Effects of certain anthelmintics on the survival and reproduction of *Euoniticellus intermedius* (Reiche) (Coleoptera: Scarabaeidae)

By

Carmen Tina Jacobs

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Declaration

I, Carmen Tina Jacobs, declare that this thesis/dissertation, which I hereby submit for the degree Master of Science (Entomology) at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

SIGNATURE: _____ DATE: _____

This manuscript is dedicated you my Marma.

Thank you for always believing in me, never giving up on me and giving me more support than I know what to do with.

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Effects of certain anthelmintics on the survival and reproduction of *Euoniticellus intermedius* (Reiche) (Coleoptera: Scarabaeidae)

Student: Carmen T. Jacobs
Supervisor: Prof. Clarke H. Scholtz
Department: Department of Zoology and Entomology, University of Pretoria, Pretoria, 0002, South Africa
Degree: Masters of Science (Entomology)

Abstract

Avermectins and milbemycins are commonly used in agro-ecosystems for the control of parasites in domestic livestock. As integral members of agro-ecosystems with importance in maintaining pasture health through dung burial behaviour, dung beetles are an excellent, non-target, bio-indicator taxon for examining potential detrimental effects of pesticide application. The current study uses the dung beetle species, *Euoniticellus intermedius* (Reiche), as a bio-indicator to test the relative toxicity of four different anthelmintics in dung residues. There have been numerous laboratory and field trials on these four anthelmintics but there has never been a laboratory trial comparing ivermectin, eprinomectin, doramectin and moxidectin under precisely the same conditions. The effects of avermectin and milbemycin toxicity are not confined to parasitic nematodes and arthropods, but also extend to a large variety of beneficial invertebrate species, which use the dung as a microhabitat and breeding resource. Over time, pesticide usage indirectly affects the rate of dung degradation through adverse effects on dung beetles. This potential problem constitutes the main focus of this study. The present and previous studies have indicated no significant effect on the survival of adults but a significant reduction in reproductive rate and reproductive success. Over time, reduced reproductive rate would result in decreased population sizes in the dung beetle community and, ultimately, a decrease in the rate of dung degradation and dung burial. It is, thus, vitally important to create awareness about the importance of dung beetles and sound farming practices for healthy agro-ecosystems.

Keywords: anthelmintics; endectocides; ivermectin; moxidectin; eprinomectin; doramectin; dung beetles; *Euoniticellus intermedius*; cattle

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Carmen T. Jacobs[*]

[*]*Scarab Research Group, Department of Zoology and Entomology, University of Pretoria*

Avermectins and Milbemycins

Introduction

The importance placed on anthelmintics to bring parasite populations under control has become a challenging arms race to produce a product that exhibits the perfect balance between host and non-target organism toxicity, and pest resistance. The need for a better product is becoming increasingly important because indications are that as fast as they are being produced, the pests are becoming resistant. Pest resistance is arguably one of the top challenges as far as protecting livestock goes and probably the main driving force behind parasite control research in the livestock industries (Sangster 1999; Wolstenholme *et al.* 2004) since resistance has been reported in many countries, in a variety of nematodes and against all currently available anthelmintics (Sutherland & Leathwick 2011).

Anthelmintics are broad-spectrum drugs that control helminth pests by removing or killing them; they are grouped according to their common chemistry and mode of action (Sangster & Dobson 2002; Vercruysse & Rew 2002). Currently, the best anthelmintics on the market are the avermectins (ivermectin, eprinomectin and doramectin) and the milbemycin (moxidectin), which are collectively known as macrocyclic lactones.

The avermectins and milbemycins are naturally produced by a strain of soil-dwelling actinomycete, *Streptomyces* (Burg *et al.* 1979; Shoop & Soll 2002). All the avermectins have a unique pharmacophore which consists of a 16-membered macrocyclic lactone

backbone (Shoop & Soll 2002) with a disaccharide chain at C.13 (Steel 1993; Vercruysse & Rew 2002). Although the avermectins are a glycosidic derivative of the pentacyclic 16-membered lactone (Chabala *et al.* 1980; Albers-Schoenberg *et al*. 1981), they do not possess the antifungal and antibacterial properties associated with the macrolide antibiotics (Burg *et al.* 1979; Chabala *et al.* 1980; Albers-Schoenberg *et al.* 1981). They act by interfering with invertebrate neurotransmission rather than inhibiting protein synthesis (Chabala *et al.* 1980; Albers-Schoenberg *et al.* 1981). As γ-aminobutyric acid (GABA) agonists (a chemical substance capable of activating a receptor to induce a full or partial pharmacological response), the avermectins act by eliminating the GABA-mediated inhibitory postsynaptic potentials and the excitatory postsynaptic potentials at the neuromuscular junction in the target organism (Fritz *et al.* 1979; Campbell 1985) thereby inhibiting nervous signal transmissions (Putter *et al.* 1981). Instead of competing with GABA by binding to its receptor, the avermectins stimulate the release of GABA from nerve-endings which in turn enhances the binding of GABA to its receptor which is situated on the post-synaptic membrane of the excitatory motorneuron in nematodes and on the post-junction membrane of the muscle cell in arthropods (Campbell 1985). This enhancement results in an increased flow of chloride ions into the cell, which in turn, results in hyperpolarisation and elimination of signal transmission (Campbell 1985).

Different chloride channel subunits in nematodes show variable sensitivity to the macrocyclic lactones and different sites of expression, which is possibly what accounts for the paralytic effects on different neuromuscular systems at different concentrations (‘http://www.merckmanuals.com’ 2013). So, in nematodes, the avermectins block transmission signals from the ventral interneurons to the excitatory motorneurons in the ventral nerve chord (Fritz *et al.* 1979; Putter *et al.* 1981; Campbell 1985) which paralyses them without causing hypercontraction or flaccid paralysis (Kass *et al.* 1980). The paralysis occurs in the pharynx, body wall, and uterine muscles and even though paralysis of pharyngeal muscle is more sensitive, paralysis (flaccid) of the body wall muscle may be critical to the host for rapid removal of the parasite (‘http://www.merckmanuals.com’ 2013). As the concentration of the macrocyclic lactone decreases, it is possible that motility may be regained; however, paralysis of the pharynx and as a result, inhibition of feeding, may last longer than body muscle paralysis and is what ultimately contributes to the parasites’ death (‘http://www.merckmanuals.com’ 2013).

In certain species of filarial nematodes, the females living in the tissue move very little as nutrients are absorbed through the cuticle. A major effect of macrocyclic lactones on these species is most likely paralysis of uterine muscles, resulting in disruption of reproduction as opposed to death ('http://www.merckmanuals.com' 2013). However, in organisms which lack a GABA system, like cestodes and trematodes, the avermectins have no effect on their cholinergic nervous systems which renders them non-toxic (Putter *et al.* 1981).

Ivermectin was the first avermectin to be introduced in 1981 (Steel 1993; Vercruysse & Rew 2002). Ivermectin (22, 23-dihydroavermectin) is a disaccharide derivative of the pentacyclic 16-membered lactone (Burg *et al.* 1979; Chabala *et al.* 1980; Campbell 1985; Römbke *et al.* 2010). The antiparasitic effect of ivermectin is extremely potent against arthropods, nematodes and acarines but has no effect on cestodes and trematodes due to them lacking a GABA system (Putter *et al.* 1981; Campbell 1985). Although potent, ivermectin is not equally active against all species and is often very stage specific (Campbell 1985) which means that a genus which is known to be susceptible to ivermectin, may not be susceptible at all life stages (Campbell & Benz 1984).

Abamectin, a combination of 80% avermectin B_{1a} and 20% avermectin B_{1b}, is the starting material for ivermectin (Shoop *et al.* 1995). It is effective against nematodes as well as acarines and, to date remains the only avermectin or milbemycin to be used in both the animal health and crop industries (Shoop *et al.* 1995).

Eprinomectin was introduced to the animal health industry in 1997 as an alternative to ivermectin since it was considered to be the only topical endectocide safe for use in lactating dairy animals (Shoop *et al.* 1996b; Vercruysse & Rew 2002). Though ivermectin has no side effects on the host and it has such a broad spectrum of activity, it cannot be used in lactating dairy animals due to the levels of residue that remain in the milk which may result in the dairy products being discarded (Shoop *et al.* 1996a, 1996b; Vercruysse & Rew 2002).

Doramectin was commercialised in 1993 (Vercruysse *et al.* 1993) and is the "friendliest" avermectin as far as discomfort during administration goes. In a study done by Grandin *et al.* (1998), 61 red Angus-cross, two year-old heifers were injected with ivermectin, doramectin or saline solution and their reactions were recorded. The animals' willingness to be re-injected was also recorded. They found that doramectin caused significantly less discomfort during administration than ivermectin, and concluded that the use of a product which causes the least discomfort during administration improves the ease of handling in the future and reduces stress in the animal (Grandin *et al.* 1998).

The milbemycins, although structurally similar to the avermectins, differ in substituents at a few of the side chains at the C-13 position and can basically be thought of as deglycosylated avermectins (Steel 1993; Sangster & Dobson 2002; Vercruysse & Rew 2002). The milbemycins, although discovered in 1973, long before the discovery of ivermectin, were originally developed for use in crop protection and only used in veterinary practices from about 1986 (Takiguchi *et al.* 1980; McKellar & Benchaoui 1996).

Moxidectin, the only milbemycin available on the market as an endectocide, was introduced in 1989 and commercialised worldwide by the early 1990's (Steel 1993; McKellar & Benchaoui 1996). The milbemycins are highly lipophilic (moxidectin is about 100 times more lipophilic than the avermectins), soluble in organic solvents and insoluble in water, and, after an initial increase in its plasma concentration, it is redistributed throughout the body fat reserves, which act as a reservoir from which it is slowly released (McKellar & Benchaoui 1996).

Various studies (Campbell 1985; Wardhaugh & Rodriguez-Menendez 1988) show that an unusual characteristic of the avermectins is, regardless of the animal or method of administration, most of the dose is excreted largely unaltered in the dung where it retains its insecticidal activity (Campbell & Campbell 1989; Steel 1993; Strong 1993) and this is where the focus of this study lies. The problem is that the avermectins' and milbemycins' mode of action is not confined to parasitic nematodes and arthropods, but also to a very large variety of beneficial invertebrate species which use the dung as a microhabitat and breeding ground and indirectly affect the rate of dung degradation over time (Strong 1993).

Previous Studies

There have been numerous laboratory and field studies done on the effects of avermectins and milbemycin in cattle dung on non-target organisms. I have selected a few studies from around the globe to illustrate the various studies being done on different aspects of dung beetle biology and the effects that the avermectins and milbemycin have on them. I have specifically chosen the countries where some of the highest concentrations of cattle (Table 1) are found ('http://www.fao.org' 2013). Texas (Fincher 1992; Fincher & Wang 1992); Argentina (Suárez *et al.* 2003, 2009; Iglesias *et al.* 2011); Mexico (Cruz Rosales *et al.* 2012); Australia (Ridsdill-Smith 1988; Doherty *et al.* 1994; Dadour 2000; Wardhaugh *et al.* 2001); France (Lumaret *et al.* 2005; Errouissi & Lumaret 2010); South Africa (Krüger & Scholtz 1997, 1998a, 1998b; Kryger *et al.* 2005); Canada (Floate *et al.* 2002; Floate 2006, 2007); the United Kingdom (Wall & Strong 1987; Strong 1993; Strong & Wall 1994; Strong *et al.* 1996; Webb *et al.* 2010); Spain (Wardhaugh & Rodriguez-Menendez 1988; Lumaret *et al.* 1993; Römbke *et al.* 2010); Japan (Iwasa *et al.* 2008) and Denmark (Madsen *et al.* 1990; Sommer *et al.* 1992). Although the methods are different in each country and have changed somewhat over the years, the results have remained more or less consistent.

1. Ivermectin

Ivermectin is the most extensively studied of all the avermectins and the first study that set the scene for interest in the field was Wall and Strong (1987). They conducted an experiment in the UK to investigate the environmental consequences of treating cattle with ivermectin. Four Friesian calves were given ivermectin in a bolus form at a concentration of 40µg/kg/day and four calves were given a placebo as the control group. The recommended dose at the time was one, or repeated subcutaneous injections of 200µg/kg ivermectin. A bolus was chosen as it remains active for many months and releases ivermectin directly into the gut. Eleven days after treatment, fresh dung was collected for a further six days. Artificial pats were made from ivermectin-treated as well as control dung and evenly spaced at 1m intervals in an alternating sequence in a 5m x 10m enclosure in a pasture. The pats and soil samples were then collected after 20, 30, 40, 50, 60, 80 and 100 days and searched for invertebrates. In contrast to the control pats, the experimental pats contained few to no

Coleoptera or Diptera. The results also indicated that there was no visible dung degradation in the ivermectin-treated dung when compared to the controls showing that even at 100 days the experimental pats were largely intact compared to the control pats which had mostly disappeared. This field trial showed that treating with a rumenal bolus, which delivers 40µg/kg ivermectin per day, was enough to disrupt the entire dung-inhabiting insect community (Wall & Strong 1987). Various subsequent studies have simulated or repeated this experiment with variable results.

a. Lethal and sub-lethal effect studies

Lumaret *et al.* (1993) studied the effects of ivermectin residues on dung beetles by running a field trial on a farm in Spain in spring. Dung was collected from a group of six steers treated with a single dose of ivermectin at the recommended dose rate of 200µg/kg body weight ivermectin 2, 4, 7, 10, 17, 24 and 31 days after treatment as well as from a control group of untreated steers. Forty dung pats of 1kg each were deposited for each series at 1m intervals in a line across a 100 x 20m plot. At the same time, four pitfall traps containing 50% ethylene glycol per series were set out and baited with dung from treated steers followed by dung from untreated steers. Two pats and a soil core from each series were collected 1, 3, 6, 9, 16 and 23 days after the start of the experiment and transferred to the lab where the number and species of beetles were recorded. Dung toxicity was assessed by recording the mortality of the dung beetles feeding on the dung and after 29 days, the numbers of larvae and pupae were recorded. No adult mortality was recorded for the duration of the study; however, 100% larval and pupal mortality was observed in dung collected on the day of treatment. No differences in offspring numbers between treated and untreated dung were observed from day six onwards. Interestingly, a delay in development was observed for beetles bred in treated dung. This was most apparent when it was found that most of the offspring in treated dung showed delayed development when compared to the control offspring. Another curious observation came from the pitfall trap data, specifically for those traps baited with dung collected 10 and 17 days after treatment where in both cases, attraction to treated and untreated dung was similar for the first three days, and then a peak of attraction occurred between days 4-6, when the dung was most attractive and still relatively fresh. The interesting part is, from day six onwards, the attraction to the treated dung persisted for 30 days while the untreated dung became unattractive after day

seven. Lumaret *et al.* (1993) proposed that increased attractiveness is a result of biochemical modifications in the dung composition, most likely due to protein degradation released by ivermectin therapy (Lumaret *et al.* 1993).

Krüger and Scholtz (1997) ran a laboratory trial to determine the lethal and sub-lethal effects of ivermectin residues in dung from animals treated with a single standard injection of ivermectin at 200µg/kg. A group of three steers was treated with ivermectin while another three were left untreated as controls. Dung was collected from the steers on 1, 2, 3, 4, 7, 14, 21 and 28 days after treatment. Laboratory-reared *Eoniticellus intermedius* were provided with 250ml of dung twice a week for two weeks and monitored for larval mortality as well as for brood ball numbers. Brood balls were counted, removed and incubated to monitor for emergence. No results regarding larval survival were reported. There was no significant difference between treated and control populations in the number of brood balls formed; however, on average, the number of adults emerging from treated brood balls was significantly lower than in the controls (similar findings in Fincher 1992). Ivermectin caused 100% mortality in offspring from 2-7 days after treatment and significantly fewer emergences from day 14 after treatment when compared to the controls. Prolonged development in treated broods (similar findings Lumaret *et al.* 1993) was also recorded, roughly 2.5 times longer for dung collected 1, 7 and 14 days after treatment and a larval developmental time of 5 weeks compared to the control of 3.5 weeks for dung collected 28 days after treatment (Krüger & Scholtz 1997).

b. Survival and reproduction studies

Ridsdill-Smith (1988) studied the effect of ivermectin on the survival and reproduction of the dung beetle *Onthophagus binodis* in Australia. Half a herd of 600 cattle was given a 200µg/kg dose of ivermectin; the other half was treated with a different anthelmintic. Dung was collected 1, 2, 4, 8 and 11 weeks after treatment. Five pairs of dung beetles, collected from the field, were placed on each of five replicate one-litre pats of treated dung on damp sand in plastic boxes. After eight days, the sand was sieved and the surviving beetles were again placed on one-litre pats of treated dung on damp sand in plastic boxes. After a further eight days the sand was sieved again and the brood balls and surviving beetles were recovered and counted. Survival of the immatures was determined from a

random sample of 100 from all replicates of each dung type. Ridsdill-Smith found that ivermectin had no influence on adult dung beetle survival. Immature survival, however, was zero for week one after treatment but steadily rose to equal that of the other anthelmintic by week eight after treatment. There was no control group to compare treatments to (Ridsdill-Smith 1988).

Fincher (1992) compared the effect of 20µg/kg and 200µg/kg ivermectin on some dung-inhabiting insects, including the introduced African dung beetle *Euoniticellus intermedius* in Texas, USA. Dung was collected from six steers; two treated with 20µg/kg ivermectin, two treated with 200µg/kg ivermectin and two untreated, the day before treatment as well as weekly after treatment for 10 weeks. Two pairs of beetles were placed in containers with soil and dung from each treatment group. After one week the surviving adults and number of brood balls were recorded. The brood balls were transferred to a separate container and monitored for emergence. The results revealed that neither dosage had any significant effect on adult survival, the same results as Ridsdill-Smith (1988) and Wardhaugh and Rodriguez-Menendez (1988), or brood ball production, when compared to the controls; however, emergence of adult *E. intermedius* from brood balls made with dung from cattle that received 200µg/kg ivermectin was reduced, but for no more than two weeks after treatment (Fincher 1992).

Cruz Rosales *et al*. (2012) evaluated the effect of ivermectin on the survival and fecundity of *Euoniticellus intermedius* adults as well as on the survival and development of *E. intermedius* from egg to adult in Mexico. Ivermectin was added to cattle dung (spiked) at three different concentrations (0.01ppm (10µg/kg), 1.0ppm (1 000µg/kg) and 100ppm (100 000µg/kg)) and given to laboratory-raised beetles. The dung was replaced every three days and weight and number of brood masses, fecundity of females, mortality of offspring and developmental time (egg – adult) were measured. They found that at low concentrations (10µg/kg), the ivermectin had no effect on the survival or fertility of the adults or on the survival of the larvae, but did record an increase in the larval development time. At the medium concentration (1 000µg/kg), which is five times the recommended dose, the survival of adults was reduced to almost half and no larvae emerged. At the highest concentration (100 000µg/kg) 100% mortality was observed and no oviposition was performed. They concluded that the prolonging of development time may cause a phase lag in the field

activity cycle which may reduce the number of *E. intermedius* individuals and the efficiency of the environmental services that they provide and that more analyses with higher concentrations between 0.01 and 0.1ppm of ivermectin are needed to establish lethal concentrations for larvae and adults of *E. intermedius* (Cruz Rosales *et al.* 2012).

c. Dung decomposition studies

Wardhaugh and Rodriguez-Menendez (1988) studied the effect of ivermectin on the development and survival of the dung beetles *Copris hispanus, Bubas bubalus* and *Onitis belial* in southern Spain. Eight calves were injected with ivermectin at 200µg/kg. Dung from the treated calves was collected the day before the treatment (control), then 1, 2, 3, 4, 8, 16 and 32 days after treatment. Ten pairs of beetles were housed in one-litre containers and supplied with control dung, and 10 pairs were supplied with dung collected on day three. Each pair was examined on a weekly basis for adult mortality as well as oviposition activity, for 60 days. Parental and immature mortality were then estimated by examining the nests. Similar procedures were followed in a second trial to compare the effects of dung collected 0, 8 and 16 days after treatment. The results showed no adult mortality, reduced egg-laying and reduced juvenile survival as did Ridsdill-Smith (1988). Interestingly, a marked reduction in adult feeding activity was observed in treatments suffering the highest mortalities, namely day 1-8 dung, and the inference was made that mortality was due to the accumulating toxic effects which suppressed feeding (Wardhaugh & Rodriguez-Menendez 1988).

