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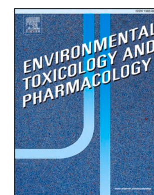

Pharmacokinetics of a long-acting subcutaneous eprinomectin injection in semi-domesticated reindeer (*Rangifer tarandus tarandus*) – A pilot study

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A R T I C L E I N F O

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A B S T R A C T

Reindeer (*Rangifer tarandus tarandus*) are exposed to the pathogenic parasitic nematode *Elaphostrongylus rangiferi* during grazing. The severity of disease is dose-dependent. Prophylactic anthelmintic treatment is needed to improve animal health and reindeer herding sustainability. Herds are traditionally only gathered once during the summer, requiring a drug with a persistent effect. In this study we investigated the suitability of long-acting eprinomectin, given as a single subcutaneous injection at 1 mg/kg bodyweight in adult reindeer and calves. Plasma and faeces concentrations were determined using ultra-high performance liquid chromatography high resolution mass spectrometry (UHPLC-HRMS). Plasma concentrations remained above the presumed effect level of 2 ng/mL for 80 days, demonstrating the drug's potential. Pharmacokinetic parameters were compared to other species using allometric scaling. Calves and adults had slightly different profiles. No viable faecal nematode eggs were detected during treatment. Eprinomectin was measurable in the reindeer faeces up to 100 days, which is of environmental concern.

1. Introduction

Infection with the parasitic nematode *Elaphostrongylus rangiferi* is of increasing concern for reindeer (*Rangifer tarandus tarandus*) in Norway as a consequence of a warming climate (Rose Vineer et al., 2021). The parasite has an indirect lifecycle with a highly temperature-dependent development in gastropods (slugs and snails) as intermediate hosts (Davidson et al., 2020), from L1 stage larvae to infective L3 stage larvae. This means that in a normal summer in a subarctic climate, *Elaphostrongylus* larvae shed that year only become infective to reindeer in the following year, in a two-year cycle. However, with increasing average temperatures, a shortening of the developmental time to a one-year cycle is predicted (Rose Vineer et al., 2021), entailing a much higher infection risk for reindeer. Exposure occurs by the accidental ingestion of L3-bearing slugs and snails during grazing. The period with the highest L3 parasite loads in gastropods is in July to August (Ciezarek, 2021), although infection can occur before and after the peak period. Disease severity is related to the number of infective L3 larvae that have been ingested. After uptake, the larvae migrate through the body to the central nervous system (CNS), where they develop further into sexually mature adults (Handeland et al., 1994). The adult parasites move then to the skeletal muscle, producing eggs that develop into L1 larvae, which in turn migrate to the lungs, are sneezed, swallowed again and finally excreted with the faeces. Adult nematodes are estimated to survive up to three years in the host (Halvorsen et al., 1985).

Infection with low parasite numbers causes little discernible disease given the majority of adult reindeer in Norway have *Elaphostrongylus* larvae in their faeces without showing clinical signs. However, higher doses can result in considerable disease and mortalities. Elaphostrongylosis in reindeer elicits a wide range of neurological clinical signs from mild head tremors and subtle hindlimb ataxia to a marked hunched back (kyphotic stance), wry neck (torticollis), hindlimb paresis and

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paralysis, and death (Davidson et al., 2020). Reduced live weights have also been reported in animals without clinical signs that had high *Elaphostrongylus* larvae burdens in their faeces (Stuut, 2021; Handeland et al., 2021). The prognosis is generally poor once clinical signs are pronounced (Davidson et al., 2020). In years with average summer temperatures, individual cases of brainworm infection are seen in some herds, but in years with warmer temperatures large-scale outbreaks have been recorded. During a recent outbreak in central Norway in 2018–2019, one herder reported losing about 50 % of the herd, with all age groups affected (Deksne et al., 2020). Animals may recover with symptomatic treatment and constant supplementary feeding during the occurrence of clinical signs, which can last months, provided the clinical disease is not too severe. Still, it may take a further year after the initial recovery before an animal is restored to full health (Isaak Danielsen, reindeer herder, personal communication). The earliest clinical signs occur 4–8 weeks post infection, long before it becomes possible to detect excreted L1 larvae in the faeces, given the parasite's prepatent period of 3–5 months (Davidson et al., 2020). Unfortunately, by the time clinical signs manifest, damage to the CNS has already occurred. This means that the window for drug treatments is limited to the period between infection of the reindeer with L3 larvae and their migration to the CNS.

Norway has semi-domesticated reindeer that are almost exclusively herded by the indigenous Sami people, in addition to wild reindeer. Both types are free-ranging throughout the year. The semi-domesticated herds are only rounded up a few times a year; the timing of which varies considerably between geographic regions. Calves are marked in late spring/summer (June-August), and in late autumn/early winter (October-January), the flocks are separated for slaughtering. Traditionally, there are no round-ups during the grazing season in late summer/early autumn. This limits the opportunities for carrying out prophylactic treatments against brainworm to the period of calf-marking. Any treatment given during this time would need to have a sufficiently long effect to provide protection for the remaining weeks/months of summer and autumn, when the risk for brainworm infections is predicted to be at its highest (Ciezarek, 2021).

