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# Effectiveness of a fixed-dose combination injectable (0.2 mg/kg doramectin + 6.0 mg/kg levamisole hydrochloride) against *Rhipicephalus microplus* and sucking lice infesting cattle☆

Andrew A. DeRosa [a,*], Aleah Pullins [a], Jezaniah Kira Tena [a], Susan Holzmer [a], Raj Packianathan [b]

[a] Zoetis, Veterinary Medicine Research and Development, 333 Portage St, Kalamazoo, MI 49007, USA
[b] Zoetis, Veterinary Medicine Research and Development, Level 6, 5 Rider Boulevard, Rhodes, NSW 2138, Australia

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ABSTRACT

Unmanaged tick and sucking lice infestations negatively impact the health and production potential of cattle. Described herein are two non-interference dose confirmation studies evaluating the efficacy of a single administration of a new fixed-dose combination injectable (FDCI) endectocide consisting of 0.2 mg/kg doramectin + 6.0 mg/kg levamisole hydrochloride, against either laboratory-induced *Rhipicephalus microplus* infestations in Australia or naturally acquired sucking lice (*Linognathus vituli*) infestations in the US. This FDCI is available as Dectomax V® in Australia and New Zealand and as Valcor® in the United States. To evaluate therapeutic efficacy against *R. microplus*, 12 calves were each exposed to 10 infestations of ~5000 larvae per infestation between Days −24 and −2. Calves were either treated on Day 0 with the FDCI or left untreated (control). Additional *R. microplus* infestations of ~5000 larvae were conducted on Day 2 and then three times weekly to also evaluate persistent efficacy of the FDCI. Tick collections were conducted daily from Day −3. Group mean live tick counts, egg production, and egg viability were analyzed for significant differences between the two groups. To determine efficacy of the FDCI against lice, 24 cattle with active sucking lice infestations based on Day −7 counts were allocated to two groups and treated on Day 0 with either saline (control) or the FDCI. Lice counts were conducted weekly from Day 14 through 42 and again on Day 56. Mean group lice counts on each count day were compared between treatment groups. In the *R. microplus* study presented here, cattle in Queensland, Australia treated with the FDCI (Dectomax V®) showed > 90 % reduction in tick counts based on arithmetic means within 48 h of treatment when compared to untreated cattle, and counts were > 95 % reduced from post-treatment Day 5 through Day 30. In the sucking lice study conducted in the US, the FDCI (Valcor®) displayed 100 % efficacy against sucking lice infestations (*L. vituli*) from first count day (Day 14 post-treatment) through Day 35 and then 99.9 % efficacy through Day 56 post-treatment. No treatment-related adverse events were reported for cattle in either study. Using *R. microplus* and sucking lice as representative ectoparasites, these studies demonstrate the ectoparasite activity of doramectin is retained in the new FDCI.

## 1. Introduction

Ectoparasites pose a significant threat to the health of cattle, resulting in significant direct and indirect losses to cattle production. Infestations can result in reduced grazing and/or rumination, automutilation due to skin irritation, decreased weight gain and milk production, and compromised hide quality (Weeks et al., 1995; Coles et al., 2003; Tasawar et al., 2008). Additionally, some ectoparasites transmit blood-borne pathogens during their feeding activities, allowing the spread of diseases between and within herds (Pérez de León et al., 2020; Muhammad et al., 2021). While it is difficult to pinpoint annual losses associated with uncontrolled cattle ectoparasites, published estimations are in the realm of US$ 2.26 billion for the United States (US) (Byford et al., 1992) and US$ $6.86 billion for Brazil (Grisi et al., 2014b). Considering the US and Brazil ranked first and second, respectively, in world beef production and accounted for a combined 39 % of the global total in 2022 (Cook, 2023), the above loss data underscore the global impact of cattle ectoparasitic infestations and consequently the value of

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* Corresponding author.
*E-mail address:* andrew.derosa@zoetis.com (A.A. DeRosa).

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successful ectoparasite control.

The unique biological and ecological profiles of cattle ectoparasites combined with the wide range of beef and dairy cattle farming systems makes finding a single solution for control challenging (Pérez de León et al., 2020). Ticks and lice are two ectoparasitic groups of major concern to cattle producers, as multiple species within each group are globally endemic and negatively affect cattle health and production if uncontrolled (Barker et al., 2017; Pérez de León et al., 2020). Where endemic, the southern cattle fever tick *Rhipicephalus microplus* is generally considered the most economically important ectoparasite due to its broad distribution, invasive nature and the high costs associated with its (1) direct feeding (e.g., reduced weight gain and milk production, hide damage) (Jonsson et al., 1998; Jonsson, 2006; Rodrigues and Leite, 2013; Grisi et al., 2014a) and (2) transmission of *Babesia bovis*, *B. bigemina* and *Anaplasma marginale* (Connell and Hall, 1972; Aguirre et al., 1994; Fuente et al., 2008). The rapid lifecycle (~3 weeks) and biological adaptability of the single-host tick *R. microplus* can make its control difficult. The sucking lice of primary concern for cattle are *Haematopinus eurysternus* (short-nosed cattle louse), *H. quadripertusus* (cattle tail louse), *Linognathus vituli* (long-nosed cattle louse) and *Solenopotes capillatus* (little blue cattle louse). General signs of lice infestation are hair loss, raw spots, reduced weight gain and ill-thrift, but heavy infestation may present with pruritus, restlessness and stress associated with pediculosis, decreased appetite and consequently decreased weight gain and milk yields, and severe anemia that can be fatal if not caught in a timely manner (Fadok, 1984; Coles et al., 2003; Otter et al., 2003; Cotter, 2019; Schlesser, 2022). Some lice species are also potential vectors for cattle bacterial pathogens. For example, *L. vituli* can transmit *Anaplasma marginale* (Hornok et al., 2010; Ketzis, 2023), and several other pathogenic bacteria (e.g., *Acinetobacter* spp., *Rickettsia* spp., and *Coxiella burnetti*) have been found in lice, suggesting these blood-feeding insects may act as potential vectors for additional bacterial infections (Reeves et al., 2006; Hornok et al., 2010; Kumsa et al., 2012).

