ivermectin in mass drug administration for mosquito control, there is a need to look at the environmental impact of the drug. We placed 1 kg dung pats from ivermectin-treated and control cattle to determine the effect of ivermectin on dung degradation and dung fauna in Tanzania. For the dung degradation study, at 15, 30 and 45 days after pats were placed in the field, we observed a total of 220 dung pats. We measured termite colonization; wet weight of the entire pat; water content; dry weight of the 10 g subsamples and organic matter from subsamples and the whole pat. For the dung fauna study, we collected fresh dung 3,10 and 29 days post treatment and put the pats out in the field to be colonized by insects before being transferred to emergence traps. We also did a semi field study where pats were collected 1,2,3 and 5 days post treatment to obtain larvae counts. We qualitatively assessed insect larvae activity in the field experiment and observed and counted larvae in the semi field study. We found that termites colonized pats from cattle treated with ivermectin more readily compared to controls, and treated pats' wet weight decreased more slowly. Dry weight of dung increased as water content decreased with no differences between the treatments. Organic matter of the subsamples did not differ in the treatments but the average mass of organic matter of the individual dung pats decreased slower in ivermectin-treated pats. Qualitatively, we observed that pats from treated cattle had less insect larvae activity as compared to controls in the field study. In the semi field study, we counted more larvae in control pats than the ivermectin pats. Our results indicate that ivermectin and its residues affect dung degradation and dung fauna in tropical savanna settings, and the environmental safety may be at risk upon mass drug administration setting.

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# Chapter 1: Introduction

Malaria is a global public health issue. Between 2000 and 2021, there have been about 2 billion malaria cases and 11.7 million deaths, most of which occurred in Sub-Saharan Africa and Asia (WHO 2022). Transmission of the disease occurs when a female *Anopheles* mosquito, infected with a malaria parasite (*Plasmodium spp.*), bites a susceptible human (Warrell and Gilles 2017). Common mosquito vectors include *Anopheles gambiae s.s.* and *Anopheles funestus,* with *P. falciparum* documented as the most common *Plasmodium* species in the tropics. The survival of these vectors is eased by favorable climatic conditions of rainfall and temperature (Gilles and Warrell 2002). There have been efforts to control malaria vectors worldwide. Such efforts include the use of indoor residual insecticide sprays and insecticide treated bed nets. These efforts target mosquitoes that prefer to feed on humans (anthropophagy) and rest indoors (endophilic). Over time, these mosquitoes have developed insecticide resistance as well as change in behavior, switching to strategies of feeding and resting outdoors (exophagy and exophilia), and feeding on non-human animals such as cattle (zoophagia) (Mutuku et al. 2011), (Thomsen et al. 2017). In 2021, the World Health Organization recommended the first malaria vaccine RTS, S. It is a virus like particle vaccine made from the protein in parasite *Plasmodium falciparum* (Beeson et al. 2022). Trials of the vaccine in children showed high efficacy in Africa. It is used together with a booster. It is now used in children in areas with high rates of malaria (Rts 2015). A variety of tools will likely be needed to continue to move toward malaria control and elimination. The development of the vaccine is promising, but still has challenges with cost and the ability to scale-up to all at-risk populations. Ivermectin, a well-known endectocide, is currently being investigated as a potential new vector control tool.

Ivermectin is an endectocide that has a broad-spectrum activity against ecto and endoparasites as well as free-living nematodes (Crump and Omura 2011). Because of its antiparasitic activity, it has a potential to control malaria vectors that feed both indoors and outdoors (Foy et al. 2011). Ivermectin has its effect when a mosquito bites an animal or human that has high enough concentrations in their blood and only works to control mosquito population when the drug is administered in mass drug administration. Some studies have shown that the malaria vector *Anopheles* is more affected by ivermectin than other mosquitoes (Gardner et al. 1993). Apart from killing the vector, the ability to reproduce, egg hatching and larvae continuity is also reduced (Fritz et al. 2009). In Asia and Africa where there are many cases of malaria, there have been trials in humans and livestock to use ivermectin for vector control. Such trials include Broad One Health Endectocide-based Malaria Intervention in Africa to humans and livestock (BOHEMIA) taking place in Kenya and Mozambique, Repeat Ivermectin Mass Drug Administrations for Malaria Control II to humans (RIMDAMAL II) that took place in Burkina Faso, Adjunctive Ivermectin Mass Drug Administration for Malaria Control to humans (MATAMAL) in Guinea Bissau, Insecticide Resistance Management in Burkina Faso and Côte d'Ivoire (REACT) in Burkina Faso and Côte d'Ivoire and Mass Drug Administration of Ivermectin and Dihydroartemisinin-piperaquine as an Additional Intervention for Malaria Elimination to humans (MASSIV) in Gambia (Ahmad et al. 2022). With mass administration of ivermectin, large amounts of cattle are treated with ivermectin at once, resulting in a lot of that moving into the environment through manure at one time raising an environmental concern.

Because of its impact on arthropods (Wilson 1993), there is a need to understand the impact of these treatments on non-target organisms to assess the environmental impact of combating malaria spread with ivermectin. While the impact in temperate settings is understood Lumaret et al. (1993); Madsen et al. (1990) where malaria is not prevalent, less is known on the impact of ivermectin in the tropical regions where it will be used against *Anopheles sp*. This thesis investigates the impact of ivermectin treatment in cattle on dung degradation and fauna.

## 1.1 Ivermectin chemistry and importance

Avermectins are complex macrocyclic lactones consisting of a macrocycle backbone for substitution with spiroketal unit, hexahydrobenzofuran unit, and disaccharide substituent at C-13 (Davies and Green 1986). This group consists of abamectin, ivermectin, doramectin and eprinomectin. They are also related to the milbemycin group. They are obtained from fermentation of *Streptomyces avermitilis* actinomycete (Campbell 1985). The fermentation reaction is associated with compounds avermectin $A_1$, $A_2$, $B_1$ and $B_2$. The compound $B_1a$ has high efficacy against endo and ecto-parasites in animals and humans and is a major component of ivermectin (22,23-dihydroavermectin B1) together with about 20% of $B_1b$ (Goa, McTavish, and Clissold 1991). Ivermectin is lipophilic and its solubility in water is low. *Streptomyces avermitilis* was first isolated from a soil sample by scientists at the Kitosato Institute in Tokyo who were working with the Merck Research laboratory (Burg and Stapley 1989). Upon isolation, it was discovered as an antihelmintic in 1979 (Miller, Hobbs, and Rutherford 1987).

Ivermectin (IVM) is able to kill various parasites without affecting hosts. It binds to specific neurotransmitters, blocking chemical transmission within the nerve-muscle synapses of arthropods involved with glutamate-gated channels (Duce and Scott 1985). It is an agonist for the activity of neurotransmitters by inhibiting the glutamate gated chloride ion channels resulting in hyper polarization of nerves involving excessive flow of chloride ions in cells (Campbell 1985). Ivermectin does not compete with Gamma-Aminobutyric Acid (GABA) a neurotransmitter, it binds to some other part of the complex (Turner and Schaeffer 1989). Inhibition of these channels occurs when the drug is at high concentration. Upon binding it stops impulse transmission and causes paralysis and death. It has the same mechanism in arthropods and nematodes. Ivermectin affects reproduction of parasites, by inhibition of oviposition (Junco et al. 2021). In humans, there is a blood-brain barrier that the drug cannot cross, and the GABA receptors are in the central nervous system while in arthropods and nematodes they are located in the peripheral nervous system (Õmura and Crump 2004).

Ivermectin is an endectocide effective against some parasites that affect cattle, dogs, swine, horses, sheep and humans. The arthropods include mites, lice and ticks to mention a few. It is also known to control nematodes such as gastrointestinal roundworms, lung worms and canine heartworm. Veterinary use of the drug started in the 1980’s (McKellar and Benchaoui 1996). The drug can be administered orally, intraruminal, subcutaneous as well as topically in animals. In humans it was first demonstrated as effective against microfilaria related to *Onchocerca volvulus* and lymphatic filariasis (Laing, Gillan, and Devaney 2017). Lymphatic filariasis is associated with the worms *Wuchereria bancrofti* and *Brugia malayi*. Ivermectin is also involved in controlling scabies, strongylidiasis and headlice. Some gastrointestinal worms in humans controlled by ivermectin include *Ascaris lumbricoides*, *Strongyloides stercoralis*, *Trichuris trichiura*, *Enterobius vermicularis* as well as hookworms and cutaneous larva migrans (Ottesen and Campbell 1994). In humans, ivermectin is absorbed in blood and excreted in faeces. The levels of the drug are high in plasma about four hours after being administered orally (Ottesen and Campbell 1994).

With time, frequent exposure of parasites to the drug can cause cases of resistance. Other factors contributing to resistance include deworming practices without considering the weight of animals which could sometimes lead to less amount of the drug administered and facilitating resistant helminths (Cruz et al. 2010). *Rhipicephalus microplus* is a common tick in cattle that is associated with decline in weight and milk and can lead to morbidity and mortality. In Argentina a study was done using larval immersion test to distinguish resistant and susceptible ticks and it was reported that the cattle tick *Rhipicephalus microplus* has developed ivermectin resistance (Alegría-López et al. 2015). This resistance has also been reported in Brazil (Martins and Furlong 2001), Mexico (Perez-Cogollo et al. 2010), Ecuador (Rodríguez-Hidalgo et al. 2017) and Colombia (Chaparro-Gutiérrez, Villar, and Schaeffer 2020). In Africa, similar findings were reported by El-Ashram et al. 2019.

Helminth infections are a great concern for livestock health. Control of nematodes involves use of antihelmintic drugs such as ivermectin and management practices. In a study in Brazil, two high concentrations of ivermectin, 630 and 700 µg/kg, were used in cattle and demonstrated resistance in *Haemonchus placei*, *Cooperia punctata* and *Oesophagostomum radiatum* (Felippelli et al. 2014). A study in Mexico also reported cases of resistance of *Haemonchus* spp. and *Trichostrongylus* spp. against ivermectin (Herrera-Manzanilla et al. 2017). To date are no reports of ivermectin resistance in mosquitoes.

## 1.2 Excretion of ivermectin and its metabolites and their impacts on the environment

Ivermectin enters the environment through dung and faecal matter. Dung consists of remaining food that herbivorous animals excrete (Gupta, Aneja, and Rana 2016). Ivermectin is released at a higher concentration in animal faeces with only 2% release in urine (Chiu and Lu 1989). Ivermectin pathways in the environment include animal and human excretion, direct deposition, land application, runoff and leaching as well as sorption. Ivermectin's insolubility in water and the affinity to organic matter in soil accounts for its accumulation (Halley et al. 1989). Upon release to the environment its rate of degradation varies mostly for soil-feces mixtures in aerobic conditions and the anaerobic conditions in manure (Liebig et al., 2010). On exposure to sunlight, it undergoes photo degradation, and this rate is higher in summer conditions in comparison to winter and hence easily eliminated in the environment (Halley, VandenHeuvel, and Wislocki 1993). Upon release in the environment, ivermectin and its metabolites can be detected for more than thirty days in the tropical dry season (Shija 2023). Ivermectin associated metabolites 3”-O-demethylivermectin (3DI) and 24-hydroxymethyl ivermectin (24OHI) are also excreted with IVM in the tropics and degrade very fast on exposure to sunlight (Shija 2023). The food remains are influenced by the cattle themselves and the plant material they feed on. Dung is an important component of the ecosystem as it adds nutrients to the environment in the decomposition process. Dung plays a role in the mineralization process of organic compounds (Lovell and Jarvis 1996). Fresh dung is mainly composed of water at 70-85 % (Lysyk, Easton, and Evenson 1985), ash (inorganic components) nutrients such as nitrogen and phosphorous, fiber which is the undigested plant matter made of cellulose, hemicellulose and lignin, and some non-structural carbohydrates (Peter Holter 2016).

