Detection of anthelmintic resistance of gastrointestinal nematodes infecting Senepol, Charbray*Senepol, and Charolais* Senepol heifers in Puerto Rico

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Detection of anthelmintic resistance of gastrointestinal nematodes infecting Senepol, Charbray*Senepol, and Charolais*Senepol heifers

Summary

The effects of four anthelmintics on nematode fecal egg counts in beef heifers was determined. Senepol [n= 22] and crossbred [Charolais*Senepol and Charbray*Senepol; n= 17] heifers were distributed among treatments with: albendazole (Valbazen®), imidazothiazole (Levasole®), doramectin (Dectomax®) and ivermectin (Ivomec®). Egg counts per gram of feces (EPG) were determined before and after administration of the drugs in order to evaluate their efficacy. Participation in the experiment was limited to animals that presented an EPG ≥ 50 and those included were treated only once (day 0) with one of the anthelmintics. The EPG was monitored on days 0, 9, 15, 22, 29, 36, 43, 60 and 90 after treatment. Animals treated with Ivomec® presented an 86% reduction in nematode egg count, while Levasole® and Dectomax® reduced the FEC by 100% and Valbazen® by 98%. In conclusion, no resistance was detected against Levasole®, Dectomax® and Valbazen®, all of which achieved a nematode egg reduction ≥95%. On the other hand, these results suggest nematode resistance to Ivomec®.

Efecto de los antihelmínticos sobre el conteo de huevos de nemátodos gastrointestinales que infectan a novillas Senepol, Charbray*Senepol y Charolais* Senepol

Resumen

Se determinó el efecto de cuatro antihelmínticos sobre el conteo de huevos de nemátodos en muestras fecales de novillas de reemplazo. Novillas (Senepol [n=22] y cruces (Charolais*Senepol y Charbray*Senepol) [n=17]) fueron distribuidas entre los siguientes tratamientos: albendazole (Valbazen®), imidazothioazole (Levasole®), doramectina (Dectomax®) e ivermectina (Ivomec®). Se seleccionó para participación en el experimento animales que presentaron ≥ 50 huevos de nemátodos por gramo de heces y estos se trataron con uno de los antihelmínticos una sola vez (día 0). Se determinó el EPG los días 0, 9, 15, 22, 29, 36, 43, 60 y 90 después del tratamiento. Animales tratados con Ivomec® tuvieron una reducción de EPG de 86% mientras que Levasole® y Dectomax® redujeron el EPG al 100% y Valbazen® al 98%. En conclusión, no se detectó resistencia contra Levasole®, Dectomax® y Valbazen®, ya que los tres redujeron los huevos de nemátodos ≥ 95%. En cambio, los resultados de esta investigación sugieren resistencia de los parásitos a Ivomec®.

Dedication

I dedicate this dissertation to my family, especially to my parents and brother, without your support and advices I would not be here. Dear family… we finally finished!

I also dedicate this work to my mentor, friend and co-advisor Dr. Melvin Pagán. Thanks for your guidance and your trust, without your help I would be in the middle of nowhere. You know how much I appreciate you. Thanks for calling me at least once a month during the last four years to check on me and to remind me every time why I had to complete this project. I truly appreciate your persistence and faith in me.

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Chapter I

Literature Review

1.1. Introduction

The beef industry contributes $25.6 million to the agriculture gross income of Puerto Rico that totaled $788.9 million (Censo Agrícola, 2011). The production efficiency of beef production is importantly affected by parasitism (Stromberg and Gasbarre, 2006). Despite the important economic impact of parasitism in the cattle industry the first and last published report of parasitism effect on bovines in Puerto Rico appeared twenty-seven years ago. Frame and Bendezú (1987) identified *Fasciola hepatica*, *Cotylophoron cotyophorum* and *Haemonchus contortus* as common parasites infecting local cattle.

Although cattle can be infected by nematodes, cestodes, and trematodes; nematodes, also known as roundworms, are considered the internal parasites that cause the greatest economical losses (Gadberry et al., 2006; Hendrix and Robinson, 2006). Natural immunity only delays the development of acquired parasitic larvae in bovines more than two years old (Gasbarre et al., 2004). Therefore, calves and heifers are at greater risk of gastrointestinal parasitism than adult cattle. Twenty years ago Waller (1994) stated that in the USA resistance of nematodes to common anthelmintics was mainly observed in intensively managed goat farms, but it was also seen in isolated, individual cattle farms. At present, nematode resistance is known to be a generalized

problem in the United States. Nematodes of the genera *Cooperia* and *Haemonchus* have demonstrated resistance to macrocyclic lactones (**ML**) in most of the examined operations in the United States (Gasbarre, 2014). These resistant parasites can cause economic losses by decreasing feed intake and feed utilization; and consequently animal productivity.

Currently, there are only three groups of broad-spectrum anthelmintics available for the control of nematodes. These groups and the active ingredient are: (1) benzimidazoles (**BZ**); (2) imidazothiazoles (levamisole, [**LEV**]) and hydropyrimidines (pyrantel/morantel); and (3) macrocyclic lactones (avermectins and milbemycins). Each group has a different mechanism of action (Coles et al., 2006). No new anthelmintics with different mechanisms of action are expected on the market in the near future. Therefore, maintain the efficacy of the three existing anthelmintic groups is essential (Coles et al., 2006). Farmers need to understand that rational use of anthelmintics is important because of the potential resistance problem and the increased public concern about the use of chemicals in food animal production (Torres-Acosta and Hoste, 2008).

1.2. **Heifer's Health**

A heifer is a young female bovine under two years of age that has not had a calf. Providing good care to calves and heifers is essential to obtain healthy adult cows. Ensuring adequate colostrum consumption within the first hours of life is crucial for the development of the neonate's immune system.

Puberty occurs once the reproductive cycle starts and the animal is able to become pregnant (Hoffmann and Plourd, 2003). Factors like breed, environment, body composition, bodyweight, sex, management, skeletal growth and nutrition can affect the age at puberty (Hafez and Hafez, 2000). In general, when heifers reach 60 to 65% of their mature weight farmers should start the reproduction cycle of heifers (Hoffmann and Plourd, 2003).

Several factors influence the development of heifers and play an important role in their performance as adults. On average, heifers should reach insemination/breeding age between 14 – 15 months of age but this is affected by nutrition, management and breed (Hoffmann and Plourd, 2003). Smaller breeds tend to mature before that age (Hoffmann and Plourd, 2003). Health problems like infections with *E. coli*, *Mycobacterium paratuberculosis* (Johne's disease), pneumonia, bovine viral diarrhea virus (BVDV) and gastrointestinal parasites can negatively affect the synchronization between age and body weight resulting in an older age at breeding (Hoffmann and Plourd, 2003).

1.3. **Parasites**

Parasitism is an association between two organisms, the parasite and the host, in which the former takes advantage from the latter. Parasites can be classified according to the place where they live. Those that live on the surface of the animal are called ectoparasites and the ones that live inside the animal, endoparasites (Hendrix and Robinson, 2006).

According to the severity of its effects parasitism can be divided into the two types: clinical and subclinical (Gadberry et al., 2006). Subclinical parasitism affects the productivity of the animal, such as milk yield and weight gain without causing apparent illness. Although in clinical parasitism early exposure to parasites is important for the build-up of natural immunity (Gasbarre, 2014). Calves and heifers with clinical parasitism may suffer from diarrhea, weight loss, grow slowly and consequently delay puberty, breeding and calving age (Gadberry et al., 2006). Young and stressed bovines are more susceptible than adults to parasitic infection. The main internal parasites that affect cattle belong to the phyla, Platyhelminthes and Nematoda.

1.3.1. **Phylum Platyhelminthes**

1.3.1.1. Class Trematoda

Trematodes have an indirect life cycle with many stages before becoming adults. The adults usually reside inside the intestines, bile ducts, lungs, blood vessels or other organ of the host (Bowman, 2009). *Fasciola hepatica* known as "liver fluke" commonly infects ruminants. Surface water and a lymnaeid snail (e.g., *Lymnaea trunculatula*) are necessary in order to complete the life cycle of trematodes. Some of the symptoms caused by *F. hepatica* are: progressive weakness, anemia, hypoproteinemia resulting in edema of the intermandibular region and abdomen. One important effect of *F. hepatica* is the condemnation of infected livers in slaughterhouses and according to the Centers for Disease

Control and Prevention (2013a), liver flukes can infect humans who consume infected liver. The presence of this parasite does not necessarily severely affect the animal's health but it can cause economic losses to producers (Bowman, 2009).