Here, we present a new fixed-dose combination injectable (FDCI) endectocide formulated with the macrocyclic lactone (ML) doramectin (0.2 mg/kg) and the imidazothiazole levamisole (6.0 mg/kg levamisole hydrochloride (HCl)) and administered at 0.04 ml/kg. Doramectin acts primarily via the activation of glutamate-gated chloride (Glu-Cl) channels (Cully et al., 1994; Krusek and Zemkova, 1994; Bloomquist, 1996; Adelsberger et al., 2000; Bloomquist, 2003), whereas levamisole acts through nicotinic acetylcholine-gated cation-selective channels (Martin and Robertson, 2007; Robertson and Martin, 2007). Combining the two anthelmintics into a single product provides two different modes of action against broad and overlapping spectra of gastrointestinal nematodes. Additionally, topical and injectable dose forms of doramectin display high and persistent efficacy against ML-susceptible sucking lice (*H. eurysternus*, *L. vituli* and *S. capillatus*) and *R. microplus* (Gonzales et al., 1993; Logan et al., 1993), with research suggesting this activity is potentiated through the activation of Glu-Cl channels similar to those found in GINs (Bloomquist, 1993; Bloomquist, 1996; Martin et al., 2021). In contrast, field and laboratory experience have clinically demonstrated that the imidazothiazole drug class, including levamisole, lacks ectoparasitic activity due to differences in the pharmacological properties of acetylcholine receptors between insects and nematodes (Pinnock et al., 1988).

The objective of this article is to report on two country-specific efficacy studies conducted as part of the registration process to demonstrate the ectoparasite activity of doramectin is retained in the new FDCI. The first study evaluated the FDCI against artificially induced infestations of *R. microplus* on beef cattle in Queensland, Australia, and the second study against naturally acquired lice infestations (*L. vituli*) on a mixture of beef and dairy cattle in Wisconsin, US. Efficacy was based on tick and louse burdens (counts and weight) as well as tick egg production (count and weight) and hatchability (count and weight).

The new FDCI is available under the tradenames Dectomax V® in Australia and New Zealand and Valcor® in the US, and we have used these tradenames throughout the manuscript to clearly identify the country in which each study was conducted. Although Valcor® and Dectomax V® are the same drug product, the reader is cautioned to not extrapolate country-specific ectoparasite indications across tradenames. For example, US studies were not conducted to support a Valcor® *R. microplus* indication, and the FDCI is therefore not indicated for this ectoparasite in the US.

## 2. Materials and methods

### 2.1. Efficacy against Rhipicephalus microplus

#### 2.1.1. Animals and study design

This study was conducted at the Queensland Animal Science Precinct (QASP; The University of Queensland) PC1 Facility in Gatton, Australia using Dectomax V®. Twelve *Bos taurus* steers (~8 months old) with no known previous exposure to *R. microplus* were purchased from a designated tick-free area. All animals weighed between 169 kg and 228 kg at treatment (Day 0), all were identified using uniquely numbered ear tags, and none had experienced recent treatment with a long-acting acaricide. Individual animal was the experimental unit as cattle were housed individually in indoor pens with raised mesh flooring and surrounding moats for physical containment. Feed (mixture of hay and custom formulated starter pellets) was offered daily in plastic feed troughs, and ad libitum access to potable water was provided via automated drinkers.

Cattle were acclimatized at the study site for four weeks prior to study start. Following acclimation, cattle were randomly allocated to pens for tick infestations. Treatment was carried out on Day 0. To assess therapeutic effect, each animal was infested multiple times prior to treatment (on Days −24, −21, −−19, −17, −14, −12, −10, −7, −5, and −2). To assess persistent efficacy, each animal was again infested with *R. microplus* larvae on Day 2 post-treatment and then three times weekly until the end of the study. Knock-down (efficacy onset) and persistent efficacy of Dectomax V® were determined through daily collection and counting of adult female ticks dropping from cattle, with collections starting on Day −3 and finishing when efficacy was < 95 %.

On Day 0, cattle were ranked based on mean adult female tick counts collected from Day −3 to Day −1. Animals were sequentially blocked and randomly allocated within block to one of two treatment groups (n = 6 animals per group). Allocation was such that each group had a similar group mean tick count and range of tick counts. All cattle were weighed, and individual weights were used to calculate treatment dose.

#### 2.1.2. Infestation and treatment

Each cattle infestation used ~5000 Non-Resistant Field Strain (NRFS) *R. microplus* larvae per animal. NRFS is a tick isolate maintained by the Biosecurity Sciences Laboratory (BSL) (Department of Agriculture and Fisheries, Coopers Plains, Queensland) that is susceptible to all known commercially available acaracides. All infestations were conducted according to standard operating procedures of the BSL and in compliance with relevant guidelines (Holdsworth et al., 2006a; APVMA, 2014; CVM, 2001). Personnel were trained in identifying and handling *R. microplus*.