Dung is occupied by various macro and micro-organisms (Adler et al. 2016). These include insects such as beetles, flies, ants and termites, nematodes, and microorganisms such as bacteria, yeast and fungi. When cattle are infected, dung may also consist of nematodes, trematodes such as flukes, cestodes such as tapeworms and protozoa when they are shed in the manure. Other species are found in dung as guests and they inhabit dung as prey and predators and these include spiders, centipedes, millipedes, earwigs, ants, ground beetles and click beetles (K. D. Floate et al. 2011). They also pave way for other organisms to enter dung and break it down further (K. D. Floate et al. 2011). These dung colonizers play a huge role in the decomposition process (Peter Holter 1979). Dung organisms are also involved in biological control of parasites. Dung breakdown is facilitated by seasons, climate, rain as well as animal activity (Dickinson, Underhay, and Ross 1981). In temperate settings, degradation of dung may take longer than in tropical settings (Merritt, Anderson, et al. 1977). Dung insect activity that helps degradation is greater when it is warm and/or wet conditions as compared to cold and/or dry conditions. Dung degradation is also affected by veterinary drug residues that are used to control livestock infections. As these products enter the environment, they contribute to less insect activity hence affecting degradation (K. D. Floate et al. 2005).

Wall and Strong (1987) showed that ivermectin released in cattle dung affects insects and lack of insects in dung was associated with a slow degradation rate. However, another study by (Wardhaugh and Mahon 1991) showed that ivermectin treated dung attracted more dung insects than untreated dung and dung degradation in treated dung was greater than untreated. Some studies have shown that the drug has an influence on faecal degradation, and slowing down of this process affects the grazing grounds of cattle. In this case, cattle tend to avoid such areas, and this may result in a negative economic impact (Anderson, Merritt, and Loomis 1984). Nitrogen from dung is released in the environment as ammonia and there are no nutrients for plant growth (Kazuhira, Hideaki, and Hirofumi 1991). Undegraded dung also serves as a conducive environment for pests that attack cattle such as stable flies and horn flies, contributing to health issues in livestock (Kunz et al. 1991). In Australia, the government had to initiate a program that introduced dung species in the country to enable dung degradation (Tyndale-Biscoe 1990). The effects are questionable in association with seasons and areas where the studies were conducted. In a study by K. Floate (1998), there was no degradation mainly because dung organisms did not have any activity on ivermectin treated pats. Wratten et al. (1993) reported that use of ivermectin did not affect dung decomposition. Madsen et al. (1990) showed slower degradation in ivermectin treated pats of 1 to 20 days post treatment. Dadour, Cook, and Neesam (1999) showed a slowed degradation in ivermectin treated pats of 7 to 10 days post treatment. Sommer and Bibby (2002) also reported slow dung decomposition in pats 2 days post treatment.

Once dung is colonized by different organisms, these contribute to an increase in soil fertility as well as biological control of parasites. According to Krüger and Scholtz (1998), the effect of ivermectin on dung fauna is linked to geographical locations as well as climate aspects such as temperature and rainfall. In association to insect activity, most Diptera feed on dung, Hymenoptera are mostly parasitoids, Coleoptera feed on dung and some feed on fungi (Skidmore et al. 1991).

### 1.2.1 Effect on Diptera

Ivermectin in dung is reported to prevent development of Diptera, by reducing the emergence of adults up to 7 weeks post treatment. This has been observed in adult and larva of *Musca domestica*, *Musca vetustissima* and *Haematobia irritans* as well as *Musca nevilli* (Allen Miller et al. 1986; Meyer 1980). Studies show that larvae of diptera are delayed to pupate and emerge to adult stage (Strong 1986). In a study by (Krüger and Scholtz 1998) some larvae in ivermectin treated dung did not pupate. Ivermectin has also been reported to reduce fertility of most flies (Strong 1993). A study by (Mahon and Wardhaugh 1991) also showed that flies that feed on ivermectin treated dung had delayed ovarian development which also corresponded with a reduced number of eggs. Reduction in productivity has also been seen in studies by (Langley and Roe 1984) where tsetse flies were fed blood with ivermectin.

Studies also suggest that Diptera are most likely to be affected by ivermectin because they are the first to appear and their growth and development is also quick (Iglesias et al. 2006). Suborders of Diptera such as Nematocera and Cyclorrhapha are affected differently by ivermectin. Nematocera have been shown to tolerate high levels of the drug, whereas Cyclorrhapha are more sensitive. This contrast has been associated with feeding behaviors (Madsen et al. 1990). Studies also show that ivermectin has an antifeedant effect, as insects face paralysis, they get powerless when it comes to feeding (Strong 1993). Another study also shows that adults that develop from ivermectin treated dung have abnormal characteristics such as abnormal wings (Clarke and Ridsdill-Smith 1990). Another effect of the drug in insects is that they face water accumulation as their malpighian tubules are affected. Molting process in insects is also a concern as affected insects fail to shed exuviae (Sheele et al. 2013).

Studies in mosquitoes fed with ivermectin treated blood from cattle showed that their survival, egg production and blood digestion is affected (Fritz et al. 2009). In mosquitoes, blood protein digestion results in products such as heme that is excreted as hematin and other nutrients required for production of eggs (Briegel 1985). There are suggestions that the reduction in egg production is associated with low hematin, and nutrients needed for egg production. Ivermectin is associated with affecting digestive responses of mosquitoes in mosquitoes fed with ivermectin blood hence the reduced digestion, hematin and low egg production (Lyimo et al. 2017).

### 1.2.2 Effect on Coleoptera

Ivermectin has been reported to reduce Coleoptera such as dung beetles in terms of their number and species richness (Ambrožová et al. 2021), (Pecenka and Lundgren 2019). There are three groups of dung beetles, mainly tunnellers, rollers and dwellers. Dwellers such as Aphodiinae mainly complete their life cycle within the pat or between the pat and soil (Cambefort and Hanski 1991). Rollers are involved in making dung balls for food and reproduction and roll the balls a distance away. Tunnellers such as Geotrupidae and Scarabaeinae mainly make tunnels and bury dung (Davis, Frolov, and Scholtz 2008). According to (K. D. Floate et al. 2011) tunnellers and rollers have a larger morphology than dwellers and are more often found in tropical settings. Some studies have shown that dung beetles fail to perform different activities due to the effect of ivermectin. Their life cycle is also affected mainly the survival of larvae and adults (Martínez et al. 2017). The larvae are more affected than adults (Lumaret et al. 2012). P. Holter (2000) suggests that larvae stages are more affected than adults since the adults have an oral part that enables filtration hence less amount of the drug is consumed in adults as compared to larvae. Studies have also shown that reproduction is affected as the number of brood balls is reduced (Cruz Rosales et al. 2012). On the other hand, the sensory ability, mainly the antennal olfactory organ and locomotion abilities is also damaged (Verdú et al. 2015). In adults the drug moves from the gut to hemolymph, and this is what causes deleterious effects (Verdú et al. 2018). Also, the drug is associated with disrupting morphology of ovary and putting vitellogenesis to an end (Verdú et al. 2020).

These activities result in oocyte resorption and slow ability to reproduce. The effects are also controversial, and some studies demonstrate that adults are not affected by the drug. Studies by (Errouissi and Lumaret 2010) showed that some beetles preferred ivermectin treated dung while others did not show such preference (Verdú et al. 2018). In some species of Coleoptera such as *Onthophagus binodis* and *Copris hispanus*, the newly emerged adults cannot survive in dung treated with ivermectin (Strong 1992). Also, the larvae of some Coleoptera may not survive in ivermectin treated dung for the first few weeks but may demonstrate normal development in a period of eight weeks later (Ridsdill-Smith and Matthiessen 1988).

## 1.3 Isoptera in dung

Isoptera, mainly termites, largely contribute to decomposition in tropical settings as compared to temperate settings (Bignell and Eggleton 2000). Termites fall into three groups based on where they reside and these include subterranean, dry-wood and damp-wood. Subterranean termites are found in soil and wood and are main pests in structures such as buildings (Thorne 1998). Dry wood termites live, feed and reproduce in wood. Dampwood termites are found in wood that has different dampness extents (Khan and Ahmad 2018). Termites are efficient at decomposition due to the gut composition mainly consisting of protists and bacteria (Eggleton 2011). The gut symbionts are involved in nitrogen fixation, acetogenesis which is the fermentation of cellulose, lignin degradation (O'Brien and Breznak 1984), (Breznak and Brune 1994). They are involved in feeding on dead plant material contributing to nutrient cycling. Most subterranean termites feed on food on the surface than buried sources. For termites, food sources such as dung have lower temperatures underneath them, termites have preference for cooler soil temperatures and dry dung enabling the subterranean termites to identify and locate their food source (Ettershank, Ettershank, and Whitford 1980).

Termites are able to detect food using thermal shadows, temperature and chemical gradients. They are not affected by dehydration and dry climatic conditions because they keep their galleries in moist conditions (Khan and Ahmad 2018). Termite activity increases contents of most elements in the mounds they build in the environment. There is less information on the effect of ivermectin on termites that colonize dung. In a laboratory study, ivermectin has been reported to affect the termite *Reticulitermes speratus* upon fumigation, preventing colony growth and disruption of the colonies (Chen et al. 2015). Another study showed that certain sublethal concentrations of ivermectin slowed termite activities such as feeding, burrowing and fighting (Mo et al. 2006).

## 1.4 Study Objectives

Given the negative impact of ivermectin on the environment in temperate settings, I investigate the impacts of ivermectin if it were to be used as a vector control tool for *Anopheles mosquitoes* in tropical settings. Specifically, in chapter 2 I investigate the effect of ivermectin treatment in cattle on dung degradation, with the hypothesis that ivermectin and its metabolites will have an effect on the rate of dung degradation. In chapter 3, I quantitatively investigate the effect of ivermectin on larval abundance in a semi-field setting with the hypothesis that ivermectin will have an effect on larval abundance and development.

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# Chapter 2: Treatment of cattle with ivermectin negatively impacts dung degradation in a tropical savanna setting.

## 2.1 Abstract

Ivermectin, an endectocide used to control ecto- and endoparasites in livestock as well as onchocerciasis, lymphatic filariasis and scabies in humans, also kills mosquitoes. Therefore, it is a candidate for mass drug administration (MDA) in humans and livestock to control malarial vectors. More than 90% of ivermectin is excreted unchanged in the dung of treated livestock, and it was previously shown in a temperate setting that ivermectin adversely affects dung degradation by targeting dung colonizers; however, studies from tropical settings, where MDA is more likely, are rare. We conducted a randomized field experiment with standardized dung pats from ivermectin-treated and control cattle to determine the effect of ivermectin on dung degradation in Tanzania. At 15, 30 and 45 days post placement, we measured termite colonization; wet weight of the entire pat; water content; dry weight; and organic matter from 10 g (wet weight) sub samples as well as organic matter of the whole pat. We found that termites colonized ivermectin pats more than controls, and wet weight decreased more slowly in the ivermectin treated pats. Dry weight increased over time as water reduced for both treatment and control pats. Additionally, organic matter loss of the sub-samples also decreased over time for both treatment and control. Total organic matter was higher in ivermectin-treated whole pats. Our results indicate that ivermectin and its residues affect dung degradation in tropical savanna settings, suggesting that dung degradation may be negatively affected upon mass drug administration in cattle. Because slow degradation negatively impacts the quality of pastureland, these non-target, environmental effects must be weighed against the benefit of curbing malaria spread.