Currently, the only anthelmintic preparation that is licensed for use in reindeer in Norway is ivermectin (Veterinærkatalogen, 2021). Ivermectin is a macrocyclic lactone (ML). The ML group comprises a large number of widely used anthelmintic drugs. The ML sub-group avermectins includes commercially available drugs such as ivermectin, abamectin, doramectin, selamectin, moxidectin and eprinomectin (McKellar and Jackson, 2004; Abongwa et al., 2017). These MLs are selective agonists of glutamate-gated chloride channels in the neurons and pharyngeal muscles of nematodes and arthropods that are not present in mammals. The activation of the channels leads to parasite paralysis and ultimately death from the inhibition of interneural and neuromuscular transmission (Lumaret et al., 2012). Avermectins are also agonists of nicotine and γ-aminobutyric acid (GABA) receptors in nematode somatic muscles cells, further increasing their anthelmintic effectivity (Lumaret et al., 2012). GABA receptors also occur in the mammalian CNS. If high enough drug amounts cross the blood-brain barrier (BBB), this mode-of-action can potentially cause neurotoxic side-effects in treated individuals.

Anthelmintic therapeutic activity is only achieved, when the ML drugs reach sufficient concentrations at the target sites during an appropriate time period. The pharmacokinetic properties describing the absorption, distribution, metabolism and excretion characteristics of a drug are dependent on its physical and chemical properties (Marley and Conder, 2002). Avermectins are lipophilic and thus extensively distributed throughout the body of mammals, concentrating in adipose tissues regardless of the route of administration (Martin et al., 2002). Slow re-transfer from these deposits into the plasma leads to prolonged terminal excretion half-lives (Canga et al., 2009). In spite of the ML's substantial lipophilicity, they do not notably cross the BBB due to the presence of multidrug efflux transporters (P-glycoprotein; P-gp) in the BBB (Kiki-Mvouaka et al., 2010). This provides protection against adverse effects on the CNS, but at the same time, hinders treatment of brain-infecting parasites.

Ivermectin is widely used as anthelmintic in farmed and companion animals, as well as humans. However, its pharmacokinetic profile is not optimal with regard to the specific requirements of elaphostrongylosis treatment in semi-domesticated reindeer, as the estimated effect duration in reindeer is only 14 days (Oksanen et al., 2014). This is too short to protect the reindeer against brainworm infections after a single application.

Three other drug preparations with longer acting anthelmintic effect in cattle and sheep (> 90 days) are commercially available, and two are approved for use in the European Union. Moxidectin is the active substance in the drugs marketed under the tradenames Cydectin® and Zemex® (European Medicines Agency, 2021). Moxidectin has similar absorption and distribution kinetics as ivermectin but has a notably prolonged mean residence time (MRT) in plasma and slower elimination, leading to an extended effect duration (Lanusse et al., 1997). There are, however, currently no moxidectin-containing products licensed for use in farmed animals in Norway (Veterinærkatalogen, 2021). The third anthelmintic drug preparation known for its long effect duration contains eprinomectin and is marketed under the tradename LongRange® (European Medicines Agency, 2018). In comparison to moxidectin, eprinomectin has a lower lipophilicity and higher affinity to P-gp. This results in a reduced affinity to body fat and decreased MRT, but a broader safety margin for adverse neurotoxic effects (Kiki-Mvouaka et al., 2010). Nevertheless, eprinomectin has proven its efficacy as an anthelmintic treatment in many ruminant species, including sheep, goat and cattle, as well as zebu, bison, camel, alpaca and red deer (Shoop et al., 1996; Gogolewski et al., 1997; Bengone-Ndong et al., 2006; Bengoumi et al., 2007; Woodbury et al., 2014; Pollock et al., 2017; Briqué-Pellet et al., 2017; Zajac and Garza, 2020). There are a number of pour-on eprinomectin-containing products licensed for use in farmed ruminants in Norway, but no s.c. products (Veterinærkatalogen, 2021).

Changing the dosage form and formulation of eprinomectin resulted in increased MRT. Pharmacokinetic studies in different species showed that the plasma MRT doubled, to about 5 days after topical application compared to per os (p.o.) application (Bengoumi et al., 2007; Wen et al., 2010). Drug delivery further increased by a factor of 2.5 with regard to the area-under-the-concentration-time-curve (AUC), with subcutaneous (s.c.) injection (Lespine et al., 2003; Aksit et al., 2016; Rostang et al., 2020). Sustained release formulations containing 5 % poly-lactide-glycolic acid, such as LongRange®, given s.c., can provide protection against nematode infections in cattle for up to 150 days (Soll et al., 2013) and in alpaca for 120 days (Pollock et al., 2017). Notably, the doses differed between the species: whereas 1 mg/kg body weight (b.w.) was sufficient in cattle to maintain plasma concentrations above the presumed effect level of 1.3 ng/mL, a dose of 5 mg/kg b.w. was required in alpacas.

Eprinomectin/LongRange® in s.c. application is licenced in North and South America for cattle and sheep (Soll et al., 2013). The formulation was, however, refused authorisation for use in domestic ruminants in the European Union in 2018 because of concerns regarding the possible effects of LongRange® on the environment and non-target species such as dung beetles (European Medicines Agency, 2018; Lumaret et al., 2012). It was concluded that the benefits of LongRange® did not outweigh the risks. The situation may, however, be assessed differently for free-ranging species such as reindeer, where anthelmintic protection for the whole summer season has to be achieved by a single treatment.