On Day 0, animals in Group T01 remained untreated and animals in Group T02 were administered Dectomax V® (1 ml/25 kg; 0.2 mg/kg doramectin + 6.0 mg/kg levamisole hydrochloride HCl) via subcutaneous (SC) injection in the left side of the neck. Study animals were observed for any abnormal responses at 1–2 h and 6–8 h post-treatment. All cattle were observed daily for general health. Personnel recording health observations or conducting assessments of efficacy did not know the treatment assignment of individual animals.

#### 2.1.3. Tick collection and viability analyses

Tick collections were conducted daily starting on Day −3. The steel mesh floor of each individual pen was washed, and adult female ticks

were collected into mesh baskets. Ticks were transported from QASP to the BSL, where they were dried, counted, and weighed to determine the total number of ticks per animal and the total weight of ticks per animal (Gonzales et al., 1993; Holdsworth et al., 2006c; Hüe et al., 2017). Random sub-samples of ~50 ticks (when available, or fewer ticks if less than 50 recovered) were weighed and incubated for 7 days (~28 °C, 85% relative humidity) for future egg weight and viability assessments. Following incubation, the eggs produced by the ticks were collected and weighed, and egg viability (%) was visually rated. Tick viability and egg production were analyzed according to standard operating procedures of the BSL (Gonzales et al., 1993; Hüe et al., 2017).

#### 2.1.4. Data analysis

Group mean tick counts at allocation were analyzed for significant differences between groups using One-Way Analysis of Variance and Tibco Spotfire® S+ Version 8.2, Tibco Software Inc. 2010 or equivalent.

Data analyses were conducted according to the guidelines published by the World Association for the Advancement of Veterinary Parasitology (WAAVP) (Holdsworth et al., 2006a). Total tick counts, total weights of ticks collected, tick survival to oviposition, total egg weights and tick egg hatchability were assessed. Percent daily survival was calculated for the Dectomax V®-treated group. Therapeutic and persistent efficacy were determined using the standard ADEQ analysis as outlined in the WAAVP guidelines for evaluating the efficacy of acaricides against ticks (Ixodidae) on ruminants (Holdsworth et al., 2006a), where ADEQ represents the number of ticks expected in the treated group if left untreated and is calculated as follows:

ADEQ = (Total pre-treatment counts in treated group/Total pre-treatment counts in control group) X Daily control count.

### 2.2. Efficacy against sucking lice

#### 2.2.1. Animals and study design

This study was conducted in Wisconsin, US using Valcor®. Forty candidate dairy and commercial beef breed cattle (*Bos taurus*) were obtained from Wisconsin, US for this study. All enrolled animals had clinical evidence of an active sucking lice infestation (*Linognathus vituli*, *Solenopotes capillatus* and/or *Haematopinus eurysternus*) defined by the observation of a minimum of 40 motile immature or adult sucking lice on Day −7. Cattle were identified using uniquely numbered ear tags. All cattle received a physical examination on Day −7, including weight, rectal temperature, thoracic auscultation, and general physical assessment. Twenty-four animals with the highest Day −7 lice counts were selected for enrollment to the study. All enrolled animals were allocated to pens and treatments according to a randomized complete block design with one-way treatment structure. Blocking was based on Day −7 lice counts and pen location. Blocks of six animals were formed based on Day −7 lice counts. Animals of the same block were randomly assigned to two pens located near each other, with three animals per pen. Within each block, pens were randomly assigned to treatments, with one pen per treatment. Four pens of three animals each were assigned to each treatment group for a total of n = 12 animals per treatment.

Enrolled cattle were > 2 months and < 1 year of age and weighed between 119 kg and 288 kg on Day −7. No enrolled animal had any apparent congenital malformation or severe illness, malady or non-weight bearing lameness, and none had formerly received a veterinary product that could either positively or negatively impact ectoparasite survival and development. Cattle were housed for the study duration inside a barn in eight separate pens of three animals each (four pens per treatment group) in a manner that prevented contact among animals in different pens. Custom feed mix (16 % protein) was fed at a rate of 2–3 % body weight per head per day with ad libitum mixed grass hay, and access to potable water was provided ad libitum via automated waters. From Day −7 onwards, all cattle were observed once daily for general health until end of study on Day 56.

Pre-treatment lice counts were conducted on Day −7 to confirm the presence of adequate infestations prior to enrollment, on Day −1 to confirm adequacy of infestation prior to treatment and on Day 0 immediately prior to treatment. On Day 0, all cattle received a single, SC administration of either saline (T01) or Valcor® (0.04 ml/kg; T02). Post-treatment lice counts were conducted for efficacy assessment.

All animals were observed for any adverse events beginning on Day 0 through the last day of counts (Day 56). Masking was achieved by separation of function. Study personnel recording observations or conducting assessments of efficacy or safety did not know the treatment assignment of pens and individual animals.