## 2.2 Introduction

Malaria is a global problem, causing about 247 million cases and 619,000 deaths around the world in 2021, with a large number of the cases associated with the African continent (WHO 2022). Nigeria, the Democratic Republic of Congo, Uganda and Mozambique have the most cases in the world, and Nigeria, the Democratic Republic of Congo, Niger and the United Republic of Tanzania have the most reported deaths in 2021, with most occurring in children under 5 years of age (WHO 2022). Malaria is transmitted through bites of the infected female *Anopheles* mosquitoes. The most common preventive strategies are the use of insecticide treated nets, residual spraying and use of repellents (C. Menéndez, D'Alessandro, and Kuile 2007). However, *Anopheles* mosquitoes are now resistant to most insecticides (Ranson et al. 2011). Additionally, in recent years, we have also seen a shift in mosquito biting behaviors such as a new preference for early evening hours, outdoor feeding and a tendency to feed on other organisms such as cattle (Reddy et al. 2011). To curb the spread of malaria, the control of *Anopheles* is a cornerstone strategy (Killeen et al. 2017). One drug that may play a role in this control is ivermectin.

Ivermectin is an antiparasitic drug used to control mites, lice, nematodes, ticks, warble flies and other parasites of livestock (Madsen et al. 1990). Additionally, it is used to treat lymphatic filariasis, scabies, human onchocerciasis and strongyloidiasis in humans (C. J. Chaccour et al. 2013). The drug is in the macrocylic lactones group and works by blocking signal transmission in the nervous system mainly by opening the chloride channels which causes hyperpolarization, flaccid paralysis and death in insects (Kavanaugh and Manning 2020). Because of its mode of action, ivermectin is effective on mosquitoes (Roadmappers 2020), and is therefore considered for mass drug administration (MDA) in humans and livestock to curb malarial spread (C. Chaccour et al. 2022). However, the impacts of this novel strategy on the environment are less understood. About 80 to 90% of the administered ivermectin is excreted in faeces (J.-P. Lumaret et al. 1993). In addition, studies show that active metabolites of ivermectin are released in dung as well (Shija 2023; Halley, VandenHeuvel, and Wislocki 1993).

Most dung fauna feed on the excrement and help the decomposition process, which returns nutrients to the soil (K. Floate 1998). However, our understanding of what ivermectin does to the dung fauna and dung degradation, is mixed and mostly from temperate regions. For example, some previous studies have demonstrated that the drug and its metabolites have an effect on most dung-colonizing invertebrates (Lee and Wall 2006; Strong 1993, 1992; Suarez 2001; Madsen et al. 1990), with the most common result being a delayed pat decomposition. However, other studies reported no adverse effects of ivermectin or its metabolites on dung fauna (Svendsen et al. 2002; Suarez et al. 2003; Kavanaugh and Manning 2020; Barth et al. 1994). Since delayed dung degradation can cause pasture fouling, an increase in pest flies, and transmission of some endoparasites in livestock (Wall and Strong 1987), there is a critical need to understand, in a real world setting, how MDA of ivermectin in cattle will affect dung fauna and degradation, especially in tropical settings, where malaria is most prevalent.

Here we study the impact of ivermectin in a tropical dry savanna setting in Tanzania, where MDA for malaria control would likely be used. In particular, we investigate the effect of ivermectin on dung degradation by qualitative observations of dung colonization, and more importantly by quantifying termite colonization, wet weight, water content, dry weight and organic matter of the dung pats. These data will help to provide evidence-based evaluation of the safety of the drug to the environment for MDA for malaria vector control.

## 2.3 Materials & Methods

### 2.3.1 Study area

We carried out the study at a field site in Sagamaganga village (S08°03.83′, E036°47.77′), about 15 km from Ifakara, a small rural town in the Kilombero District, Morogoro Region, south central Tanzania. We performed the study in the dry season from 1st July 2022 to 28th August 2022. In preparation, we cleared the field site from brush and then fenced the area to prevent the entry of other animals, including poultry.

### 2.3.2 Cattle

We haphazardly selected N = 22 Tanzanian short horn zebu cattle from two households. We recorded the weight, age and sex of the cattle 1 week prior to the ivermectin treatment (see below). Weight was estimated by tape measurement of the trunk circumference by a trained veterinarian. The selected individuals ranged from 1 to 4 years of age and weighed from 90 to 351 kg. The cattle were without exposure to antiparasitic drugs in the 3 months leading up to the experiment.

### 2.3.3 Ivermectin administration

For logistical reasons, one household served as the control and the other as treatment. We used the Ivomec brand where each 1 ml contains 10 mg of ivermectin. We administered the drug subcutaneously on 8th July 2022 after we calculated the required dosage for each cattle based on weight. The dosage was at the rate of 200 µg/kg body weight. Administration of the drug was approved by the National Institute for Medical Research in Tanzania.

### 2.3.4 Data collection

Starting the day after ivermectin administration, and for a total of 5 days, we collected two fresh dung pats from each of the 22 cattle on a daily basis. We homogenized the two pats from a cow, sub sampled 1 kg from the homogenate dung and distributed it to random positions in the field. We placed the pats two meter distance from one another on a wire mesh and covered them with a chicken wire mesh cage to exclude birds, but not insects. Overall, we collected 44 pats per day and randomly placed them in the field , and continued for five days.

#### *2.3.4.1 Dung sampling and wet weight measurements*

On days 15, 30 and 45 after the pats were placed in the field, we measured them for wet weight, dry weight (sub sample, see below) and organic matter loss (sub sample, loss on ignition, see below). We measured all pats in the field to establish wet weight. The data were entered in ODK collect. After measuring wet weight, we took a 10 g sub-sample. We sent the 10 g sub-samples upon collection to the Ifakara Health Institute laboratory, where they were stored in a refrigerator at 4°C until they were processed for dry weight measurements and loss on ignition.

#### *2.3.4.2 Dry weight measurement*

We began by re-weighing the 10 g sub samples. Then we homogenized the sub sample by grinding with a mortar and pestle. Afterwards we placed the homogenized sub sample in a crucible which we previously weighed to the nearest 0.01g. We placed the crucible with the dung sample in an oven for 6 hours at 110°C to remove all the moisture content (K. Floate 1998). After drying the samples, we placed them in a desiccator with an active desiccant for 30 minutes to cool. We then measured and recorded the dry weight.

We calculated the percentage dry matter $p_{\mathrm{dry}}$ as the remaining weight of a sample after drying:

$$p_{\mathrm{dry}} = 100 \times \frac{(m_{s1} + m_c) - m_c}{(m_{s2} + m_c) - m_c}$$

where $m_c$ was the weight of the empty crucible, and $m_{s1}$ and $m_{s2}$ were the dry and wet samples, respectively.

#### *2.3.4.3 Organic matter measurement using loss on ignition*

We determined organic matter in the dung pat using the loss on ignition method: we heated the dried samples from the previous step in a per-heated furnace at 500°C overnight (12 hours) and then cooled it in a desiccator for 30 minutes. We then calculated the weight of the remaining ashes (Iglesias et al. 2006; Heiri, Lotter, and Lemcke 2001).

Percent LOI was calculated as:

$$p_{\mathrm{loi}} = 100 \times \frac{(dwc_{110} - dwc_{500})}{dw_{110}}$$

where $dwc_{110}$ is the crucible and sample weight at 110°C, $dwc_{500}$ is the crucible and sample weight at 500°C, and $dw_{110}$ is the original dry weight of the sample as obtained from the previous step.

#### *2.3.4.4 Total organic matter*

From the sub sample organic matter loss that we obtained above, we extrapolated to the full pat to get the total organic matter in the individual pats by combining wet weight and the lost organic matter:

$$\text{Total organic matter} = \text{wet weight} \times (dwc_{110} - dwc_{500})/10$$

where $dwc_{500}$ is the crucible and sample weight at 500°C and $dwc_{110}$ is the crucible and sample weight at 110°C.

#### *2.3.4.5 Qualitative observations*

After the pats were placed in the field, we recorded visitations of different arthropods in the first three hours and the following day after deposition for five consecutive days of collection. Weekly observations of the arthropods continued, and pats were recorded for larvae by turning the pats on the underside where they were attached to soil. The larvae observations were compared in the two treatments.

#### *2.3.4.6 Determining termite presence*

We observed the dung pats for built mounds, tunnels, hollow space in the dung and added soil as well as the presence of termites from the time the pats started drying. We also recorded the presence on day 15 and 30.

### 2.3.5 Statistical Analysis

We used R version 4.2.1 (R Core Team and others 2020) to perform data analysis. To account for the longitudinal nature of our data sets with repeated measures of individual pats, we used mixed models to study the effect of ivermectin on our outcomes (wet weight, water content, dry weight, organic matter and termite infestation). Specifically, we used generalized linear mixed effects models with random intercepts for individual dung pats and treatment x time interactions as fixed effects using the lme4 package (Bates et al. 2014). We used the emmeans package (Lenth 2021) to extract estimated marginal means and to compute standard errors and confidence intervals.

## 2.4 Results

For this study all the 220 samples were measured for wet weight, water content, dry weight, and organic matter. A few dung pats (<5%) were excluded from the analysis along the different sampling days because the action of termites was complete, and the dung pats degraded completely. Pat loss occurred on day 15 for 2 samples and on day 30 for 3 samples.

### 2.4.1 Qualitative observations of timing and presence of fauna

In the first few hours after which dung was placed in the field, we observed visits from some Diptera, mainly Calliphoridae and Muscidae. The dung pats remained moist on the underside that was in contact with the soil, but the pats formed a dried crust on the upper side. Once that crust appeared we observed Coleoptera such as Scarabaeidae and Staphylinidae. These were present for a few days until the pats were no longer moist. Isoptera that were present throughout the season also arrived after drying of the pats. Additionally, we observed that the eggs and larvae of dung beetles appeared a few days after the pats were placed in the field.

We observed termites, adult beetles and larvae of dung beetles feeding on the liquid and fiber content of pats. As the pats continued to dry, they became less attractive to most arthropods (see Figure 3 and 4, IVM and control pats drying a week after being placed in the field). IVM pats also lose moisture slowly as compared to control pats as seen (Figure 3 and Figure 4), however this also may depend on the pat size. After two weeks, the pats were mostly dry and crusty and seemed not to favor any larva development. Qualitatively, we observed more Coleptera larvae in the control pats versus the ivermectin pats.