1.3.1.2. Class Cestoda

Cestodes are commonly known as tapeworms and their life cycle usually requires at least two hosts for completion. Some members of the Taeniidae family are zoonotic, humans being the definitive host (where sexual reproduction of the parasite occurs) and animals like pigs, cattle, and sheep serve as intermediate hosts (Bowman, 2009). There are several members within this group but *Taenia saginata* is the one that infects cattle. After extra-intestinal migration this worm resides in the muscle, hence the risk to infect humans who consume raw beef (Bowman, 2009). As with *F. hepatica*, infection of an animal with *T. saginata* can result in condemnation of muscle resulting in economic losses to the producer and the industry (CDC, 2013b).

1.3.2. **Phylum Nematoda**

Nematodes are successful animal parasites because of their adaptive capacity (Grencis and Hartnett, 2011). The body form varies little among nematodes, which makes identification and taxonomic classification difficult (Bowman, 2009). The life cycle of nematodes can be generalized as consisting of four stages: adult, pre-infective, infective and pre-adult; and it can also be

separated by four transitions: contamination, development, infection and maturation (Bowman, 2009).

Nematodes are considered to be the internal parasites that cause the greatest economical losses in livestock (Gadberry et al., 2006). In the US more than $2 million per year are loss in productivity and operational costs due to infection of gastrointestinal nematodes in beef and dairy cattle (Stromberg and Gasbarre, 2006). Parasitic nematodes can be classified in two groups according to the place where they reside, gastrointestinal (**GI**) nematodes and tissue-dwelling nematodes (Grencis and Harnett, 2011). Tissue- dwelling nematodes generally belong to the Filarioidea superfamily of the Order Spirurida. Gastrointestinal nematodes that commonly infect cattle generally belong to the Trichostrongyloidea superfamily of the Order Strongylida.

1.3.2.1. **Order Strongylida**

Strongyloidea, Trichostrongyloidea, Ancylostomatoidea and Metastrongyloidea are the four superfamilies grouped in this order. The life cycle of members of these four superfamilies is direct except some genera of the Metastrogyloidea (i.e. *Dirofilaria immitis*) (Bowman, 2009). Eggs of all these nematodes have the same appearance under the microscope, therefore larval stages are used for identification.

1.3.3. **Gastrointestinal nematodes**

Most common gastrointestinal nematodes that infect cattle have a direct life cycle. In the host, adult nematode produce eggs that eventually are expelled within feces. A larva hatches from the egg and molts two times before it becomes a third-stage larva (L_3) also known as infective stage (Hendrix and Robinson, 2006). Infection occurs when the animal consumes the third-stage larva while eating grass, and the larva completes its cycle in the gastrointestinal tract of the host (Gadberry et al., 2006). A summary of the general life cycle can be observed in Figure 1.1.

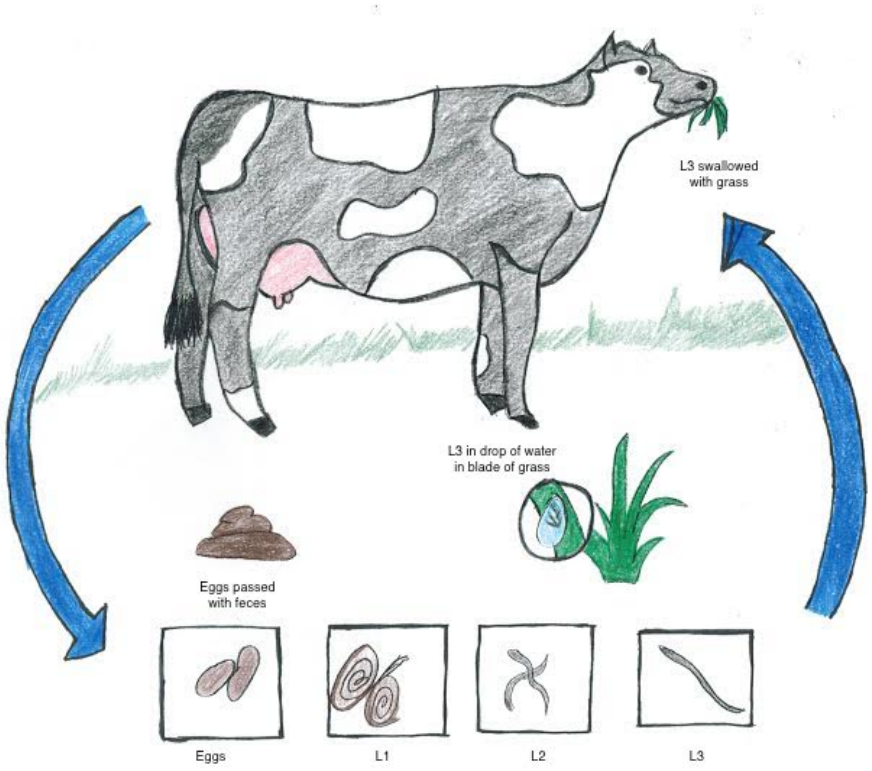

Figure 1. 1: General life cycle of gastrointestinal nematodes (Adapted from Bowman, 2009).

The Family Trichostrongyloidea includes *Ostertagia spp., Haemonchus spp., Trichostrongyles spp.,* and *Cooperia spp.*, which commonly infect ruminants and are responsible for causing parasitic gastroenteritis (**PGE**). Subclinical PGE affects the herd causing dramatic production losses (Strickland, 2012). Symptoms of PGE include: weight loss, diarrhea, pale mucous membranes, dehydration, rough hair coat and submandibular edema (Bowman, 2009). According to Mulcahy et al. (2004), some of the pathology associated with helminthic infection results from loss of blood due to parasite feeding, competition for nutrients, nutritional dysfunction, interference with gut motility and/or neuromuscular control and blockade of the intestine.

The nematode of major importance in cattle is *Ostertagia ostertagi*, commonly known as “brown stomach worm”. *Ostertagia ostertagi* causes major economic losses in the beef and dairy industries (Mulcahy et al., 2004; Gasbarre, 2014). Young animals infected with *O. ostertagi* can develop chronic abomasitis and exhibit profuse watery diarrhea, anemia and hypoproteinemia, which can be clinically appreciated as submaxillary edema (“bottle jaw”) (Taylor et al., 2007). Animals with a severe infection will look emaciated but have intact appetite. Necropsy of severely infected animals will reveal a mucosa dotted with grayish white, pinhead- to pea-sized nodules with a worm protruding from a small opening (Bowman, 2009). Importantly, *Ostertagia ostertagi* has the ability to undergo arrested development and enter a latent phase until conditions are ideal for its development.

Haemonchus contortus and *Haemonchus placei* nematodes infect the abomasum of ruminants (Taylor et al., 2007). The female worm has a white, egg-filled uterus that spirals around the blood–filled gut, for which this nematode is known to have a barber pole appearance. If the parasite load with *H. placei* is high the bovine host will suffer anemia (Taylor et al., 2007). This parasite is more common in small ruminants in which it can be extremely detrimental. High egg counts (> 10,000 EPG) are typical of haemonchosis (infection with *Haemonchus*) (Bowman, 2009). Paleness of the skin and mucous membranes can be used as a sign of infection with *H. contortus*. In small ruminants the use of FAMACHA (derived from name of the creator of this system) helps to identify animals that need antiparasitic treatment (Bowman, 2009). However this method cannot be used accurately in bovines.

Nematodirus helvetianus can also infect cattle and can be identified as to genus and species using a fecal flotation technique. An increase in numbers of infections with *N. helvetianus* in the United States has been reported (Gasbarre, 2014). High *Nematodirus helvetianus* loads can be severely pathogenic to young animals (Gasbarre, 2014).

Infections with *Trichostrongylus* are usually asymptomatic but when the parasite load is sufficiently high the animals can develop watery diarrhea (Taylor et al., 2007). Necropsies of infected animals do not show obvious lesions in the small intestines and the parasites themselves are difficult to notice because of their relatively small size (Bowman, 2009).