#### 2.2.2. Infestations and treatment

Cattle enrolled in this study had naturally established infestations of immature and adult sucking lice (*L. vituli*, *H. eurysternus* and/or *S. capillatus*) based on Day −7 and Day −1 lice counts conducted by personnel with extensive experience in lice identification. On Day 0, all enrolled animals received a single SC administration (1 ml/25 kg) of either sterile water (Group T01; control) or Valcor® (Group T02; 0.2 mg doramectin + 6.0 mg levamisole HCl) in the lateral midline of the left side of the neck using a sterile disposable syringe and 18-gauge x ¾ inch sterile disposable needle. A new syringe and needle were used for each animal, and all dose volumes > 10 ml were administered at two injection sites.

#### 2.2.3. Lice counts

Pre-treatment counts were conducted to confirm adequacy of infestation prior to enrollment and on Day 0 immediately prior to treatment for efficacy calculations. Post-treatment lice counts were conducted on Days 14, 21, 28, 35, 42, and 56 following a randomized order list generated using SAS® Release 9.4 (SAS Institute, Cary, NC) on each counting day. Counts were conducted on all animals on the same calendar day.

Lice counts were conducted by observing and recording the number of motile sucking lice at the following predilection sites: Poll (including the occipital area and ears extending to the top of the neck; ~ area = 10 cm × 20 cm), eyes (~ area = 20 cm × 30 cm), muzzle band (~ area = 5 cm × 25 cm), cheeks (~ area = 10 cm × 20 cm), dewlap (~ area = 10 cm × 20 cm), topline (~ area = 10 cm × 30 cm) and withers (~ area = 10 cm × 30 cm). For bilateral regions, counts were conducted on each side of the animals. The entire area of each sampled predilection site was visually examined for motile sucking lice.

Animals were restrained for lice count using a squeeze chute. To limit fomite transfer of lice between pens, all study personnel performing lice counts wore fresh gloves and smocks at each pen. If a halter was used it was changed between pens, and all equipment used on an animal underwent louse removal procedures prior to the next animal to minimize the chance of lice being transferred between individual animals.

#### 2.2.4. Data analysis

Total lice counts were averaged (arithmetic mean) over each pen and then log transformed (ln (average count +1)) prior to analysis. The log-transformed average counts were analyzed using a general linear mixed model separately for each time point. The model included the fixed effect of treatment and the random effects of block and error. Back-transformed (geometric) least squares (LS) means (GLSMs) were calculated along with 95% confidence intervals by treatment at each time point. Minimums and maximums were calculated by treatment at each time point. Treatment comparisons were done at each time point using a 2-sided 5% level of significance.

Efficacy for each observation period was calculated using Abbott's formula and GLSMs.

Abbott's formula for percent efficacy = 100 * (GLSM Sterile Water – GLSM Valcor®) / GLSM Sterile Water.

Physical examinations were summarized by treatment and time point using frequency distribution tables. Continuous measures including heart rate, rectal temperature and respiration rate were

summarized with descriptive statistics (means, standard deviation, minimum, maximum). Body weights were summarized by treatment and time point using descriptive statistics.

Abnormal general health observations were listed by animal, time point and treatment. The number of animals with abnormal observation was summarized by treatment and time point.

## 3. Results

### 3.1. *Rhipicephalus microplus*

#### 3.1.1. *Efficacy assessments*

Cattle in each group had similar levels of tick infestation at allocation, with Day −3 to −1 mean tick counts ranging from 33.7–300.3 for Group T01 and 42.3–297.3 for Group T02. Mean tick counts for each group at allocation were not significantly different from each other (Untreated, 163.1 ± 105.2; Dectomax V®, 156.5 ± 99.1; $p > 0.05$).

Tick counts for Dectomax V®-treated cattle decreased within 24 h of treatment (Fig. 1A), indicating a rapid therapeutic effect, and continued to decline over the next 48 h. Compared to untreated cattle, daily tick survival for Dectomax V®-treated cattle was < 10 % by post-treatment Day 3, < 5 % by Day 5, decreased to < 1 % on Day 9 through Day 29 and then increased to ~3 % on Day 30. (Fig. 1B).

Table 1 shows the arithmetic mean efficacy of Dectomax V® against *R. microplus* based on tick burden, egg production and egg hatchability. Based on count data, Dectomax V® was highly effective in controlling tick infestations, with > 95 % reduction in tick counts from Day 5 through 30 and > 98 % from Day 7 through 30. Egg counts and egg hatchability were also reduced by > 98 % from Day 3 through 30. When efficacy is calculated based on tick weight data, Dectomax V® treatment resulted in > 98 % reduction in tick burden, egg production and egg hatchability from Day 3 through 30, demonstrating 30-day protection against development of viable cattle ticks on FDCI-treated cattle.

#### 3.1.2. *Health observations*

Daily observations of cattle throughout this study did not reveal any treatment-related adverse events.