#### 2.4.2 Ivermectin treated pats were more likely to be infested with termites

Overall, we found a significant effect of ivermectin on the probability of termite pat infestation on day 15. Specifically, ivermectin treated pats were more likely to be infested by termites at day 15 (odds ratio: 23.2 (3.9 to 136.2); df = ; $z = -3.48$; $p < 0.001$) as compared to day 30 (odds ratio:4.7 (0.9 to 23.3); df = ; $z = -1.89$; $p = 0.059$; see Figure 1). In other words, the probability of a termite infestation in pats from ivermectin treated cattle decreased from 15 to 30 days while the reverse occurred in pats from control cattle (Figure 1).

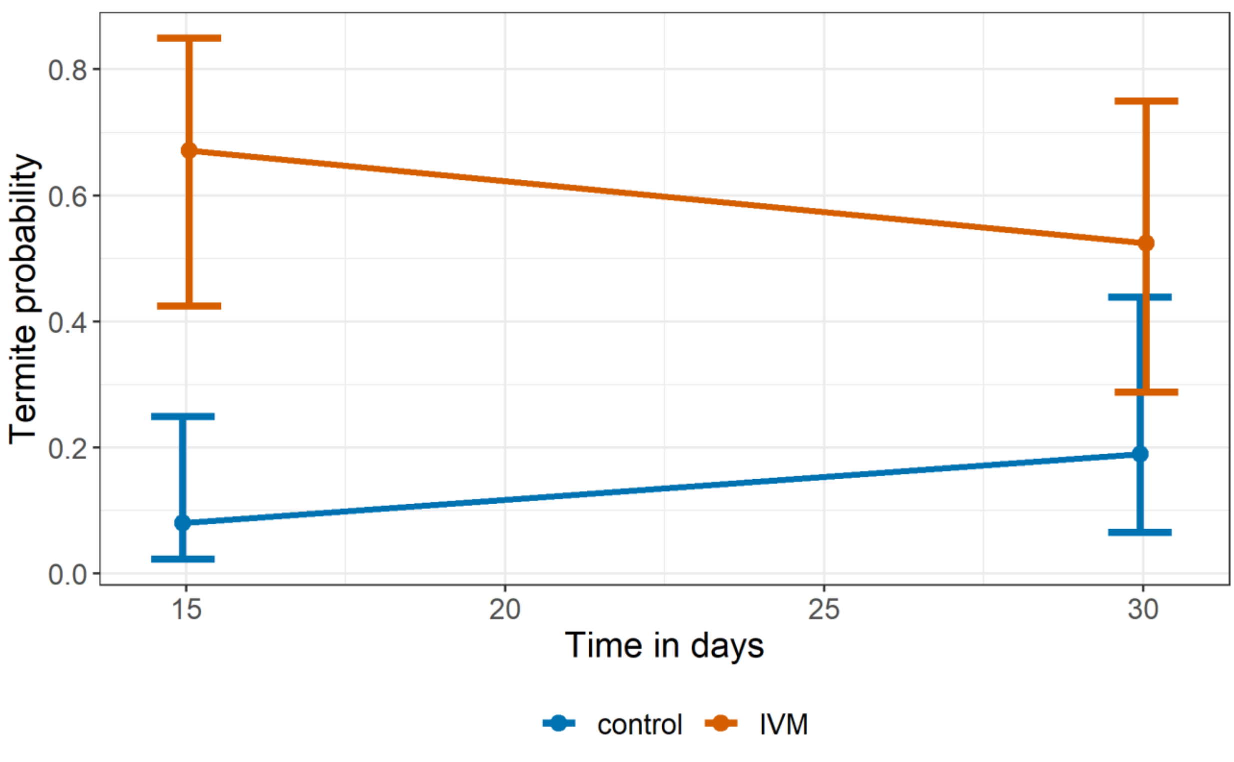


*Figure 1: Termites preferred ivermectin (IVM) treated pats compared to control pats. The vertical lines represent the 95% confidence intervals for the termite probability while the points indicate*

*the probability of infestation. These results are significant showing termite infestation at a higher probability in ivermectin treated dung on day 15 and a more likely although not significant preference on day 30*

### 2.4.3 Wet weight decreased slower in ivermectin treated pats, irrespective of termite presence

Both treatment and control wet weights decreased from Day 0 to Day 30. However, by Day 15, there was a significant effect of treatment, where the control pats were already significantly lighter than treatment pats (control pats = 291.9 g (275.7 to 308.1) versus treatment pats = 337.2 g (321.0 to 353.3), with a mean difference: -45.3 g (-68.2 to -22.4); df = 435.1; t =-3.89; $p < 0.001$, Figure 2).

The difference was maintained on Day 30 (control pats = 277.8 g (261.6 to 294.1) versus treatment pats = 317.0 g (300.7 to 333.2), with a mean difference: -39.2 g (-62.1 to -16.2); df = 437.8; t = -3.35; $p < 0.001$). Lastly, the difference was also maintained although to a smaller extent on Day 45 (control pats = 238.5 g (222.2 to 254.7) versus treatment pats = 262.9 g (246.7 to 279.2), with a mean difference: -24.5 g (-47.4 to -1.5); df = 437.8; t =-2.09; p = 0.037); see Figure 2).

Because IVM seems to enable the presence of termites, we tested whether termites affected the wet weight decrease, but we did not find a relationship between termite presence and wet weight decrease on Day 15 (controls: 11.1 g (-7.8 to 30.0) IVM: 20.3 g (2.8 to 37.9); mean difference: -9.2 g (-35.0 to 16.6); df = 214; t =-0.7; p = 0.482, data not shown)

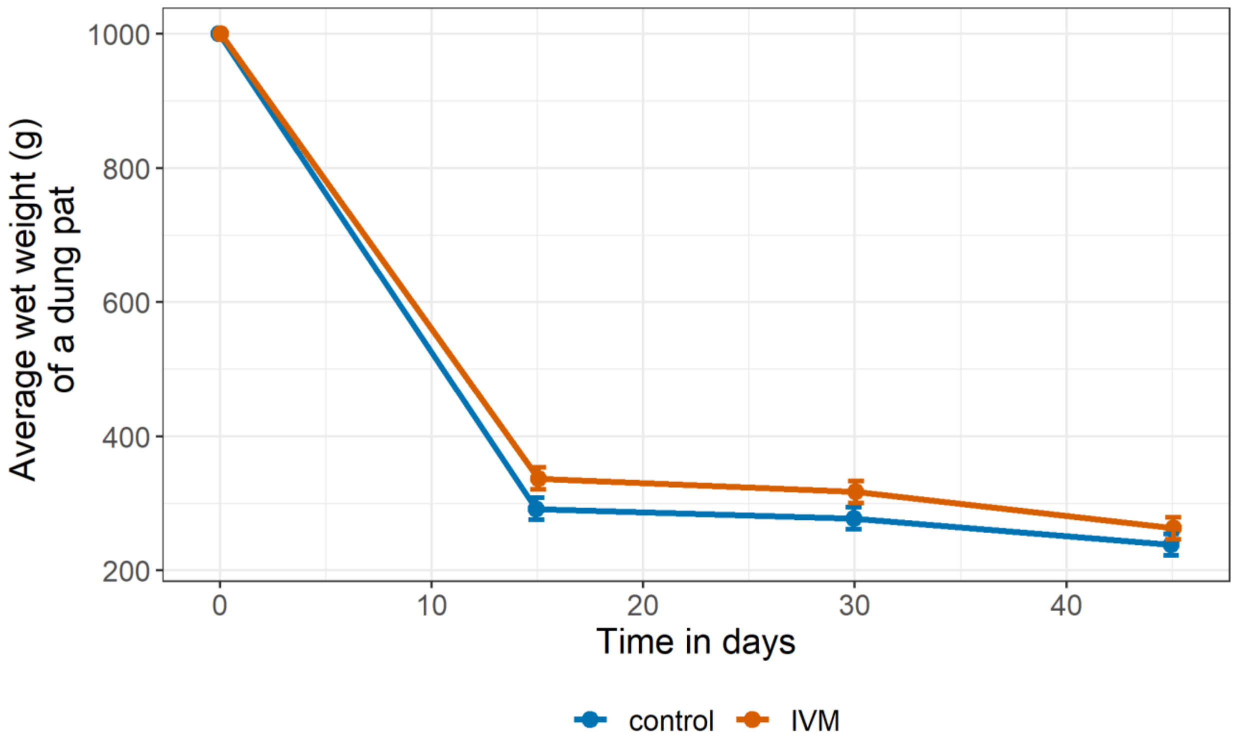


*Figure 2: Ivermectin (IVM) treated pats displayed overall slower wet weight decrease in comparison to control, with the largest effect occurring from day 0 to15. The vertical lines represent the 95% confidence intervals for the wet weight while the points indicate the means of the wet weight. These results are significant showing that ivermectin affects dung degradation by slowing wet weight decrease.*

*Figure 3: IVM pat a week after being placed in the field.*

*Figure 4: Control pat a week after being placed in the field.*

### 2.4.4 Water content decreased similarly in treatment and control pats

Water content (%) decreased over the three time points for both treatment and control dung pats. Both treatments did not differ in water content at days 15 control pats = 16.4 (14.5 to 18.2) versus treatment pats = 15.7 (13.9 to 17.6), with a mean difference: 0.6 (-2.0 to 3.3); df = 643.9; t =0.47; p = 0.639, days 30 (control pats = 13.6 (11.7 to 15.5) versus treatment pats = 12.4 (10.6 to 14.3), with a mean difference: 1.2 (-1.5 to 3.8); df = 644.1; t = 0.88; p = 0.381) or day 45 (control pats = 6.5 (4.6 to 8.3) versus treatment pats = 6.0 (4.1 to 7.9), with a mean difference: 0.5 (-2.2 to 3.1); df = 643.9; t =0.35; p = 0.724); see Figure 5).

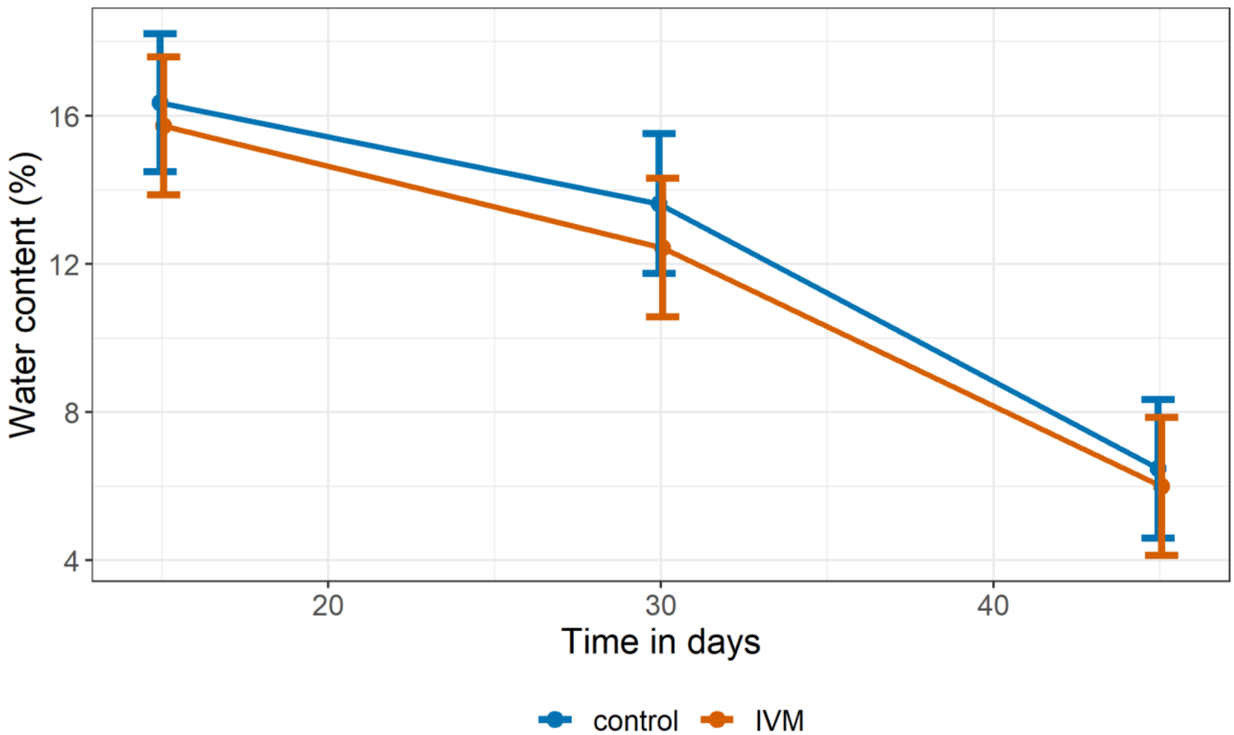


*Figure 5: Ivermectin (IVM) did not affect water content (%) on day 15, 30 and 45. The vertical lines represent the 95% confidence intervals for the water content (%) while the points indicate the means of the water content. These results show that ivermectin and control pats water content decrease similarly.*

