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Therapeutic efficacy of eprinomectin extended-release injection against induced infections of developing (fourth-stage larvae) and adult nematode parasites of cattle

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ABSTRACT

The therapeutic efficacy of eprinomectin in an extended-release injection (ERI) formulation was evaluated against induced infections of developing fourth-stage larval or adult gastrointestinal and pulmonary nematodes of cattle in a series of six studies under two identical protocols (three each for developing fourth-stage larvae or adults) conducted in the USA, Germany or the UK (two studies at each location, one per stage).

Each study initially included 16 nematode-free cattle. The cattle were of various breeds or crosses, weighed 109–186.5 kg prior to treatment, and were approximately 4–7 months old. The animals were blocked based on pre-treatment bodyweight and then randomly allocated to treatment: eprinomectin ERI vehicle (control) at 1 mL/50 kg body weight or eprinomectin 5% ERI at 1 mL/50 kg bodyweight (1.0 mg eprinomectin/kg) for a total of eight and eight animals in each group. Treatments were administered once on Day 0 by subcutaneous injection in front of the shoulder.

In each study, cattle were infected with a combination of infective third-stage larvae or eggs of gastrointestinal and pulmonary nematodes. Inoculation was scheduled so that the nematodes were expected to be fourth-stage larvae or adults at the time of treatment. For parasite recovery, all study animals were humanely euthanized and necropsied 14–15 (adult infections) or 21–22 days after treatment (developing fourth-stage larval infections).

When compared with the vehicle-treated control counts, efficacy of eprinomectin ERI against developing fourth-stage larvae and adults was ≥98% ($p < 0.05$) for the following nematodes: *Dictyocaulus viviparus*, *Bunostomum phlebotomum*, *Cooperia curticei*, *C. oncophora*, *C. surnabada*, *C. punctata*, *Haemonchus contortus*, *H. placei*, *Nematodirus helvetianus*, *Oesophagostomum radiatum*, *Oes. venulosum*, *Ostertagia leptospicularis*, *O. ostertagi*, *O. circumcincta*, *O. pinnata*, *O. trifurcata* (developing fourth-stage larval infections only), *Strongyloides papillosus*, *Trichostrongylus axei*, *T. colubriformis*, and *Trichuris ovis* (adult infections only).

All animals accepted the treatment well. No adverse reaction to treatments was observed in any animal in any study.

1. Introduction

As documented in numerous publications, eprinomectin, the most recent commercialized compound of the macrocyclic lactone class of parasiticides used in

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a 0.5% pour-on formulation at 0.5 mg eprinomectin/kg bodyweight, is highly effective against infections with gastrointestinal and pulmonary nematodes, chorioptic and sarcoptic mange mites, biting and sucking lice infestations, and larvae stages of cattle grubs (Shoop et al., 1996; Barth et al., 1997; Gogolewski et al., 1997a, 1997b; Holste et al., 1997, 1998; Pitt et al., 1997; Williams et al., 1997; Yazwinski et al., 1997; Schönberg et al., 2000; Watson and Forbes, 2000; Campbell et al., 2001; Schoett et al., 2002; Rehbein et al., 2005). Dose titration studies have demonstrated that eprinomectin represents some threefold greater potency against gastrointestinal nematodes than ivermectin (Shoop and Soll, 2002), and egg count reductions occurred faster in cattle treated with eprinomectin than in animals treated with other topical macrocyclic lactones products (Baggott et al., 1999). The eprinomectin pour-on formulation has a persistent activity of up to 28 days against important bovine nematodes, e.g., *Cooperia* spp., *Nematodirus helvetianus* and *Ostertagia ostertagi* (Cramer et al., 2000; Holste et al., 2002) and hence has been used successfully in strategic programs for controlling gastrointestinal nematodes and lungworms in cattle (Batty et al., 1999; Epe et al., 1999; Dorny et al., 2000).

The suitability of eprinomectin as an injectable anthelmintic for cattle has been demonstrated in titration experiments using an experimental propylene glycol/glycerol formal (60:40) based formulation (Shoop et al., 2001).