### 3.2. *Sucking lice*

#### 3.2.1. *Efficacy assessments*

The study was considered valid since at least five sterile water-treated cattle (T01), with at least one animal from each of the four pens, met the criteria for adequacy of infestation at each post-treatment efficacy observation. Of note, whereas all T01 cattle were infested with *L. vituli* at each efficacy evaluation, only two T01 animals were positive

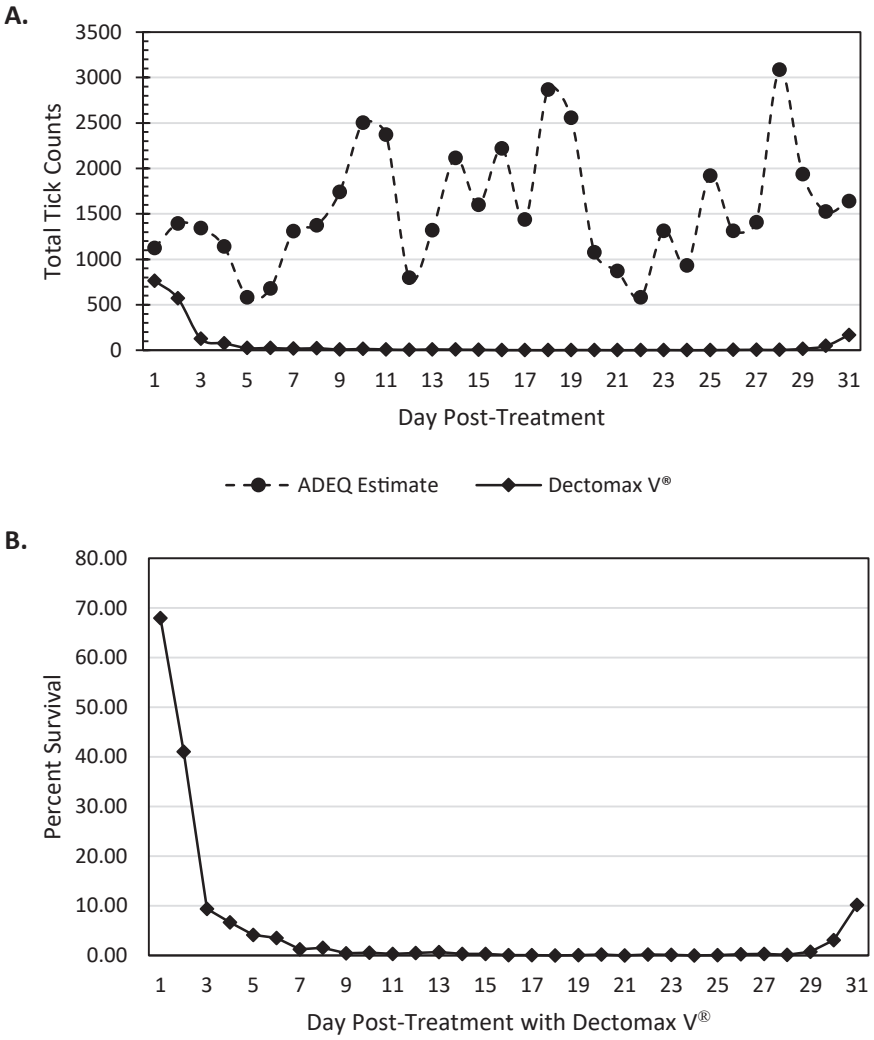


**Fig. 1. A.** Total daily tick counts from cattle treated with the new fixed-dose combination injectable Dectomax V® (0.2 mg/kg doramectin + 6.0 mg/kg levamisole hydrochloride) compared to the ADEQ estimate*. *ADEQ estimate is the estimated tick count if cattle were left untreated (Holdsworth et al., 2006). **B.** Percent survival of ticks following administration of the fixed-dose combination injectable (Dectomax V®; 0.2 mg/kg doramectin + 6.0 mg/kg levamisole hydrochloride).

**Table 1**
Arithmetic mean reduction in tick burden, egg production and egg hatchability based on count and weight data from cattle treated with Dectomax V® (0.2 mg/kg doramectin + 6.0 mg/kg levamisole hydrochloride).

| Day post-treatment[a] | Percent efficacy (Count data) | | | Percent efficacy (Weight data) | | |
|---|---|---|---|---|---|---|
| | Tick burden | Egg production | Egg hatchability | Tick burden | Egg production | Egg hatchability |
| **1** | 32.01 | 75.68 | 75.3 | 77.91 | 77.20 | 76.84 |
| **2** | 58.92 | 91.92 | 91.92 | 90.76 | 92.42 | 92.42 |
| **3** | 90.61 | 98.92 | 98.96 | 98.39 | 98.89 | 98.93 |
| **4** | 93.34 | 99.62 | 99.65 | 99.10 | 99.60 | 99.64 |
| **5** | 95.87 | 99.86 | 99.88 | 99.56 | 99.86 | 99.87 |
| **6** | 96.46 | 99.95 | 99.96 | 99.70 | 99.95 | 99.96 |
| **7** | 98.78 | 99.95 | 99.96 | 99.80 | 99.94 | 99.96 |
| **8** | 98.47 | 99.98 | 100.00 | 99.87 | 99.98 | 100.00 |
| **9** | 99.54 | 99.98 | 99.98 | 99.95 | 99.98 | 99.98 |
| **10** | 99.44 | 100.00 | 100.00 | 99.96 | 100.00 | 100.00 |
| **11** | 99.66 | 99.95 | 99.94 | 99.92 | 99.94 | 99.94 |
| **12** | 99.5 | 99.99 | 100.00 | 99.95 | 99.99 | 100.00 |
| **13** | 99.32 | 100.00 | 100.00 | 99.94 | 100.00 | 100.00 |
| **14** | 99.72 | 99.99 | 99.99 | 99.92 | 99.99 | 99.99 |
| **15** | 99.69 | 100.00 | 100.00 | 99.97 | 100.00 | 100.00 |
| **16** | 99.95 | 99.99 | 99.99 | 99.98 | 99.99 | 99.99 |
| **17** | 99.93 | 99.97 | 99.97 | 99.97 | 99.97 | 99.97 |
| **18** | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| **19** | 99.92 | 100.00 | 100.00 | 99.99 | 100.00 | 100.00 |
| **20** | 99.81 | 99.9 | 99.89 | 99.90 | 99.89 | 99.89 |
| **21** | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| **22** | 99.83 | 99.86 | 99.88 | 99.85 | 99.86 | 99.88 |
| **23** | 99.85 | 99.86 | 99.88 | 99.84 | 99.86 | 99.88 |
| **24** | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| **25** | 99.95 | 99.99 | 99.99 | 99.98 | 99.99 | 99.99 |
| **26** | 99.77 | 99.89 | 99.88 | 99.88 | 99.88 | 99.88 |
| **27** | 99.64 | 99.88 | 99.88 | 99.88 | 99.87 | 99.88 |
| **28** | 99.9 | 99.9 | 99.9 | 99.92 | 99.90 | 99.90 |
| **29** | 99.23 | 99.64 | 99.64 | 99.65 | 99.63 | 99.63 |
| **30** | 99.92 | 98.43 | 99.43 | 99.53 | 98.38 | 98.38 |