So far, only a few studies have investigated the applicability of MLs in reindeer. Ivermectin nematocidal effectivity and pharmacokinetics were compared after 200 μg/kg b.w. s.c. or p.o., or 500 μg/kg pour-on, showing that s.c. dosing was superior (Oksanen et al., 1993). In one additional experiment, the endectocidic efficacy of 200 μg/kg b.w. moxidectin s.c. in reindeer was elucidated and found to be slightly lower than that of ivermectin against warble fly *Hypoderma tarandi* (Oksanen

and Nieminen, 1998). Eprinomectin has not previously been tested in reindeer.

The aim of the present study was to investigate the feasibility of using the sustained release formulation LongRange® to reach sufficient eprinomectin plasma concentrations in reindeer for long-term protection against nematode infections. By determining the pharmacokinetic characteristics after a single s.c. application, we wanted to evaluate if this drug preparation could maintain sufficiently high eprinomectin levels over time to protect against brainworm and thus solve one of the problems in treating free-living semi-domesticated reindeer. At the same time, we analysed eprinomectin concentrations in faeces to estimate the ecotoxicological impact of the treatment.

2. Materials and methods

2.1. Experimental animals and sampling

Four adult, four-year old female reindeer and one male and one female calf (aged 4–5 months at study start) that had been accustomed to handling and to being housed for periods of up to a week in individual indoor/outdoor pens (corridors; 1–2 m × ~40 m) at the approved research animal facility (approval number 089) at the Department of Arctic and Marine Biology, UiT – the Arctic University of Norway), were included in the study. The project was approved by the Norwegian Food Safety Authority under licence no. 23936. The Norwegian Medicines Agency gave authorisation to import and use LongRange® in an experimental study in reindeer. The pharmaceutical wholesaler VESO assisted with importing the product directly from Boehringer Ingelheim in Canada (Burlington, ON, Canada). There was a slight delay in arranging the customs export from Canada, which stalled the study start by a month to the end of September.

The four adult reindeer, with b.w. ranging from 99 to 130 kg, were housed individually in stalls for 24 h prior to the experiment, and the calves, b.w. 54 and 59 kg, were housed for five days in “Lulla” calf stalls (Reime Landteknikk, Nærbø, Norway) that were placed in a 40 m^2 room with expanded metal floors. The animals had rubber mats to lay down on, and ad lib access to water and “FK Reinfôr BAS”, a pellet feed made for domestic reindeer, as well as to lichens and branches as treats. They were catheterised under sedation using an intramuscular (i.m.) injection of xylazine (Rompun 20 mg/mL; Bayer Animal Health GmbH, Leverkusen, Germany) at 0.87–1 mg/kg b.w. for the adults (Veterinærkatalogen, 2022a), or medetomidine hydrochloride (Zalopine 10 mg/mL; Orion Pharma, Espoo, Finland) at 0.1 mg/kg b.w. for the calves (Arnemo et al., 2014). An Equivet HiFlow Longterm catheter (16 G, 3 in.; Kruuse, Langeskov, Danmark) was placed in the jugular vein on the left side of the shaved neck. The catheter was sewn in place using non-absorbable suture material (Supramid 2/0, B. Braun Melsungen AG, Melsungen, Germany). A 30 cm connecting tube (Argon Medical Devices, Plano, TX, USA) was attached to the catheter via a Luer lock connection, feeding into a three-way tap (Kruuse) for sampling. In calves, also the extension tube was sewn in place to reduce drag on the intravenous catheter. The catheters and tubes were flushed with 5 mL sterile physiological saline and 1.5 mL heparinised saline solution (heparin 100 IU/mL; Leo Pharma AS, Lysaker, Norway) after each sampling, and at least twice daily to ensure continued patency. After the operation, the sedation was reversed using i.m. atipamezole (Antisedan vet. 5 mg/mL; Orion Pharma) at 0.16–0.35 mg/kg b.w. for the adults and 0.46–0.52 mg/kg b.w. for the calves (Veterinærkatalogen, 2022b).

Once all animals were fully recovered (after 3 h in adults and just over 1 h in calves), a single s.c. injection of either LongRange® (1 mg/kg b.w.) or physiological saline was placed just in front of the right shoulder. They were administered within a few minutes of each other. The injection site was monitored daily in the first week and thereafter during blood-sampling. Any changes to the hair or skin overlying the injection site were noted.

Blood samples were taken with decreasing frequency, from every two hours during the first 12 h, to daily, then weekly, then fortnightly and finally monthly intervals (Fig. 1). During the first study period with short intervals during sampling, the reindeer were tethered in the stalls to allow easy access. The catheters were removed on day seven, and the animals were moved to outdoor enclosures for the remaining study period. The outdoor area consisted of a 41,000 m^2 forest and grassland area that was divided into 15 enclosures. The adult female reindeer and the calves were kept in two separate enclosures (each about 2000 m^2). The enclosures were switched weekly to fortnightly to ensure fresh snow or pastures. The animals were fed reindeer pellets ad libitum, while fresh water or snow was available depending on the season. Blood samples were taken from all animals at all scheduled time points with the exception of the female adult 3, for which samples could not be drawn from the catheter at 6–18 h post injection.