Madsen *et al.* (1990) conducted field as well as laboratory experiments in Denmark to show how treating cattle with ivermectin affects the fauna and decomposition of dung pats. Dung was collected from three heifers on 1, 10, 20 and 30 days after treatment with the recommended dose of 200µg/kg ivermectin as well as from three untreated heifers. Sixty 1kg pats of dung from treated cattle were placed at 2m intervals in an alternating sequence across a field and insect activity was observed for 5-15 min after deposition. Samples were taken within 2-3 months and the insects in the dung pats were identified and counted in the lab. The disappearance rate of the dung pat was estimated as a percentage on five collection occasions 3, 14, 35, 53, and 86 days after being deposited. For the laboratory trial, dung was collected from the same ivermectin-treated heifers at the same time intervals and exposed at the same field site for 7, 45 and 62 days after which they were collected and bioassayed with

Musca autumnalis. In another trial, fresh dung was collected from the ivermectin treated heifers at different intervals (1-33 days) after treatment and tested against *M. domestica* as well as *M. autumnalis*. The results from the field trial showed that ivermectin had an effect on beetle larvae 1-10 days after treatment but that the number of larvae was not affected by ivermectin applied 20-30 days before collection. The decomposition rate was significantly delayed when compared to control dung but also depended on variables such as climate, season, soil type, faunal inhabitants and microclimate. The results from the laboratory bioassays showed a 95-100% mortality rate in *M. domestica* as well as *M. autumnalis* for dung collected one day after treatment. There was no clear reduction in excreted ivermectin placed in the field for 7-62 days and the 62-day assay was obscured by natural mortality. Most of the variance found in this experiment was attributed to seasonal conditions (Madsen *et al.* 1990).

Sommer *et al.* (1992) ran a field trial in Denmark to assess the impact of ivermectin residues on dung fauna and the resulting effect on dung degradation. Twenty-four heifers were divided into three groups of eight. One group was treated with 0.2 mg/kg subcutaneous ivermectin, another group with 0.5 mg/kg pour-on formulation of ivermectin and the third group was left untreated, as the control. Dung was collected from the heifers on 1, 2, 5, 13, 14, 28, 29, 42 and 43 days after treatment. Twelve 1kg pats of dung from each treatment were placed in an ungrazed field and three replicates were sampled after 3, 17, 31 and 45 days of field exposure to check for arthropods. According to the arthropods found in the treated dung, Sommer *et al*. (1992) found no significant difference between the residues found in the pour-on and injectable formulations even though the injectable formulation was 2.5 times the dose of the pour-on formulation; however, dung collected from cattle 1-2 days after treatment with the injectable formulation led to delayed dung degradation for up to 45 days but no effect was observed from dung collected 13-14 days after treatment. Dung collected from cattle 1-2 days after treatment with the pour-on formulation led to delayed dung degradation for up to 13-14 days after treatment which was a similar result to that of Wardhaugh and Rodriguez-Menendez (1988) and Madsen *et al.* (1990).

Iglesias *et al*. (2011) evaluated the local effects of ivermectin on dung fauna and degradation under different meteorological and biological conditions in the same area in Argentina in 2011. The experiment was carried out as a trial on four naturally parasitized

calves. Two were treated with 0.2 mg/kg ivermectin and the other two were left untreated as the control. Dung was collected 3, 7, 14, 21 and 28 days after treatment and the ivermectin concentration was determined chemically by high-pressure liquid chromatography (HPLC), the organic matter percentages were analysed and the organisms in the pats were collected and counted. Meteorological data were simultaneously recorded at weekly intervals for the duration of the trial. Ivermectin concentrations were highest in samples taken three days after treatment and lowest in samples taken 28-60 days after treatment. The results showed fewer arthropods, however not statistically significant, were found in the dung of the calves treated with ivermectin (Iglesias *et al.* 2011).

d. Community structure studies

Krüger and Scholtz (1998a, b) conducted a large-scale field study to determine the ecotoxicological effect of ivermectin on the dung beetle community structure under drought (Krüger & Scholtz 1998a) and high rainfall (Krüger & Scholtz 1998b) conditions. Both studies were carried out in the same summer-rainfall area in South Africa. A breeding herd of 80 cows was split into two and half the herd was treated with a single recommended dose of 200µg/kg ivermectin while the other half remained untreated. Insects were collected directly from dung pats, as opposed to pit-fall traps, to simulate a more natural situation, as well as from artificial 1kg dung pats that were standardized to eliminated as many variables as possible. Collection occurred at monthly intervals for three months. The results showed a large effect on the dung beetle community in the form of significantly lower species richness and evenness as well as increased species dominance in treated dung during drought (Krüger & Scholtz 1998a). During high rainfall, however, fewer beetle and fly larvae were found in the pats after seven days, but no effect of ivermectin was detected after a year (Krüger & Scholtz 1998b). This suggests that these ecotoxicological effects are likely to be more severe in times of drought than under more favourable conditions.

Kryger *et al*. (2005) carried out a long-term, large-scale field study in South Africa to assess the effect of ivermectin on the structure of dung beetle communities. One herd of 25 heifers was treated with ivermectin at 200µg/kg and the other herd of 25 heifers was left untreated. Dung beetle communities were monitored over the entire summer by pitfall trap

sampling. No observable effects of ivermectin on the dung beetle communities was found as the disparities between treated and untreated dung were insignificant and most probably due to differences in microclimate. Species richness and diversity were also unaffected and ecologically similar to the control communities. This study showed that treatment with ivermectin under extensive farming conditions in the South African Highveld can be considered safe with regard to the dung beetle communities under high rainfall (Kryger *et al.* 2005).

Strong *et al*. (1996) carried out a comparative field trial to examine the effects of ivermectin and fenbendazole on dung colonizing Diptera and Coleoptera in the UK. Twelve calves were divided into three groups of four and each group was treated with either ivermectin, fenbendazole or left untreated. Dung was collected 7, 14, 21 and 42 days after treatment and searched for invertebrates. Forty-five days after treatment, 18 pitfall traps containing water and soap were set out and baited with dung from treated calves followed by dung from untreated calves. Although there were no significant differences in adult beetle numbers between the treated and untreated dung, not only was there a significant difference in larval and pupal numbers found between the ivermectin and fenbendazole treated and untreated dung, but the larvae found in the ivermectin treated dung were inhibited in their development. Pitfall trapping showed no significant difference in adult beetle numbers between treated and untreated dung, although a trend toward higher numbers of beetle attractions to the treated dung was noted (Strong *et al.* 1996).

Römbke *et al*. (2010) carried out a field study in Spain to see the effects of ivermectin on the structure and function of dung and soil invertebrate communities. Dung was collected 2, 3, 4 and 7 days after treating cattle with 200µg/kg ivermectin as well as from untreated cattle. Standardised, artificial dung pats were distributed in a field and collected 2, 4, 7, 14 and 28 days later. The dung fauna was sampled and all animals were identified and recorded. They observed a significantly lower abundance of adult dung beetles on the dung from cattle treated with ivermectin compared to the control group. They also noted that although adult dung beetles were attracted to the ivermectin-spiked dung, the rate of degradation was slower than for the control dung (Römbke *et al.* 2010).

e. Dung attractiveness studies

Errouissi and Lumaret (2010) studied the effects that ivermectin have on the attractiveness of dung treated with ivermectin to dung beetles. They performed a two-year study in France to assess the field effects of ivermectin residues on the attractiveness of dung to dung-colonizing insects. They used pitfall traps baited with dung from cattle treated with a slow-release bolus of ivermectin as well as dung from untreated cattle. The pitfall traps were placed out at one-week intervals for a total of five weeks. They found that the ivermectin-contaminated dung showed a significant attractive effect which highlighted the danger of wide-spread ivermectin use as this potentially puts the dung beetles' offspring, and indirectly, future beetle generations survival at risk (Errouissi & Lumaret 2010).

2. Eprinomectin

Lumaret *et al*. (2005) examined the larvicidal activity of eprinomectin residues on the dung-inhabiting fly *Neomyia cornicina* in France. Five heifers were treated with a topical pour-on eprinomectin solution at the recommended dose of 500µg/kg and five remained untreated. Faecal samples were collected from the heifers before treatment, on the day of treatment and daily for seven days, as well as 9, 12, 15, 20, 25, 29 and 41 days after treatment. Eprinomectin concentrations were measured using HPLC. Bioassays were performed by depositing *N. cornicina* eggs in containers filled with 250g of dung and sand. The eggs were left to incubate in the laboratory and left until the emergence of adult flies. Eprinomectin concentration in the dung was highest on day three after treatment and slowly dropped to almost undetectable on day 29. Eprinomectin residues in dung had a significant effect on *N. cornicina* as no emergences were observed on the dung from days 1 – 11 but after day 12 the first flies emerged. No significant differences were observed from day 20 – 41 post treatment (Lumaret *et al.* 2005).

3. *Doramectin*

There are very few publications on the toxicity of doramectin against non-target dung-inhabiting organisms and they are comparisons with ivermectin (Dadour 2000; Suárez *et al.* 2003; Webb *et al.* 2010) and moxidectin (Suárez *et al.* 2009) which is discussed in section five. I could not find any studies done on doramectin alone.

4. *Moxidectin*

Fincher and Wang (1992) tested the effects of moxidectin on two introduced African species of dung beetle namely, *Euoniticellus intermedius* and *Onthopagus gazella*. In Texas, Holstein steers were injected with moxidectin at the recommended dose of 200µg/kg and dung was collected 0, 1, 2, 3, 7, 10, 14, 21, 28, 35 and 42 days after treatment. Two pairs of each dung beetle species were placed in 40*l* buckets filled with moist soil and a 400g dung pat from each steer. Fresh dung was added after a week and the parents were removed. The buckets were monitored for progeny and sex and numbers were recorded. After five weeks the buckets were emptied and the number of complete brood balls was recorded to calculate percentage emergences. There were no significant differences between the mean number of brood balls produced by either species or on the emergence of progeny between treated and untreated dung. There also seemed to be no effect on the sex ratio for either species. They concluded by stating that moxidectin seemed to be a compound which is compatible with beneficial dung-burying beetles when used at the recommended dose (Fincher & Wang 1992).

Iwasa *et al*. (2008) examined the effects of moxidectin on non-target coprophilous insects in cattle dung in field as well as laboratory trials in Japan. Dung from cattle treated with the recommended dose of 500µg/kg moxidectin was collected 1, 3, 7, 14, 21, 28 and 35 days after treatment and was placed in a container with three mating pairs of the dung beetle *Caccobius jessoensis*. The same was done for dung from untreated cattle. Brood balls were collected, counted, weighed and incubated to record adult emergences. Artificial dung pats, made from dung collected from cattle treated with 500µg/kg moxidectin as well as untreated dung, were placed in a field and collected after a month, from which brood balls were

collected and incubated to record adult emergences. Moxidectin concentration was determined using HPLC and the results showed that concentrations were at maximum levels three days after treatment, showed a marked decline by day seven and none was detectable by day 21. No significant differences were found between the control and the treated cattle dung with regards to numbers and weight of brood balls as well as emergence rates. Results of the field study, again, showed no significant differences between the control and the treated cattle dung. They concluded that moxidectin has no, or at most, the least effect compared to other avermectins on non-target coprophagous insects (Iwasa *et al.* 2008).

5. Comparative studies: comparisons of two products

The following are comparative studies between ivermectin and doramectin (Dadour 2000; Suárez *et al.* 2003; Webb *et al.* 2010); ivermectin and moxidectin (Doherty *et al.* 1994; Strong & Wall 1994); moxidectin and doramectin (Suárez *et al.* 2009); and moxidectin and eprinomectin (Wardhaugh *et al.* 2001).

Dadour (2000) examined the impact that abamectin and doramectin have on the survival and reproduction of the dung beetle *Onthophagus binodis*. This study was done in Australia and abamectin, rather than ivermectin, was chosen because it was the first avermectin sold commercially for the treatment of endoparasites in Australia. Dung was collected 1, 3, 6, 9, 18, 24, 34 and 42 days after 150 heifers were divided into 3 groups and treated with either 200µg/kg doramectin, 200µg/kg abamectin or nothing to serve as the control group. Five pairs of *O. binodis* were given fresh dung once a week from each treatment and monitored for 4 weeks for adult survival and brood ball numbers. The brood balls were then incubated and monitored daily for emergence. Doramectin concentrations were determined using HPLC. Significant adult mortality was observed from abamectin-treated dung 3-6 days after treatment and in doramectin-treated dung at nine days after treatment. This study confirmed an interesting response in dung beetles. Whereas abamectin residues had no effect on adult mortality in sexually mature beetles but rather sexually immature (newly emerged) beetles, which go through a period of intense feeding during which they are exposed to maximum abamectin residues, were found to be much more affected by the residues. In contrast to other studies (Fincher 1992; Krüger & Scholtz 1997)

brood ball production was also significantly lower in beetles fed on dung from cattle treated with abamectin for up to 42 days after treatment. Brood ball production was also significantly lower in beetles fed on dung from cattle treated with doramectin, but only for 3-6 days after treatment. The enhanced brood mass in beetles fed on dung from doramectin-treated cattle at 24-34 days after treatment could not be explained. According to the HPLC results, doramectin reached maximum concentration on day three after treatment following a linear decline with an elimination half-life of 15 days (Dadour 2000).

Suarez *et al.* (2003) compared the effects of ivermectin and doramectin on the invertebrate colonisation of cattle dung in Argentina. Dung was collected from two groups of steers treated with either the recommended dose of 200µg/kg doramectin or 200µg/kg ivermectin and from a third untreated group 3, 7, 16, and 29 days after treatment. Fifty artificial pats were formed and placed in three rows in a 2m alternating sequence and collected after 7, 14, 21, 42, 100 and 180 days for analysis in the laboratory. Ivermectin and doramectin residue concentrations were also determined using HPLC. No significant differences were found in the numbers of adult beetles, regardless of the treatment. Faecal residue concentrations for both ivermectin and doramectin were highest in the first few days and remained relatively high throughout the experimental period. Doramectin concentrations were higher than ivermectin concentrations as the results show that after 180 days of exposure to environmental conditions, dung collected 27 days after ivermectin treatment still showed 56% residue compared to dung collected from doramectin treatment which showed 75% residue (Suárez *et al.* 2003).

Webb *et al*. (2010) assessed the abundance and dispersal of dung beetles in response to ivermectin and doramectin treatment on pastured cattle in Scotland by running a two-year field trial. Three groups of cattle were treated with the recommended dosage of 500µg/kg ivermectin, 500µg/kg doramectin and the last group was left untreated. Dung was collected from cattle five days prior to, and two days after treatment. Eight dung-baited pitfall traps were set in two grids of four in each field, with traps spaced approximately 8m apart within each grid. Traps were emptied and re-baited every 7–10 days in the first year and every 14 days in the second year. On each sampling date, two traps from each of the two grids in each study field were selected, and the individuals of each *Aphodius* species were counted and pooled across those four traps. In the field-scale study, significantly more beetles were

trapped in fields grazed by cattle treated with an avermectin than in fields where cattle remained untreated. The colonising trials, however, indicated that *Aphodius* beetles preferred colonising dung from untreated cattle rather than dung from cattle treated with doramectin and that *Aphodius* dung beetles can discriminate between dung from untreated cattle and dung from cattle treated with doramectin at a spatial scale of at least 70 m (Webb *et al.* 2010).

Doherty *et al.* (1994) compared the larvicidal activities of different concentrations of moxidectin and abamectin on *Onthophagus gazella* to assess the level of threat they pose to dung fauna and consequently, dung degradation, in Australia. Dung pats were made from dung spiked with 4, 8, 16, 32, 64, 128, 256 and 512µg/kg of either moxidectin or abamectin and fed to pairs of lab-reared *O. gazella*. The beetles were removed 18 days later, leaving only the brood balls for another 35 days. Brood balls, larvae, pupae and adults were then counted. Although oviposition was not affected by either treatment, larval survival was affected by all concentrations of abamectin and by all concentrations of moxidectin over 128µg/kg. In fact, moxidectin at 256 and 512µg/kg produced survival comparable to 4 and 8µg/kg abamectin (Doherty *et al.* 1994).

Strong and Wall (1994) compared the relative effects of ivermectin and moxidectin on the colonisation of dung by dung-inhabiting insects in England. Dung was collected from cattle 2, 7, 14 and 21 days after being treated with either ivermectin 200µg/kg or moxidectin 200µg/kg as well as from untreated cattle. Ninety-six artificial dung pats were randomly placed at 1m intervals in a 12 x 8m grid, collected again 7, 14, 21 and 28 days after deposition, and analysed for invertebrates. There was no significant difference between the three treatments in adult Scarabaeidae numbers showing that neither ivermectin nor moxidectin residues repel colonizing adult beetles. However, dung collected from ivermectin-treated cattle up to seven days after treatment, showed high larval mortality which moxidectin and the control did not (Strong & Wall 1994).

Suarez *et al*. (2009) showed the effects of moxidectin and doramectin faecal residues on the activity of dung colonizing insects by depositing dung from cattle treated with moxidectin, dung from cattle treated with doramectin and control dung from untreated cattle on a field. Comparisons of dung degradation were inconclusive; however, total numbers of

insects recovered from control pats were significantly higher than in treated pats. Furthermore, a lower adverse effect was observed for moxidectin compared to doramectin with no significant degradation of moxidectin or doramectin being observed (Suárez *et al.* 2009).

Wardhaugh *et al*. (2001) compared eprinomectin to moxidectin by examining the survival and development of *Onthophagus taurus* when fed on dung from treated cattle in Australia. Dung was collected from three groups of six heifers 3, 7, 14, 21, 42 and 70 days after being treated with either eprinomectin or moxidectin as well as from an untreated group. *O. taurus* were paired up and fed treated and untreated dung for 10 days, after which brood balls were counted, stored and checked weekly until emergences were complete. The newly emerged adults were fed on the same treated dung for seven days and then transferred to a container where they were fed untreated dung. Numbers of live and dead beetles were recorded after a further 10 days. The results showed that moxidectin had no effect on the survival or development on the beetles but the opposite was found to be true for eprinomectin. High juvenile mortality and suppressed brood ball production, among those that survived, were recorded. They concluded by designing a model that simulated the effects of eprinomectin residues and suggested that a single treatment of eprinomectin is capable of reducing the next generation by 25-35% (Wardhaugh *et al.* 2001).

6. Comparative studies: comparisons of all four products

I could only find the results of two laboratory studies which show comparative results between ivermectin, moxidectin, doramectin and eprinomectin but neither of them are under the same laboratory conditions (Floate *et al.* 2002; Floate 2007). Floate (2006) also wrote a review about the global environmental effects of faecal residues left by treatment of cattle with ivermectin, doramectin, moxidectin and eprinomectin on non-target dung-inhabiting species.