[a] Treatment; Dectomax V® (0.2 mg/kg doramectin + 6.0 mg/kg levamisole hydrochloride) at 1 ml/25 kg. n = 6 animals.

for *S. capillatus*, and none were positive for *H. eurysternus*. Consequently, only *L. vituli* infestations were considered sufficient for efficacy evaluation. Back-transformed GLSM lice counts for Days 14, 21, 28, 35, 42 and 56 are listed in Table 2. Compared to T01 cattle, GLSMs for T02 cattle were significantly ($p < 0.0001$) reduced on all count days. Efficacy of Valcor® in treating *L. vituli* infestations was 100 % on Day 14

**Table 2**
Geometric least squares mean (GLSM) reduction in lice counts on cattle treated with Valcor® (0.2 mg/kg doramectin + 6.0 mg/kg levamisole hydrochloride (HCl)) compared to control (sterile water-treated) cattle.

| Study Day | Treatment[a] | Minimum | Maximum | GLSM[b] | 95% Confidence Interval | Efficacy[c] (%) | p-Value |
|---|---|---|---|---|---|---|---|
| **14** | **T01 Sterile Water** | 53.3 | 157.7 | 83.5 | 53.0–131.2 | 100.0 | < 0.0001 |
| | **T02 Valcor®** | 0.0 | 0.0 | 0.0 | -0.4–0.6 | | |
| **21** | **T01 Sterile Water** | 58.7 | 141.7 | 96.3 | 65.2–142.0 | 100.0 | < 0.0001 |
| | **T02 Valcor®** | 0.0 | 0.0 | 0.0 | -0.3–0.5 | | |
| **28** | **T01 Sterile Water** | 53.3 | 126.0 | 87.1 | 60.0–126.4 | 100.0 | < 0.0001 |
| | **T02 Valcor®** | 0.0 | 0.0 | 0.0 | -0.3–0.4 | | |
| **35** | **T01 Sterile Water** | 68.3 | 148.0 | 101.1 | 72.6–140.7 | 100.0 | < 0.0001 |
| | **T02 Valcor®** | 0.0 | 0.0 | 0.0 | -0.3–0.4 | | |
| **42** | **T01 Sterile Water** | 61.7 | 117.0 | 85.8 | 62.0–118.7 | 99.9 | < 0.0001 |
| | **T02 Valcor®** | 0.0 | 0.3 | 0.1 | -0.2–0.5 | | |
| **56** | **T01 Sterile Water** | 56.7 | 99.7 | 70.3 | 54.6–90.3 | 99.9 | < 0.0001 |
| | **T02 Valcor®** | 0.0 | 0.3 | 0.1 | -0.2–0.4 | | |

[a] Treatment; Dectomax V® (0.2 mg/kg doramectin + 6.0 mg/kg levamisole hydrochloride) at 1 ml/25 kg. n = 4 pens per treatment group, with n = 3 animals per pen at each timepoint.
[b] GLSM, geometric least squares mean.
[c] Efficacy = 100 * (GLSM Sterile Water – GLSM Valcor®) / GLSM Sterile Water.

through Day 35 post-treatment and remained at 99.9 % on Days 42 and 56 post-treatment (Table 2).

*3.2.2. Animals and health observations*

The 24 animals (20 steers and 4 females) enrolled on study consisted of three beef and twenty-one dairy breed cattle weighing between 123.5 kg and 301 kg on Day 0. Day 0 mean weights for T01 and T02 cattle were 189.2 ± 55.7 kg and 198.5 ± 61.9 kg, respectively. Pre-treatment (Day −7) physical examinations showed animals in each treatment group were of good health.

No treatment-related adverse events were reported for any animal throughout the study. Three animals had abnormal general health observations recorded throughout the study. One animal in the sterile water-treated group was observed to have pinkeye and one in this same group had symptoms indicative of bovine respiratory disease; both conditions resolved after treatment. One animal in the Valcor®-treated group had diarrhea and signs of coccidiosis that resolved after treatment. These observations were made both prior to and after treatment administration and are typical for the animals and housing conditions represented in the study.