### 2.4.5 Dry weight changes were similar for treatment and control pats

Dung dry weight increased over the three time points for both treatment and control dung pats for the 10 g subsamples (Figure 6). Additionally, ivermectin treated pats and control did not differ in dry weight at days 15 control 83.6 (81.8 to 85.5) ; IVM: 84.3 (82.4 to 86.1); mean difference: -0.6 (-3.3 to 2.0); df = 643.9; t = -0.47; p = 0.639), days 30 control: 86.4 (84.5 to 88.3); IVM: 87.6 (85.7 to 89.4); mean difference: -1.2 (-3.8 to 1.5); df = 644.1; t = -0.88; p = 0.381), or days 45 control: 93.5 (91.7 to 95.4); IVM: 94.0 (92.1 to 95.9); mean difference: -0.5 (-3.1 to 2.2); df = 643.9; t = -0.35; p = 0.724; see Figure 6).

Importantly, this increase in dry weight was not because there was an increase in organic weight, but rather that each subsample across time possessed a progressively higher proportion of dry matter (see discussion).

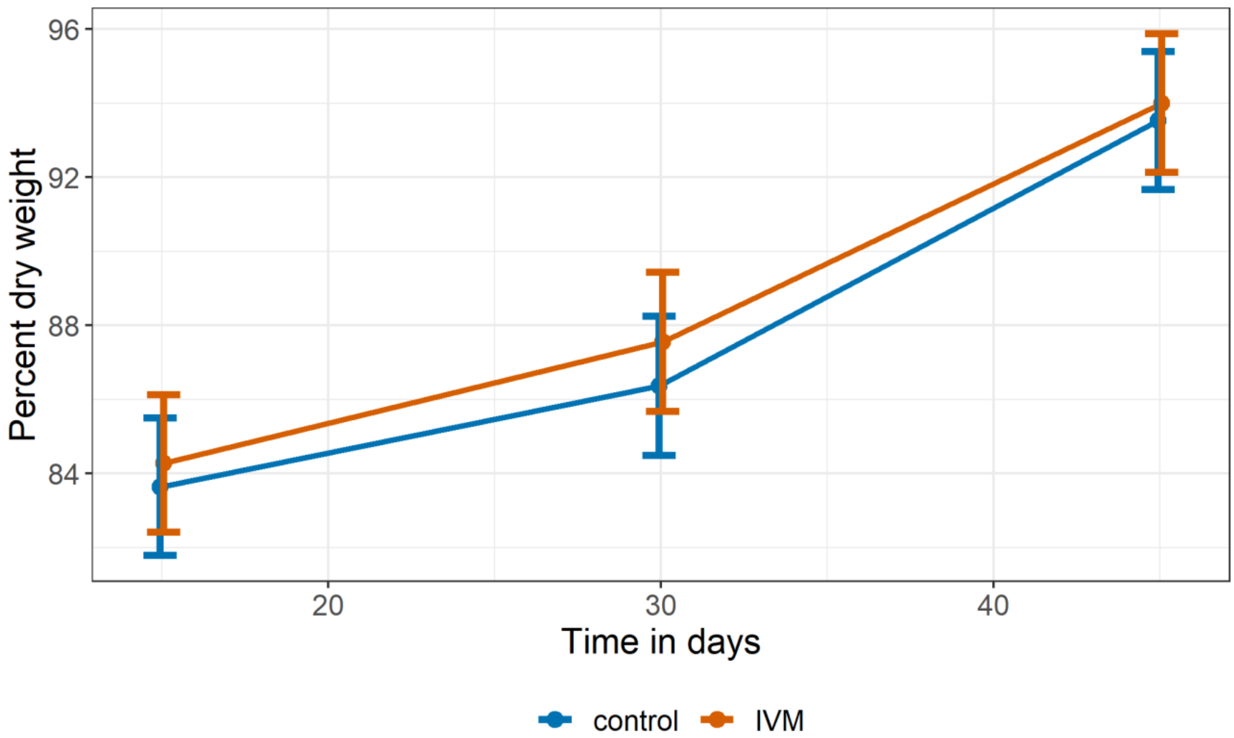


*Figure 6: Ivermectin (IVM) did not affect percent dry weight on day 15, 30 and 45. The vertical lines represent the 95% confidence intervals for dry weight while the points indicate the means of the dry weight. These results show that ivermectin and control pats dry weight increase similarly.*

### 2.4.6 Organic matter content decreased similarly between IVM treated and untreated pats

There was no effect of treatment on organic matter (Figure 7). Ivermectin treated pats and control did not differ in loss on ignition at days 15 (control: 59.9 (57.2 to 62.6) ; IVM: 59.8 (57.1 to 62.5); mean difference: 0.0 (-3.8 to 3.8); df = 631.8; t = 0.02; p = 0.983), days 30 (control: 46.5 (43.7 to49.2) ; IVM: 47.1 (44.4 to 49.8); mean difference: -0.6 (-4.5 to 3.2); df = 632.8; t = -0.31;p = 0.758), or days 45 (control: 45.6 (42.9 to 48.3); IVM: 44.8 (42.1 to 47.5); mean difference: 0.8 (-3.0 to 4.6); df = 632.1; t = 0.41; p = 0.682; see Figure 7).

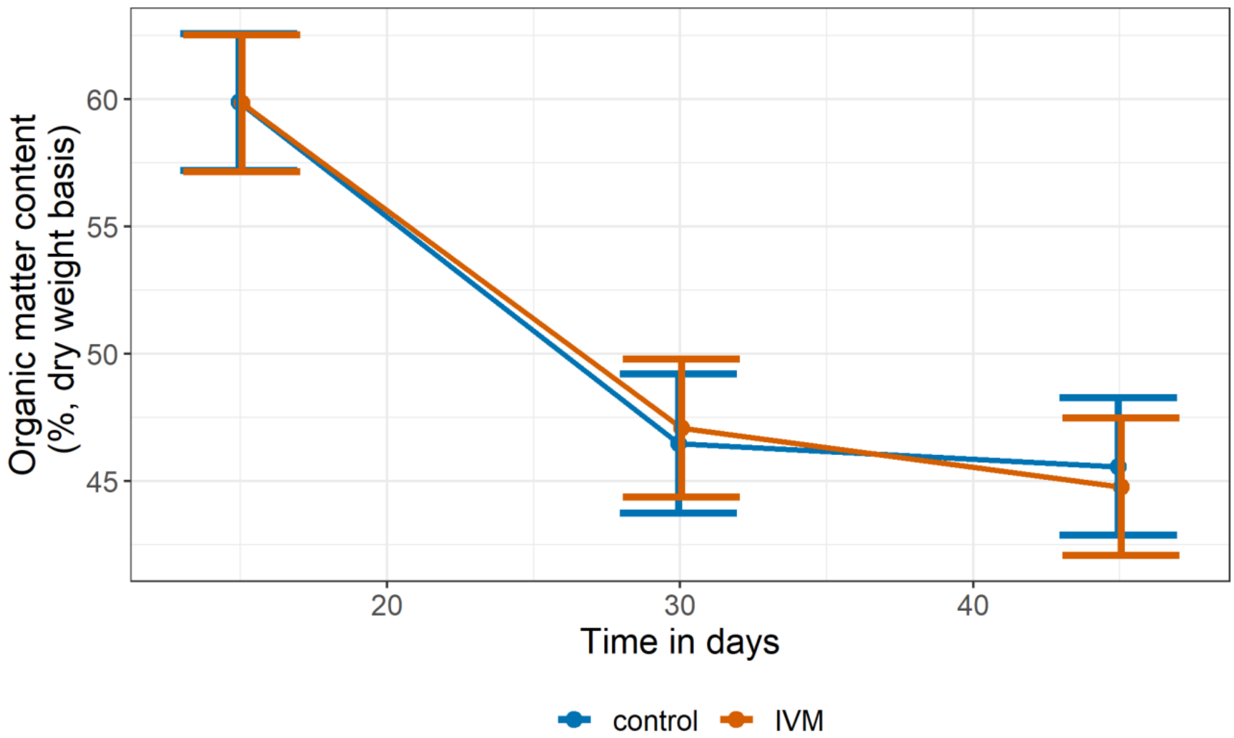


*Figure 7: Ivermectin (IVM) did not affect organic matter content, dry weight basis on day 15, 30 and 45. The vertical lines represent the 95% confidence intervals for percentage loss on ignition while the points indicate the mean values from ignition loss. These results show that ivermectin and control pats decrease similarly.*

### 2.4.7 Total organic matter decreased slower in ivermectin pats

Both treatment and control total organic matter decreased from Day 0 to Day 30. However, there was a significant effect of treatment on total organic matter loss over time, with control pats = 144.1 (135.5 to 152.7) remained versus treatment = 166.1 (157.5 to 174.7) for a mean difference: -22.0 (-34.1 to -9.8); df = 563.5; $t = -3.55$; $p < 0.001$. By day 30, 106.8 (98.1 to 115.5) remained in control pats, and 125.2 (116.5 to 133.8) remained in the IVM treated pats for a mean difference: -18.4 (-30.6 to -6.1); df = 568.1; $t = -2.93$; $p = 0.003$. On day 45, the difference remained but it was no longer significant: 100.5 (91.9 to 109.2) remained in control pats, and 107.1 (98.5 to 115.7) remained in IVM treated pats (mean difference: -6.6 (-18.8 to 5.6); df = 564.9; t = -1.06; p = 0.291