During catheterisation, blood was drawn with a 5 mL syringe and transferred to a 4 mL BD vacutainer® tube containing K2E (7.2 mg EDTA) as anti-coagulant (Becton Dickinson Medical, Franklin Lakes, NY, USA). After catheter removal and reduction of the sampling frequency to weekly intervals, blood was sampled directly from the jugular vein into 10 mL BD vacutainer® tubes containing K2E using an 18 G needle (BD vacutainer® precision single sample needle, 18 G x 1.5 in.) with a BD vacutainer® One-use holder. The tubes were gently inverted to ensure sufficient mixing and then kept on a slant at room temperature (RT) for 1 h before centrifugation (2000*g*, 10 min, RT) was carried out. The plasma was transferred to 2 mL Nalgene cryogenic vials (Thermo Fisher Scientific, Rochester, NY, USA) and stored at − 80 °C until analysis for eprinomectin.

Faecal samples were collected daily from mats placed behind the animals in their stalls during the first week of the study. After the animals had returned to their outdoor enclosures, they were restrained during sampling. The faecal samples were taken directly from the rectum with a gloved hand, with the same frequency as the blood sampling (Fig. 1). The collected faeces were divided. One sample was kept refrigerated (2–6 °C) and analysed for parasites within 48 h, the other was frozen (−20 °C) within 2 h after sampling and stored until analysis for eprinomectin. The reindeer calves were given a single 15 mL dose of albendazole p.o. (Valbazen vet® 19 mg/mL; Zoetis, Farum, Danmark) on day 98 for tapeworm (*Moniezia* spp.) treatment.

2.2. Preparation of plasma samples

Plasma samples were thawed at RT and 200 μL-aliquots transferred to Phree solid-phase extraction (SPE) tubes (1 mL; Phenomenex, Torrance, CA, USA) for protein precipitation and phospholipid removal. Acetonitrile (ACN, gradient quality; Romil, Cambridge, UK) (1 mL), containing 1 % formic acid, was added to the plasma on-column and aspirated and dispensed carefully several times to mix the solution and precipitate the proteins. The samples were then filtered through the Phree columns by applying a gentle vacuum. The filtrates were evaporated to dryness at 60 °C under a stream of nitrogen and residues dissolved in 200 μL ACN/water (50:50) by vortexing. The samples were transferred to HPLC vials with fixed inserts (Thermo Fisher Scientific) and stored at − 20 °C until analysis. Positive control samples were included in each round of sample preparation by spiking blank reindeer plasma with 10 μL of an eprinomectin standard solution in ACN/water (50:50) to reach a concentration of 13.5 ng/mL eprinomectin B1a in plasma. The eprinomectin reference standard was purchased from Sigma-Aldrich (Merck, Darmstadt, Germany) and comprised 90.4 % eprinomectin B1a (Figs. 2) and 4.9 % of isomeric eprinomectin B1b.

2.3. Preparation of faecal samples

Faecal samples were thawed at RT. Aliquots of 0.2 g were weighed into 10 mL-polypropylene tubes (Sarstedt, Nümbrecht, Germany) and left to air-dry overnight. ACN (1.25 mL) was added, and the samples were vortexed for 2 min and shaken for 40 min on an orbital shaker

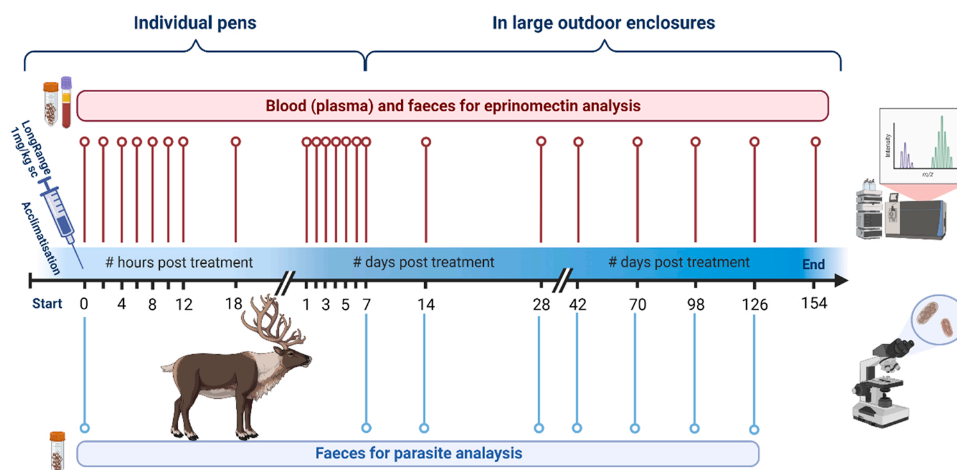

Fig. 1. Study design and sampling frequency. Blood and faecal samples were taken at regular intervals from four adult reindeer and two calves for the analysis of eprinomectin concentrations over time. Faeces were also analysed for the presence of parasites. Three adults and both calves were injected s.c. with LongRange® (1 mg/kg b.w.), whilst the fourth adult received saline as control.