Floate *et al*. (2002) compared the effects of ivermectin, doramectin, eprinomectin and moxidectin on the natural assemblage of insects developing in dung as well as the effects on the dung degradation in Canada. Pour-on formulations of ivermectin, doramectin, eprinomectin and moxidectin were applied to four groups of heifers at the recommended

dose of 500µg/kg and dung was collected 1, 2, 4 and 6 weeks after treatment. Artificial dung pats were then randomly deposited in a block design in a pasture adjacent to grazing cattle and collected again after eight days to analyse insect populations. To monitor dung beetle activity, dung-baited pitfall traps were placed in the centre and at either end of the study site. Based on the number of species affected and duration of suppression, the results showed that, in descending order of adverse effect, doramectin > ivermectin > eprinomectin >> moxidectin. Treatment of cattle with doramectin, ivermectin, eprinomectin or moxidectin reduced levels of insect activity in the dung but moxidectin was the least likely to affect the natural insect assemblage associated with cattle dung (Floate *et al.* 2002).

Floate (2006) wrote a review on the environmental effects of endectocide use in cattle in Canada. He raised concerns that the use of endectocides may reduce the insect diversity and lead to the accumulation of undegraded dung on pastures, as reduced insect activity can lead to reduced dung pat degradation. He validated this statement by saying that ivermectin, doramectin, moxidectin and eprinomectin residues reduce insect activity in dung from treated animals for weeks to months after application. In terms of toxicity, the overall comparison of the avermectins and milbemycin, he suggested that, on the basis of number of species affected and duration of suppression, doramectin > ivermectin > eprinomectin >> moxidectin in descending order of adverse effect, which is what he reported experimentally in 2002 (Floate 2006).

Floate (2007) compared the field effects of ivermectin, doramectin, eprinomectin and moxidectin residues on the attractiveness of dung to dung colonizing insects over 3 years in Canada. Pitfall traps were set in spring and autumn and re-baited weekly for a month in each season. Insect captures were compared between pitfall traps baited with dung from untreated cattle and dung from cattle treated with doramectin, eprinomectin, moxidectin or ivermectin at the recommended dose of 500µg/kg. Two-fold and up to six-fold differences in captures between control and treated dung were observed. More specifically, doramectin showed 11 of 29 cases of attraction and 11 of 29 cases of repellence, eprinomectin tended to repel insects with 19 of 29 cases of repellence, while ivermectin (17 of 25 cases) and moxidectin (17 of 18 cases) showed a strong attractive effect. He concluded that emergence of offspring from field-colonized dung should not be used as a measure of residue toxicity, standardised laboratory test should still be the preferred method, but rather as a measure of 'insect

activity' which is a composite measure of residual toxicity, the number and species composition and the mortality factors such as predation, competition and parasitism (Floate 2007).

Methodologies of administration

The avermectins are currently the only compounds which effectively control the important ecto- and endoparasites of cattle simultaneously (Vercruysse & Rew 2002). There are a variety of ways to administer avermectins to cattle namely; subcutaneously, in the form of an intramuscular injection and topically in the form of a pour-on. Each has its own pros and cons, so what is the best way to treat for parasites?

a. Pour-on

Gayrard *et al*. (1999) compared the plasma concentration profiles of ivermectin and doramectin pour-on formulations in France. A group of 12 cattle were treated with a single dose of ivermectin at the recommended dose of 500µg/kg and another group of 12 cattle were treated with a single dose of doramectin pour-on at the recommended dose of 500µg/kg. Blood samples were taken on the day of dosing (day 0) as well as 1, 2, 4, 8, 12, 24, 36, 48 and 72 hours after dosing as well as 4, 5, 7, 10, 15, 20, 25, 30, 40, and 50 days post-treatment. The samples were analysed for ivermectin and doramectin using HPLC. There was no significant difference between the observed maximum concentration (12.2 ± 6.0ng/ml; 12.2 ± 4.8ng/ml) and the time to maximum concentration (3.4 ± 0.8 days; 4.3 ± 1.6 days) for ivermectin and doramectin respectively. However, doramectin led to a 45% higher overall exposure compared to ivermectin and the relative bio-availability (rate of absorption) was lower for doramectin than for ivermectin which resulted in the longer persistence of doramectin in the plasma (Gayrard *et al.* 1997).

Lumaret *et al*. (2005) determined the plasma and faecal concentrations of pour-on eprinomectin in cattle following treatment at the recommended dose of 500µg/kg by using

HPLC. The maximum plasma concentrations were recorded at two days after treatment and the maximum faecal concentrations were recorded at three days after treatment. Eprinomectin remained detectable in the plasma as well as the faeces until 29 days after treatment (Lumaret *et al.* 2005).

b. Injection

Lo *et al.* (1985) reported the effects of formulation on the efficacy of ivermectin. Based on the solubility properties of ivermectin, they proposed that the formulation affects its distribution after subcutaneous injection by reporting that it was absorbed three times more slowly when administered in a propylene glycol-glycerol solvent when compared to an aqueous-based solvent (Lo *et al.* 1985). Hayes *et al*. (1994) found that the rate of absorption of moxidectin into the bloodstream increased dramatically when administered in an aqueous injectable-based formulation when compared to an oil-based formulation (Hayes *et al.* 1994).

Lumaret *et al.* (1993) measured ivermectin concentrations in dung from cattle treated with a single dose of injectable ivermectin at the recommended dose rate of 200µg/kg by using HPLC. Chemical analyses of the ivermectin concentration in fresh dung indicated that the concentration of ivermectin increased daily on days 1-4 after treatment, reaching a peak of elimination on day five followed by a rapid decrease until day 12 where after the concentration was under the detection limit (Lumaret *et al.* 1993).

c. Comparisons

One would expect that the injectable formulations would be more effective than the pour-on formulation but this is not always the case.

Miller *et al*. (1981) investigated the effectiveness of ivermectin (then called MK-933) against pest flies (horn flies, stable flies, face flies and house flies) administered in

different ways namely; oral capsules which deliver doses between 1 and 200µg/kg ivermectin per day, daily subcutaneous injections of 5 or 10µg/kg ivermectin per day, a single injection of 200µg/kg ivermectin, rumenal boluses or tablets which deliver 50µg/kg ivermectin per day. They found that ivermectin is effective at low doses. Daily oral doses of less than 1µg/kg ivermectin per day killed all horn flies and 5µg/kg ivermectin per day killed all face flies, 60% of stable flies and 90% of house flies. Oral doses of 20µg/kg ivermectin per day were effective in killing 90% of stable flies. Daily 5µg/kg ivermectin per day subcutaneous injections were enough to completely inhibit fly development in the dung and a single subcutaneous injection of 200µg/kg ivermectin controlled horn fly populations for up to four weeks (Miller *et al.* 1981).

In the Denmark field trial by Sommer *et al.* (1992) the concentration of subcutaneous ivermectin was compared to the pour-on formulation of ivermectin using high-pressure liquid chromatography. Although there was no significant difference between the residue concentrations of the pour-on and injectable formulations, even though the injectable formulation was 2.5 times the dose of the pour-on formulation, the injectable formulation led to a longer period of delayed dung degradation than the pour-on formulation (Sommer *et al.* 1992).

Herd *et al*. (1996) examined the persistence of ivermectin in faeces by comparing the faecal residues following different modes of administration namely, sustained-release bolus, pour-on and injectable formulations in Ohio, USA. They emphasise the importance of formulation and route of administration in drug concentration determination, persistence and ecotoxic potential. A group of 16 cattle was divided into four groups and given the following treatments; Group 1 animals were given a slow-release bolus containing 1.72g of ivermectin which was designed to release 12.7mg/day for 135 days; Those in Group 2 were treated with an ivermectin pour-on formulation at the recommended dose of 0.5mg/kg (500µg/kg); Group 3 cattle were injected subcutaneously with ivermectin at the recommended dose of 0.2mg/kg (200µg/kg); and animals in Group 4 were left untreated as the control group. Determination of ivermectin plasma and faecal residues was done by HPLC. Maximum plasma concentrations in Group 2 (pour-on) and Group 3 (injection) were significantly higher than that in Group 1 (SR bolus) and the time to attainment of maximum plasma concentration in Group 1 was significantly greater than in Group 2 and Group 3. Maximum

faecal concentrations were highest in Group 2 followed by Group 1 and Group 3. The time to attainment of maximum faecal concentration was significantly shorter in Group 2 followed by Group 3. The time to attainment of maximum faecal concentration in Group 1 was significantly longer. All faecal concentrations recorded, regardless of mode of administration, were well above concentrations which are lethal or even sub-lethal to beneficial dung-breeding invertebrates. They concluded by stating that the SR bolus and pour-on formulations are likely to be more ecotoxic to non-target organisms than the injectable formulation judging from their higher faecal concentrations and that the SR bolus formulation is of particular concern due to the persistent excretion of toxic concentrations for prolonged periods of time (Herd *et al.* 1996).

Lonneux *et al*. (1997) compared the efficacy of injectable and pour-on formulations of ivermectin and moxidectin in naturally infected cattle under similar conditions on a farm in Belgium. Sixty-five cattle were divided into five groups and given the following treatments; Those in Group 1 remained untreated as the controls; Group 2 animals were treated with an ivermectin pour-on at the recommended dose of 500µg/kg; Group 3 cattle were treated with a 0.5% pour-on formulation of moxidectin; Those in Group 4 received a subcutaneous ivermectin injection at the recommended dose of 0.2mg/kg (200µg/kg); and the animals in Group 5 received a subcutaneous moxidectin injection at the recommended dose of 0.2mg/kg (200µg/kg). Halfway through the experiment there were still infected cattle in the ivermectin and moxidectin pour-on groups as well as in the injectable moxidectin group, whereas all the cattle from the injectable ivermectin group were free of infection. By the end of the experiment, all treated cattle were free of infection (Lonneux *et al.* 1997).

It is a common misconception that helminth populations can increase within the host alone. In fact, there are numerous variables which contribute to its lifestyle e.g. climate and the hosts' surroundings (living conditions) and management, not to mention that the lifecycle of the helminth is completed outside of the host (Vercruysse & Rew 2002). This ultimately means that not only cattle condition but good farming practices are equally important to ensure happier, healthier, pest free animals, as prevention is always better than cure.

Resistance

Introduction

Sangster and Dobson (2002) define resistance as the helminths' ability to survive doses of drug that would normally kill them. Pest control by means of chemicals is the main reason for resistance as resistant worms carry the resistant alleles which they pass onto the next generation (Sangster 1999; Sangster & Dobson 2002; Wolstenholme *et al.* 2004). Essentially, the better the drugs work, the more likely resistance is to develop as, over time, treatment leads to the survival and reproduction of resistant pests and the dilution of susceptible genes (Sangster 1999).

The lifestyle of grazing animals burdened by intensive farming practices puts them at an extremely high risk of exposure to helminth infections at pasture. Any future intensification of pasture-based systems will increase the risk of helminth-borne diseases even further, which represents substantial economic and welfare consequences to the global livestock industry (McKellar & Jackson 2004; Morgan *et al.* 2013).

The livestock industry is the largest agricultural sector in South Africa contributing around 49% of the total agricultural output in this country. Almost 80% of the agricultural land in South Africa is suitable for extensive livestock farming. South Africa produces roughly 85% of its red meat requirements, while the remaining 15% is imported from Namibia, Botswana, Swaziland, Australia, New Zealand and Europe ('http://www.info.gov.za' 2013, 'http://www.nda.agric.za' 2013)

Cattle are found throughout the country and feedlots account for approximately 75% of all beef produced in the country. The total number of cattle in South Africa at the end of May 2013 was estimated at R13 915 million. These comprised various beef cattle breeds, approximately 80% of the total number of cattle in the country, as well as dairy cattle, which make up the rest. Eastern Cape Province with 3,305 million, 2,776 million in KwaZulu-Natal Province, 2,308 million in the Free State Province and the remaining provinces share 5,526 million of the total cattle population in the country ('http://www.nda.agric.za' 2013).

The red meat industry is one of the fastest growing industries in the South African agricultural sector, contributing approximately 15.9% to the gross value of agricultural production in South Africa during 2011/12 ('http://www.nda.agric.za' 2013).

The South African dairy industry is economically important with over 4 000 milk producers employing 60 000 farm workers and providing 40 000 people with indirect jobs within the value chain such as milk processing. Milk production is the sixth largest agricultural industry in the country. The gross value of milk produced in South Africa during 2011 is estimated at R9 224 million ('http://www.nda.agric.za' 2013).

Between the first quarter of 2012 and the first quarter of 2013, the gross production value of beef increased slightly by 1% from nearly R4.2 billion to R4.2 billion. Between the first quarter of 2012 and the first quarter of 2013, the gross production value of milk increased by 6%, from R 2.6 billion to R2.7 billion. Gross farming income from all agricultural products for the year ended 30 June 2013 is estimated at R178 050 million, of which income from animal products amounted to R83 637 million ('http://www.nda.agric.za' 2013).

The total gross value of animal products for 2012/2013 was estimated at R83 687 million which is roughly 46.4% of the total gross agricultural production (total production during the production season valued at the average basic prices received by producers), which was estimated at R180 360 million ('http://www.nda.agric.za' 2013).

Expenditure on intermediate goods and services in the agricultural sector during 2012/13 is estimated at R100 047 million, which represents a rise of 11.6% from R89 632 million in 2011/12. Large increases occurred in expenditure on seed and plants (18.0%), fuel (15.3%), packing material (14.8%) and dips and sprays (13.6%) ('http://www.nda.agric.za' 2013).

The contribution of agriculture to value added for the year ended 31 December 2012 is estimated at R72 731 million which represents 2.6% of the total value added to the economy ('http://www.nda.agric.za' 2013).

Replacing cattle farming with game ranching is becoming more common and proving to be an increasingly significant factor in the farming sector, in general, in South Africa (van der Waal & Dekker 2000; Cloete *et al.* 2007). South Africa has larger variety and a bigger number of game species than most countries. The practice of game farming has grown into a viable industry with great economic potential. Cloete *et al.* (2007) assessed the financial implications of switching from cattle to game farming and their results showed that game ranching can be more profitable per hectare than cattle (Cloete *et al.* 2007).

So, if the demand is so great and South Africa has all the resources, what is the problem? A literature search for studies done on game farming and pest management revealed a huge research gap. When humans interfere with an ecosystem the obvious results are stress due to overcrowding which leads to a decrease in productivity and an increased chance of contracting disease which could result in death (Malan *et al.* 1997). Mixed farming increases the chances of cross-infection of parasites between cattle and game as they compete for grazing and the chances of interactions increase (Madzingira *et al.* 2002). It goes without saying that intensive high-value game farming also leads to high parasite loads and the use of anti-parasitics with the cycle of resistance repeating itself, but further studies need to be done in this regard.

Records of resistance

In a review (McKellar & Jackson 2004), a record of first resistance of the most effective anthelmintic compounds with the lowest toxicity from 1940 to the present (Kaplan 2004; McKellar & Jackson 2004) is presented and when reviewed with regard to date of introduction and date of first instance of resistance, an startling picture starts emerging. With the exception of a few, the resistance free period is around a decade (Table 2). The prevalence of resistance is on the rise with records of resistance to virtually every group of pesticide known to man (Malan *et al*. 1997).

The way forward

Most recently, Wall and Beynon (2012) wrote a review on the impact of macrocyclic lactone parasiticides. They reported that macrocyclic lactone residues from parasiticide treatments may play an important role in the loss of coprophilous insects which may, in turn, delay pat decomposition. They added that field studies show contradicting results which reflect confounding factors such as weather conditions, pat moisture content, pat location, time of year, dung insect species phenologies, timing and method of application which play an important role in whether the results seen in experimental and laboratory data have any impact on the economically important process of dung decomposition. The timely removal of dung from pastures by insects and weathering is both functionally and economically important and if appropriate decomposition does not occur, cattle farmers may suffer considerable economic losses as a result of pasture fouling, increases in dung breeding pest fly populations and a higher transmission of livestock endoparasites. The benefits of rapid dung removal are therefore rather substantial; not only does it reduce such losses, but it helps to return nutrients to the soil, particularly nitrogen, a large proportion of which would otherwise be lost as ammonia (Wall & Beynon 2012).

As the genetic basis for parasite resistance is not well understood (Wolstenholme 2012), resistance management by means of scientific collaboration, improved grazing management and monitoring as well as the standardisation of resistance diagnosis techniques (Sangster 1999; Sangster & Dobson 2002; Wolstenholme *et al.* 2004) is essential to develop effective strategies to minimise the impact of anthelmintic resistance as there is currently no effective alternative to the chemical control of intensively grazed livestock pests (Wolstenholme *et al.* 2004). Although grazing management techniques such as prevention, evasion and dilution offer a fast and easy solution to improved livestock pest control, there is no fool-proof technique that currently allows full elimination of anthelmintics (Barger 1997). It is important to note that no method should be considered as a single or unsustainable process but rather combinations of different methods to control pests, the more choices one has, the better (Barger 1997; Sangster & Dobson 2002).

Although it is difficult to recommend a control programme that will suit all forms and styles of livestock farming, a standardised procedure for the testing of anti-parasitics

needs to be produced in order to accurately compare the toxicity of various products. And, of course, the best scenario would be to farm holistically without the need for any pesticides.

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Figures and Tables

Table 1. Total cattle numbers * Downloaded from FAOSTAT

Domain	Country	Item	Element	Year	Value (head)
Live Animals	Brazil	Cattle	Stocks	2011	212815311
Live Animals	India	Cattle	Stocks	2011	210824000
Live Animals	United States of America	Cattle	Stocks	2011	92682400
Live Animals	China, mainland	Cattle	Stocks	2011	82886000
Live Animals	Ethiopia	Cattle	Stocks	2011	53382194
Live Animals	Argentina	Cattle	Stocks	2011	48000000
Live Animals	Pakistan	Cattle	Stocks	2011	35568000
Live Animals	Mexico	Cattle	Stocks	2011	32936334
Live Animals	Sudan (former)	Cattle	Stocks	2011	29618000
Live Animals	Australia	Cattle	Stocks	2011	28506169
Live Animals	Colombia	Cattle	Stocks	2011	25156068
Live Animals	Bangladesh	Cattle	Stocks	2011	23121000
Live Animals	United Republic of Tanzania	Cattle	Stocks	2011	21300000
Live Animals	Russian Federation	Cattle	Stocks	2011	19967863
Live Animals	France	Cattle	Stocks	2011	19085561
Live Animals	Nigeria	Cattle	Stocks	2011	18871399
Live Animals	Kenya	Cattle	Stocks	2011	18173500
Live Animals	Venezuela (Bolivarian Republic of)	Cattle	Stocks	2011	17350000
Live Animals	Indonesia	Cattle	Stocks	2011	14824000
Live Animals	Myanmar	Cattle	Stocks	2011	14088043
Live Animals	South Africa	Cattle	Stocks	2011	13688328
Live Animals	Germany	Cattle	Stocks	2011	12562600
Live Animals	Paraguay	Cattle	Stocks	2011	12437120
Live Animals	Canada	Cattle	Stocks	2011	12155000
Live Animals	Uruguay	Cattle	Stocks	2011	11808000
Live Animals	Turkey	Cattle	Stocks	2011	11369800
Live Animals	New Zealand	Cattle	Stocks	2011	10020917
Live Animals	Madagascar	Cattle	Stocks	2011	10000000
Live Animals	United Kingdom	Cattle	Stocks	2011	9933000
Live Animals	Niger	Cattle	Stocks	2011	9552611
Live Animals	Mali	Cattle	Stocks	2011	9438182
Live Animals	Uzbekistan	Cattle	Stocks	2011	9093700
Live Animals	Iran (Islamic Republic of)	Cattle	Stocks	2011	8600000
Live Animals	Burkina Faso	Cattle	Stocks	2011	8566448
Live Animals	Bolivia (Plurinational State of)	Cattle	Stocks	2011	8400439
Live Animals	Uganda	Cattle	Stocks	2011	8103300
Live Animals	Chad	Cattle	Stocks	2011	7650000
Live Animals	Nepal	Cattle	Stocks	2011	7226050
Live Animals	Thailand	Cattle	Stocks	2011	6680000