1.4. **Anthelmintics**

There are three groups of broad-spectrum anthelmintic drugs in use against gastrointestinal nematodes. The mechanism of action of these drugs involves either selective binding of the drug to receptors in the nervous system of the parasite or disruption of important metabolic routes of the parasite (James et al., 2009). In United States, apromimately more than $500 million are spent in anthelmintics to control gastrointestinal infection with nematodes (Stromberg and Gasbarre, 2006).

1.4.1. Macrocyclic Lactones (**ML**)

Macrocyclic lactones were introduced in the early 1980's and they are still the leading worldwide antiparasitic agents used in livestock (González-Canga et al., 2009). This group includes avermectins and milbemycins which are naturally occurring fermentation products of several actinomycetes of the genus *Streptomyces* (Barragry, 1987). Ivermectin, abamectin, doramectin, eprinomectin and selamectin are the available forms of the avermectin group. The most commonly used avermectin is ivermectin. Milbemycin oxime (i.e. Interceptor®) and moxidectin (i.e. Advantage Multi®) are the milbemycins commercially available. As a category, MLs are effective at low doses, are very safe and provide broad-spectrum activity against nematodes, including most larvae and adult forms. Macrocyclic lactones are also active against arthropod parasites (Bowman, 2009; González-Canga et al., 2009).

Ivermectin and doramectin are toxic to the parasites by potentiating their glutamate-gated chloride ion channels. The permeability to chloride ions is increased causing hyperpolarization of nerve cells resulting in paralysis and death of the parasites (Papich, 2010). Mammals are not commonly affected by ivermectin because of the absence of glutamate-gated chloride channels. Macrocyclic lactones inhibit neurotransmission of nematodes inducing flaccid paralysis and also interfere with nematodes' feeding, movement and egg laying ability (González-Canga et al., 2009). However, ML does not have ovicidal properties and only eliminates larval stage parasites. The route of administration affects bioavailability of the drug. The greatest bioavailability is achieved with subcutaneous injection (**SC**). The lowest values for area under the curve (AUC; a measure of total systemic exposure to the drug) are obtained after topical application of the product (González-Canga et al., 2009; Turner, 2014). When ivermectin is administered SC, it has anthelmintic activity against common gastrointestinal nematodes, lasting for approximately 10 days (González-Canga et al., 2009). The licking behavior of animals to be treated should be evaluated before using the pour-on product. Because after topical administration a substantial amount of the preparation can access the systemic circulation by oral consumption what contributes to the development of parasite resistance (González-Canga et al., 2009).

In pre-slaughter meat animals, ivermectin withdrawal times are 35 days for SC injection and 48 days for topical application ("pour-on") (Papich, 2010). No

withdrawal time has been established in the case of milk for market (NADA # 128-409, 2014).

Some studies suggest that ML resistance in nematodes arises from mutations in the glutamate-gated chloride ion channel receptors (Blackhall et al., 1998; Njue and Prichard, 2004). In their review paper Kotze et al. (2014) conclude that no mutation has been identified that can explain the resistant phenotypes observed in most field nematode isolates.

1.4.2. Benzimidazoles (**BZ**)

Benzimidazoles represent a large family of broad-spectrum drugs. They were first marketed as fungicidal agents in the 1960's and subsequently have proven to be effective against parasitic nematodes of domestic animals (Bowman, 2009; Merck, 2012). The BZs bind to intracellular β-tubulin in parasites, preventing the microtubule formation required for cell division (Papich, 2010; Kotze et al, 2014). The molecules of this class have higher affinity for nematode tubulin than for mammalian tubulin. Therefore BZs provide selective activity against parasites (Bowman, 2009). Benzimidazoles can inhibit fumarate reductase, which blocks mitochondrial function, depriving the parasite of energy and hence causing its death (Bowman, 2009). One notable characteristic of BZs is their ovicidal capacity in addition to their inhibition of egg production (Bowman, 2009). Albendazole is the newest BZ and it has a potent broad-spectrum activity while offering a wide safety margin in cattle when used according to label

specification. The withdrawal time of albendazole is 27 days for meat and not established for milk from treated animals (Papich, 2010).

Resistance to BZs is less prevalent in parasitic nematodes of cattle than in those of small ruminants and horses. The molecular mechanism associated with resistance to BZs in most trichostrongylid nematode species is the possession of a single nucleotide polymorphism (**SNP**) in the isotype-1 β-tubulin gene of the parasite. This SNP, known as F200Y, is a substitution at codon 200 of tyrosine for phenylalanine, encoded by a change from TTC to TAC (Kotze et al., 2014). Two other SNPs associated with BZs resistance have been discovered at codons 167 and 198 in the isotype-1 β-tubulin gene, but F200Y still appear to be the most important with respect to BZ resistant phenotypes (Kotze et al., 2014).

1.4.3. Imidazothiazoles (**LEV**) and Hydropyrimidines

Tetramisole was the first imidazolthiazole discovered in 1966. The molecule was a racemic mixture of two optical isomers but only the L-isomer (levamisole) showed anthelmintic activity (Bowman, 2009). Levamisole is a cholinergic agonist targeting the nicotinic acetylcholine receptors (**nAChRs**) of nematodes. The nAChRs have five subunits that together form a transmembrane ion-channel and different combinations of these subunits allow receptors to have different pharmacological properties (Kotze et al., 2014). Opening of nAChRs channels produces depolarization, allowing the entry of calcium through these channels increasing the sarcoplasmic calcium, which

produce a spastic muscle contraction (Martin et al., 2012). Consequently, the parasite cannot maintain its location and is eliminated within the feces.

Contrary to BZ, LEV does not have ovicidal properties and only eliminates larval stage parasites. After oral administration of LEV the withdrawal time is 2 days for meat animals (NADA # 091-826, 2014). No withdrawal time for milk from treated dairy cows has been established (Papich, 2010).

1.5. **Anthelmintic use and development of resistance**

Parasite control is needed in heifers that are raised on pastures (Gasbarre, 2014). Adult cows may have low fecal egg counts but that does not mean that the animals are free of parasites. Thus, a good deworming program should be established.

In a beef cow-calf operation, calves should be dewormed for the first time at weaning and some producers may need second treatment at the end of the grazing season (Gasbarre, 2014). It is important that a deworming program be tailored according to the farmers' needs, the weather, and herd health. The goal with grazing animals is to maintain low levels of parasite transmission on the pastures. According to Gasbarre (2014), the use of more than one class of anthelmintic during deworming should be encouraged. Using combinations of drugs will slow the development of parasite resistance. Importantly, this practice should be initiated while the anthelmintic products are still effective.

Selective pressure applied against the parasite genome is the cause for resistance development (Gasbarre, 2014). Under-dosing animals with

deworming drugs can significantly promote the development of resistance. Therefore, it is essential to weight all animals or to estimate bodyweight using a girth tape, in order to calculate the therapeutic dose of specific products. Use of pour-on products can also be related to the development of parasite resistance due to variation in the amount of drug that is absorbed through the skin and how it is distributed among individual animals (Leathwick, 2013). Not achieving the desired drug tissue levels contributes to selection for parasite drug resistance (Gasbarre, 2014).

Regular use of broad-spectrum anthelmintics has resulted in the worldwide development of anthelmintic resistant nematode populations (Taylor et al., 2002). Resistance is encountered when a greater frequency of nematodes within a population can tolerate given doses of an anthelmintic when compared to the normal population. Offspring of resistant nematodes are capable of inheriting this characteristic (Prichard et al., 1980). An anthelmintic is fully effective if not a single worm survives after the treatment. However, if any viable eggs are observed in the feces some resistant worms are still present in the animal. Viable eggs observed after anthelmintic administration are considered resistant to the treatment (Coles et al., 2006).

Anthelmintic resistance has been reported in parasitic nematodes that infect almost all species of domestic animals and in some of those parasitic to human beings (Jabbar et al., 2006). Variable degrees of resistance to all major groups of anthelmintics have been reported in different species of GI nematodes (Jabbar et al., 2006). These authors published a summary of resistance reported

instances in small ruminants by genus and species of nematode, anthelmintic resisted, and country, including literature references. This summary demonstrates that resistance is a worldwide problem for small ruminants. Gasbarre (2014) also stated that resistant GI nematodes infecting ruminants occur throughout the world. Surtherland and Leathwick (2010) suggested that determining resistance in cattle is more challenging than in small ruminants because of lesser egg output in feces and differences in the type of therapeutic product used.