In order to extend the persistent activity of eprinomectin against endoparasites, an injectable formulation has been developed which releases the active in concentrations to provide effective control of nematode infections in cattle for up to 150 days after treatment (Soll et al., 2013). In this extended-release formulation, eprinomectin is released from a matrix formed with poly(d,l-lactide-*co*-glycolic)acid (PLGA). PLGA is known as a safe and effective biodegradable material which has been assessed as a drug delivery system for extended release applications of various pharmaceutical compounds in human and veterinary medicines including macrocyclic lactones (Lewis, 1990; Miller et al., 1999; Clark et al., 2004; Winzenburg et al., 2004).

The studies reported here were designed to determine the therapeutic efficacy of eprinomectin extended-release injection (ERI) against established infections with normally developing larval and adult gastrointestinal and pulmonary nematode parasites of cattle.

2. Materials and methods

A total of six controlled studies were conducted, two each in the USA (Studies 1 and 4), in Germany (Studies 2 and 5), and in the UK (Studies 3 and 6). Three studies each were conducted under the same protocol to confirm the efficacy either against developing fourth-stage larval (Studies 1–3) or against adult nematode parasites (Studies 4–6).

The studies were designed and conducted to comply with the regulatory requirements of both the FDA/CVM and the European Medicines Agency/Committee for Medicinal Products for Veterinary Use, and according to relevant guidelines for good clinical practices (GCPs) and for establishing the efficacy of cattle anthelmintics.

The studies were performed as blinded studies, i.e., all personnel involved in collecting data were masked to the treatment assignment of the animals.

2.1. Experimental animals

The animal descriptions and details are presented in Table 1. A total of 48 (32 male, 16 male castrate) healthy, ruminating Holstein, Braunvieh (Brown Swiss), Limousin, Limousin cross, or Brown Swiss cross cattle were included in each series of three studies. The animals weighed 109–167.4 kg prior to treatment (Day −1) and were 4–6 months old in Studies 1–3; animals used in Studies 4–6 weighed 124.5–186.5 kg prior to treatment (Day −1) and were approximately 5–7 months old. Animals had not been treated previously with an avermectin or milbemycin product and were not shedding gastrointestinal nematode eggs and lungworm larvae as confirmed by standard fecal examination techniques (quantitative fecal egg count and Baermann techniques) 15–36 days prior to initial larval inoculation.

In all studies, cattle were held indoors to preclude unintentional nematode infection and were housed individually, for a minimum of two weeks before treatment until the end of the study. The environmental conditions were identical for all animals within a study. The animals were fed according to local practice, the same for each animal within each study. Drinking water was available at all times. Animals were handled with due regard to their welfare and in compliance with Merial Institutional Animal Care and Use Committee (IACUC) approvals, any applicable local regulations, and requirements of any local IACUC.

2.2. Induced nematode infections

The cattle were inoculated with a combination of infective third-stage larvae or eggs (*Trichuris ovis*) of the following nematodes: *Dictyocaulus viviparus*, *Bunostomum phlebotomum*, Chabertia ovina, *Cooperia curticei*, *C. oncophora*/surnabada, *C. punctata*, *Haemonchus contortus*, *H. placei*, *Nematodirus helvetianus*, *Ostertagia leptospicularis*, *O. ostertagi*/lyrata, *O. circumcincta*/pinnata/trifurcata, *Oesophagostomum radiatum*, *Oes. venulosum*, *Strongyloides papillosus*, *Trichostrongylus axei*, *T. capricola*, *T. colubriformis* and/or *Trichuris* spp. (sheep origin). Generally, parasites used were recent field isolates as defined per VICH GL 7 (FDA Guidance 90), Effectiveness of anthelmintics: general recommendations (Vercruysse et al., 2001); however, the *D. viviparus* isolate used in Studies 3 and 6 and the *O. radiatum* isolates used in Studies 2–6 were laboratory isolates. The number of infectious stages administered for each challenge was in accordance with the WAAVP Guidelines for Testing of Anthelmintics in Ruminants (Wood et al., 1995). The inoculation schedule was designed such that at day of treatment (=Day 0) nematodes were fourth-stage larvae in Studies 1–3 and adults or inhibited larvae in Studies 4–6 (Wood et al., 1995). The schedule of inoculation and the actual number of larvae/eggs administered to each animal in each study are shown in Table 2.