Domain	Country	Item	Element	Year	Value (head)
Live Animals	Ireland	Cattle	Stocks	2011	6493000
Live Animals	Kazakhstan	Cattle	Stocks	2011	6175300
Live Animals	Spain	Cattle	Stocks	2011	5923200
Live Animals	Italy	Cattle	Stocks	2011	5832457
Live Animals	Poland	Cattle	Stocks	2011	5761878
Live Animals	Peru	Cattle	Stocks	2011	5689173
Live Animals	Afghanistan	Cattle	Stocks	2011	5524000
Live Animals	Viet Nam	Cattle	Stocks	2011	5436600
Live Animals	Ecuador	Cattle	Stocks	2011	5358904
Live Animals	Zimbabwe	Cattle	Stocks	2011	5060000
Live Animals	Somalia	Cattle	Stocks	2011	4850000
Live Animals	Cameroon	Cattle	Stocks	2011	4845000
Live Animals	Egypt	Cattle	Stocks	2011	4803000
Live Animals	Guinea	Cattle	Stocks	2011	4672000
Live Animals	Angola	Cattle	Stocks	2011	4586570
Live Animals	Ukraine	Cattle	Stocks	2011	4494400
Live Animals	Japan	Cattle	Stocks	2011	4230000
Live Animals	Central African Republic	Cattle	Stocks	2011	4182000
Live Animals	Belarus	Cattle	Stocks	2011	4151300
Live Animals	Cuba	Cattle	Stocks	2011	4059100
Live Animals	Netherlands	Cattle	Stocks	2011	3885350
Live Animals	Chile	Cattle	Stocks	2011	3758547
Live Animals	Nicaragua	Cattle	Stocks	2011	3750000
Live Animals	Cambodia	Cattle	Stocks	2011	3406972
Live Animals	Guatemala	Cattle	Stocks	2011	3388220
Live Animals	Republic of Korea	Cattle	Stocks	2011	3353353
Live Animals	Senegal	Cattle	Stocks	2011	3345540
Live Animals	Morocco	Cattle	Stocks	2011	3037930
Live Animals	Zambia	Cattle	Stocks	2011	3000000
Live Animals	Dominican Republic	Cattle	Stocks	2011	2950000
Live Animals	Botswana	Cattle	Stocks	2011	2750000
Live Animals	Honduras	Cattle	Stocks	2011	2650000
Live Animals	Belgium	Cattle	Stocks	2011	2534950
Live Animals	Philippines	Cattle	Stocks	2011	2518400
Live Animals	Azerbaijan	Cattle	Stocks	2011	2412305
Live Animals	Namibia	Cattle	Stocks	2011	2350000
Live Animals	Mongolia	Cattle	Stocks	2011	2339700
Live Animals	Turkmenistan	Cattle	Stocks	2011	2200000
Live Animals	Eritrea	Cattle	Stocks	2011	2065000
Live Animals	Benin	Cattle	Stocks	2011	2058000
Live Animals	Austria	Cattle	Stocks	2011	2013281
Live Animals	Romania	Cattle	Stocks	2011	2001105
Live Animals	Tajikistan	Cattle	Stocks	2011	1896894
Live Animals	Algeria	Cattle	Stocks	2011	1790140
Live Animals	Panama	Cattle	Stocks	2011	1728748
Live Animals	Mauritania	Cattle	Stocks	2011	1700000

Domain	Country	Item	Element	Year	Value (head)
Live Animals	Yemen	Cattle	Stocks	2011	1654000
Live Animals	Iraq	Cattle	Stocks	2011	1600000
Live Animals	Côte d'Ivoire	Cattle	Stocks	2011	1582652
Live Animals	Switzerland	Cattle	Stocks	2011	1577407
Live Animals	Denmark	Cattle	Stocks	2011	1567971
Live Animals	Lao People's Democratic Republic	Cattle	Stocks	2011	1538000
Live Animals	Sweden	Cattle	Stocks	2011	1511846
Live Animals	Portugal	Cattle	Stocks	2011	1503000
Live Animals	Ghana	Cattle	Stocks	2011	1498000
Live Animals	Haiti	Cattle	Stocks	2011	1455000
Live Animals	Costa Rica	Cattle	Stocks	2011	1380000
Live Animals	Czech Republic	Cattle	Stocks	2011	1343686
Live Animals	Kyrgyzstan	Cattle	Stocks	2011	1338583
Live Animals	Mozambique	Cattle	Stocks	2011	1280000
Live Animals	Sri Lanka	Cattle	Stocks	2011	1191850
Live Animals	Syrian Arab Republic	Cattle	Stocks	2011	1158000
Live Animals	Rwanda	Cattle	Stocks	2011	1143231
Live Animals	Malawi	Cattle	Stocks	2011	1110560
Live Animals	Georgia	Cattle	Stocks	2011	1049400
Live Animals	El Salvador	Cattle	Stocks	2011	1015140
Live Animals	Serbia	Cattle	Stocks	2011	936570
Live Animals	Malaysia	Cattle	Stocks	2011	925000
Live Animals	Finland	Cattle	Stocks	2011	914053
Live Animals	Norway	Cattle	Stocks	2011	864139
Live Animals	Democratic Republic of the Congo	Cattle	Stocks	2011	748000
Live Animals	Lithuania	Cattle	Stocks	2011	748000
Live Animals	Hungary	Cattle	Stocks	2011	682000
Live Animals	Tunisia	Cattle	Stocks	2011	655730
Live Animals	Burundi	Cattle	Stocks	2011	653580
Live Animals	Guinea-Bissau	Cattle	Stocks	2011	650000
Live Animals	Lesotho	Cattle	Stocks	2011	650000
Live Animals	Greece	Cattle	Stocks	2011	627000
Live Animals	Swaziland	Cattle	Stocks	2011	625000
Live Animals	Democratic People's Republic of Korea	Cattle	Stocks	2011	580000
Live Animals	Armenia	Cattle	Stocks	2011	571400
Live Animals	Sierra Leone	Cattle	Stocks	2011	568700
Live Animals	Bulgaria	Cattle	Stocks	2011	544456
Live Animals	Albania	Cattle	Stocks	2011	492000
Live Animals	Slovenia	Cattle	Stocks	2011	470151
Live Animals	Slovakia	Cattle	Stocks	2011	467125
Live Animals	Bosnia and Herzegovina	Cattle	Stocks	2011	455258
Live Animals	Croatia	Cattle	Stocks	2011	446000
Live Animals	Israel	Cattle	Stocks	2011	432000
Live Animals	Saudi Arabia	Cattle	Stocks	2011	400000
Live Animals	Gambia	Cattle	Stocks	2011	398472
Live Animals	Puerto Rico	Cattle	Stocks	2011	380000

Domain	Country	Item	Element	Year	Value (head)
Live Animals	Latvia	Cattle	Stocks	2011	379500
Live Animals	Oman	Cattle	Stocks	2011	339500
Live Animals	Congo	Cattle	Stocks	2011	335000
Live Animals	Fiji	Cattle	Stocks	2011	312000
Live Animals	Togo	Cattle	Stocks	2011	311334
Live Animals	Bhutan	Cattle	Stocks	2011	306190
Live Animals	Djibouti	Cattle	Stocks	2011	296000
Live Animals	The former Yugoslav Republic of Macedonia	Cattle	Stocks	2011	265299
Live Animals	Estonia	Cattle	Stocks	2011	236300
Live Animals	Republic of Moldova	Cattle	Stocks	2011	215951
Live Animals	Libya	Cattle	Stocks	2011	197000
Live Animals	Luxembourg	Cattle	Stocks	2011	192535
Live Animals	Jamaica	Cattle	Stocks	2011	168000
Live Animals	Vanuatu	Cattle	Stocks	2011	160000
Live Animals	Timor-Leste	Cattle	Stocks	2011	155000
Live Animals	China, Taiwan Province of	Cattle	Stocks	2011	136152
Live Animals	Guyana	Cattle	Stocks	2011	112800
Live Animals	United Arab Emirates	Cattle	Stocks	2011	105177
Live Animals	Papua New Guinea	Cattle	Stocks	2011	94000
Live Animals	Belize	Cattle	Stocks	2011	91200
Live Animals	New Caledonia	Cattle	Stocks	2011	90000
Live Animals	Montenegro	Cattle	Stocks	2011	87183
Live Animals	Guadeloupe	Cattle	Stocks	2011	75000
Live Animals	Iceland	Cattle	Stocks	2011	72773
Live Animals	Jordan	Cattle	Stocks	2011	67600
Live Animals	Lebanon	Cattle	Stocks	2011	65000
Live Animals	Cyprus	Cattle	Stocks	2011	56915
Live Animals	Suriname	Cattle	Stocks	2011	55245
Live Animals	Comoros	Cattle	Stocks	2011	50000
Live Animals	Cabo Verde	Cattle	Stocks	2011	46500
Live Animals	Liberia	Cattle	Stocks	2011	39800
Live Animals	Occupied Palestinian Territory	Cattle	Stocks	2011	39625
Live Animals	Gabon	Cattle	Stocks	2011	37500
Live Animals	Kuwait	Cattle	Stocks	2011	35000
Live Animals	Trinidad and Tobago	Cattle	Stocks	2011	33000
Live Animals	Samoa	Cattle	Stocks	2011	30000
Live Animals	Réunion	Cattle	Stocks	2011	26500
Live Animals	Martinique	Cattle	Stocks	2011	18477
Live Animals	Malta	Cattle	Stocks	2011	15074
Live Animals	Antigua and Barbuda	Cattle	Stocks	2011	14600
Live Animals	Solomon Islands	Cattle	Stocks	2011	14500
Live Animals	French Guiana	Cattle	Stocks	2011	14300
Live Animals	Micronesia (Federated States of)	Cattle	Stocks	2011	14000
Live Animals	Dominica	Cattle	Stocks	2011	13500
Live Animals	Tonga	Cattle	Stocks	2011	11300
Live Animals	Saint Lucia	Cattle	Stocks	2011	11000

Domain	Country	Item	Element	Year	Value (head)
Live Animals	Barbados	Cattle	Stocks	2011	10800
Live Animals	Qatar	Cattle	Stocks	2011	10063
Live Animals	Bahrain	Cattle	Stocks	2011	10000
Live Animals	Montserrat	Cattle	Stocks	2011	9800
Live Animals	United States Virgin Islands	Cattle	Stocks	2011	8200
Live Animals	French Polynesia	Cattle	Stocks	2011	7300
Live Animals	Mauritius	Cattle	Stocks	2011	6596
Live Animals	Liechtenstein	Cattle	Stocks	2011	6200
Live Animals	Equatorial Guinea	Cattle	Stocks	2011	5200
Live Animals	Saint Vincent and the Grenadines	Cattle	Stocks	2011	5100
Live Animals	Sao Tome and Principe	Cattle	Stocks	2011	5100
Live Animals	Grenada	Cattle	Stocks	2011	4450
Live Animals	Falkland Islands (Malvinas)	Cattle	Stocks	2011	4300
Live Animals	Saint Kitts and Nevis	Cattle	Stocks	2011	3500
Live Animals	British Virgin Islands	Cattle	Stocks	2011	2400
Live Animals	Cayman Islands	Cattle	Stocks	2011	2061
Live Animals	Faroe Islands	Cattle	Stocks	2011	2000
Live Animals	China, Hong Kong SAR	Cattle	Stocks	2011	1600
Live Animals	Brunei Darussalam	Cattle	Stocks	2011	852
Live Animals	Bahamas	Cattle	Stocks	2011	750
Live Animals	Saint Helena, Ascension and Tristan da Cunha	Cattle	Stocks	2011	700
Live Animals	Seychelles	Cattle	Stocks	2011	660
Live Animals	Bermuda	Cattle	Stocks	2011	650
Live Animals	Singapore	Cattle	Stocks	2011	200
Live Animals	Guam	Cattle	Stocks	2011	140
Live Animals	Cook Islands	Cattle	Stocks	2011	130
Live Animals	Niue	Cattle	Stocks	2011	115
Live Animals	American Samoa	Cattle	Stocks	2011	110
Live Animals	Wallis and Futuna Islands	Cattle	Stocks	2011	60
Live Animals	Saint Pierre and Miquelon	Cattle	Stocks	2011	35
Live Animals	Greenland	Cattle	Stocks	2011	15

Table 2. Anthelmintics: resistance free years

Anthelmintic	Year introduced	First reported resistance	Resistance-free years	References
Phenothiazine	1940	1957	17	(Manson-Bahr 1940; Drudge *et al.* 1957)
Piperazine	1957	1966	9	(Kushner *et al.* 1957; Norton & De Beer 1957; Drudge *et al.* 1988)
Bephenium	1958	1970	12	(Rawes & Scarnell 1958; Commey & Haddock 1970)
Thiabendazole	1961	1964	3	(Brown *et al.* 1961; Gordon 1961; Drudge *et al.* 1964; Hotson *et al.* 1970; Le Jambre *et al.* 1976)
Haloxon	1962	1969	7	(Brown *et al.* 1962; Andersen *et al.* 1969)
Parbendazole	1967	1975	8	(Batte & Moncol 1968; Berger 1975)
Levamizole	1968	1976	8	(Coles *et al.* 1974; Le Jambre *et al.* 1976)
Mebendazole	1972	1983	11	(Chaia *et al.* 1972; Hughes 1983; McKenna & Hughes 1986)
Albendazole	1972	1990	18	(Theodorides *et al.* 1976; Knight & Colglazier 1977; Morris & Taylor 1990)
Oxibendazole	1973	1989	16	(Theodorides *et al.* 1973; Tolliver *et al.* 1993)
Fenbendazole	1974	1989	15	(Baeder *et al.* 1974; Yadav 1990)
Oxfendazole	1977	1983	6	(Borgsteede 1977; Middelberg & McKenna 1983)
Ivermectin	1981	1985	4	(Carmichael *et al.* 1987; Steel 1993; Vercruysse & Rew 2002)
Moxidectin	1989	1995	6	(Steel 1993; Leathwick 1995; McKellar & Benchaoui 1996)
Doramectin	1993	1996	3	(Vercruysse *et al.* 1993; Vermunt *et al.* 1996)
Eprinomectin	1997	2006	9	(Shoop *et al.* 1996b; Vercruysse & Rew 2002; Mason & McKay 2006)
		Average	**9.5**	

Chapter 2: Testing for relative toxicity of four anthelmintics (ivermectin, eprinomectin, doramectin and moxidectin) using the dung beetle species, *Euoniticellus intermedius* (Reiche) (Coleoptera: Scarabaeidae), as a bio-indicator.

Carmen T. Jacobs[*], *Adrian L.V. Davis*[*], *Clarke H. Scholtz*[*]

[*]*Scarab Research Group, Department of Zoology and Entomology, University of Pretoria*

Introduction

Avermectins and milbemycins are widely used in agro-ecosystems for the control of parasites in domestic livestock (Davies & Green 1986; Ridsdill-Smith 1993; Shoop *et al.* 1995; Wardhaugh *et al.* 2001). Whilst relative toxicity of different anti-parasitics and the development of resistance by parasites are issues of importance to farmers, relative toxicity to non-target species is of concern for global conservation efforts (Wall & Strong 1987; Madsen *et al.* 1990; Lumaret *et al.* 1993; Halley *et al.* 1993; McKellar 1997; Wardhaugh *et al.* 2001; Lumaret & Errouissi 2002). As integral members of agro-ecosystems with importance in maintaining pasture health through dung burial behaviour (Fincher 1981; Yokoyama *et al.* 1991; Nichols *et al.* 2008), scarabaeine beetles are an excellent, non-target, bio-indicator taxon for examining potential detrimental effects of pesticide application (Spector 2006; Nichols *et al.* 2008; Jacobs *et al.* 2010). Therefore, the current study uses the dung beetle species, *Euoniticellus intermedius* (Reiche), as a bio-indicator to test the relative toxicity of four different anthelmintics in dung residues.

The avermectins (ivermectin, eprinomectin and doramectin) are one of two groups of broad-spectrum anthelmintics comprising derivatives of the macrocyclic lactones (Burg *et al.* 1979; Albers-Schoenberg *et al.* 1981; Putter *et al.* 1981; Steel 1993; Vercruysse & Rew 2002). They are obtained from the fermentation products of an actinomycete, the soil fungus *Streptomyces avermitilis* (Burg *et al.* 1979; Albers-Schoenberg *et al.* 1981; Putter *et al.* 1981; Steel 1993; Vercruysse & Rew 2002) and are termed endectocides owing to their

ability to kill both endo- and ectoparasites (McKellar & Benchaoui 1996; Shoop et al. 1996a). In many invertebrate parasites, they act as an agonist by stimulating uncontrolled γ-aminobutyric acid (GABA) flow between nerve endings and nerve or muscle receptors, which increases the flow of chlorine ions into cells resulting in hyperpolarization, elimination of neural signal transmission, and paralysis (Campbell 1985). Different sites along different neuromuscular transmission systems may show variable sensitivity to avermectins so that effects may vary with differences in pesticide concentrations (‘http://www.merckmanuals.com’ 2013). However, paralysing effects can also be reversed by picrotoxin, which regulates the closing of the chloride ion channels (Kass *et al.* 1980; Campbell 1985).

In nematodes, both neural and muscular paralysis (hypercontraction or flaccid) have been recorded (Fritz *et al.* 1979; Kass *et al.* 1980; Putter *et al.* 1981; Campbell 1985). Cessation of activity, without hypercontraction or flaccid muscular paralysis (Kass *et al.* 1980), results from inhibition of neural signals from the ventral interneurons to the excitatory motoneurons in the ventral nerve chord. Inhibition of body functions results from paralysis of the pharyngeal, body wall, and uterine muscles (‘http://www.merckmanuals.com’ 2013). Short duration of flaccid paralysis of the body wall muscle may be critical for rapid expulsion of the pesticide (‘http://www.merckmanuals.com’ 2013). As the concentration decreases, it is possible that motility may be regained, although in the event of survival, paralysis of uterine muscles may result in disruption of reproduction (‘http://www.merckmanuals.com’ 2013). However, paralysis of the more sensitive pharyngeal muscles and inhibition of feeding may be even more critical as it may last longer than that of body wall muscle paralysis and may ultimately cause the death of nematode parasites (‘http://www.merckmanuals.com’ 2013). Although similar work has not been conducted on dung beetles, observations of immobile individuals with their legs extended suggest that they are also susceptible to hypercontraction caused by pesticide residues in dung as well as reduced survivorship and reproductive output (Fisher & Mrozik 1992). By contrast, avermectins are non-toxic to endoparasitic cestode and trematode Platyhelminthes, which lack a GABA system (Putter *et al.* 1981).

The first avermectin (ivermectin) was introduced in 1981 (Steel 1993; Vercruysse & Rew 2002). Although it actively controls arthropods, nematodes and acarines (Putter *et al.* 1981; Campbell 1985), it is not equally effective against all species. It is, also, often stage specific (Campbell 1985), which means that it may not be effective against all life history stages of a susceptible taxon (Campbell & Benz 1984). Even though ivermectin has no side effects to the host, it cannot be used in lactating dairy animals due to the levels of residues in the milk, which may result in the dairy products being discarded (Shoop *et al.* 1996a, 1996b; Vercruysse & Rew 2002). Eprinomectin was introduced to the animal health industry in 1997 as an alternative to ivermectin and is the only topical endectocide safe for use in lactating dairy animals (Shoop *et al.* 1996a; Vercruysse & Rew 2002). Doramectin was commercialised in 1993 (Vercruysse *et al*. 1993) and improves the ease of handling of livestock as it causes significantly less discomfort during administration than ivermectin (Grandin *et al.* 1998, 1999).