Resistance of *Cooperia spp.* to the ML has become common in New Zealand (Familton et al., 2001), is an important problem in Brazil (Anziani et al., 2001, 2004; Fiel et al., 2001) and is present in the UK (Stafford and Coles, 1999) and the USA (Gasbarre et al., 2004). Also, in Argentina, *Cooperia oncophora* was also found to be resistant to ML (Mejía et al., 2003).

The widespread resistance of *Cooperia spp.* against ML includes areas where *Haemonchus spp.* is also resistant to ML. By contrast there appears to be a delayed and less widespread resistance of *Ostertagia ostertagi* to ML (Gasbarre, 2014).

1.6. **Detection of anthelmintic resistance (AR)**

Parasite resistance is suspected when there is an apparent poor clinical response to anthelmintic treatments. However, several factors should be taken in account before deciding on a diagnosis of parasite resistance (Taylor et al., 2002). Anthelmintics can fail to give good results due to factors such as faulty

drenching equipment or under-dosing due to imprecise assessment of bodyweight (Taylor et al., 2002). There is no simple method to detect parasite resistance and the definitive identification requires killing of cattle (Gasbarre, 2014). However, slaughtering animals can be impractical for field studies. Several of the available methods used to detect AR in live animals will be briefly discussed.

1.6.1. Fecal egg count reduction test (**FECRT**)

This method provides an estimation of anthelmintic efficacy by comparing fecal egg counts (**FEC**) before and after treatment (Taylor et al., 2002). The test only measures effects on egg production by mature worms. Nematode eggs in feces are counted at the time of treatment and 10 to 14 days after treatment. If the interval between treatments is less than 10 days, the egg production may be suppressed leading to an overestimation of anthelmintic efficacy with the BZ anthelmintics (Taylor et al., 2002). It has been suggested that 10 to 14 days is the optimal interval to allow sufficient time for the drug to work but also to ensure insufficient time for reinfection and consequent parasite patency (Gasbarre, 2014).

This test is sensitive if 25% or more of the exposed worms are resistant (Jabbar et al., 2006). Naturally or experimentally infected animals can be used as test subjects and the treatment should be administered at the correct label dose rate (Coles et al., 2006). One of the disadvantages to the FECRT is that the results may not accurately estimate anthelmintic efficacy because the

nematode egg output does not always correlate well with actual worm numbers (Jabbar et al., 2006).

1.6.2. The egg hatch test (**EHT**)

Coles et al. (2006) summarized the protocol for this test, which is used for the detection of BZ resistance based on the ovicidal activity of these drugs (Jabbar et al., 2006). The percentage of eggs hatching in the presence of discriminating doses of BZs indicates the proportion of resistant eggs in the sample. However, discriminating doses have not been established for eggs of nematodes infecting cattle.

1.6.3. Larval development assay

Nematode eggs or L_1 larvae are exposed to different concentrations of anthelmintics placed in agar wells in a small test tube containing nutrient medium. The effect of the drugs on the subsequent development into L_3 larvae is measured (Jabbar et al., 2006). This test is claimed to be more sensitive than FECRT and EHT and can detect resistance when only 10% of the worm population carries resistance genes (Jabbar et al., 2006). The test appears to be successful in detecting resistance against BZ and LEV anthelmintics in ruminants.

Because parasite resistance is now a worldwide problem and no anthelmintic with a different mechanism of action is expected in the market it is essential to maintain the efficacy from the three existing groups (Coles et al.,

2006). We hypothesized that nematode resistance to common anthelmintic is an existing problem in Puerto Rico. The objective of the study was to determine if resistant gastrointestinal nematodes were infecting beef heifers using the FECRT method to detect resistance.

Chapter II

Detection of anthelmintic resistance of gastrointestinal nematodes infecting Senepol, Charbray*Senepol, and Charolais* Senepol heifers in Puerto Rico

Abstract

The effect of anthelmintics on the nematode egg count in fecal samples from beef heifers was determined. Heifers, (Senepol [n= 22], and crossbred (Charbray*Senepol and Charolais*Senepol) [n= 17]), from the Isabela Agricultural Experimental Sub-Station of the University of Puerto Rico, were distributed among the following four treatments: albendazole (Valbazen®), imidazothiazole (Levasole®), doramectin (Dectomax®) and ivermectin (Ivomec®). The heifers were 15 months old and weight 263 kg on average. A modified version of McMaster technique, combining concentration and flotation procedures, was used to determine the egg counts per gram of feces (**EPG**) before and after administration of the drugs at the dose recommendations of the manufacturer and thus evaluate their efficacy. Only animals that presented an EPG ≥ 50 were included and treated once (day 0) with one of the anthelmintics. The EPG was monitored on days 0, 9, 15, 22, 29, 36, 43, 60 and 90 after treatment. Body weight (after a 12-hour fast) was also recorded at every sampling date. Animals treated with Ivomec® presented an 86% reduction in nematode eggs, while Valbazen® by 98%. Levasole® and Dectomax® reduced the FEC by 100%. In conclusion, although no resistance against Levasole®, Dectomax® and Valbazen® was detected based on a nematode egg reduction ≥95%, our results suggest resistance to ivermectin in this beef cattle herd.

Efecto de los antihelmínticos sobre el conteo de huevos de nemátodos gastrointestinales que infectan a novillas Senepol, Charbray*Senepol y Charolais* Senepol

Resumen

Se determinó el efecto de cuatro antihelmínticos sobre el conteo de huevos de nemátodos en muestras fecales de novillas de reemplazo. Novillas (Senepol [n=22] y cruces (Charolais*Senepol y Charbray*Senepol) [n=17]) de la Sub-Estación Experimental de Isabela de la Universidad de Puerto Rico fueron distribuidas entre los siguientes tratamientos: albendazole (Valbazen®), imidazothioazole (Levasole®), doramectina (Dectomax®) e ivermectina (Ivomec®). En promedio las novillas tenían 15 meses de edad y pesaban 263 kg. Mediante la técnica McMaster, modificada por combinar técnicas de concentración y flotación, se contabilizó los huevos de nemátodos por gramo de heces (EPG) antes y después de tratar los animales con los antihelmínticos en la dosis recomendada por el fabricante una sola vez (día 0). Solamente animales que presentaron un EPG ≥ 50 fueron incluidos. Se determinó el EPG en los días 0, 9, 15, 22, 29, 36, 43, 60 y 90 después del tratamiento. En cada muestreo los animales fueron pesados luego de un ayuno de 12 horas. Los animales tratados con Ivomec® tuvieron una reducción de 86% en EPG y Valbazen® de 98%. Levasole® y Dectomax® redujeron el EPG al 100%. En conclusión, no se detectó resistencia contra Levasole®, Dectomax® y Valbazen®, en vista de sus reducciones de huevos de nemátodos ≥ 95%, pero estos resultados sugieren resistencia de los parásitos a ivermectina en este hato.

Introduction

In most beef production operations parasitism is one of the primary causes of production losses. Subclinical parasite infection is common in beef cattle; and causes indigestion, poor feed conversion and inadequate weight gain (Gadberry et al., 2006).

There are only three groups of anthelmintics with broad spectrum available for the control of gastrointestinal nematodes in heifers (Coles et al., 2006). These drugs can be classified according to active ingredient: Benzimidazoles (**BZ**), macrocyclic lactones (**ML**) and imidazothiazole (**LEV**). Each group has a different mechanism of action. However, ML (i.e. ivermectin), have been dominating the market since its introduction in the early 1980s (Sutherland and Leathwick, 2011). No new drugs with different modes of action are expected in the near future, therefore knowing the level of anthelmintic resistance (**AR**) and maintaining the efficacy of the existent anthelmintics is essential (Coles et al., 2006).

Formulation of the product and route of administration affect the absorption and distribution of the anthelmintic in the animal (Lifschitz et al., 2000). Broad-spectrum anthelmintics should quickly remove resident worm burdens. If the drug has persistent activity, it will also prevent the establishment of newly ingested infective larvae for a specific period of time following treatment.