## 4. Discussion

The data from the two studies described herein demonstrate doramectin when co-formulated with levamisole in this new FDCI retains a high and persistent level of efficacy against *R. microplus* and *L. vituli*, providing empirical evidence that levamisole in the FDCI does not interfere with the ectoparasite activity of doramectin. In Australia, tick counts for cattle treated with Dectomax V® were reduced compared to tick counts for control cattle by > 90 % within 72 h post-treatment, and tick counts remained > 95 % reduced from post-treatment Day 5 through Day 30. These data support a Dectomax V® label indication in Australia for 30-day prevention of viable *R. microplus* development, exceeding the 28-day prevention of viable *R. microplus* development on the Australian Dectomax® injectable for cattle label indication (Zoetis, 1996). In the US, the FDCI Valcor® was effective in treating established lice infestations, resulting in 100 % efficacy against *L. vituli* from the first day of evaluation (Day 14) through Day 35 and then 99.9 % efficacy through Day 56 post-treatment. Daily observations of cattle in both studies did not reveal any treatment-related adverse events. These data illustrate doramectin retains a high level of efficacy against important cattle ectoparasites and highlight the value Valcor® and Dectomax V® bring to their respective markets.

*Rhipicephalus microplus* is an important single-host tick that mainly infests cattle and related bovids but can also be found on other domesticated species (e.g., horses, donkeys, goats, dogs) and some free-living or captive wild mammals (e.g., white-tailed deer, red deer) (Spickler, 2022). In Australia, *R. microplus* has a high prevalence and broad distribution, and it subsequently renders a large negative impact on the cattle industry. There are an estimated 8–9 million cattle in tick endemic areas within Australia (Playford, 2005; Sackett and Holmes, 2006), and the per head cost of infestation was valued at ~ AUD $15 more than 15 years ago (Sackett and Holmes, 2006), with inflation likely driving that higher today. The costs associated with *R. microplus* cattle infestation encompass both reduced income and increased expenses, and the national economic loss for Australian producers has been estimated in the range of ~ AUD $146 M to AUD $239 M (Commission, 1975; Sackett and Holmes, 2006). Consequently, the Australian cattle producer is more vested – and more willing to invest – in a management solution for *R. microplus* populations than their US counterparts, who have operated under the umbrella of a *R. microplus* eradication program since 1907 (Graham and Hourrigan, 1977; Walker, 2011). These market differences underly the rationale for the *R. microplus* indication on Dectomax V® for Australia and the absence of this indication on the US Valcor® label.

In endemic regions, acaracides or endectocides are often used to treat and control *R. microplus* on cattle, but not all treatments routinely prevent reinfestation. In Davey et al., (2005), cattle infested with all life stages of *R. microplus* (larvae, nymphs, and adults) were treated with a single SC injection of either ivermectin or moxidectin at 200 μg/kg. Based on kill and the reproductive capacity of any surviving female ticks, therapeutic control was calculated to be ≥ 99% for both endectocides. However, when efficacy at time of treatment was analyzed for each developmental stage, both ivermectin and moxidectin provided significantly ($p < 0.006$) greater control of adults or nymphs (≥ 99.7 %) than larvae (≤ 98.4 %). Further analysis revealed the efficacies of ivermectin and moxidectin were only 23.3 % and 79.8 %, respectively, at two weeks post-treatment and were 0.0 % and 19.5 %, respectively, at four weeks post-treatment. Unfortunately, these data are not outliers but either align closely or exceed those of earlier studies investigating these and other endectocides (Cramer et al., 1988; Remington et al., 1997; Aguilar-Tipacamu and Rodriguez-Vivas, 2003; George and Davey, 2004).

One concern when using products with incomplete efficacy against immature stages is the chance of them escaping detection during inspection and thereby decreasing the likelihood of a successful eradication program (Graham and Hourrigan, 1977; Davey et al., 2005). To avoid this situation, many acaracides are applied repeatedly and frequently to ensure ongoing control of *R. microplus* infestations. However, some *R. microplus* populations have developed resistance to multiple classes of acaricides and endectocides in response to the strong selection pressure associated with their intense use (George and Davey, 2004; Abbas et al., 2014; Klafke et al., 2017; Rodriguez-Vivas et al., 2018), an unsurprising consequence of recurrent treatments. Indeed, the problem has become so widespread that *R. microplus* has been ranked as one of the most resistant arthropods globally (Pérez de León et al., 2020).

Data from the current study suggest doramectin in Dectomax V® provides a solution to the incomplete and short-lived efficacies experienced with some products against ML-susceptible *R. microplus*. The ten tick infestations prior to treatment were scheduled (Day −24 through Day −2) to ensure all lifecycle stages were present on cattle at the time of treatment, and the study duration provided sufficient time for larvae not killed at treatment to develop to adults. Considering the approximately 21 days required for larvae in the last pre-treatment infestation to feed and develop to engorged adults (Arocho Rosario et al., 2022), larvae from the last infestation (Day −2) before treatment should have developed to adults by ~ Day 19 if they survived treatment on Day 0. Mean adult tick counts for Dectomax V®-treated cattle on Days 17 through 21 ranged from 0 to 1.8, and efficacy (based on counts or weight) was > 99%. Additionally, efficacy remained > 95 % through Day 30 post-treatment, even though cattle were reinfested multiple times per week following treatment on Day 0. Importantly, female ticks from Dectomax V®-treated cattle also showed > 98 % reduction in egg production and egg hatchability from post-treatment Day 3 through Day 30, highlighting the additional advantage of decreased pasture contamination and thereby parasite transmission.