The Milbemycins are the second group comprising the macrocyclic lactones. They may be considered as deglycosylated avermectins (Steel 1993; Vercruysse & Rew 2002). Discovered in 1973, they were originally developed for use in crop protection and only used in veterinary practices from the mid 1980's (Takiguchi *et al.* 1980; McKellar & Benchaoui 1996). Moxidectin, the only milbemycin available on the market as an endectocide, was introduced in 1989 and was commercialised worldwide by the early 1990's (Steel 1993; McKellar & Benchaoui 1996). Milbemycins are highly lipophilic, soluble in organic solvents but insoluble in water. Moxidectin is about 100 times more lipophilic than the avermectins and, after an initial increase in its plasma concentration, it is redistributed throughout the body fat reserves, which act as a reservoir from which it is slowly released (McKellar & Benchaoui 1996).

Various studies show that, regardless of the animal or method of administration, most of the avermectin or milbemycin dosage is voided, largely unaltered in the dung, where it retains its insecticidal properties (Steel 1993; Strong 1993; Vercruysse & Rew 2002). Thus, the effects of avermectin and milbemycin toxicity are not confined to parasitic nematodes and arthropods, but also extend to a large variety of beneficial invertebrate species, which use the dung as a microhabitat and breeding resource. Over time, pesticide usage could indirectly affect the rate of dung degradation through adverse effects on dung

beetles (Strong 1993). This potential problem constitutes the main focus of this study. Of the four anthelmintics tested here, some of their effects on dung beetles have been examined in various separate (Fincher 1992; Krüger & Scholtz 1997; Kryger *et al.* 2005; Iwasa *et al.* 2008; Römbke *et al.* 2010; Errouissi & Lumaret 2010; Iglesias *et al.* 2011; Cruz Rosales *et al.* 2012) or comparative trials (Wardhaugh *et al.* 2001; Floate *et al.* 2002; Suárez *et al.* 2003, 2009; Floate 2006, 2007). However, there has never been a laboratory trial comparing ivermectin, eprinomectin, doramectin and moxidectin under precisely the same conditions.

Materials and Methods

Bio-indicator species

Euoniticellus intermedius (Reiche) is a small, tunnelling, dung beetle species that is widespread in African savannas and has been successfully introduced into Australia for the biological control of cattle dung (Hanski & Cambefort 1991). Because *E. intermedius* has a relatively short life-cycle, high fecundity, and is easily bred in the laboratory, it has been used in toxicity tests of various animal health products over the last two decades in both South Africa (Krüger & Scholtz 1997; Krüger *et al.* 1999; Kryger *et al.* 2006) and Australia (Fisara 1994, 1995, 1996). Adults of *E. intermedius* show a strong association with cattle dung (Davis 1994) and feed on the liquid dung fractions. By contrast, the larvae feed on the solid components inside dung ovoids ("brood balls") formed by the adults in the soil beneath the dropping (Hanski & Cambefort 1991). Each "brood ball" contains a single larva. Adults live for about 45 days and duration of the immature stages is about 30 days. Females are capable of producing up to 120 offspring in a lifetime (Doube 1991).

Anthelmintic treatment and bio-indicator protocol

Ivermectin, eprinomectin, doramectin and moxidectin were administered to cattle as subcutaneous injections at their registered dosage rates. Dung for the laboratory trials was collected 1, 7, 14, 21and 28 days after treatment. Another group of cattle remained untreated to serve as a control group. The control group had not been treated with any antiparasitic product for at least five weeks or with an anthelmintic bolus for at least five months prior to

collection of their dung. Dung from individual animals, supplied by CEVA Animal Health (Pty) Ltd, was frozen at -20ºC and stored at ≤-20ºC until needed. Each unit of dung was thawed and thoroughly mixed before use.

Laboratory colonies of *E. intermedius* were established by collecting adult beetles from the field (North-West Province, rural townships) where neither antiparasitic nor anthelmintic drugs are used. Only beetles from the insectary-raised F1 generation were used in experiments. Beetles were reared at 26-27ºC with a 12h photoperiod and approximately 60-70% relative humidity. Beetles were kept in 1 L gauze-topped plastic buckets, three-quarters filled with compact, moist, sandy soil from the collecting area.

To investigate the effects of ivermectin, eprinomectin, doramectin and moxidectin on fecundity and fertility, 25 pairs (one male and one female) of ten-day old unmated *E. intermedius* from the F1 generation were randomly selected and placed in 1 L gauze-topped plastic buckets, three-quarters filled with compact, moist, sandy soil. Five pairs were each provided with 250 mL of thawed control dung whereas four sets of five pairs were, respectively, provided with dung from treatments, twice a week for 15 days.

The soil, in which the beetles were kept, was sieved after 8 days and again after 15 days. Beetles and brood balls were removed and counted. Beetles were placed on their backs and watched for movement. Beetles that did not show any leg movement for 5 minutes were considered dead. Live beetles were returned to the containers with fresh soil and fed again with 250 mL of thawed dung. Beetles still alive at the end of this 15 day period were considered to have survived the treatment.

The brood balls removed on day 8 (week 1) and day 15 (week 2) were counted and placed between layers of moist soil. Numbers of F2 adult beetles emerging from these brood balls were counted and provided with fresh dung from untreated cattle to assess their breeding capacity as regards F2 brood production and emergence of F3 generation beetles. The endpoints F1 adult survival, number of F1 and F2 brood balls, plus F2 and F3 adult emergences were analysed using Generalised Linear Models (GLM) based on either a factorial or one-way ANOVA from STATISTICA® (Version 11, Statsoft, Tulsa, Oklahoma).

Post-hoc tests (Tukey's HSD) were conducted to identify which treatments or days were significantly different from one another.

Results

Overall adult mortality

Although adult F1 mortality in treated dung was greater than natural mortality in control dung (Fig. 1), there was appreciable variation. Thus, there was no significant effect of different pesticide treatments on adult survival. There were, also, no significant differences between treatments although moxidectin was clearly less detrimental to *E. intermedius* than other tested anthelmintics.

Overall reproductive output

There were significant differences between overall F1 brood ball production and overall F2 emergences (Figs 2A, 2B) as these varied with dung treatment and time after treatment. Numbers of F1 brood balls were clearly greater than F2 emergences of *E. intermedius* from both control and treated dung. However, proportional F2 mortality was greater in treated than control dung for both individual treatments, and in combined treatments over 1-28 days (Table 1). Despite the variation, overall decline in brood ball production was not significant between treatments (Fig 2A), perhaps because of anomalously high production in ivermectin-treated dung. However, a significant decline in F2 emergences was recorded with Tukey's HSD tests indicating significantly higher decline in avermectin treated dung than in moxidectin treated dung, which was higher but not significantly different to that in control dung. Over time data for four combined pesticide treatments yielded significant parallel declines in brood production and F2 emergences (Fig. 2B) that seemed to be diminishing by a slight but significant climb in numbers only after 21-28 days (Tukey's HSD).

Week 1 versus week 2 reproductive output

Control dung results only

Patterns of F1 brood production and F2 emergences of *E. intermedius* in untreated control dung showed complementary patterns in that there were steep declines from day 1, which levelled out to significantly lower numbers from days 7-28, except for the anomalous steep increase in both brood production and emergences on day 28 in Week 2 that were not significantly different from figures for day 1 (Figs 3A, 3B – Tukey's HSD). Proportional differences for natural mortality based on numbers of F2 emergences compared to numbers of broods indicated a fairly uniform trend of natural mortality for the first 14 days, with a decline by day 28 and anomalous results for day 21 (Table 2).

Pesticide dung results only

Overall, Week 1 reproductive output was mostly lower than that of week 2 in both control and treated pads. Week 1 results from treated pads showed steep declines from day 1, which levelled out to significantly lower numbers from days 7-28, as in control pads. However, for Week 2 pads there was, again, an anomalous increase on days 14 -28 although numbers remained significantly different from day 1 for broods but did not differ significantly from day 1 for F2 emergences of *E. intermedius*. Proportional differences for mortality based on numbers of F2 emergences compared to numbers of broods indicated a trend to increasing (up to Day 14) then declining mortality across time, which was, mostly, much greater than that shown by natural mortality in untreated control pads (Table 2).

Discussion

General trends

Reliance on anthelmintics has become a race to produce a product that exhibits the perfect balance between toxicity to parasites and non-toxicity to non-target organisms. From a purely agricultural point of view, the need for improved products is increasingly important because it appears that as fast as new products are released, pests develop resistance. Elimination of pest resistance is arguably one of the top challenges for protection of livestock.

Current endectocides all show varying degrees of toxicity to both target ('http://www.merckmanuals.com' 2013) and non-target beneficial organisms including the experimental animal used in this study as a bio-indicator. Unlike in parasitic nematodes (Fritz *et al.* 1979; Kass *et al.* 1980; Putter *et al.* 1981; Campbell 1985), there have been no tests on the precise chemo-physiological action of the various endectocides in dung beetles. However, various publications have recorded dung beetle responses to pesticide residues in dung that include non-significant increases in adult mortality and significantly reduced reproductive output (Fincher 1992; Wardhaugh *et al.* 2001; Suárez *et al.* 2003; Cruz Rosales *et al.* 2012) as well as observations of dung beetles with their legs extended that could represent hyper-contraction, loss of motility and feeding disruption.

Although all four of the present pesticide products have been previously tested for detrimental effects on non-target organisms (Fincher 1992; Krüger & Scholtz 1997; Wardhaugh *et al.* 2001; Floate *et al.* 2002; Suárez *et al.* 2003, 2009; Kryger *et al.* 2005; Floate 2006, 2007; Iwasa *et al.* 2008; Römbke *et al.* 2010; Errouissi & Lumaret 2010; Iglesias *et al.* 2011; Cruz Rosales *et al.* 2012) this is the first study that examines their effects under the same experimental conditions on the same non-target organism, *Euoniticellus intermedius*. Although there is some variation in the results, in general, the three avermectins were shown to have greater detrimental effects than the milbemycin, moxidectin. Moxidectin has also been shown to be less toxic to dung beetles than doramectin and ivermectin in a previous study (Barber *et al.* 2003). This may be related to its lipophilic properties since dung beetles have well-developed fat bodies and moxidectin

may become attached to these organelles from which it is released slowly, thus occurring in lower concentrations than the avermectins (Barber *et al.* 2003). In an experiment done by Floate *et al*. in 2002, they ranked pour-on formulations of ivermectin, doramectin, eprinomectin and moxidectin in descending order of adverse effect (Floate *et al.* 2002). The results were similar to a review done by Floate in 2006, showing that based on the number of species affected and duration of suppression, doramectin > ivermectin > eprinomectin >> moxidectin in descending order of adverse effect, and they concluded that moxidectin was the least likely to affect natural insect assemblage associated with cattle dung (Floate *et al.* 2002; Floate 2006). The findings here are quite different; in descending order of adverse effect ivermectin > eprinomectin > doramectin > moxidectin.

Specific trends

In the present study, there are steep declines in F1 brood production and F2 emergences of *E. intermedius* from control dung following day 1. These are repeated for both Week 1 and Week 2 results, suggesting a natural decline in fecundity over time that must be taken into account in interpreting trends from pesticide-treated dung. However, reproductive success in control dung was, on average, higher than that from dung containing pesticide residues. Furthermore, out of 40 combinations (week 1, week 2) for each pesticide (ivermectin, eprinomectin, doramectin, moxidectin) and each day (1, 7, 14, 21, 28), F1 brood ball production in control dung was greater in 32 instances and emergence of F2 individuals was greater in 34 instances (see Appendix 1).

The numbers of F2 emergences was consistently lower than the numbers of F1 broods. As this trend was true for results from both control and treated dung, it could be due to natural mortality or a combination of both natural and pesticide-induced mortality in the case of treated dung. For the most part, proportional mortality in control dung was consistently much lower than in treated dung. Thus, results suggest relatively much greater susceptibility to mortality for immatures that are feeding and developing in dung infused with pesticide residues. In rank order moxidectin was the least toxic to *E. intermedius* followed by doramectin, eprinomectin and ivermectin, with an overall 17% difference in toxicity between the least and most toxic endectocides.

As the concentration of drug residues might be expected to decline with time after administration, one might expect the detrimental effects on non-target organism to also decline. In the present study, after initial declines in reproductive output after day one, there was no change in mean brood ball production and emergences across days, 7, 14, 21 and 28 for week 1 results. However, the decline from day 1 in week 2 results was followed by steep increases in brood production emergences from day 14 or 21. These patterns were repeated in eight separate tests for the four pesticides (see Appendix 1). It is unclear if these anomalous patterns relate to the differences in the dung or the bio-indicators used for Week 2 experiments. Could it be due to the death of females with the lowest fecundity?

Conclusions

It is clear that residues of avermectins in dung and to a lesser extent, milbemycins, are detrimental to the non-target dung beetle species, *Euoniticellus intermedius*. This implies that there could be an effect that extends to the entire natural assemblage of insects inhabiting and feeding on the dung of cattle treated with avermectin or milbemycin products. The present and previous studies have indicated no significant effect on the survival of adults but a significant reduction in reproductive rate and reproductive success (Fincher 1992; Wardhaugh *et al.* 2001; Suárez *et al.* 2003; Cruz Rosales *et al.* 2012). Over time, reduced reproductive rate would result in decreased population sizes in the dung beetle community and, ultimately, a decrease in the rate of dung degradation and dung burial. Low dung beetle density or the absence of dung beetles can, potentially, favour increased populations of dung-breeding flies, assuming they are less influenced by the pesticide residues. It could certainly reduce burial of dung leading to potentially greater exposure to cestode and trematode eggs in dung, which are endoparasites that are not affected by avermectin treatment. It is, thus, vitally important to create awareness about the importance of dung beetles and sound farming practices for healthy agro-ecosystems.

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Figures and Tables

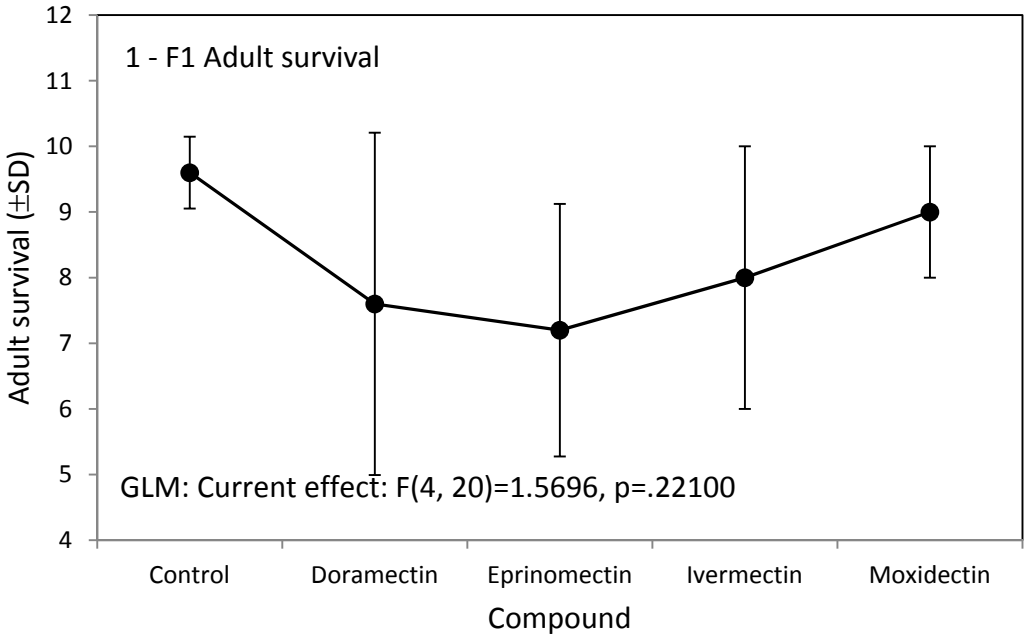
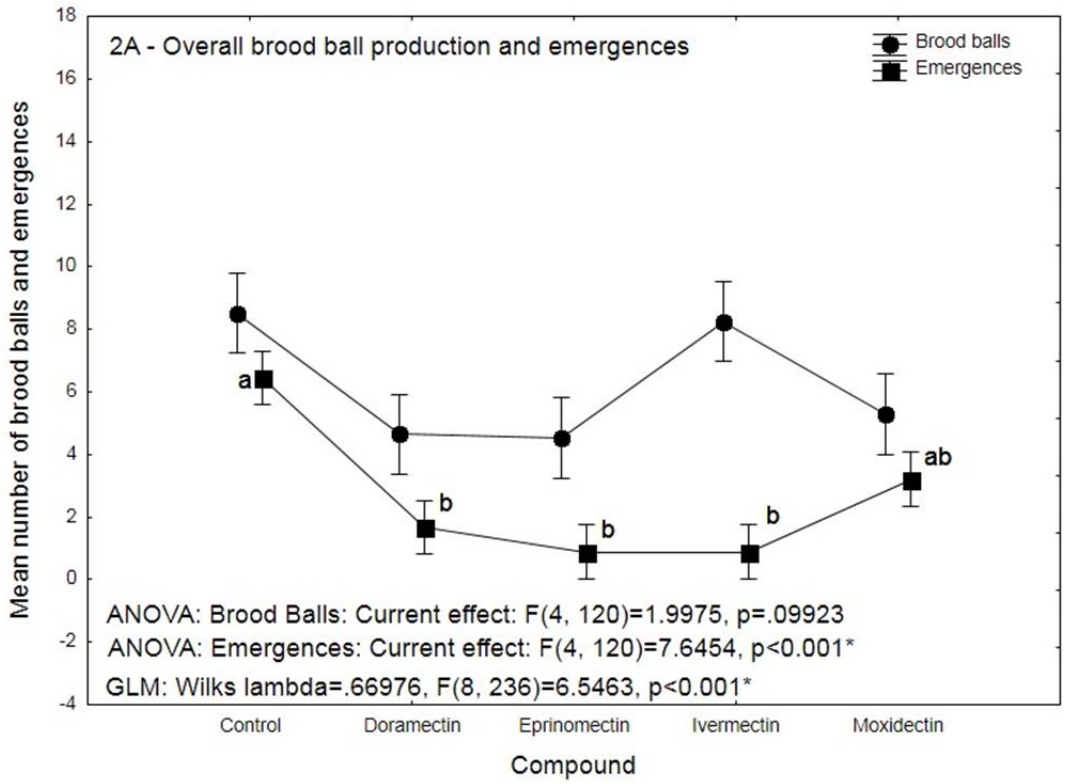
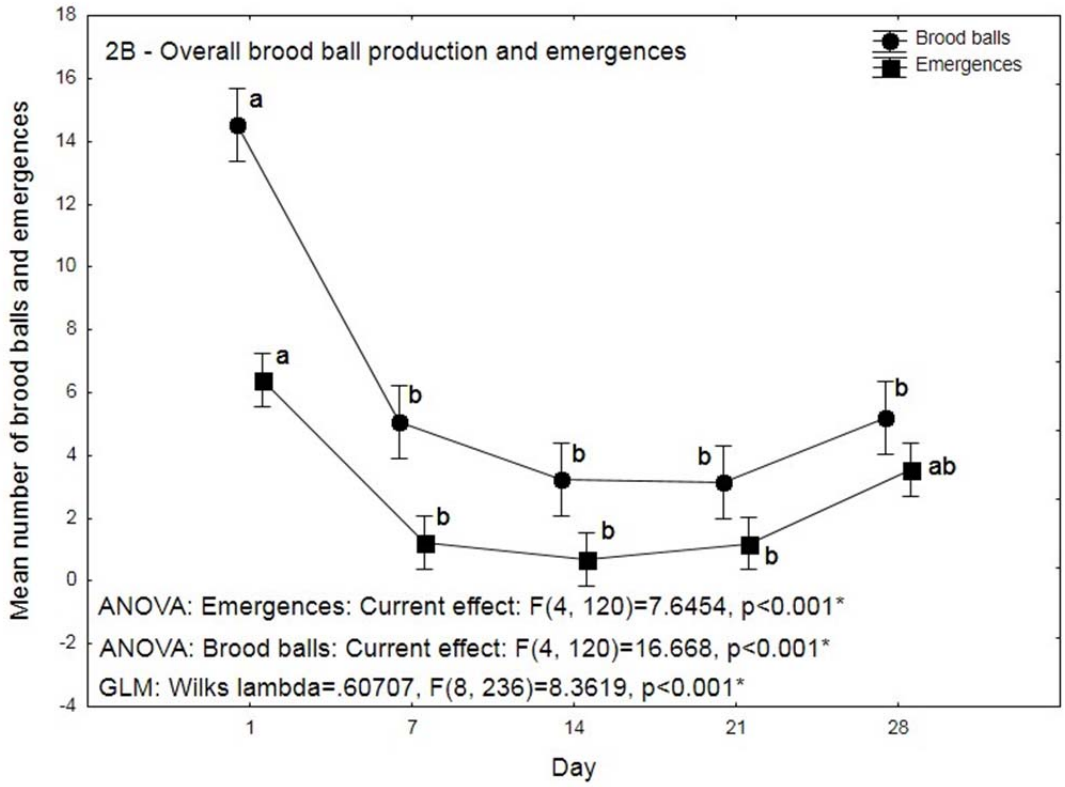


Figure 1. Mean number (±SD) of F1 adult *Euoniticellus intermedius* surviving 15 days in untreated control dung or dung containing different types of pesticide residues.