In cattle, AR has been increasing during recent years (Gasbarre et al., 2009). Currently, published studies in cattle indicate that AR is common in Argentina, Brazil, Europe and the USA (Coles et al., 2006; Soulsby, 2007;

Gasbarre et. al, 2009b; Gasbarre, 2014). In Puerto Rico published studies evaluating the presence of anthelmintic resistance in nematodes infecting replacement beef heifers are lacking. We hypothesized that nematode resistance to common anthelmintic is an existing problem in Puerto Rico. The objective of the study was to determine if resistant gastrointestinal nematodes were infecting beef heifers using the FECRT method to detect resistance.

Materials and Methods

Facilities, animals and experimental design

The experiment was performed in the northern region of Puerto Rico at the Isabela Agricultural Experimental Sub-station of the University of Puerto Rico (18°28'24.4"N, 67°00'56.0"W) and use Senepol [n= 22] and crossbred [Charolais*Senepol and Charbray*Senepol; n= 17] heifers that on average were 15 months old and 263 kg of bodyweight. Heifers were managed under a rotational grazing system with concentrate supplementation. The experiment was conducted from January through April, 2010.

Anthelmintic doses were administered as recommended by the manufacturers. Ivomec® and Dectomax® are formulated to deliver 200 mcg ivermectin/ kg of body weight when administered SC at a dose of 0.2 mg/kg. One bolus of Levasole® administered orally is recommended for animals weighting between 204 and 341 kg. The dose for oral Valbazen® is 10 mg/kg of bodyweight.

By obtaining a sample of approximately 10 g of feces directly from the rectum the initial fecal egg count (**FEC**) was determined at day 0 and at days 9, 15, 22, 29, 36, 43, 60, and 90 after treatment. Initially a group of 65 heifers were sampled to determine the eggs per gram of feces (**EPG**) values and only animals with ≥ 50 EPG (n= 39 heifers) were used for the experiment. Heifers were individually weighted after a 12-hour fast using a portable scale at the time when fecal samples were collected.

Treatments

Heifers were blocked by breed and weight and then randomly distributed into one of the four treatments. The active ingredient in treatment 1 was imidazothiazole, the commercial name of which is Levasole® (Schering-Plough Animal Health, New Jersey). A bolus of Levasole® contains 2.19 g of levamisole hydrochloride. Recommended doses depend on the animals' weight range. The boluses were administrated orally.

Dectomax® (Pfizer, New York) and Ivomec® (Merial, New Jersey) were used for treatment 2 and 3, respectively. These drugs have macrocyclic lactones as their active ingredient; and are different commercial products but with similar mechanism of action. Dectomax® contains 1% w/v (10 mg/mL) of doramectin and Ivomec® contains 1% w/v (10 mg/mL) of ivermectin; the manufacturers of both recommend subcutaneous injection of the drug. Treatment 4 was oral administration of Valbazen® (Pfizer, New York) which has 11.36% w/v (11.36

mg/mL) of albendazole as the active ingredient. Treatments were individually administered to each heifer only once at day 0.

Diagnostic analyses

*Fecal egg count (**FEC**)*

Fecal egg count was performed within 5 hours after fecal collection in order to assure good condition of the eggs (Taylor et al., 2002). Feces were collected on days: -1 (to determine pre-treatment parasite load), 0 (day of treatment; initial EPG for reduction test), 9, 15 (final EPG for reduction test), 22, 29, 36, 43, 60 and 90 after treatment.

Fecal egg count was performed using a modified McMaster technique as previously described by Mejía et al. (2003). Part of the protocol was adapted to the laboratory facilities; the centrifugation and flotation techniques were combined to increase the probabilities of observing nematodes eggs (Taylor et. al, 2007)

The fecal samples were processed individually; 3 g of fresh feces were combined with 42 mL of distilled water. Using the cone of paper water cup the mixture was filtered through layers of gauze (Tyco Healthcare Group, Massachusetts) and the filtration product was collected into a 50 mL centrifuge tube. The tubes were centrifuged (GMI, Inc., Minnesota – Model: Damon IEC HN-SII) during 2 min at 12, 247x g. After centrifugation, the supernatant was discarded and the precipitate was dissolved with 35 mL of saturated sodium chloride flotation solution. Approximately 1 mL of the solution was transferred

into the two chambers of the McMaster slide. After 2 min the slides were observed under the microscope (Olympus, Illinois - Model: BX40) at 10X magnification. The total number of eggs seen inside the McMaster slide was multiplied by a factor of 50. In this experiment anthelmintic resistance was considered to exist when a value lower than 95% reduction in FECRT was obtained (Coles et al., 2006).

Fecal egg count reduction test (**FECRT**)

The fecal egg count reduction test was used with all anthelmintic groups to detect AR. By comparing fecal egg counts of ruminants before and after treatment the FECRT provides an assessment of anthelmintic effectiveness. The drug efficacy for all treatments was calculated as a percentage by using the formula:

$$Eff = \left(\frac{(pre - post)}{pre}\right) \times 100$$

pre is the average fecal egg count before and post is the fecal egg count after treatment

Statistical Analysis

Statistical Analysis of FECRT

From the FECRT data the arithmetic means (percentage reduction of FEC) and 95% confidence intervals were calculated. The arithmetic means of were analyzed by proc mixed program (SAS software). An additional statistical

analysis was carryout to determine interaction between EPG and genotype. The statistical model used to calculate the overall anthelmintic efficacy was:

$$Y_i = \mu + A_i + \varepsilon$$

Where

Y_i = observations pertaining to the fecal egg count reduction after treatment

μ = overall mean

A_i = treatment effect on fecal egg count reduction

ε = standard error

Statistical analysis to determine nematode resistance

A value of 1 was assigned to animals with a FECRT of ≤ 95% and a value of 2 to animals with a FECRT of ≥ 95%. The data thus coded were analyzed by a glimmix procedure (SAS software). The formula used to determine anthelmintic resistance was:

$$E(Y) = \frac{e^{\beta 0 + \alpha i}}{1 + e^{\beta 0 + \alpha i}}$$

Where:

E (Y) = expected value of the nematode population with resistance

B_0 = Parasite resistance

αi = Independent treatment (Valbazen®, Dectomax®, Ivomec® and Levasole®)

H0 = p ≤ 0.05

Ha = p ≥ 0.05

Statistical Analysis for Body Weight

The arithmetic means of body weight per treatment were analyzed by glimmix procedure (SAS software, Version 9.1). The statistical model was:

$$Y_{ij} = \mu + a_i + \varepsilon_{ij}$$

Where

Y_{ij} = *j*th observation pertaining to the *i*th anthelmintic

μ = overall mean

α_i = fixed effect of anthelmintic treatment ((Levasole®, Ivomec®, Valbazen®, and Dectomax®)

Results

To evaluate the reduction on fecal egg count and anthelmintic resistance of gastrointestinal nematodes to four different anthelmintics a total of 39 beef heifers were initially used. The raw data of EPG values classified by heifers, genotype, and treatment are presented in the appendix A (Table A 1). No interaction was found between EPG and genotype (p=0.98). In Figure 2.1 the percentages of FECRT are presented. Heifers treated with Ivomec® presented an 86.3 ± 5.7% reduction of nematode egg numbers, those treated with Levasole® and Dectomax® each showed 100 ± 5.1% reduction and those given Valbazen® a 98.3 ± 5.1% reduction. Only Ivomec® failed to reduce the FEC by ≥ 95%.

In Figure 2.2 the percentage of heifers that presented a resistant nematode population against each of four different anthelmintics is shown.

Nematode population of heifers treated with Ivomec® were 25 ± 15.3% resistant, whereas the corresponding value for heifers treated with Dectomax® was 9 ± 8.6%, with Valbazen® was 10± 9.4, and no resistance was detected against Levasole®.

Animals were weighed at every visit and no significant ($p=0.95$) effect of treatment on this variable was found. The appendix B (Table B 1) presents the bodyweight of the heifers from the study with genotype and treatment during the experimental period. The mean weights and standard errors per treatment were: Dectomax® 266±24.2 kg; Ivomec®, 267±32.6 kg; Levasole®, 266±2.2 kg; and Valbazen® 258±25.2 kg. Figure 2.3 presents the curves of the body weight change by treatment over the course experiment. Time did have a significant effect on weight ($p<0.0001$). Also a statistical difference ($p=0.0047$) was detected between genotypes in mean weight. Senepol heifers weighed 247±17.1 kg overall and crossbred heifers 35 kg more at 282±20.9 kg. Figure 2.4 shows the mean weights by genotype during the 90 days of experimentation.