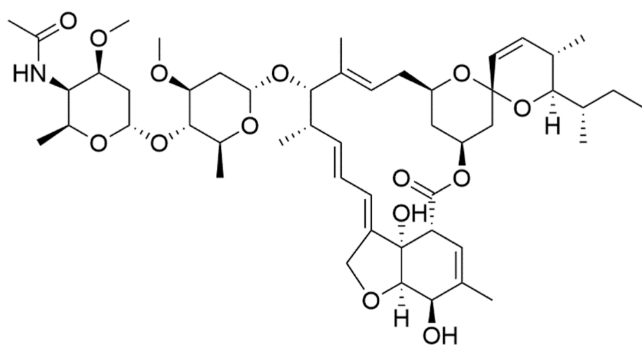

Fig. 2. Molecular structure of eprinomectin B1a.

(Medline Scientific, Chalgrove, UK) at 175 min^{-1}. The mixtures were centrifuged for 20 min (3000g, 20 °C) (Beckman Coulter, Brea, CA, USA) and 300 μL supernatant was transferred to a new tube. Water (1 mL) and triethylamine (1 μL) (≥ 99.5 %; Merck) were added to the supernatant, and SPE was performed using an Oasis PRiME® HLB SPE 96 well-plate (Waters Corporation, Milford, MA, USA). After loading onto the SPE plate, the samples were washed twice with 500 μL 23 % ACN. Subsequently, eprinomectin was eluted with 300 μL ACN/water (70:30). The eluates were evaporated to dryness at 60 °C under a nitrogen stream, and the residues were dissolved in ACN/water (50:50), transferred to HPLC vials with fixed inserts and stored at − 20 °C until analysis. Positive control samples were included in each round of sample preparation by spiking blank faeces with 10 μL of the eprinomectin B1a standard solution in ACN/water (50:50) to reach a concentration of 12.5 ng/g eprinomectin B1a in faeces.

2.4. Analysis of eprinomectin by UHPLC-HRMS

The eprinomectin concentrations in the processed plasma and faecal samples were analysed using a Vanquish Horizon ultrahigh-performance liquid chromatography instrument (Thermo Fisher Scientific, Waltham, MA, USA) connected to a Q-Exactive high-resolution mass spectrometer (Thermo Fisher Scientific) (UHPLC-HRMS), equipped with a HESI-II heated electrospray interface. The sample vials were maintained at 15 °C in the UHPLC autosampler. Chromatography was performed on a 100 mm × 2.0 mm i.d. Luna C18 (2)-HST column (2.5 μm, Phenomenex) at 30 °C. The capillary and probe heater temperatures were kept at 270 and 300 °C, respectively. Other instrumental settings included an AGC target of 3×10^6, maximum inject time of 256 ms, spray voltage of 3.5 kV, S-lens level of 90 %, sheath gas flow of 35 units and auxiliary gas flow of 10 units. The HRMS was operated in positive-ion, full-scan mode (m/z 800–1000) using a mass resolution set to 70,000. Chromatographic separation was achieved using a gradient of water (A) and ACN/water (95:5, v/v) (B), with both mobile phases containing 5 mM ammonium acetate and 5 mM acetic acid. The flow rate was 0.3 mL/min and the sample injection volume 2 μL. The column was eluted isocratically for 0.5 min with 50 % B, before a linear gradient was applied, increasing to 99 % B over 7.5 min. After flushing the column with 99 % B for 1 min, the mobile phase composition was returned to the initial conditions at 50 % B and equilibrated for 1.9 min. The total run time was 11 min. Quantification was achieved using matrix-matched calibration curves for plasma or faeces in the concentration range 0.87–256 ng/mL eprinomectin B1a.

2.5. Determination of pharmacokinetic parameters

The eprinomectin plasma and faeces concentrations measured in the reindeer were used to construct individual concentration-time curves. Moreover, the plasma concentration data were fed into the PKSolver add-in program in Microsoft Excel® (Zhang et al., 2010), allowing the determination of relevant pharmacokinetic (PK) parameters by non-compartmental analysis after s.c. application: area under the curve (AUC), mean residence time (MRT), terminal elimination half-life ($t_{½}$), time to maximum concentration (T_{max}), and maximum concentration (C_{max}). The PK parameters were calculated for each reindeer individually, before mean values with standard deviations were derived for the adult reindeer (n = 3) and the calves (n = 2). Eprinomectin residues were not detected in the adult negative control reindeer.

Plasma AUCs after a single topical or s.c. eprinomectin application were retrieved from literature for a number of different ruminant species. Using Dose (D)/AUC = Clearance (CL)/Bioavailability (F), the observable plasma clearances after extravascular application were calculated, which were subsequently applied in b.w.-dependent allometric scaling (log CL ~ log b.w.) (Obach et al., 1997).

2.6. Parasite egg and larvae analysis

Effects of the LongRange® treatment on the numbers of gastrointestinal nematode (GIN) eggs in the faeces of reindeer were evaluated by determining the faecal egg counts (FEC) using a modified McMaster flotation method (Taylor et al., 2015). The analysis had a sensitivity of 20 eggs per gram (EPG). Parasite larvae were analysed using a modified

Baermanns method (Handeland et al., 2019) with an estimated sensitivity of 1 larva per gram (LPG) based on the sample volumes used. Eggs belonging to the order Strongylida were counted, including genera in the families *Trichostrongyloidae* and *Strongyloida*, which cannot be morphologically distinguished. In addition, the faeces were analysed for eggs typical for the orders Enoplida (genus *Capillaria*), Trichocephalida (genus *Trichuris*) and Cyclophyllidea (genus *Moniezia*). It was not possible to obtain sufficient amounts of faeces from the adult reindeer on day 98. The chemical analysis was prioritised, followed by the McMaster method, and lastly the Baermann method. No samples were available for parasite analysis on day 154.