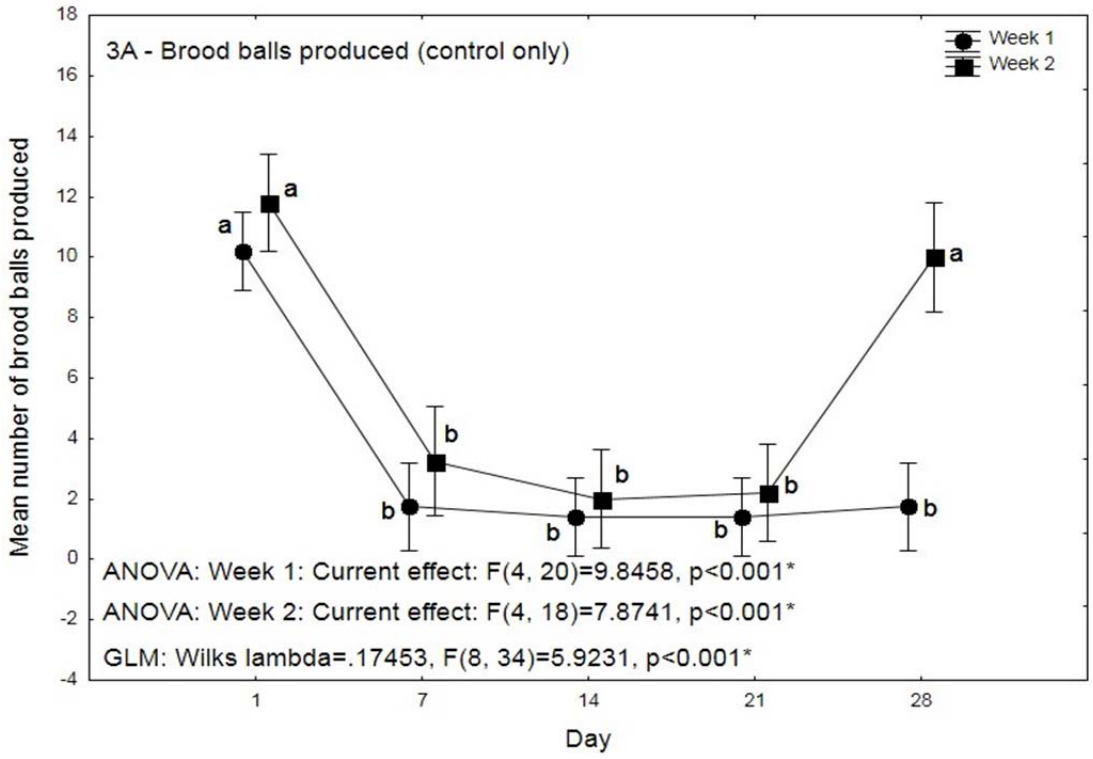
Figures 2A, 2B. Overall numbers of F1 brood balls (±SE) and F2 *E.intermedius* emergences (±SE) from untreated control dung and dung containing four different pesticide residues.

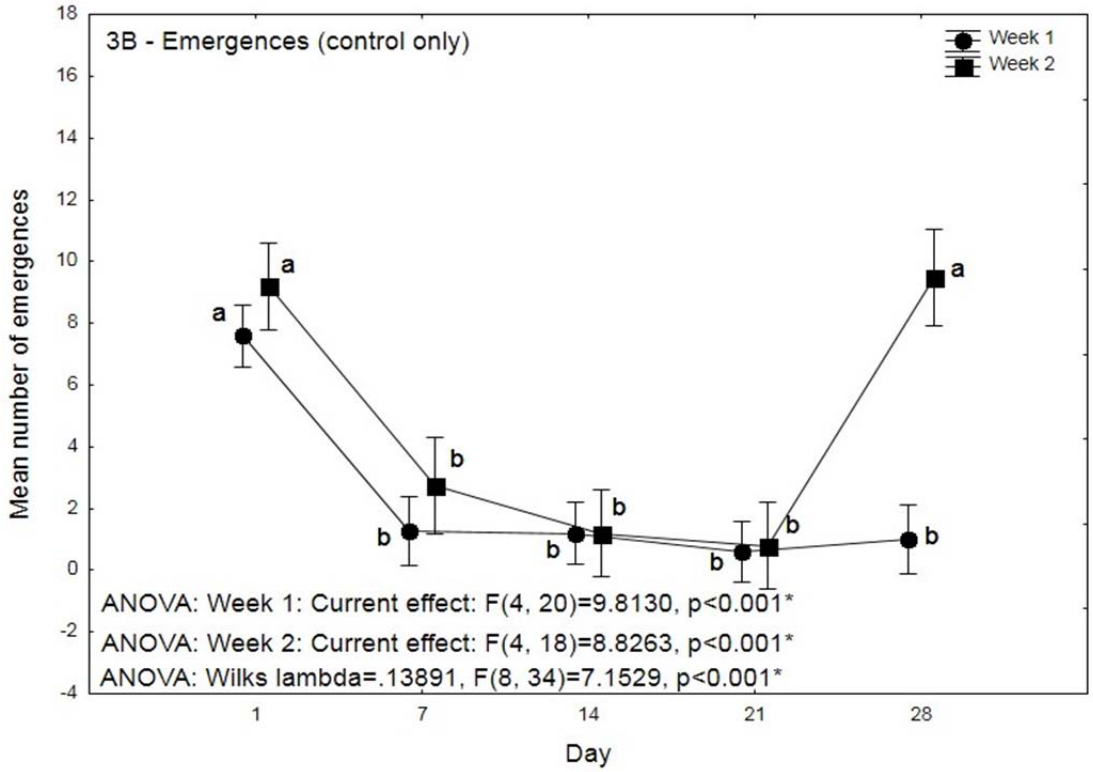
Table 1. Percentage overall mortality of F2 immatures.

	Control	Doramectin	Eprinomectin	Ivermectin	Moxidectin
% Mortality	29.72	68.59	72.29	77.74	60.13
% Mortality (- natural mortality)	-	38.87	42.57	48.02	30.41

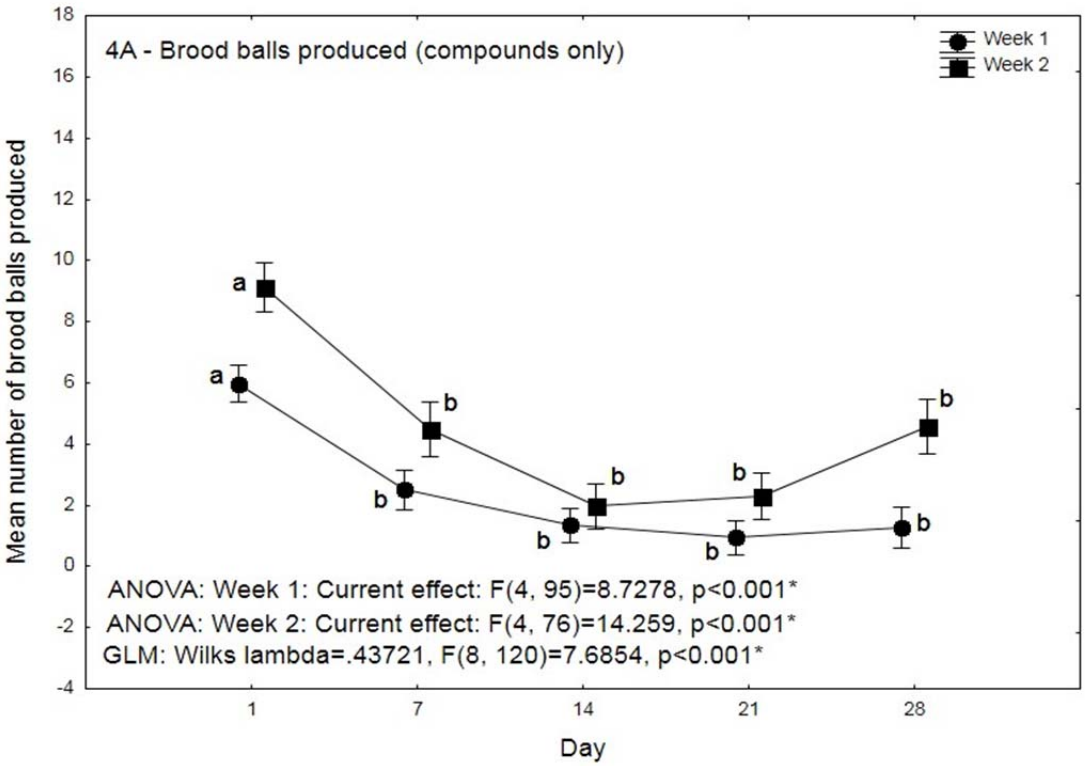
Table 2. Percentage F2 immature mortality over days.

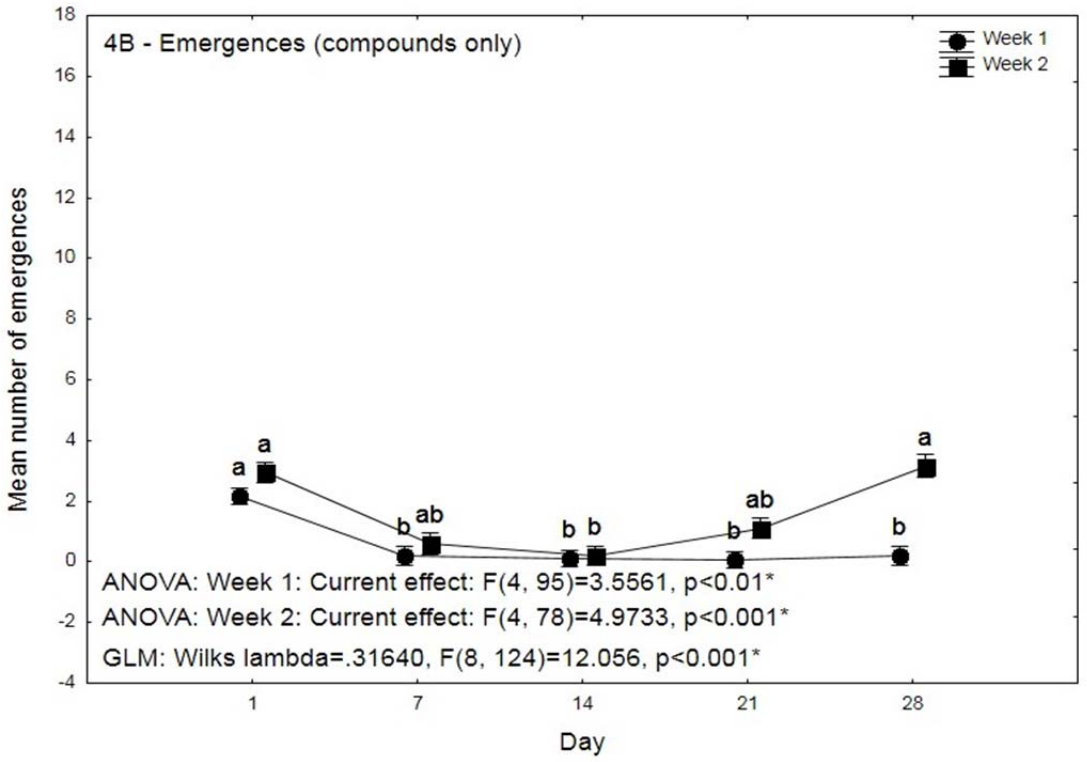
	Day 1	Day 7	Day 14	Day 21	Day 28
% Mortality (overall)	58.62	74.45	74.99	64.56	35.83
% Mortality (control only)	23.64	23.81	29.41	61.11	10.64
% Mortality (compounds only)	67.37	87.12	86.39	65.42	42.13





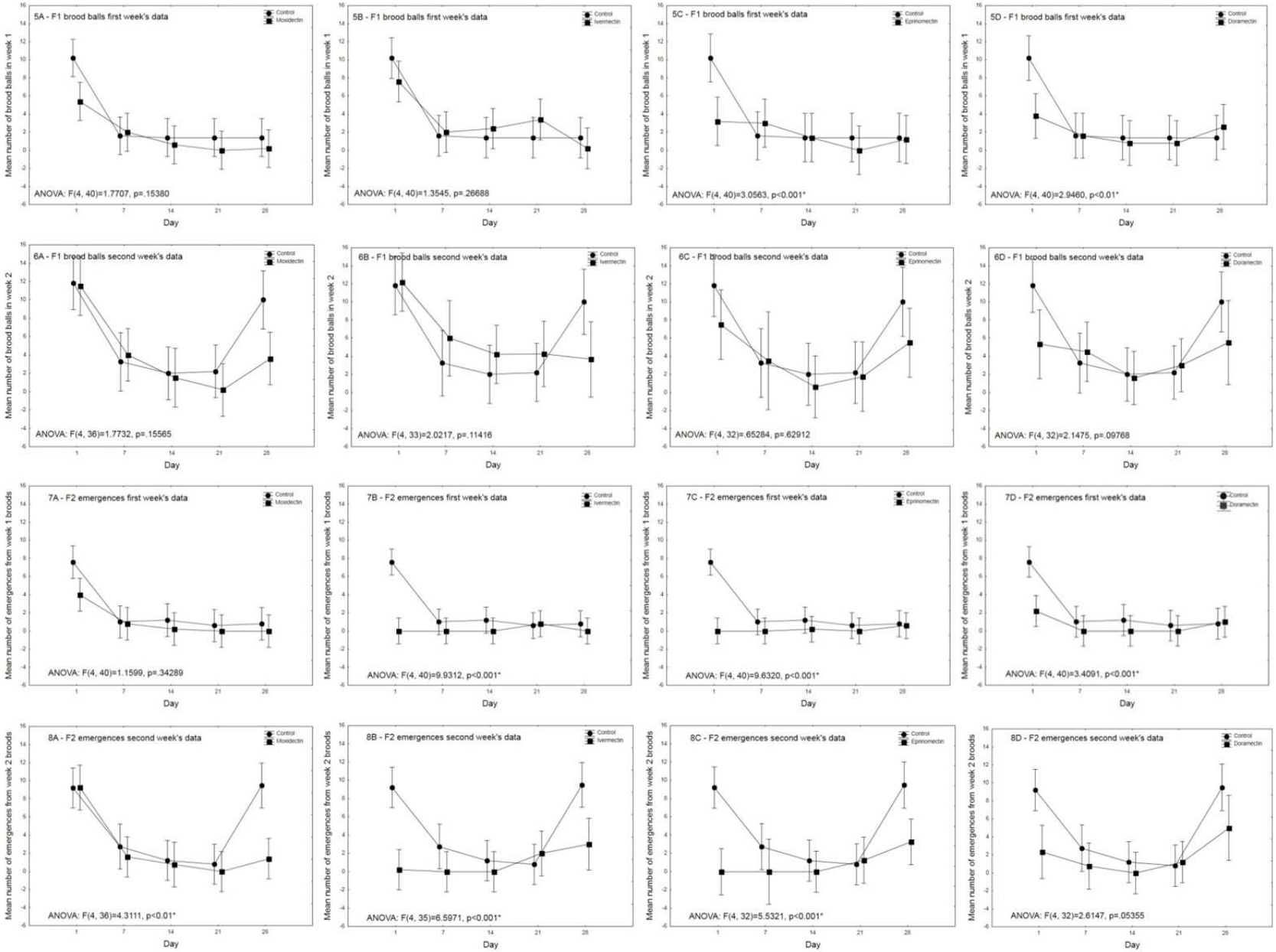
Figures 3A, 3B. Data for F1 brood balls (±SE) and F2 *E. intermedius* emergences (±SE) from untreated control dung.





Figures 4A, 4B. Combined data for F1 brood balls (±SE) and F2 *E. intermedius* emergences (±SE) from dung treated with doramectin, eprinomectin, ivermectin or moxidectin.

Appendix 1. Figures 5A – 8D. Summaries of the variablity shown by F1 brood ball production and F2 *E. intermedius* emergences over week 1 and week 2 for from untreated control dung or dung containing moxidectin, ivermectin, eprinomectin or doramectin residues.



Chapter 3: Survival and reproduction of *Euoniticellus intermedius* (Reiche) (Coleoptera: Scarabaeidae) in dung following treatment of cattle with an unregistered ectoparasiticide

Carmen T. Jacobs[*], *Adrian L.V. Davis*[*], *Clarke H. Scholtz*[*]

[*]*Scarab Research Group, Department of Zoology and Entomology, University of Pretoria*

Introduction

Euoniticellus intermedius (Reiche) is a small, tunnelling, dung beetle species that is widespread in African savannas and has been successfully introduced into Australia for the biological control of cattle dung (Hanski & Cambefort 1991). Because *E. intermedius* has a relatively short life-cycle, a high fecundity, and is easily bred in the laboratory, it has been used in toxicity tests of various animal health products over the last two decades in both South Africa (Krüger & Scholtz 1997; Krüger *et al.* 1999; Kryger *et al.* 2006) and Australia (Fisara 1994, 1995, 1996).

Adults of *E. intermedius* show a strong association with cattle dung (Davis 1994). They are known to feed on the liquid dung fractions whereas the larvae feed on the solid components inside dung ovoids (brood balls) formed by the adults in the soil beneath the dropping. Each brood ball contains a single larva. Adults live for about 45 days and duration of the immature stages is about 30 days. Females are capable of producing up to 120 offspring in a lifetime (Doube 1991).

This was a pilot study to test a new ectoparasiticide molecule at a very early stage of development, hereafter known as '*product x*'. This molecule is intended to be included in a new treatment for cattle ectoparasites. The route of administration has not yet been decided. It is absorbed systemically and excreted via the faeces in the treated animal. It has both contact and systemic (feeding) effects on insects. Thus, its potential

influence on dung beetles could be from their eating toxic dung or by being in contact with excreted, chemically unchanged product in the dung.

The objective of this study is to evaluate the *in-vitro* sensitivity of dung beetle species to *product x* in the early stages of development by testing survival and reproduction of *E. intermedius* after exposure to this ectoparasiticide via dung of treated cattle.

Materials and Methods

All of the tested cattle were held at Intervet facilities [Malelane Research Unit, Intervet SA (Pty) Ltd.]. For the pilot study, a group of cattle were treated with *product x* at 5mg /kg bodyweight. This was administered to the cattle in four different ways: subcutaneously (treatment 1, T1), as a topical solution (T2), as a topical suspension (T3), and intravenously (T4). Dung was collected from these cattle on day 1 (D1), day 7 (D7), day 14 (D14), day 21 (D21) and day 28 (D28) post treatment. Another group of cattle remained untreated to serve as a control group. The control group had not been treated with any antiparasitic product for at least five weeks or with an anthelmintic bolus for at least five months prior to collection of their dung. Dung from individual animals was supplied by the sponsor frozen at -20ºC and stored at ≤-20ºC until needed. Each unit of dung was thawed and thoroughly mixed before use.