Discussion

According to the present results, Ivomec® did not achieve an adequate reduction of the nematode egg count of ≥ 95%. This is indicative of resistance to Ivomec® in the nematode population of heifers from the Isabela Agricultural Experimental Sub-Station of the University of Puerto Rico. The FECRT analysis detected no resistance against Dectomax®, Valbazen® and Levasole®, as these treatments achieved a reduction of nematode eggs numbers ≥ 95%.

In addition to determining nematode resistance to a treatment when defined as not achieving a reduction of ≥ 95% in the FECRT; resistance was also determined with a binomial statistical model. None of the heifers treated with Levasole® showed nematodes with resistance to this product. However, Dectomax®, Valbazen® and Ivomec® treatments presented evidence of nematode resistance. Evaluating resistance with this alternative method allowed early identification of heifers with nematodes resistant to Dectomax® and Valbazen® which were not detected using the FECRT.

Negative effects on body weight have been described in animals infected with ML resistant *Haemonchus* (Borges et al., 2013). However, in the heifers used for this study no effects of anthelmintic treatment on body weight were evident. The only relevant significant effect noted on weight was that of genotype crossbred heifers being heavier than Senepol heifers during all of the experimental periods, most likely due to hybrid vigor. There was a significant effect of time on bodyweight, which was expected as these heifers were in a stage of growth and development. Heifers from all the treatments presented a reduction on bodyweight at day 9 post-treatment most likely due to stress from initial experimental handling and treatment administration. Stress occasioned by handling, inflammation created at the injection site, and the irritation of the oral mucosa from administration of oral treatments potentially caused anorexia post-treatment, which can explain the reduction in bodyweight at day 9.

Anthelmintic resistance has been a big problem in small ruminants for decades and is now a worldwide problem of ruminants in general (Gasbarre,

2014). Populations of resistant nematodes have been described in New Zealand, Argentina, Australia, Germany, India, Kenya, Pakistan, Brazil, and recently in the USA and Mexico (Jabbar et al., 2006; Gasbarre et al., 2009a; Edmonds et al., 2010; Surtherland and Leathwick, 2010; Becerra-Nava et al., 2014; Gasbarre, 2014). The time interval used in determining the efficacy of the anthelmintic product is key. Ten to fourteen days have been suggested as the optimal interval in which is sufficient to allow for the drug to work but also ensures insufficient time for reinfection and consequent establishment of the parasite within the host (Gasbarre, 2014). The same pattern was detected in our population, in the data shown in appendix A (Table A 1) there is a reduction in the EPG from day 0 to day 15 suggesting efficacy of the products (Dectomax®, Valbazen®, and Levasole®). After day 36, heifers had an increase in the EPG count most likely due to reinfection as described in the literature.

In the heifer population studied, resistance was only detected against Ivomec®, which did not reduce the nematode egg count by ≥ 95%. Ivomec® is a member of the ML group which was introduced in the early 1980's as antiparasitic agents. Ivomec® still remains the leading worldwide antiparasitic agent for livestock (González-Canga et al., 2009). Because of the accessibility and easy use of this product, most farmers in Puerto Rico have heavily relied on ivermectin as their main and often only drug to treat helminthes. When nematodes are frequently exposed to a drug individuals that already have a mutation that provides them with anthelmintic resistance will have an evolutionary advantage (Coles, 2005). The rate of development of resistance will

be affected by how efficient the surviving nematodes are in completing their life cycle, which involves survival on the pasture, ability to migrate on forage and infectivity when ingested (Coles, 2005). Presumably, the development of resistance against Ivomec® observed is related to its frequent use for helminthic control.

In the present study an early stage of resistance against Dectomax® and Valbazen® was also detected. Misinformation and frequent indiscriminate use will result in the development of anthelmintic resistance against these products and also against Levasole®, which at present did not presented resistance in the nematode populations of the heifers studied. Maintaining the efficacy of Dectomax®, Valbazen® and Levasole® treatment against gastrointestinal nematodes is crucial for protecting the health of heifers at the Isabela Agricultural Sub-Station. According to Geary et al. (2012) combinations of two or more anthelmintics are being used routinely to manage AR in ruminants and to expand the range of efficacy in Australia and New Zealand. Combined products can delay the emergence and spread of resistance and also control the population of parasites that are already resistant (Geary et al., 2012). Protocols of treatment with combined products are suggested for use on farms, like the one of the present study, in which anthelmintics are still effective in reducing nematode egg counts. Contrary to such recent suggestions, the Food and Drug Administration (**FDA**) states that "*in regulatory terms, these are combinations with highly completely overlapping indications*" and that none of the combined drugs, proposed by previous publications, are currently approved in the USA (FDA,

2014). These opposed points of view regarding the use of combinations of products reflect the multifactorial nature of AR and the fact that detection of resistance is more complicated than first believed.

One common principle of control stated in all the recently published, in which the FDA concurs is the need to establish a subpopulation of worms in "refugia" to maintain efficacy of the existent products (Coles, 2005; Dobson et al., 2012; FDA, 2014; Gasbarre, 2014; Geary et al., 2012; Kornele et al., 2014). Refugia is a term that refers to the proportion of the total nematode population that is not selected for anthelmintic treatment (Kornele et al., 2014). Refuge nematodes are beneficial in maintaining a fraction of susceptible parasites on the farm (FDA, 2014). Eighty percent of parasite eggs are shed by 20% of the animals in a herd ("heavy shedders") (FDA, 2014). Identification and slaughter of these animals or treating individual heavy shedders can also help to slow the development of AR. Monitoring FEC of the animals will help to identify heavy shedders within the herd.

The present results indicate that three out of the four anthelmintics tested are still effective within the herd. Using FECRT to monitor efficacy of the anthelmintic products and to detect problems of resistance at an early stage to maintaining resistant nematode population at acceptable levels. Even though a problem of resistance to Ivomec® was found, the other three anthelmintics are still efficacious at this farm. With the establishment of refugia nematodes and treatment of heavy shedder animals the efficacy of these products can be prolonged and the development of resistance slowed.

Conclusion

Resistance of the gastrointestinal nematodes against ivermectin is an exiting problem in heifers at the Isabela Agricultural Experimental Sub-station of the University of Puerto Rico. No significant effect of the anthelmintic treatments on body weight was noted. Crossbred heifers were significantly heavier than pure Senepols during the entire length of the experiment. Dectomax® and Levasole® appeared to be the most efficient treatments to reduce the nematode egg count under the conditions of this experiment, followed by Valbazen®. Ivomec® did not reduce the FEC by ≥ 95% suggesting nematode resistance to this drug.