3. Results

3.1. Reindeer health and welfare

The animals adapted well to the housing conditions and showed no signs of stress. The catheterisation was successful, and the animals recovered rapidly from the procedure. After removing the catheters, the wounds healed without complications. Feed intake was normal, and the growth rates of the calves were as expected during the study period. The reindeer tolerated the injection of 1 mg/kg b.w. LongRange® s.c. without any visible issues.

On day 7 post treatment, one adult reindeer developed a skin reaction around the injection site, where a circular area, 2 cm in diameter, of alopecia appeared. The skin was slightly raised and harder in texture than in the surrounding area of the neck. There was no evidence of hyperaemia or infection at the injection site. Comparable skin reactions were not observed in the other animals. The symptoms regressed in the second study week, and were not observable by the next sampling point on day 14, with the hair growing back with time.

3.2. Performance of the UHPLC-HRMS method for eprinomectin detection in reindeer plasma and faeces

The characteristics of the newly developed eprinomectin method were determined with regard to common method validation guidelines (International Organization for Standardization, 2018). Eprinomectin was identified by its specific chromatographic retention time and accurate mass in the UHPLC-HRMS analysis.

The method was linear ($R^2 \geq 0.98$) in the concentration range 0.87–256 ng/mL in plasma and in faeces, using matrix-matched calibration curves. Eprinomectin B1a eluted with a retention time of 7.02 min (Fig. 3a) and was detected as protonated molecule at *m/z* 914.5260 (Fig. 3b). The selectivity was satisfactory, allowing to discriminate the target substance from interferences in the matrices. The sensitivity of the method was defined by the limit of detection (LOD = 0.6 ng/mL in plasma and 7.1 ng/mL in faeces), which was established from the calibration curve as LOD = 3 ×SD/m, using the slope (m) and the standard deviation of the y-intercept of the regression line (Magnusson and Örnemark, 2014). The uncertainty of the method was described by precision measuring in plasma (CV_{plasma} = 9.2 %; 30 ng/mL; n = 5; inter-day) and faeces (CV_{faeces} = 8.4 %; 32 ng/g; n = 4; intra-day). The accuracy of the UHPLC-HRMS method was determined by calculating recovery rates in plasma (79.8 ± 12.8 %; 13.5 ng/mL; n = 6) and in faeces (71.8 ± 14.8 %; 12.8 ng/g; n = 5) based on the positive control sample included in each analysis. The analytical performance parameters were in the recommended ranges and the method was considered as suitable for the applications planned in this study.

3.3. Eprinomectin concentration-time profiles in plasma and faeces of reindeer after a single s.c. dose

Calves and adults showed slightly different plasma concentration-time profiles after receiving one injection of 1 mg/kg b.w. LongRange®. There was also a considerable variation between the individual animals in both groups as expected for small sample numbers (Fig. 4).

The overall profiles were, however, comparable in all animals: the eprinomectin plasma concentrations increased during the first week after the application to the maximum concentration (C_{max}), decreased during the subsequent period, before a second, lower maximum was reached at about day 28. In the adult reindeer, a third small concentration peak was discernible at about day 70. The highest C_{max} was detected in the male calf. The eprinomectin plasma concentrations in the calves were about a factor 2 higher than in the adult animals in the initial phase of the study, but decreased faster. Eprinomectin concentrations above the LOD of the UHPLC-MS method were not detectable at day 98 in any of the reindeer, even though it was still detectable at low levels in the faeces of the adults. At day 126, eprinomectin