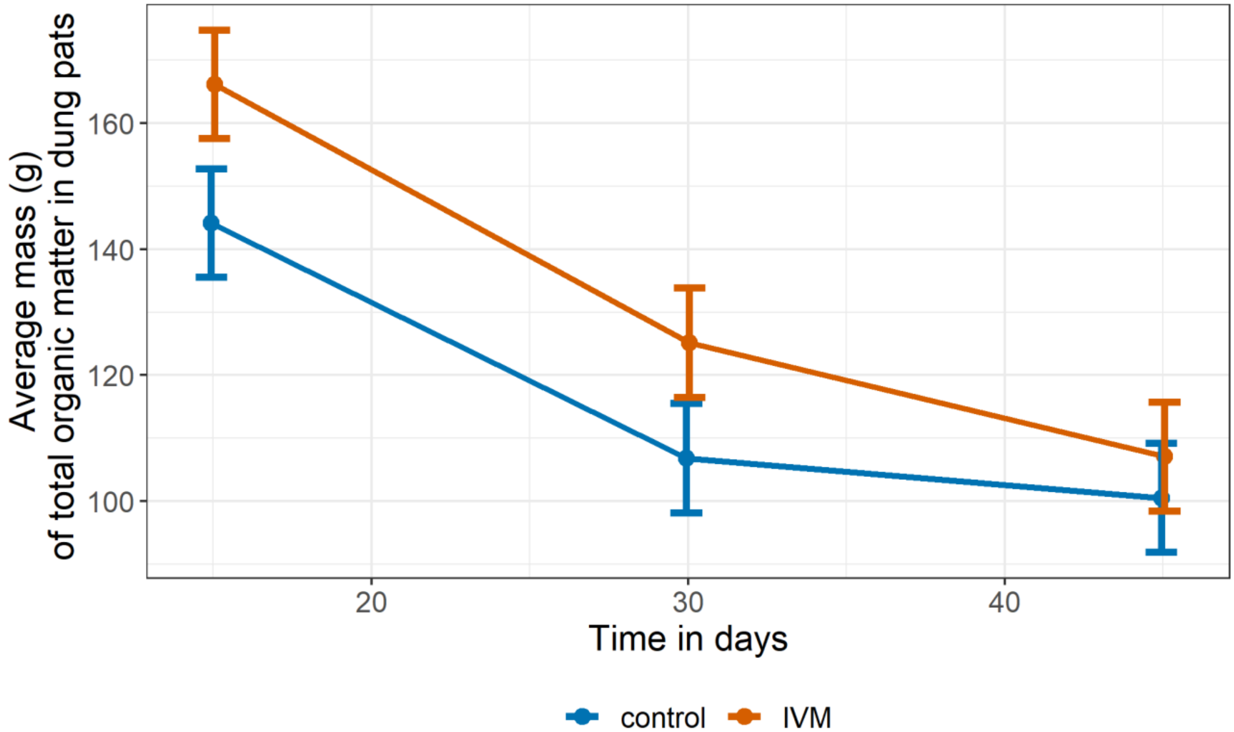


*Figure 8: Ivermectin (IVM) affects the average mass (g) of total organic matter on day 15, 30 and 45 in the dung pats. The vertical lines represent the 95% confidence intervals for total organic matter while the points indicate the mean values of the total organic matter. These results show that ivermectin significantly affects the total organic matter in dung.*

## 2.5 Discussion

Here we used cattle dung pats in a field experiment to determine the impact of ivermectin on dung degradation in Tanzania. In our study we found that after dung pats were placed in the field, they were visited and colonized by various arthropods. In particular we observed flies, beetles and termites on the pats. Qualitatively, we observed less larval activity in ivermectin treated pats compared to control pats, though quantitatively termites infested treated pats more than control pats (Figure 1). Concurrently, and in line with what we would expect if fewer insects colonize the pats (Madsen et al. 1990; Kavanaugh and Manning 2020), we observed that ivermectin treated pats degraded more slowly as measured by their wet weight (Figure 2) and the overall organic matter (Figure 8). These results suggest that ivermectin slows or prevents the colonization of the pats by arthropods, which then considerably slows dung degradation in tropical savanna settings and organic matter is almost similar in the treatments by day 45. Our findings have implications for plans on mass administration of ivermectin in this area, as any benefit of controlling *Anopheles* populations must be balanced against these non-target environmental effects.

When we placed pats in the experimental field, we saw activity of Diptera, Coleoptera and Isoptera in our study. Coleoptera and Isoptera dominated, a result similar to a previous study looking at the effects of seasons on dung deposition during the dry season (Omaliko 1981). The colonization of pats with these insects is crucial: beetles have been found to remove dung (Kavanaugh and Manning 2020), and as adult termites, flies and beetles make their ways in and out of the pats and feed, they facilitate the drying of the pats (Doube 2018; R. Menéndez, Webb, and Orwin 2016) and the decomposition process (Kadiri, Lumaret, and Floate 2014; J. Lumaret, Kadiri, and Bertrand 1992). Because of the importance of dung organisms for nutrient cycling, the loss of larval activity in ivermectin treated pats that we observed qualitatively compared to the control pats in the first few days before pats dry out could be problematic. For example, it was suggested that most of the dung degradation is due to dipteran larvae (Madsen et al. 1990). According to O'HEA et al. (2010) larvae and adult insect stages contribute to reducing dung pats and also facilitate the degradation process by late decomposers as well promoting soil conditioning and recycling. In other words, while insect larvae may not significantly contribute to decomposition, they are involved in direct respiration of organic matter, mixing and aerating dung, and breaking the dung into smaller particles which enables microbial decomposition (Sommer et al. 1992; Holter 1979).

In contrast to beetles, we observed termites throughout the entire dry season, further corroborating their ability to tolerate harsh dry conditions (Ferrar and Watson 1970). Termites can be especially important, as in addition to tunneling, termites also add up soil contents in the dung in attempt to make their mounds (Whitford, Steinberger, and Ettershank 1982). Their feeding and tunneling activities facilitate microbial activity, which then helps with organic matter break down into the necessary nutrients that are then taken up by plants (Fox-Dobbs et al. 2010). In that way, termites help nutrients become available, which also improves grazing activities in grasslands (J. Lumaret 1995). Despite the documented importance of termites in dung degradation, in our data we did not find an association between termite infestation and dung degradation, measured as wet weight.

Additionally in light of the qualitative lower insect activity in ivermectin treated pats, it is surprising that termites seemed to associate primarily with treated pats. To our knowledge, this is the first time that this has been observed in termites, though a similar behavior has been observed in dung beetles (Wardhaugh and Rodriguez-Menendez 1988). There the authors suggested that some volatile metabolites of ivermectin in the dung as well as changes in the gut flora of cattle caused by ivermectin may be involved in the bias to ivermectin dung (Wardhaugh and Mahon 1991). In our study it may be that, given the overall lower rates of colonization by insects, perhaps the termites simply face less competition in ivermectin treated pats. If adults can bear the exposure to ivermectin, this would enable them to colonize and exploit pats more easily in absence of competition. For example, if Dipteran larvae are particularly sensitive to ivermectin (Madsen et al. 1990; Suarez et al. 2003), then more resources remain for the termites. Further research of the phenomenon is needed before we can draw conclusions.

In the first 15 days, the wet weight of control pats decreased from 1 kg to c. 290 g, whereas the ivermectin treated pats only decreased from 1 kg to c. 340 g, a difference of almost 14%. We then continued to track wet weight across time to determine faecal degradation. We have shown that standardized 1 kg pats decreased in both the ivermectin and control pats. In this study ivermectin treated pats had the wet weight decreasing more slowly than the control pats. Overall, we see the decrease in control pats from 1 kg to c 290 g, then 277 g and further to 238 g and IVM pats from 1 kg to c 337 kg and slightly decreasing to c 317 g and further to c 260 g. By day 30 there was a

12% difference in the decrease of the two treatment pats close to that at day 15. Although the difference became smaller, a gap of 9% remained at the end of the study but was not statistically significant. In contrast to the wet weight of the whole pat, the dry weight of the 10 g sub samples increased (Figure 6) presumably because of moisture loss due to the dry weather with a mean of about 27°C for our study area. That meant that the wet weight of the 10 g sub sample was closer to the dry weight. In support of this, a similar study by (Dickinson and Craig 1990) also reported a reduction in water content of dung associated with dry weather and low humidity in the atmosphere. Water content decreased over time with no significant differences in the treatments as well mostly contributed to by the intense hot climatic conditions in these tropical settings (Figure 5). Crucially, though, this drying out of pats and water content occurred at a similar rate in both treated and control pats, indicating that the impact of ivermectin on insect colonization and the associated bioturbation may have had little effect on the dry weight and water content.

Regardless, dry weight from subsamples might therefore be a poor indicator of dung degradation, because it excludes weight of soil and other materials that may be brought in or disturbance by living organisms (Tixier et al. 2016). In this study the wet weights of the dung pats and the total organic matter seem to be good indicators of the degradation process. If we looked at the organic matter of the 10 g sample expressed as organic matter content in % dry weight basis, we observed a decrease in organic matter from day 15 to 30 in both treatments before it remained relatively stable from day 30 to day 45. We did not find any statistically significant treatment differences in organic matter for the sub samples. However, if we extrapolate the organic matter measured in the sub sample to the whole pat, we find a narrowing difference over time: The difference between ivermectin treated and control pats is largest on day 15, still significant at day 30, but not significant on day 45. While this extrapolation to total organic matter is coming with some uncertainties, for example, we do not know if the sub samples were representative for the whole pats especially as insects moved these dung pats and termites leaving hollow spaces in dung with sometimes traces of soil and less dung or soil remaining as the samples, we find these data to be compelling evidence that ivermectin given to cattle decreases dung degradation. Since there were no differences in organic matter content dry weight basis, the differences in total organic matter are mostly driven by the differences in wet weight. Even if our calculations were uncertain, wet weight alone can be used to measure the degradation process (Wall and Strong 1987; Slade et al. 2007). In pats where dung organisms are involved in degradation, they break down dung and facilitate microbial activity causing rapid organic matter loss signified by a decline in wet weight as seen in control pats (Wall and Strong 1987).

Accordingly, Beynon et al. (2012), J. Lumaret (1995), and Wall and Strong (1987) all used wet weight to measure pat decomposition, and, just as in our study, reported control pats as lighter than experimental pats, suggesting control pats degrade more rapidly than the treated ones. Other studies done in temperate settings also show that dung degradation is reduced more in ivermectin treated pats versus control (Madsen et al. 1990; Sommer et al. 1992; K. Floate 1998). A few studies, however, failed to find an effect of ivermectin on dung degradation. (Barth et al. 1994) did not find differences in area of pats, and Suarez et al. (2003) did not find differences in wet weight. At first glance this latter discrepancy is puzzling, but a closer reading of the study by Suarez et al. (2003) shows that their study was conducted during severe drought, and they did find an effect of ivermectin in an earlier study done under different climatic conditions (Suarez 2001). In conjunction with their method of pat preparation (15 centimeter flat pats), this could mean that pats provided a high surface area for the sun to aid the breakdown of ivermectin and its metabolites

(Shija 2023). It remains to be seen if pat area, as used in Barth et al. (1994), is a good measure for dung degradation.