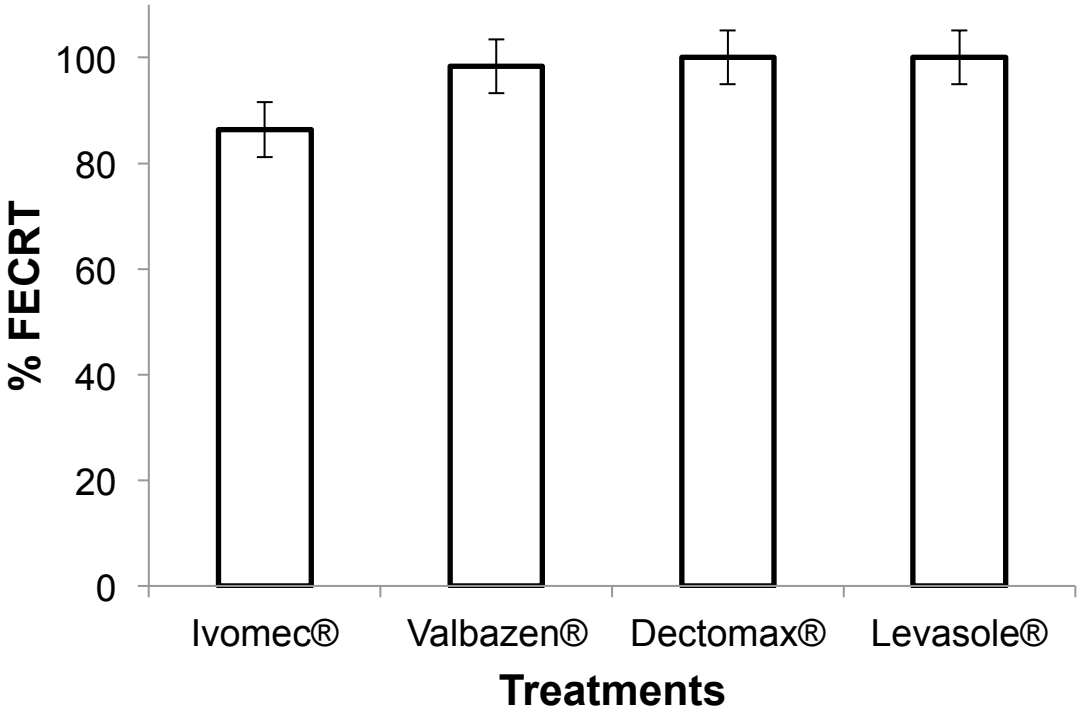

Figure 2. 1: The effects of different anthelmintics on the reduction rate of nematode egg counts of beef heifers.

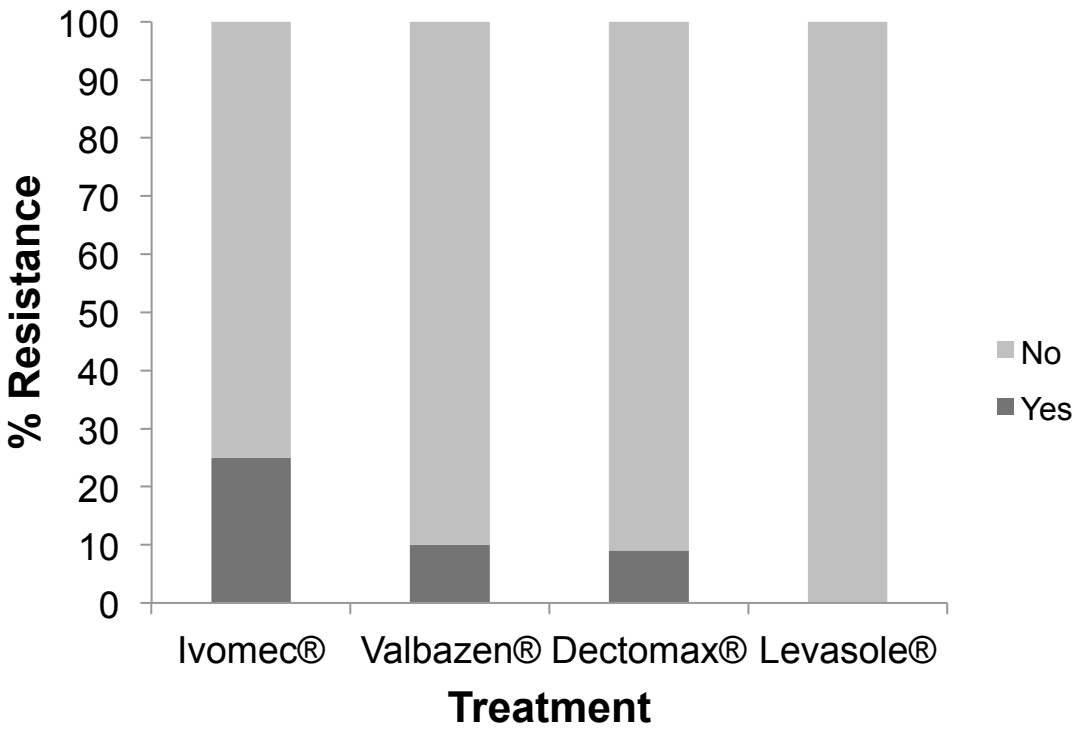

Figure 2. 2: The effects of different anthelmintics on the development of nematode resistance.

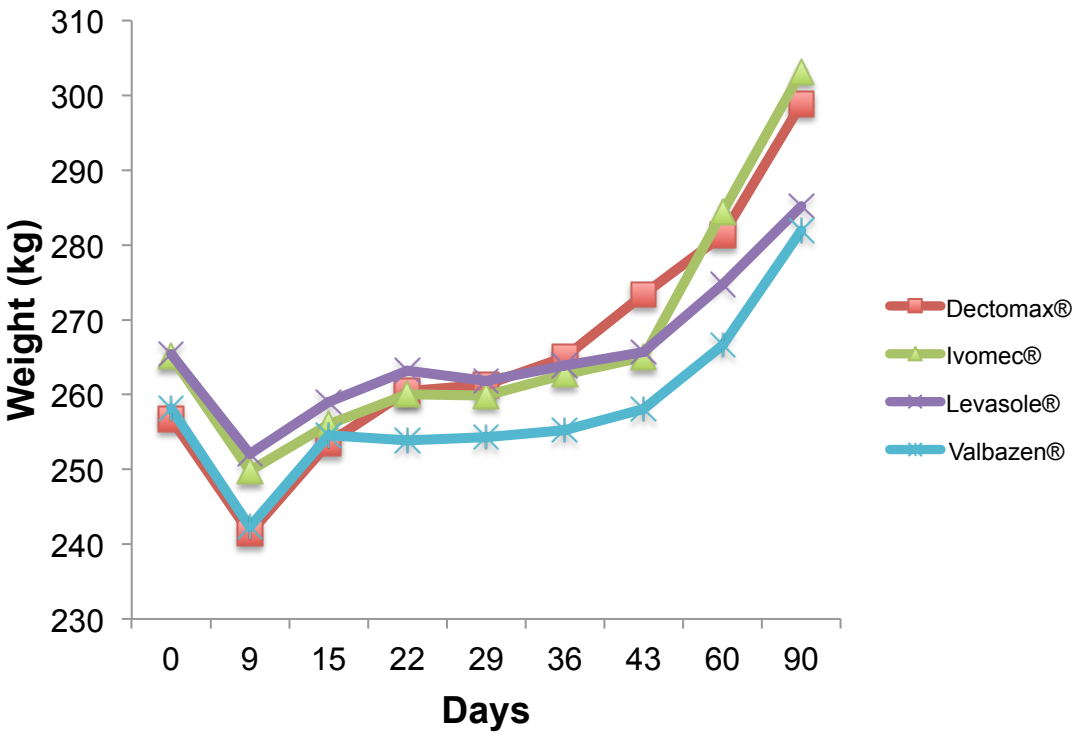

Figure 2. 3: Means of body weight by treatment from day 0 until day 90 of heifers treated at day 0 with four different anthelmintics. The *y-axis* was adjusted for a better appreciation of the data.