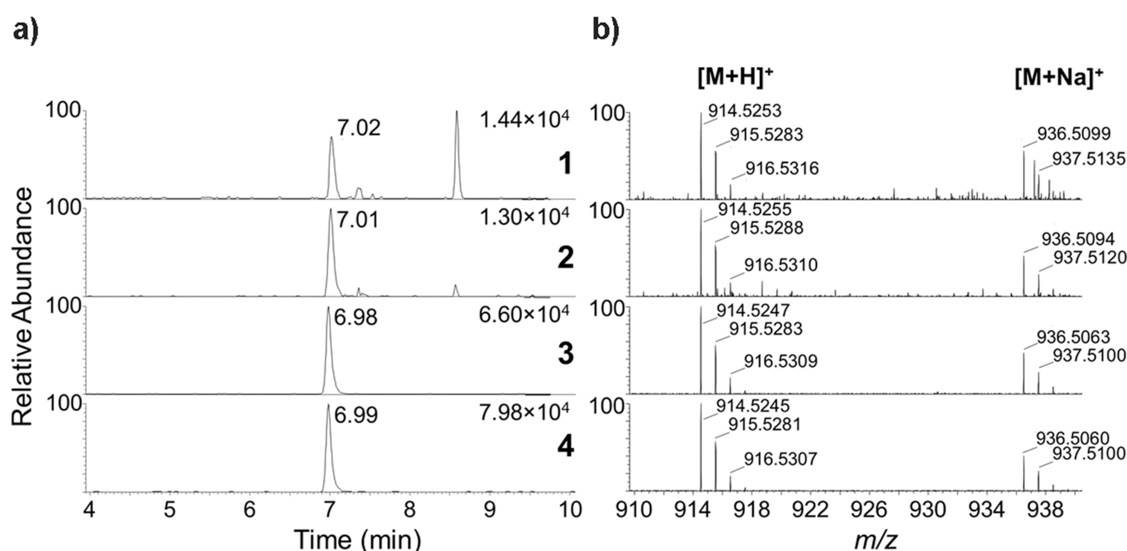

Fig. 3. a) LC–HRMS extracted ion chromatograms (± 5 ppm) of protonated eprinomectin B1a (*m/z* 914.5260) and b) mass spectra of the peak at 6.96–7.02 min. Individual traces show 1: blank reindeer faeces sample spiked with 12.8 ng/g eprinomectin B1a; 2: reindeer faeces sample containing 14.1 ng/g eprinomectin B1a; 3: blank reindeer plasma sample spiked with 13.5 ng/mL eprinomectin B1a; 4: reindeer plasma sample containing 11.1 ng/mL eprinomectin B1a. The intensities of the highest peak in each chromatogram are indicated in the upper right-hand corners (arbitrary units).

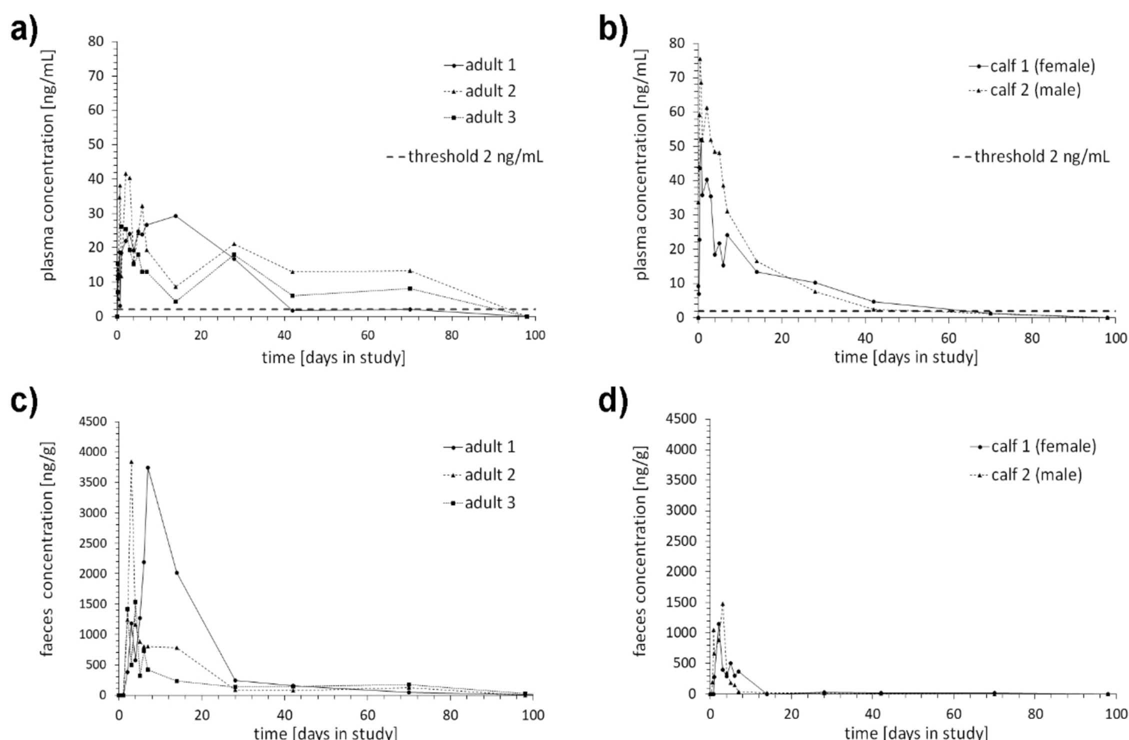

Fig. 4. Concentration-time curves after a single s.c. application of eprinomectin (LongRange®) in, respectively, the plasma (a, b) and faeces (c, d) of three adult female reindeer and two calves (one male, one female). The grey horizontal lines in the plasma graphs (a, b) show the 2 ng/mL level, which considered an estimate for the lower limit of anthelmintic activity.

concentrations above LOD were not determined in the faeces of either age group.

In both age groups, the eprinomectin concentration-time profiles in faeces peaked three times. In the adults, the concentrations were highest at about day 3, day 7 and day 70, and in the calves at about day 1, day 4 and day 28, although the differences between the animals were considerable. In all but one of the treated animals eprinomectin was measurable in faeces at 24 h after the s.c. application, whilst in one adult (A1) it was first detectable at 48 h.