We conducted our study during the dry season, and these results cannot easily be extrapolated to the wet season. For example, dry conditions may impact the dung insects and negatively impact the microbial community (Errouissi and Lumaret 2010; Vessby 2001). The loss of dung diversity due to dry conditions may then exacerbate the effects of ivermectin (Beynon et al. 2012). On the other hand, increased sun irradiation during the dry season may quickly degrade ivermectin and its metabolites Shija (2023), lessening the impact of ivermectin treatments on dung degradation. Either way, there is a critical need to conduct similar studies in wet seasons in the tropics to rule out the dry condition effect on microbial activity and investigate further the ivermectin effect during this period that is crucial to malaria spread (Kija, Kimera, and Mnyone 2022; Katusi et al. 2022). Of course, malaria is prevalent year-round in our study area (Mapua et al. 2022), and our study represents an important first step if we want to evaluate the environmental impact of mass drug administration to curb the spread of malaria.

Although ivermectin could serve as an immediate and cheaper method for tropical mosquito control (C. Chaccour et al. 2023), it may also be responsible for unintended environmental effects. Slow dung degradation affects pasture quality to the point where economic losses are incurred (K. D. Floate et al. 2005). In addition, undegraded dung limits the feeding area of livestock (Wall and Strong 1987), and slowly degrading dung facilitates the activities of some livestock pests (MacDiarmid and Watkin 1972). Even though we did not measure the impact of our artificial pats on pasture quality, our data supports further investigations in these tropical settings, especially in the wet season. Also, it is unknown what level of difference in degradation rate is environmentally relevant. Significant difference statistically does not equal real world significance.

In summary, our results from tropical Tanzania add to previous studies from temperate regions demonstrating that mass drug administration of ivermectin may negatively affect dung degradation. Humans and human activities generally contribute to environmental contaminants such as antiparasitics, herbicides and antibiotics. Importantly these are all beneficial but their massive use could also lead to unintended negative consequences in human, animal, plant and environmental health. A One health approach is essential in facing challenging diseases, however, impacts of such approaches should be weighed in all the intersections before implementation.

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# Chapter 3: Ivermectin and its biological metabolites affects larval development and abundance in a tropical savanna setting

## 3.1 Abstract

Vector control has been an effective tool for malaria control for years; however, insecticide resistance and change in mosquito behavior affect vector control, so it is important to develop novel tools to support future vector control strategies. Ivermectin, a drug used in livestock and humans to control a variety of parasites, targets the nervous system of arthropods, including *Anopheles* mosquitoes, making it a suitable tool for malaria control through mass drug administration. In studies in temperate regions, ivermectin has been reported to affect the dung insect community; however, less is known in tropical settings where malarial cases are prevalent. We set up field and semi field experiments to determine the effect of ivermectin on dung fauna abundance and diversity in Tanzania. At 3, 10 and 29 days post treatment for both ivermectin-treated and control cattle, we collected dung pats and placed them in the field to observe insect emergence. On 1, 2, 3 and 5 days post treatment, we placed dung in a semi field area away from intense climatic settings in the field and counted larvae weekly. We found that ivermectin treated dung had fewer Coleoptera larvae than control pats in both field and semi-field settings. These results indicate that ivermectin and its residues affect dung insect larvae, mainly by disrupting their life cycles and causing a decline in their abundance in dung in a tropical dry setting. Therefore, mass drug administration for malaria may negatively affect dung communities. Since ivermectin is excreted in the dung of treated livestock, dung insects are at a high risk of facing a tremendous effect of the treatment, which should be considered before the use of ivermectin for malaria control in mass drug administration.

## 3.2 Introduction

Since 2000, the use of long-lasting insecticidal nets (LLINs) and indoor residual residual spraying for malaria vector control has contributed to the reduction of about 663 million malaria cases (Bhatt et al. 2015). According to the World Health Report, about 94% of all malaria deaths are linked to Africa (WHO 2019). Malaria affects communities, causing a work force reduction and further contributing to economic impacts. Because Africa continues to have the greatest malaria burden (Snow et al. 2017), the World Health organization has put strategies in place such as targeting malaria burden by 90 % by 2030 (WHO 2015).

Regardless, malaria continues to affect the African continent disproportionately (Badmos et al. 2021). Residual malaria transmission, which is the remaining low malaria transmission despite the use of common control methods, has been a challenge to vector control efforts (WHO 2017; Abraham, Massebo, and Lindtjørn 2017). This is mostly caused by a change in mosquito behavior (Killeen et al. 2017; Kenea et al. 2016; C. Chaccour and Killeen 2016; Killeen 2014). *Anopheles* mosquitoes are now resistant to most insecticides such as pyrethroids (Ranson and Lissenden 2016). Therefore, new interventions are required to reduce the malarial vector population.

Ivermectin was introduced in the 1980s to control parasites and nematodes in humans and livestock (Crump and Omura 2011). In insects, ivermectin affects neuromuscular systems, causing deleterious effects (Canga et al. 2008). *Anopheles* mosquitoes are susceptible to ivermectin (Kobylinski et al. 2010), and the drug causes mortality, knockdown, sterility and immobility

(Kobylinski, Foy, and Richardson 2012; Fritz et al. 2009; Pampiglione et al. 1985). Ivermectin is therefore a potential control tool for malaria vectors (WHO 2016; C. J. Chaccour et al. 2013). Indeed, there have been efforts to use mass drug administration for control of mosquitoes in tropical settings; however, less is known about the effect of the drug on non-target organisms.

Because most of ivermectin and its metabolites are excreted in the dung of treated livestock (Chiu and Lu 1989), dung colonizers such as insects, bacteria and fungi that participate in decomposition are likely to be affected (Holter 1979). For example, insects such as dung beetles are involved in the decomposition of dung, facilitate microorganism activity (Holter 1979), assist in the aeration process, and allow microorganisms such as bacteria to continue degradation (Bang et al. 2005), all of which contributes to soil fertility. In summary, dung organisms recycle nutrients and prevent dung accumulation in the environment (Beynon, Wainwright, and Christie 2015; Maldonado et al. 2019).

There is mixed evidence detailing the effects of ivermectin on dung fauna. For example, studies in temperate regions have shown that ivermectin in dung reduces the number of adult Diptera (mostly the Cyclorrhapha) and Coleoptera larvae (Iglesias 1998). Additionally, it was shown that ivermectin also affects the ecological activity of dung organisms like dung beetles such as aeration, adding nutrients to soil and contributing to improved pasture quality and reducing gases that contribute to global warming (Correa et al. 2022; Jean-Pierre Lumaret et al. 2012; Nichols et al. 2008). Overall, it seems that effects on insects include reduced egg production, oviposition and larval development (Strong 1992). However, it was also reported that the larvae of Nematocera are unaffected (Sommer et al. 1992). Importantly most studies focusing on the impact of the drug on dung fauna have mostly been done in temperate regions and there is less information on such effects in a tropical region, where malaria is highly present, but where higher solar irradiation may quickly degrade ivermectin (Shija 2023).

Here we study the impact of ivermectin on dung fauna in a tropical dry setting of Tanzania. In particular, we investigate the effect of the drug on insect activity and larval abundance. These data will help inform decision making on mass drug administration in Africa for malaria vector control.

## 3.3 Materials and Methods

### 3.3.1 Field preparations

We conducted the experiments at the research farms of the Ifakara Heath Institute. We assigned cattle randomly to one of two treatments: treatment and control. The cattle involved were Tanzanian short horn zebu, the local indigenous breed. We tested the impact of ivermectin on dung fauna using 200 µg/kg body weight dose of ivermectin, the dosage is commonly used to control some parasitic infections in cattle (Crump and Omura 2011). A veterinarian administered ivermectin subcutaneously based on weight-dosage calculations. Administration was approved by the National Institute for Medical Research in Tanzania. After the cattle were treated, we collected fresh dung pats from cattle on different days and then put the pats out in the field and semi-field setting (see below) for exposure.

### 3.3.2 Experimental design

We used standard methods (Jochmann et al. 2011) to assess the effects of ivermectin on dung insect communities. We collected 20 (10 treated and 10 control) random composite samples from the cattle on day 3, 10 and 29 after IVM administration, collecting a total of 60 pats across this study. We placed the dung pats in the field at least 2 m apart for 1 day to be colonized by insects. Then we placed the pats on a 25 cm × 25 cm plastic net (8–10 mm mesh) to facilitate retrieval and covered them with a chicken wire mesh cage to exclude birds but not insects. We observed the pats daily. Following the 1 day field exposure period, we collected the dung pats and placed them in an emergence cage to recover insects (Suarez et al. 2003). It was originally expected that the emerged insects would be collected for enumeration and identification upon emerging, but the hot climatic conditions hindered this. Therefore, we adopted a semi-field approach (see below).

##### *3.3.2.1 Semifield dung pats*

Because of the dry climatic season, we also performed a semi field experiment, where we also collected fresh dung pats after cattle treatment. We collected 10 (5 treated and 5 control) random pats on day 1, 2, 3 and 5 after administration, collecting a total of 40 pats across this study. We left the pats to be colonized by insects for one day upon being placed on a 25 cm × 25 cm plastic net (8–10 mm mesh) in a semi field setting. After the one day period for insect colonization, the pats were placed in containers and covered with a mesh netting. We watered the pats every 2 weeks to keep the dung moist and facilitate larval growth and survival. We counted and recorded the number of larvae that were found in the pats on a weekly basis and observed their development.

### 3.3.3 Statistical Analysis

We analyzed data using R version 4.2.1. We used Poisson mixed models to analyze larval counts. We used generalized linear mixed effect models to study the effect of ivermectin on insect counts with random intercepts of the scaled days and experiment type (Bates 2010). We also obtained the estimated marginal means (emmeans) to derive standard errors and confidence intervals (Lenth 2021).

## 3.4 Results

### 3.4.1 Qualitative description of type and order of fauna in the field dung pats

A variety of arthropods colonized dung after the 1 Day exposure. We observed arthropods from Coleoptera families of Scarabaeidae and Staphylinidae, Diptera families of Muscidae and Calliphoridae, Hymenoptera family of Formicidae, mostly the safari ants (Dorylus), and the Isoptera family of Termitidae. Other smaller species of Collembola and Acari were also observed.

Adult insects, mainly dung beetles and flies, occupied both ivermectin and control pats in the first few days to a week after the pats were placed, depending on the size of the pat. The following day after pats were laid, we observed Coleoptera larvae. The adults (mainly Coleoptera) could colonize the pat up to a week, depending on the size of the dung, to the time it loses its moisture. As soon as the pats dried, no adult dung beetles were observed, and then termites took over the dung pats. Qualitatively there were more larvae observed in the first week in the control than the treated pats. Dung pats dried up very quickly in this tropical region dry season.

### 3.4.2 Ivermectin treated pats had few larvae compared to control pats

We found a significant effect of treatment on larvae counts, with the difference between treatment and control increasing over time (Figure 9). In particular, we found a significant effect of treatment on larvae from day 1 to day 124, (mean counts on day 124 (95%): control pats = 82.1 (78.1 to 86.2), treatment pats = 11.9 (11.3 to12.6), corresponding to an increase by 6.89 (6.66 to 7.12) times; df = ∞; z = 112.92; $p < 0.001$.