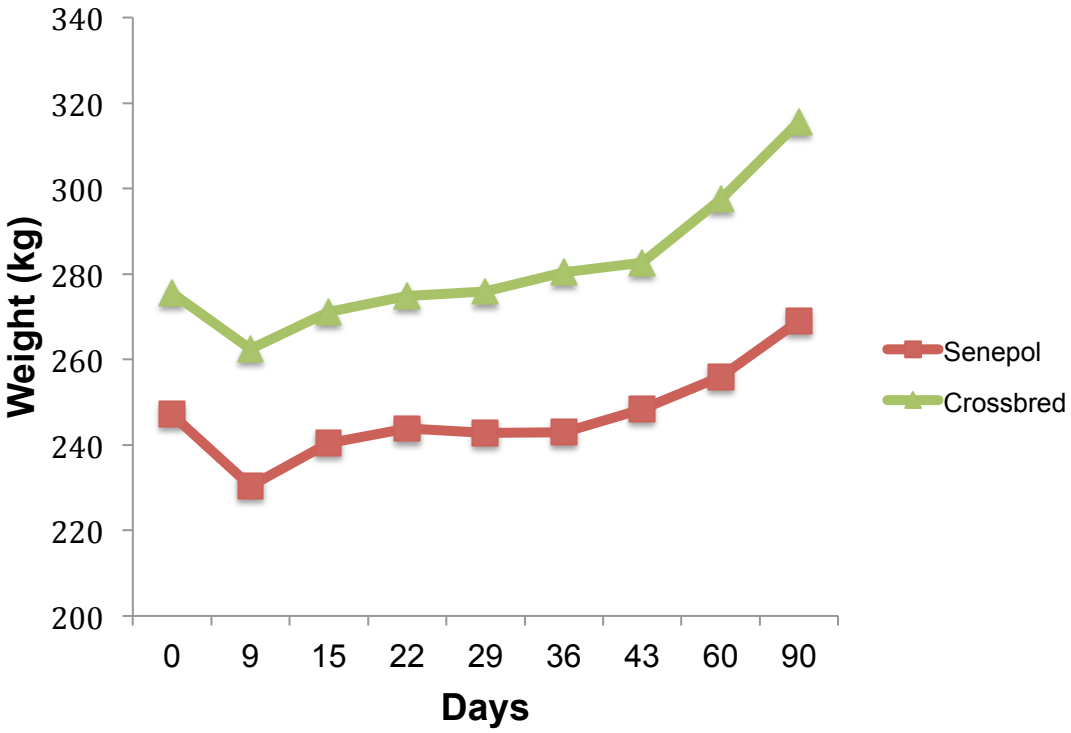

Figure 2. 4: Means of body weight by genotype during the experimental period. The *y-axis* was adjusted for a better appreciation of the data.

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APPENDICES

Appendix A: Count of nematode eggs per gram (EPG) in heifers during the experimental period

Table A 1: Values of nematode EPG by treatment obtained on day 0 and day 9, 15, 22, 29, 36, 43, 60, and 90 post-treatment

			EPG by day of collection								
Treatment	**Genotype***	**Animal**	**0**	**9**	**15**	**22**	**29**	**36**	**43**	**60**	**90**
Dectomax®	CB	1358	150	0	0	100	0	0	0	0	.
Dectomax®	CB	1374	200	0	0	0	0	0	0	0	0
Dectomax®	CB	1375	50	0	0	0	0	0	0	0	0
Dectomax®	CB	1423	150	200	0	50	50	100	0	100	150
Dectomax®	CB	1427	50	0	0	0	0	.	0	0	0
Dectomax®	SEN	5139	50	0	0	0	50	0	0	.	0
Dectomax®	SEN	5160	50	0	0	0	0	0	0	0	0
Dectomax®	SEN	5168	200	0	0	0	0	0	0	0	0
Dectomax®	SEN	5182	100	0	0	0	0	0	50	0	0
Dectomax®	SEN	5205	250	50	.	0	0	50	50	350	100
Dectomax®	SEN	5207	300	0	0	0	0	100	500	0	50
Ivomec®	CB	1405	50	50	0	100	50	50	0	50	0
Ivomec®	CB	1428	50	0	0	0	.	0	0	0	0
Ivomec®	SEN	5134	50	50	50	50	50	100	250	0	0
Ivomec®	SEN	5146	50	0	0	0	.	0	0	0	0
Ivomec®	SEN	5170	300	0	0	0	0	0	0	0	0
Ivomec®	SEN	5200	550	150	50	50	50	50	100	0	0
Ivomec®	SEN	8110	50	0	0	0	0	0	50	0	0
Ivomec®	SEN	8115	100	0	0	0	0	0	50	0	0
Levasole®	CB	1361	100	0	0	0	0	0	0	0	50
Levasole®	CB	1367	150	0	0	0	0	0	50	0	0
Levasole®	CB	1383	50	0	0	0	0	0	0	0	50
Levasole®	CB	1420	150	0	0	0	0	0	50	150	50
Levasole®	CB	1436	200	0	0	0	0	0	50	50	0
Levasole®	SEN	5138	50	0	0	0	50	0	0	0	0
Levasole®	SEN	5166	100	0	0	100	0	50	100	.	250
Levasole®	SEN	5194	50	0	0	0	0	0	50	0	0
Levasole®	SEN	5209	100	0	0	0	0	50	.	50	50
Levasole®	SEN	8129	250	0	0	0	0	0	0	100	250
Valbazen®	CB	1369	50	0	0	100	0	0	0	0	50
Valbazen®	CB	1392	50	0	0	0	0	100	0	.	0
Valbazen®	CB	1409	150	0	0	0	0	0	.	0	0
Valbazen®	CB	1412	200	0	0	0	50	0	0	100	50
Valbazen®	CB	1447	50	0	0	0	0	0	0	50	0
Valbazen®	SEN	5147	250	0	0	0	0	0	0	0	150

Valbazen®	SEN	5164	250	0	0	0	0	0	0	.	50
Valbazen®	SEN	5167	50	0	0	0	0	0	0	50	0
Valbazen®	SEN	5180	300	0	50	0	0	0	0	0	50
Valbazen®	SEN	5204	100	0	0	100	0	0	0	0	50

Genotype*: SEN refers to Senepol and CB to crossbred (Senepol*Charbray or Senepol*Charolais).

Appendix B: Bodyweight of heifers by treatment during the experimental period

Table B 1: Bodyweight of heifers by treatment during the experimental period

			Weight (Kg) by day								
Treatment	**Genotype***	**Animal**	**0**	**9**	**15**	**22**	**29**	**36**	**43**	**60**	**90**
Dectomax®	CB	1358	307	291	298	298	302	311	311	323	343
Dectomax®	CB	1374	264	257	268	264	273	284	284	296	316
Dectomax®	CB	1375	252	239	252	261	261	264	268	282	298
Dectomax®	CB	1423	325	307	325	330	336	339	341	364	388
Dectomax®	CB	1427	261	248	264	271	280	284	291	309	332
Dectomax®	SEN	5139	282	264	282	284	286	286	291	296	314
Dectomax®	SEN	5160	255	232	246	255	257	259	273	275	293
Dectomax®	SEN	5168	243	225	234	284	241	241	250	264	273
Dectomax®	SEN	5182	214	202	209	207	211	218	273	236	245
Dectomax®	SEN	5205	232	216	227	230	232	234	234	257	275
Dectomax®	SEN	5207	166	150	157	159	166	164	166	162	175
Ivomec®	CB	1405	286	273	273	289	284	286	277	302	327
Ivomec®	CB	1428	250	239	246	250	250	259	259	284	305
Ivomec®	SEN	5134	268	255	270	273	277	268	282	298	309
Ivomec®	SEN	5146	248	229	220	211	221	230	239	250	273
Ivomec®	SEN	5170	221	205	216	214	214	216	214	236	250
Ivomec®	SEN	5200	214	200	207	214	205	.	221	230	255
Ivomec®	SEN	8110	325	296	316	316	323	323	332	346	350
Ivomec®	SEN	8115	298	280	288	277	277	270	284	296	307
Levasole®	CB	1361	259	241	257	261	.	266	275	284	293
Levasole®	CB	1367	300	273	291	291	296	302	305	318	261
Levasole®	CB	1383	261	259	256	264	261	257	261	259	261
Levasole®	CB	1420	284	273	282	277	273	284	284	296	309
Levasole®	CB	1436	273	275	264	277	277	277	277	296	309
Levasole®	SEN	5138	254	239	243	252	261	273	282	305	286
Levasole®	SEN	5166	305	286	293	300	293	298	302	314	325
Levasole®	SEN	5194	200	186	202	204	198	200	193	207	214
Levasole®	SEN	5209	211	205	205	211	207	205	209	202	214
Levasole®	SEN	8129	307	284	298	298	300	296	289	300	309
Valbazen®	CB	1369	311	294	314	316	309	314	323	332	348
Valbazen®	CB	1392	268	255	257	266	268	277	273	277	302
Valbazen®	CB	1409	289	275	291	286	280	280	291	305	330
Valbazen®	CB	1412	282	268	281	273	280	275	277	286	300
Valbazen®	CB	1447	236	218	230	218	225	232	250	259	.
Valbazen®	SEN	5147	284	271	284	284	284	284	274	284	298
Valbazen®	SEN	5164	243	227	239	241	241	230	232	245	252
Valbazen®	SEN	5167	248	232	241	241	241	248	243	255	277

Valbazen®	SEN	5180	232	211	227	227	229	227	230	239	257
Valbazen®	SEN	5204	189	173	182	186	186	186	189	184	189

Genotype*: SEN refers to Senepol and CB to crossbred (Senepol*Charbray or Senepol*Charolais).