Laboratory colonies of *E. intermedius* were established by collecting adult beetles from the field (North-West Province, rural townships) where neither antiparasitic nor anthelmintic drugs are used. Only beetles from the insectary-raised F1 generation were used in experiments. Beetles were reared at 26-27ºC with a 12h photoperiod and approximately 60-70% relative humidity. Beetles were kept in 1 L gauze-topped plastic buckets, three-quarters filled with compact, moist, sandy soil from the collecting area.

To investigate the effects of *product x* on fecundity and fertility, 25 pairs (one male and one female) of ten-day old unmated *E. intermedius* from the F1 generation were randomly selected and placed in 1 L gauze-topped plastic buckets, three-quarters filled

with compact, moist, sandy soil. Five pairs were each provided with 250 mL of thawed control dung whereas four sets of five pairs were, respectively, provided with dung from treatments T1-T4, twice a week for 15 days.

The soil, in which the beetles were kept, was sieved after 8 days and again after 15 days. Beetles and brood balls were removed and counted. Beetles were placed on their backs and watched for movement. Beetles that did not show any leg movement for 5 minutes were considered dead. Live beetles were returned to the containers with fresh soil and fed again with 250 mL of thawed dung. Beetles still alive at the end of this 15 day period were considered to have survived the treatment.

The brood balls removed on day 8 (week 1) and day 15 (week 2) were counted and placed between layers of moist soil. Numbers of F2 adult beetles emerging from these brood balls were counted and provided with fresh dung from untreated cattle to assess their breeding capacity as regards F2 brood production and emergence of F3 generation beetles. The endpoints F1 adult survival, number of F1 and F2 brood balls, plus F2 and F3 adult emergence were analysed using Generalised Linear Models (GLM) based on either a factorial or one-way ANOVA from STATISTICA® (Version 10, Statsoft, Tulsa, Oklahoma). Post-hoc tests (Tukey's HSD) were conducted to identify which treatments or days were significantly different from one another.

Results

Adult survival

There was no significant difference in F1 adult survival between the control dung and dung from treated cattle (Fig. 1A). Except for day 28 in dung from treated cattle, neither were there significant differences between results over time (Fig. 1B). Of 20 paired control versus treatment combinations (see Appendix 1 - F1 adult survival), survival was lower in the antiparasitic treatments than in the controls in ten cases whereas there was no difference in four cases. Thus, mortality in control dung exceeded that in antiparasitic treatments in six cases.

F1 brood ball production and F2 emergence

There were significant differences between control and treated cattle in overall F1 brood ball production (Figs 2A, 3A) and emergences of F2 adults from brood balls (Figs 4A, 5A). For week 1 results, F1 brood balls and F2 emergences were lower in all treatments and significantly so for T1, T2, and T4 (Figs 2A, 4A). For week 2 results, F1 brood balls and F2 emergences were also lower in all treatments and significantly so for T2, and T4 (Figs 3A, 5A). Over time, after treatment with *product x*, there were no significant differences in brood production and emergences for either week 1 or week 2 results (Figs 2B-5B). However, significant declines from days 1-28 were recorded for results from control dung. Even so, out of 40 paired control versus treatment combinations, brood ball production was lower in all but seven results for cattle treated with *product x* (see Appendix 1 – F1 broods week 1 and F1 broods week 2) and emergence of F2 beetles was lower in all but six results (see Appendix 1 – F2 beetles week 1 and F2 beetles week 2).

F2 brood ball production and F3 emergence

There were also significant differences between control and treated dung in overall F2 brood ball production (Fig. 6) and emergences of F3 adults from broods (Fig. 7). F2 brood ball construction was lower in all treatments and significantly so for T1, T2, and T4 (Fig. 6A). F3 emergences were significantly lower in all four treatments (T1-T4) (Fig. 7A). Again, over time after treatment with *product x*, there were no significant differences in brood ball production or emergences (Figs 6B, 7B). However, significant declines from days 1-28 were recorded for results from control dung. Even so, of 20 paired control versus treatment combinations, F2 brood ball production (see Appendix 1 - F2 broods) was lower in all but three results for cattle treated with *product x*. For F3 adults (see Appendix 1 – F3 beetles), only two records from treated dung exceeded those from the control dung.

Residue concentration in dung

Over time, concentrations of *product x* residues in dung differed between treatments (Fig. 8) although there were few significant differences in patterns of beetle response to *product x* (Figs 9A – 15D). Residues declined over days 1-28 in T3 and T4 but showed little change in T1 and T2. Concentrations were much higher in T4 than T3 and higher in T1 than in T2, by day 28, with concentrations in T3 and T4 below those in T1 and T2. Although endpoints describing breeding success (brood ball production and emergence) in each treatment variously showed some marked declines in numbers at different times, these were significant only in the case of some Day 14 results in T4 (F1 brood balls first week, F2 emergence first week, F2 brood balls, and F3 emergence;Fig. 10D – 15D). There were also significant differences in survival of adults between different days after treatment (Fig. 9A, 9C).

Overall statistical analysis

Differences between treatments are significant for all endpoints except F1 adult survival when results for control dung are included. By contrast, few significant differences occur when they are excluded (only F1 brood ball production and F2 emergence for week 1). Thus, most of the variance is contributed by differences between control and treated dung (Tables 1, 2) indicating that *product x* had a strong deleterious effect on breeding by *E. intermedius* with different methods of administering the ectoparasiticide having little significant influence (Appendix 2).

Over time, differences between treatments are significant for four endpoints when results for control dung are included (F1 adult survival, F1 brood ball production and F2 emergence for week 1, F3 emergences). However, they are significant for all endpoints when results for control dung are excluded. Considering that slopes of decline over time in control pads are greater than in treatment pads (Figs 1-7) this result contradicts what one would expect intuitively.

In a number of cases, between-treatment trends and between-days trends are not consistent resulting in significant interactions. These mostly comprise the same variables whether or not controls are included or excluded (F1 brood ball production and F2 emergences for week 1, F2 brood balls and F3 emergences).

Discussion

Adult survival was, apparently, not compromised by *product x* but overall breeding success was significantly lower in dung from treated cattle than in control dung. There were limited differences between treatments despite differences in the route of administration and despite differences in faecal residues over time. Although one would intuitively expect no decline in control results over time, they, in fact, declined to converge with those for treated dung over days 1-28. This decline remains unexplained.

Adult survival ranged from no mortality on day 1 in control dung to some mortality on days 1-28 in all treatments, including later controls. No significant differences were found between any of the treatments and the control. The significant differences in results over time are solely due to results for day 28 for T1 and T3. However, as mortality of adults was greater in control dung than in treated dung in 50% of paired cases, it may be concluded that adult mortality in dung from treated cattle is indistinguishable from natural mortality in control dung.

Breeding success in dung of cattle treated with *product x* was consistently lower than in dung of untreated cattle. However, different routes of administering *product x* to cattle apparently produce a limited difference in response to *product x* residues in their dung. Breeding success in T3 was consistently greater than in the other treatments. Although, on average, breeding success was lower than that in control dung these differences were mostly not significant. Except for week 2 results for T1, breeding success in treatments T1, T2, and T4 was significantly lower than in control dung in all instances.

In two treatments, little change in faecal concentrations was observed over time after treatment of cattle (T1, T2) whereas residue levels declined over time in the other two treatments (T3, T4). One would predict similar depressed reproductive success in response to similar levels of *product x* residues and increases in reproductive success as levels of antiparasiticide residues fall over time. However, for the most part, there were few significant differences in breeding success over time and these showed no consistent

pattern that could be ascribed to changes or lack of changes in levels of *product x* residues over time.

Theoretically, there should be no decline in brood ball production and breeding success in controls over time. As the experiments were conducted on young, laboratory-bred F1 beetles that were used soon after emergence from brood balls, this decline would not reflect declining fecundity due to aging in the experimental population. Furthermore, there are no similar parallel declines in results yielded from dung of treated cattle. As *product x* is extremely lipophilic it will remain on surfaces, especially plastic. Therefore some form cross-contamination over time may be a more likely explanation. How this might have occurred is uncertain. Errors in labelling of experimental dung have been ruled out. Furthermore, procedures to avoid cross-contamination were strictly adhered to during experiments. However, although equipment was thoroughly washed with detergent and water between each phase of the bioassay as per standard Scarab Research Group laboratory protocol, it now transpires that this may have been insufficient to remove *product x* residues. Liquids containing ethanol and propanol may be necessary for this purpose, such as the surface disinfectant Bacillol®.

Nevertheless, despite the patterns of convergence, overall breeding success in dung from the cattle treated with *product x* was significantly lower than in dung from untreated cattle. However, *product x* administered as topical suspension (T3) demonstrated the least severe effects with F3 adult emergence representing the only endpoint significantly differing from the controls. This finding matches well with the measured residues in dung of treated cattle with T3 resulting in the lowest initial concentration and a continuous decline.

As there is no clear correlation between *product x* concentrations in dung and endpoints describing breeding success (brood ball production and emergence), it is not possible to determine the threshold concentration under which deleterious effects on dung beetles would be unlikely. Equally, it is impossible to assess the how long deleterious effects on dung beetle reproduction persist after treatment of cattle with *product x*. In contrast, adult beetle survival seems not to be affected even at the highest concentration measured, namely 733.3 ng/g.

Conclusion

One may conclude that *product x* has no detectable effect on adult beetle mortality but may strongly influence breeding capacity, leading to reduced brood production. This influence persists into the second generation produced from treated dung.

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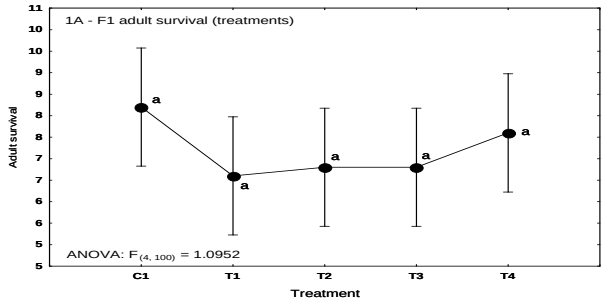
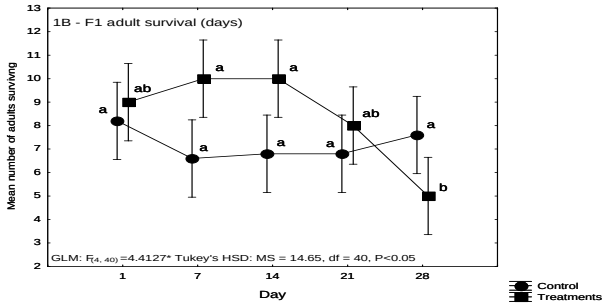
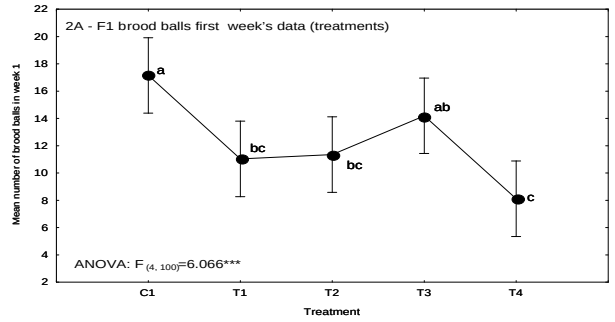
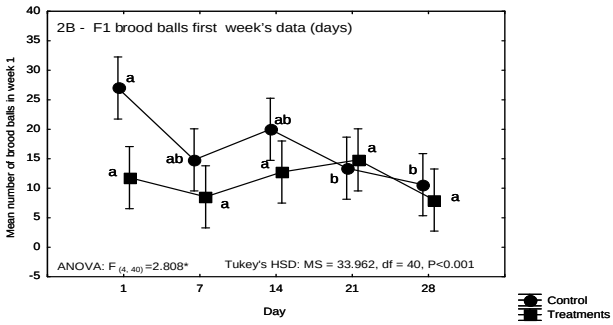
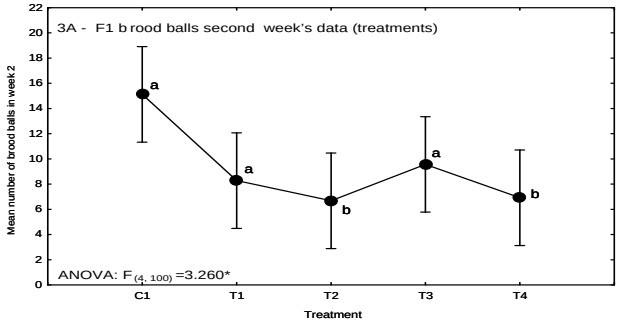
Figures and Tables

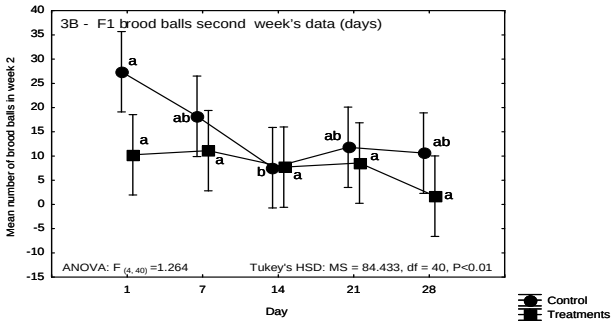


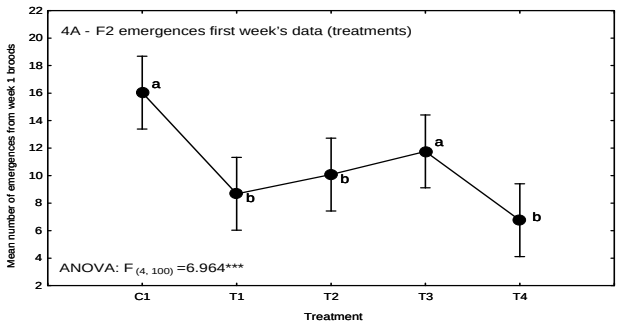


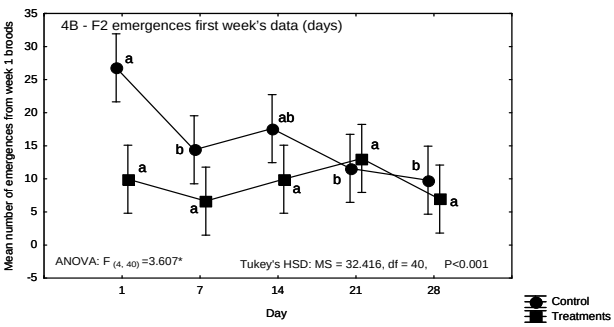


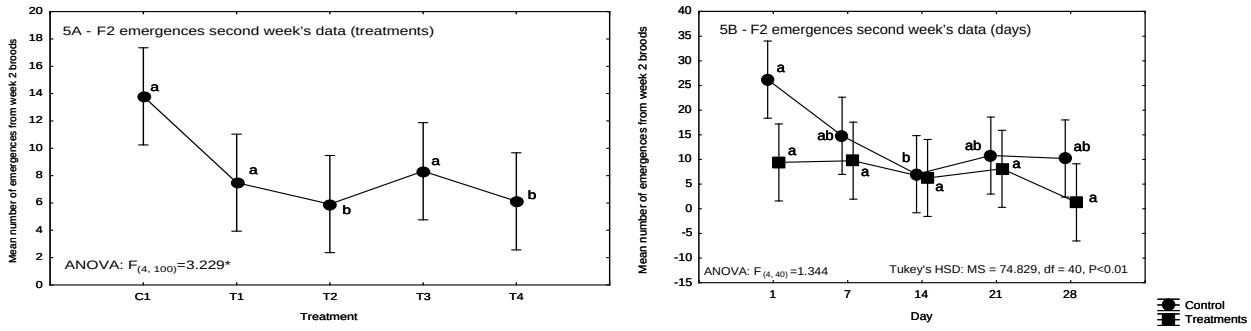






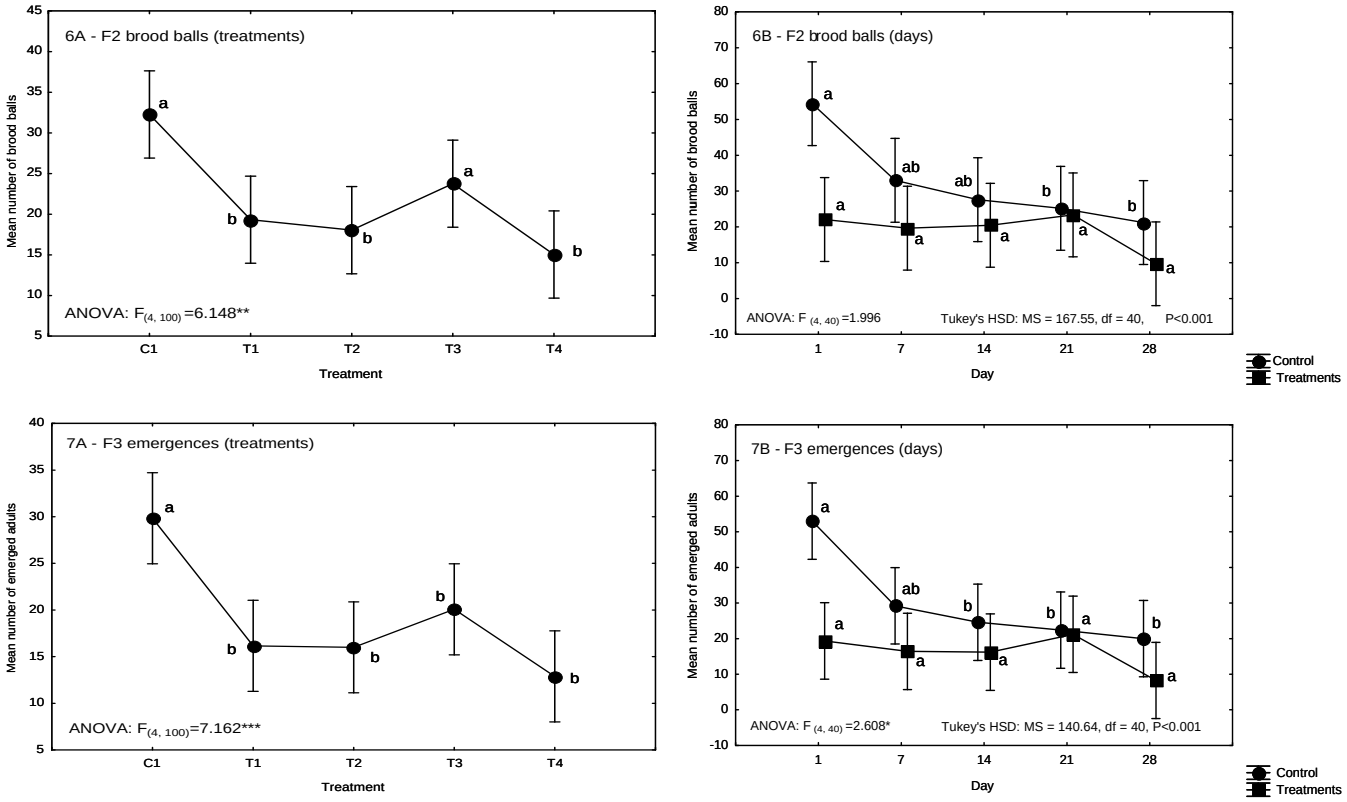






Figures 1A-5A. Comparisons between results for *Euoniticellus intermedius* (F1 generation) from dung of untreated cattle (C1 = control) and those from dung of cattle treated with *product x* using four different routes of administration (T1-T4). **Figures 1B-5B.** Comparisons between results for *Euoniticellus intermedius* from dung 1, 7, 14, 21 and 28 days after treatment with *product x*. **Figures 1A, 1B**. Mean number of F1 adult surviving 15 days; **Figures 2A, 2B**. Mean number of F1 brood balls produced during week one; **Figures 3A, 3B**. Mean number of F1 brood balls produced during week two; **Figures 4A, 4B**. Mean number of F2 adults emerging from week one brood balls; **Figures 5A, 5B**. Mean number of F2 adults emerging from week two brood balls.