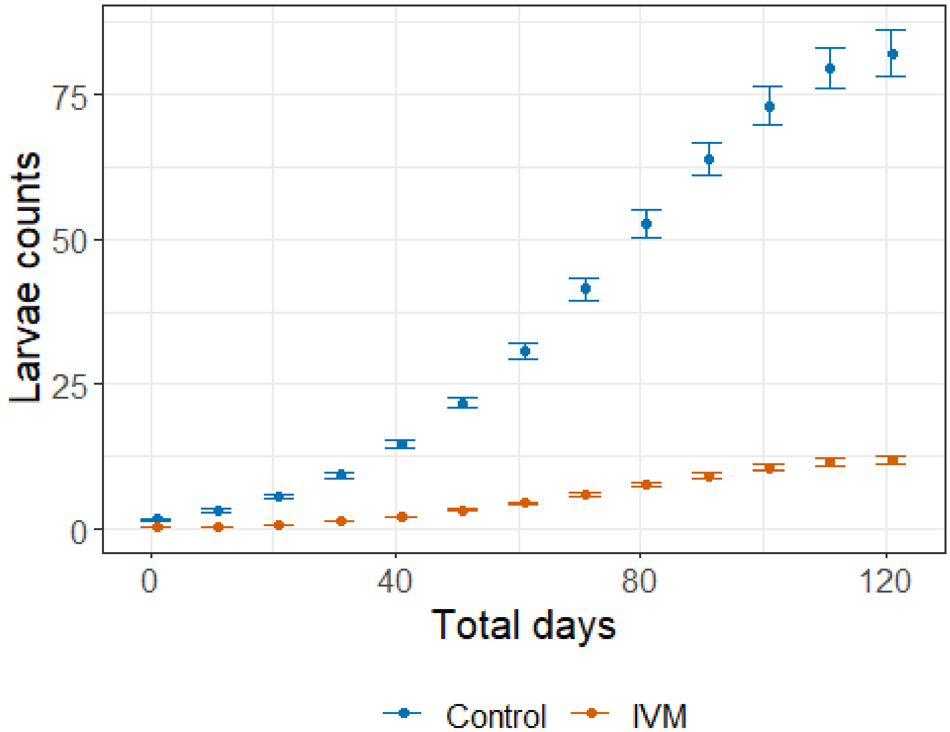


*Figure 9: There were fewer larvae counts in ivermectin (IVM) treated pats in comparison to control. The vertical lines represent the 95% confidence intervals for the rates of larvae counts while the points indicate the means of these counts. These results are significant showing ivermectin negatively impacts larvae abundance*

## 3.5 Discussion

Here we placed cattle dung pats in a field and semi field setting to determine the effect of ivermectin on dung fauna abundance and diversity in tropical settings mainly focusing on larval abundance. We found a significant effect of ivermectin on dung fauna larval abundance (Figure 9). In particular, we counted more larvae in control versus ivermectin treated pats, showing a negative impact of the drug similar to studies by Pérez-Cogollo et al. (2018) and O'HEA et al. (2010). This suggests that ivermectin affects insect abundance as well as the whole insect developmental process, and consequently mass drug administration for malaria control will negatively affect dung insect communities calling for further critical considerations on the evaluation of environmental safety.

In this study, we found that dung pats were occupied by firstly Diptera (flies) and Coleoptera (dung beetles) similar to observations by McLintock and Depner (1954), followed by Isoptera (termites). We also observed that in the tropical dry season, dung pats dry up quickly and that together with ivermectin and its metabolites could affect survival of the dung organisms similar to a study by (Ambrožová et al. 2021; Jean-Pierre Lumaret et al. 1993). We observed adult organisms in both pats in the first few days before drying out, indicating adult organisms such as beetles and termites are perhaps not affected by ivermectin (Pérez-Cogollo et al. 2018; Iwasa et al. 2005; Yoshida et al. 2004). Adult dung beetles prefer feeding only on fresh dung, explaining their disappearance after the pats became dry and larvae feed on older dung (Holter 2016).

In both the field and semi field settings of the study, we concurrently observed more larvae in the control versus ivermectin pats, despite only doing observations and not counts in the field. In both cases, the number of larvae increases with time; however, in the field, numbers quickly decreased as the dung lost its moisture, which occurs quickly in dry tropical settings (Jean Pierre Lumaret and Kirk 1987). This also explains the inability of larvae to survive weeks after (Holter 2016). Moisture content is very important for dung beetle development (Errouissi et al. 2004). In summary, in the tropics ivermectin and its metabolites and also the dry conditions greatly affect larval development, causing survival competition for the larvae to complete their life cycle.

In spite of the quick drying of the field pats, we still qualitatively observed only a few larvae in the treated pats, which means ivermectin and its metabolites could have had an effect before the drying process. This effect was further exemplified in the semi field setting where we performed larval counts (Figure 9). Although ivermectin degrades very fast in tropical settings on exposure to sunlight as compared to temperate settings (Shija 2023; Halley et al. 1989), the effects of the drug on dung fauna were clear even before the drying of the pats in the field experiment with low larvae counts. That result was more stark in the semi field setting where the dung pats were not exposed to the intense sunlight that would facilitate photo degradation: we also found low larvae counts in ivermectin treated pats.

In the tropics, the concentration of ivermectin and its metabolites decreases over time in dung, with the metabolite 3″-O-demethylivermectin (3DI) going to undetected levels on the upper dung layer in a week's time post dung placement (Shija 2023). Thirty days later, 3DI goes to undetectable levels in the lower layer as well. Ivermectin also decreases to about 65% on the upper part of the dung and about 31 % on the lower side of the dung pats over the course of three days, although this difference of course depends on depth of the dung pats in the field. Adult dung beetles and larvae feed on the moist, lower part of dung, which could still contain enough concentrations of ivermectin and the associated metabolites to impact their survival and fitness, which would explain our observation of fewer larvae in ivermectin dung and no larvae on the upper part of dung.

In this study Diptera (flies) were only observed for one day, when the dung pats were still moist on the top layer. Afterwards, we did not observe Diptera development, as the conditions for their development were not favorable. For example, Campobasso, Di Vella, and Introna (2001) reports that high temperatures affect Diptera development. We observed average daily field temperatures of 27°C. Coleoptera larvae in this study were most affected, which agrees with earlier studies (Martínez et al. 2017; O'HEA et al. 2010; Jean-Pierre Lumaret et al. 1993). Coleoptera, mainly dung beetles, are important and contribute to burying dung in soil helping nutrient cycling in the environment also reducing methane emission through their burying activities (Shah and Shah 2022). Although adult beetles do not seem to be affected, the lower larvae count in ivermectin treated pats could be associated with a reduction in reproductive ability (Strong et al. 1996; Ridsdill-Smith 1993). Iglesias (1998) also reports a few larvae in treated pats up to 30 days after pat deposition which also matches with our studies. Apart from Diptera and Coleoptera, Isoptera (termites) occupied the dung pats throughout the entire period in the field experiment. In this study, we have observed that termites can endure the hot climate and that ivermectin and its metabolites do not seem to affect them, though we did not assess the impact on developing larvae. Additionally, it may be that the dry dung pats have ivermectin metabolite contents that cause the attraction or even the absence of other organisms in search of dung resources.

Although our results show that adult dung insects (mostly dung beetles, flies and termites) do not seem to be as impacted by ivermectin as compared to larvae (Suarez et al. 2003; Strong et al. 1996), the nature of the study in field setting allowed for them to move from one area to another, which made it difficult in our data collection and analysis. Further research should be done in an enclosed area to determine if the activities of adult dung insects in the tropics are affected by ivermectin treatment.

Our study was conducted during a tropical dry season where there is less insect activity. Therefore, it would be important to perform this experiment during the tropical wet season since the hot dry conditions in the field setting could have had a greater effect (Holley and Andrew 2019; Tyndale-Biscoe and Walker 1992) than the effects of ivermectin and its photo degradation process causing stressful conditions for the dung communities.

Ivermectin is a strong tool for malaria vector control, as seen in the decline in malaria cases after its use in trials (Foy et al. 2019; Alout et al. 2014) However, as we have shown here, the mass drug administration of ivermectin would also greatly impact the dung insect communities of the treated livestock. Since ivermectin seems to affect dung insects, a decline in dung insect activity would result in delaying the decomposition process because the feeding, aerating activities and also microbial activity that contribute to degradation are hindered with less insect activity that help reach the inner parts of the dung (Pecenka and Lundgren 2018; Strong 1992; Madsen et al. 1990). In the end, these negative ecological effects of ivermectin need to be balanced against the benefit to human health. More research is needed to compare the activity of dung insects in the two seasons of the tropical climate, which is the rainy and dry seasons, to establish when mass drug administration has the least impact.

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# Chapter 4: Final Discussion and Conclusion

We determined dung degradation using different methods including wet weight, water content, dry weight and organic matter which is the most proposed method (Jochmann et al. 2011) and found a gradual wet weight decrease, high organic matter and also less insect activity demonstrated by few larvae counts in ivermectin dung as compared to control pats in the tropical dry season in Tanzania. With these observed consequences, dung remains in the environment for a long period indicating poor degradation. Results from this research confirm that ivermectin is a threat to the degradation process and insect activity. These results are also supported by other studies mostly from temperate regions (Iglesias et al. 2006; Christian Sommer and Bibby 2002; Dadour, Cook, and Neesam 1999; C. Sommer et al. 1992). Ivermectin is important in controlling arthropods but excessive use of the drug impacts dung organisms and the ecological services they provide and for a case where the drug is being considered for use in mass drug administration to control malaria vectors, there is a need to look deeper at such environmental effects and also assess its great potential.

When the dung pats are still moist, they are occupied by dung organisms, mostly dung beetles, and these adults do not seem to be affected by ivermectin evidenced these adults actively contributing to moving and aerating the soil at that point, presumably helping decomposition. Termites seem to contribute to the degradation process of dung, although their action starts later when the dung pats are dry. Perhaps with a reduced ivermectin concentration at that time this does not seem to affect them and they surprisingly prefer ivermectin treated pats.

Although our results showed the impact of the drug on the environment, it should be noted that with the increase in temperature in dry seasons of the tropics, assessment of the impact of ivermectin and its metabolites may also have been affected. That calls for further research in a tropical wet season and compare the seasons in terms of the impacts seen and the rates of malaria spread. Also, there is a need to study the impact of the drug on the adult dung organisms, mainly Coleoptera, physiologically in terms of foraging, movement and reproduction. Termite physiology should also be assessed and the ivermectin concentrations that they can tolerate due to there intense degrading activities. To our knowledge, this is the first study recording termite preference to ivermectin dung and there is a need to determine if it is ivermectin or the metabolites that termites are attracted to. Additionally, this field study was done in an area close to termite mounds and which soil conditions support termites. Conditions therefore could have contributed to the intense dung degrading and therefore investigations should also be done in termite free areas to determine what happens to the degradation process.

Malaria is endemic in Tanzania, and it is a common problem in the Kilombero Valley region where we did our study. Most malaria vectors prevalent in the area include *Anopheles gambiae*, *Anopheles funestus* and *Anopheles arabiensis* (Smith et al. 1993). Livestock activities are also common in the area as it is now being occupied by pastoralists (Mayagaya et al. 2015). There is an increase in the use and awareness of veterinary drugs, pharmacies, drug shops in many areas in Tanzania (Keyyu et al. 2003). Ivermectin is a common drug used for most worm infections in livestock. Despite the use of the drug in such settings, malaria is still prevalent in the valley.

Using ivermectin to reduce malaria transmission has a lot of socioeconomic benefits in Africa. Despite the advantages it presents, environmental health should also be considered apart from animal and plant health. Chemical accumulation in the environment affects organisms as well as the overall economy. With such implications comes a great need to consider biodiversity protection due to the services provided that could benefit animal and plant health.

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