Like ticks, sucking lice also feed on the blood of their host and can be highly problematic for cattle producers. Even moderate infestations in cattle can result in significant decreases in average daily weight gains (Byford et al., 1992), and it has been estimated that livestock producers in the US may lose up to US $125 M per year due to direct performance losses and associated wear and tear to facilities (Drummond et al., 1981; Tarpoff, 2018). *L. vituli, H. eurysternus,* and *S. capillatus* are the three species primarily responsible globally for sucking louse infestations on cattle (Georgi, 1985; Urquhart et al., 1987; Nafstad and Grønstøl, 2001; Cortinas and Jones, 2006; Walker, 2007; Juneau and Kaufman, 2021; McKiernan et al., 2021). Topical application of insecticides can be highly effective against the adult and nymph stages, but lice eggs are not affected by most insecticides, and a follow-up treatment two to three weeks after the initial application is required for the complete elimination of all lice stages (Thomas, 2011; Warren, 2019). Additional insecticide treatments are also required to prevent re-infestation, a

critical aspect of louse control in the field as new cattle can be regularly introduced into herds or pens of previously treated animals (Villeneuve and Daigneault, 1997).

In the last ~30 years, cattle producers have shifted away from traditional insecticides (Nafstad and Grønstøl, 2001). Pyrethroids are an improvement in that they lack the residual and ecological problems associated with the older insecticides and are available in various formulations allowing ease of application, but they lack efficacy against the egg stage of sucking lice and multiple treatments are required to ensure elimination of an infestation (Nafstad and Grønstøl, 2001; Thomas, 2011; Warren, 2019). In contrast to insecticides, a single administration of some MLs (e.g., doramectin, ivermectin, moxidectin) provides rapid, complete and long-term control of sucking lice (Townsend, 2000). At its US approval, Dectomax® (0.2 mg/kg doramectin) was shown to be 100 % effective in treating existing infestations of *H. eurysternus*, *L. vituli* and *S. capillatus* within 7 days of administration, and doramectin-treated cattle had significantly ($p < 0.05$) lower lice counts than control cattle from Day 7 through Day 28 (Logan et al., 1993; FDA, 1996). Additional studies have also reported doramectin to be highly effective against naturally acquired and introduced infestations of sucking lice on cattle (Lloyd et al., 1996; Phillips et al., 1996; Villeneuve and Daigneault, 1997).

The study herein confirms doramectin remains efficacious in treating ML-susceptible sucking lice infestations on cattle when formulated with levamisole in the novel FDCI endectocide, Valcor®. A single SC administration of Valcor® was 100 % effective in treating existing *L. vituli* infestations within 14 days of treatment, and control cattle maintained significantly ($p < 0.05$) higher mean lice counts than Valcor®-treated cattle for the entire duration of the study (through Day 56). These data align well with those reported for doramectin against sucking lice infestations when administered to cattle as a single-active endectocide (Logan et al., 1993).

## 5. Conclusions

Ticks and sucking lice can affect cattle health and production in various ways, requiring rapid and complete control and optimally, persistent activity. The data presented here demonstrate the ectoparasitic efficacy of doramectin is retained when the drug is administered SC to cattle as part of a newly developed combination endectocide consisting of 0.2 mg/kg doramectin + 6.0 mg/kg levamisole HCl. This FDCI, approved under the trade names of Valcor® in the US and Dectomax V® in Australia and New Zealand, is a tool cattle producers can confidently use in accordance with regional approved indications to obtain a high and persistent level of efficacy against these economically important ectoparasites.

## Ethics approval and consent to participate

*R. microplus* efficacy study: All animals were handled in compliance with Department of Agriculture and Fisheries Animal Ethics Committee (SA 2017/12/627 approved 17 January 2018) and any applicable local regulations. The study complied with the VICH GL9 Good Clinical Practice Guidelines (June 2000) (CVM, 2001), the APVMA Data Guidelines – Efficacy and target animal safety general guidelines (Part 8, 01 July 2014) (APVMA, 2014) and the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for evaluating the efficacy of acaricides against ticks (*Ixodidae*) on ruminants (Holdsworth et al., 2006b).

Sucking lice efficacy study: Procedures for animal use were approved by the Zoetis Ethical Review Board. The study complied with the WAAVP guidelines for evaluating the efficacy of ectoparasiticides against biting lice, sucking lice and sheep keds on ruminants (Holdsworth et al., 2006c).

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## CRediT authorship contribution statement

**Andrew A DeRosa:** Conceptualization, Methodology, Supervision, Writing, Interpretation. **Aleah Pullins:** Supervision, Investigation. **Jezaniah Kira Tena:** Writing – review & editing, Writing – original draft, Methodology. **Susan Holzmer:** Supervision, Investigation. **Raj Packianathan:** Writing – review & editing, Writing – original draft, Supervision, Investigation.

## Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests. Andrew A. DeRosa reports a relationship with Zoetis Inc that includes: employment. Aleah Pullins reports a relationship with Zoetis Inc that includes: employment. Jezaniah Kira Tena reports a relationship with Zoetis Inc that includes: employment. Susan Holzmer reports a relationship with Zoetis Inc that includes: employment. Raj Packianathan reports a relationship with Zoetis Australia Pty Limited that includes: employment.

## Data availability

The dataset supporting the conclusions of this article is included within the article.

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