Data points followed by different letters differed significantly ($P< 0.05$, Tukey's HSD).



Figures 6A-7A. Comparisons between results for *Euoniticellus intermedius* (F2 generation) from dung of untreated cattle (C1 = control) and those from dung of cattle treated with *product x* in four different routes of administration (T1-T4). **Figures 6B-7B.** Comparisons between results for *Euoniticellus intermedius* from dung voided 1, 7, 14, 21 and 28 days after treatment with *product x*. **Figures 6A, 6B**. Mean number of F2 brood balls produced during weeks one and two; **Figures 7A, 7B**. Mean number of F3 adults emerging from brood balls produced in weeks one and two.

Data points followed by different letters differed significantly ($P< 0.05$, Tukey's HSD).

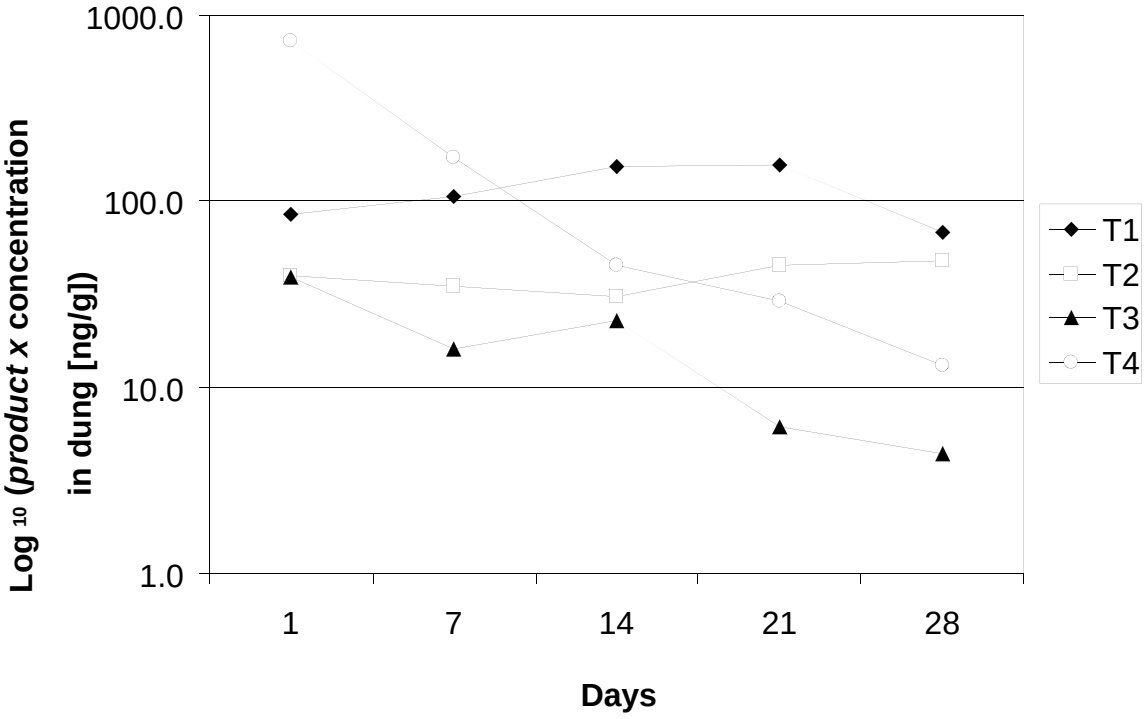
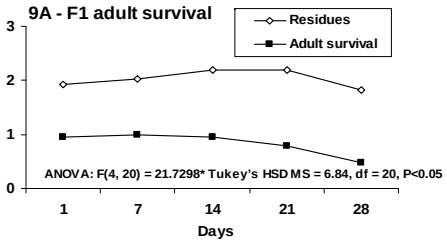
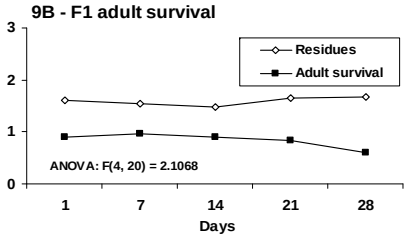
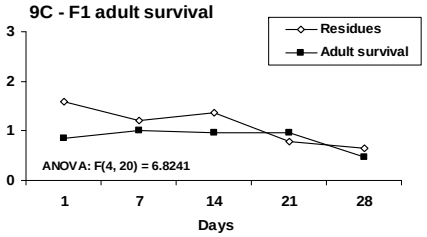


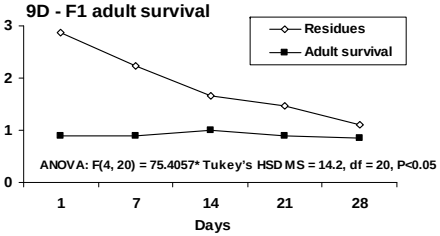
Figure 8. Concentration of *product x* residues remaining in dung voided 1-28 days after treatment in four different routes of administration (T1-T4). Residue concentrations measured in ng/g and log_{10} transformed.

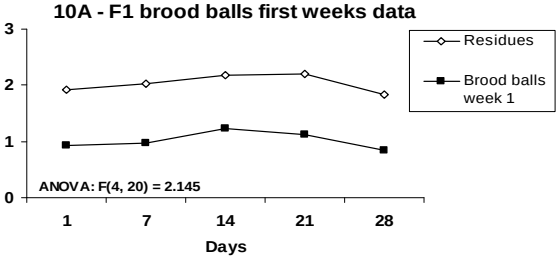
Treatment 1	Treatment 2	Treatment 3	Treatment 4

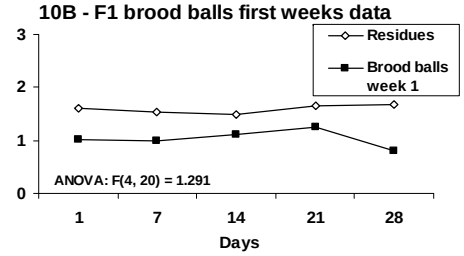


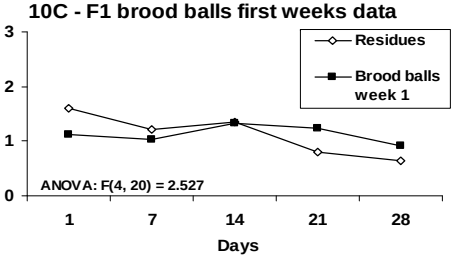


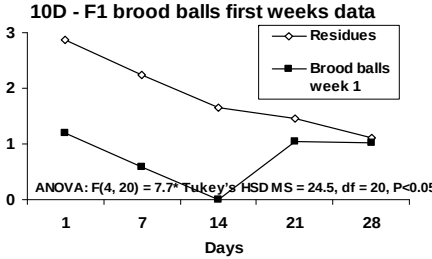


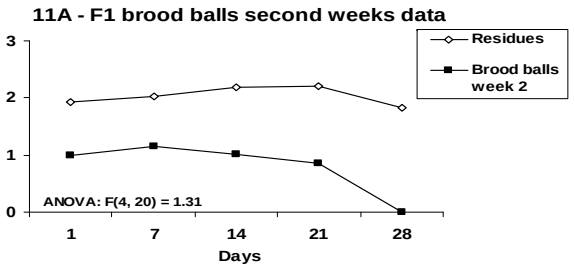


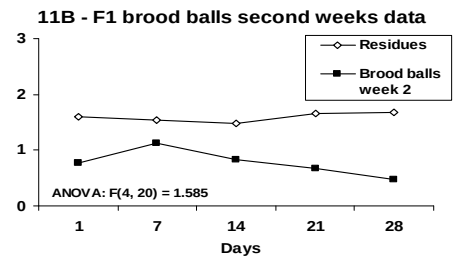


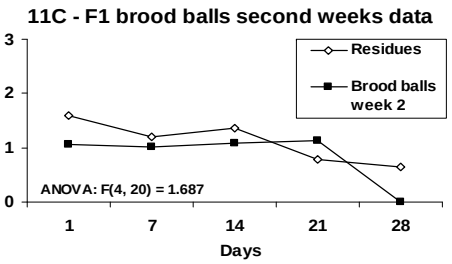


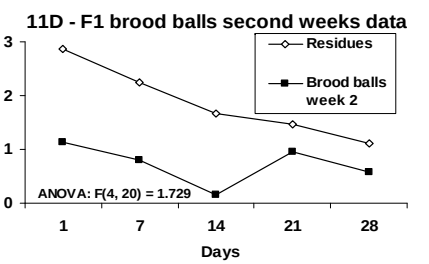


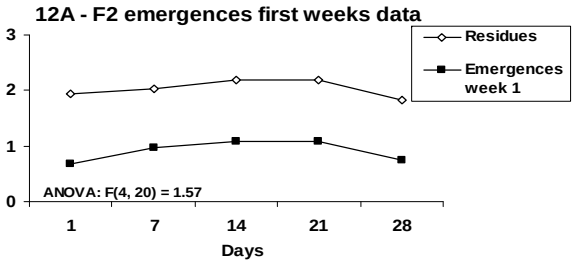


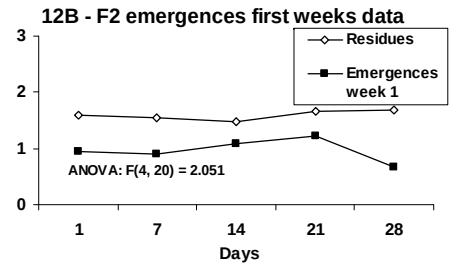


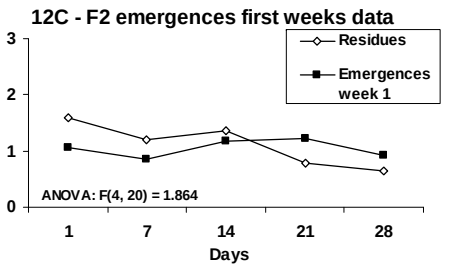


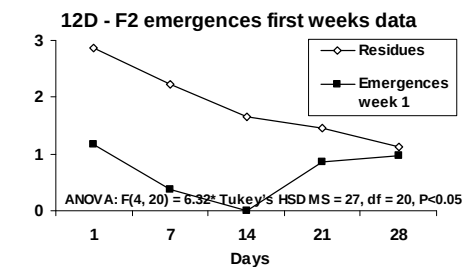


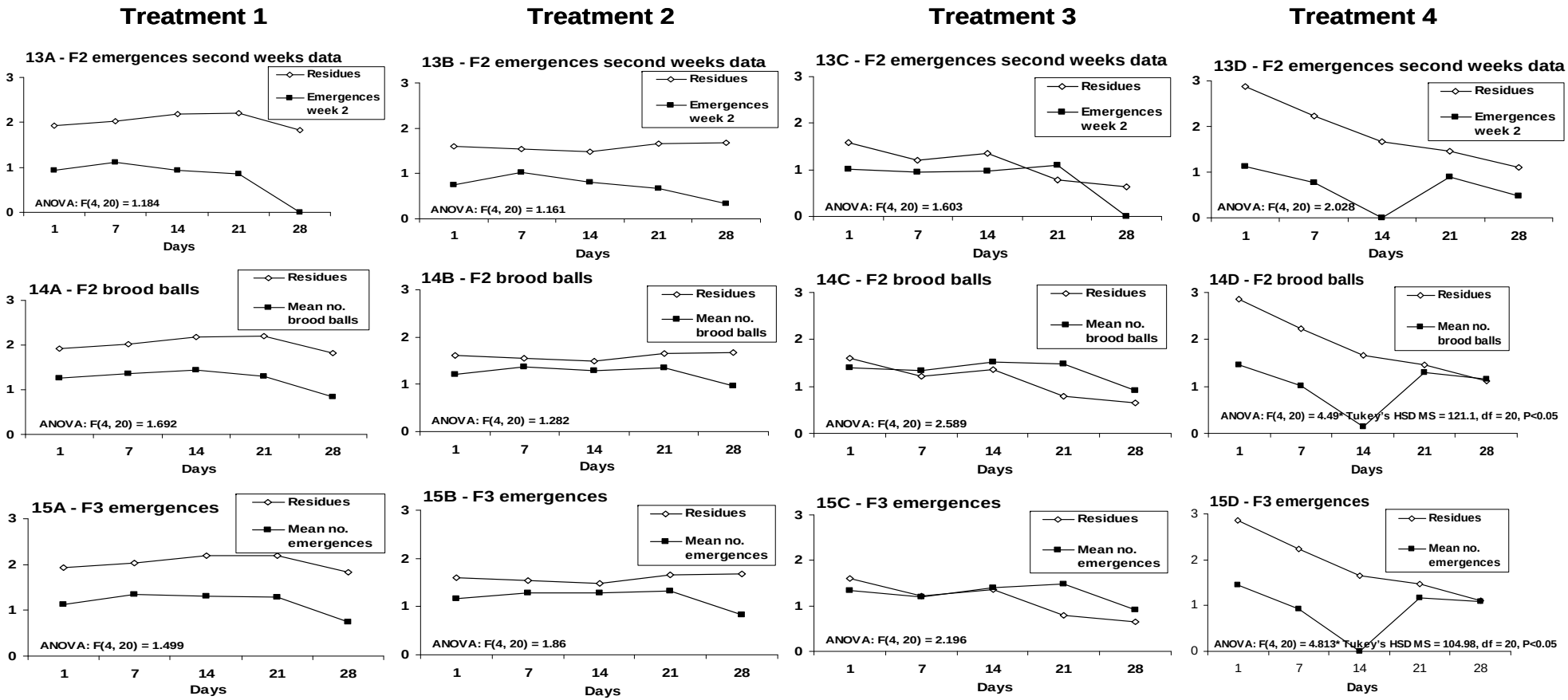












Figures 9A – 15D. Patterns of dung beetle response to *product x* residues in dung voided from 1-28 days after treatment in four different routes of administration (T1-T4). Both numbers for beetles and residue concentrations (ng/g) $\log_{10}$ transformed. GLM one way ANOVA results for dung beetles only.

Table 1. *F*-values obtained for comparisons between results for *Euoniticellus intermedius* (F1-F3 generations) from dung of untreated cattle (control) and those from dung of cattle treated with *product x* (T1-T4), which was voided on five different occasions over time after treatment (1, 7, 14, 21 and 28 days).

Controls included	Treatments	Days	Treatments*Days
F1 adult survival	1.095	5.857*	1.570
F1 brood balls first week's data	6.066***	2.808*	2.489**
F1 brood balls second week's data	3.260*	1.264	1.036
F2 emergences first week's data	6.964***	3.607*	2.656*
F2 emergences second week's data	3.229*	1.344	1.005
F2 brood balls	6.148**	1.996	1.982*
F3 emergences	7.162***	2.608*	2.216*

*P<0.05, **P<0.01, ***P<0.001

Table 2. *F*-values obtained for comparisons between results for *Euoniticellus intermedius* (F1-F3 generations) from dung of cattle treated with *product x* (T1-T4), which was voided on five different occasions over time after treatment (1, 7, 14, 21 and 28 days).

Controls excluded	Treatments	Days	Treatments*Days
F1 adult survival	0.630	3.215*	0.328
F1 brood balls first week's data	3.349*	3.575*	2.392*
F1 brood balls second week's data	0.576	3.535*	0.891
F2 emergences first week's data	2.741*	3.392*	2.323*
F2 emergences second week's data	0.482	3.520*	0.793
F2 brood balls	2.135	3.815**	1.980
F3 emergences	1.726	3.894*	1.992*

*P<0.05, **P<0.01, ***P<0.001

Appendix 1. Average numbers from control versus treatment dung (each of the F1 brood, F2 and F3 values represents mean data from five replicates).

	F1 adult Survival*		F1 broods Week 1		F1 broods Week 2		F2 beetles Week 1		F2 beetles Week 2		F2 broods		F3 beetles	
	Control	**T1**	**Control**	**T1**	**Control**	**T1**	**Control**	**T1**	**Control**	**T1**	**Control**	**T1**	**Control**	**T1**
1	10	7	27.0	8.6	27.4	9.8	26.8	4.8	26.2	8.4	54.4	18.4	53.0	13.2
7	10	8	14.8	9.4	18.2	14.0	14.4	9.2	14.8	13.2	33.0	23.4	29.2	22.4
14	7	9	20.0	17.2	7.6	10.4	17.6	12.0	7.0	8.6	27.6	27.6	24.6	20.6
21	7	6	13.4	13.2	11.8	7.2	11.6	11.8	10.8	7.2	25.2	20.4	22.4	19.0
28	7	3	10.6	6.8	10.6	0.0	9.8	5.6	10.2	0.0	21.2	6.8	20.0	5.6
	Control	**T2**	**Control**	**T2**	**Control**	**T2**	**Control**	**T2**	**Control**	**T2**	**Control**	**T2**	**Control**	**T2**
1	10	6	27.0	10.2	27.4	5.8	26.8	8.8	26.2	5.6	54.4	16.0	53.0	14.4
7	10	9	14.8	10.0	18.2	13.4	14.4	8.0	14.8	10.8	33.0	23.4	29.2	18.8
14	7	8	20.0	12.6	7.6	6.6	17.6	12.4	7.0	6.4	27.6	19.2	24.6	18.8
21	7	7	13.4	17.6	11.8	4.6	11.6	16.6	10.8	4.6	25.2	22.2	22.4	21.2
28	7	4	10.6	6.4	10.6	3.0	9.8	4.6	10.2	2.2	21.2	9.4	20.0	6.8
	Control	**T3**	**Control**	**T3**	**Control**	**T3**	**Control**	**T3**	**Control**	**T3**	**Control**	**T3**	**Control**	**T3**
1	10	5	27.0	13.0	27.4	11.6	26.8	11.4	26.2	10.4	54.4	24.6	53.0	21.8
7	10	10	14.8	11.0	18.2	10.6	14.4	7.0	14.8	9.0	33.0	21.6	29.2	16.0
14	7	9	20.0	21.2	7.6	12.4	17.6	15.4	7.0	9.4	27.6	33.6	24.6	24.8
21	7	7	13.4	17.6	11.8	13.2	11.6	16.8	10.8	12.8	25.2	30.8	22.4	29.6
28	7	3	10.6	8.2	10.6	0.0	9.8	8.2	10.2	0.0	21.2	8.2	20.0	8.2
	Control	**T4**	**Control**	**T4**	**Control**	**T4**	**Control**	**T4**	**Control**	**T4**	**Control**	**T4**	**Control**	**T4**
1	10	7	27.0	15.4	27.4	13.8	26.8	14.8	26.2	13.2	54.4	29.2	53.0	28.0
7	10	7	14.8	3.8	18.2	6.4	14.4	2.4	14.8	6.0	33.0	10.2	29.2	8.4

	F1 adult Survival*		F1 broods Week 1		F1 broods Week 2		F2 beetles Week 1		F2 beetles Week 2		F2 broods		F3 beetles	
14	7	9	20.0	0.0	7.6	1.4	17.6	0.0	7.0	0.6	27.6	1.4	24.6	0.6
21	7	8	13.4	10.8	11.8	9.2	11.6	7.2	10.8	7.8	25.2	20.0	22.4	15.0
28	7	7	10.6	10.6	10.6	3.8	9.8	9.4	10.2	3.0	21.2	14.4	20.0	12.4

*F1 adult survival after two weeks from a starting point of 10 individuals (5 male, 5 female).

Appendix 2. Regression pictures showing similarities between treatments and differences between treatments and controls.