3.4. *Eprinomectin pharmacokinetics in reindeer after a single s.c. application*

Pharmacokinetic analysis of the plasma concentration data showed that the time point (T_{max}) of the maximum plasma concentration (C_{max}) was reached within about 0.5 days in the calves and 1.5 days in the adults, with the exception of one female adult (A1) that had a delayed absorption, possibly due to the skin reaction at the injection site. The shorter time period to T_{max} in the calves may have contributed to the higher C_{max} as compared to the adults (Table 1). The differences in the concentration-time profiles between the two age groups (Fig. 4) had, however, little effect on their total exposure to eprinomectin as demonstrated by comparable mean AUC_{0-t} values, reaching 746 ng*d/mL ± 8.4 % in the calves versus 889 ng*d/mL ± 18.3 % in the adult reindeer in the time interval from study start to the last measurable concentration above LOD. The variabilities in both groups for the mean AUC_{0-t}, as well as for the mean C_{max} (63.6 ± 18.6 % in calves and 32.2 ± 20.6 % in adults), were rather low against the background of the small

Table 1
Pharmacokinetic parameters for plasma and faeces after a single s.c. application of 1 mg/kg b.w. LongRange® (eprinomectin) in adult reindeer and calves.

	Adult reindeer					Calves			
Parameter	A1[#]	A2	A3	Mean	SD	C1	C2	mean	SD
Plasma									
$t_{1/2}$ [d]	13.5	57.2	47.3	*39.3*	*18.7*	14.5	11.4	*12.9*	*1.53*
T_{max} [d]	14	2.0	1.0	*5.7*	*5.9*	0.8	0.5	*0.6*	*0.1*
C_{max} [ng/mL]	29.3	41.5	26.1	*32.3*	*6.64*	51.8	75.4	*63.6*	*11.8*
AUC_{0-t} [ng*d/mL]	849	1105	712	*889*	*163*	683	809	*746*	*62.9*
$AUC_{0\text{-inf,obs}}$ [ng*d/mL]	889	2202	1261	*1451*	*553*	711	831	*771*	*59.5*
$AUC_{0-t}/AUC_{0\text{-inf,obs}}$ [%]	95.6	50.2	56.4	*67.4*	*20.1*	96.0	97.4	*96.7*	*0.70*
$MRT_{0\text{-inf,obs}}$ [d]	21.6	91.9	77.4	*63.6*	*30.3*	21.5	15.2	*18.4*	*3.14*
Faeces									
$t_{1/2,faeces}$ [d]	13.7	16.8	26.9	*19.1*	*5.66*	61.8	8.11	*35.0*	*26.9*
$T_{max,faeces}$ [d]	7.0	3.0	4.0	*4.7*	*1.7*	2.0	3.0	*2.5*	*0.5*
$C_{max,faeces}$ [ng/g]	3740	3845	1536	*3040*	*1064*	1150	1474	*1312*	*162*
$AUC_{0-t,faeces}$ [ng*d/g]	49,677	23,685	19,272	*30,878*	*13,414*	7920	4408	*6164*	*1756*
C_{max} plasma/faeces	0.008	0.011	0.017	*0.011*[*]	–	0.045	0.051	*0.048*[*]	–
AUC_{0-t} plasma/faeces	0.017	0.047	0.037	*0.029*[*]	–	0.086	0.184	*0.121*[*]	–

[#] Animal A1 developed alopecia in the skin around the injection site at day 7.
[*] Value was calculated from the means (not from the individual data).

sample size. Because of the multi-phase plasma concentration-time profile, the determination of the terminal half-life ($t_{1/2}$) of eprinomectin in reindeer was determined by the data obtained for the late time points (> 42 days) in the study period (Fig. 4). The sensitivity (LOD) of the analytical method was thus of great importance. In this respect, notable differences between the individual $t_{1/2}$ were to be expected (Table 1). The delineation of the elimination phase of eprinomectin was also decisive for the estimation of the $AUC_{0\text{-inf,obs}}$, which in dependence of $t_{1/2}$ predicted the expected exposure during the whole study period. Whereas the shorter $t_{1/2}$ determined for the calves and the adult A1 resulted in good congruency (> 95 %) of AUC_{0-t} and $AUC_{0\text{-inf,obs}}$, the longer $t_{1/2}$ calculated for the adults A2 and A3 lead to assumption that the AUC_{0-t} predicted only about 50 % of the expected total $AUC_{0\text{-inf,obs}}$. Consequently, the derived mean residence time ($MRT_{0\text{-inf,obs}}$), representing the average time of a molecule in the body, for eprinomectin in the reindeer showed the same dependencies.

The $t_{1/2,faeces}$ of unchanged eprinomectin in the faeces varied considerably between the individual animals. It can, however, be expected that the ability of the analytical method to determine low substance concentrations (LOD) at later time points in the study had a substantial impact on the result. The difference in $t_{1/2,faeces}$ was especially pronounced for the two calves (Table 1). In contrast, $C_{max,faeces}$ (1312 ng/g ± 12.3 %) in the calves were comparable and showed a higher variability in the adult animals (3040 ng/g ± 35.0 %). $T_{max,faeces}$ was in all animals at about day 3, with the exception of the adult A1 with the delayed absorption profile. Generally, the eprinomectin concentrations measured in faeces were much higher than those in plasma, determined in the same animals at the same time points (Fig. 4). In the adult reindeer, the ratio of the individual C_{max} in faeces and plasma ranged from 59 to 128, while it was at 20–22 in the calves, demonstrating the greater variability in the adults. The ratio of the AUC_{0-t} in faeces and plasma mirrored this, ranging from 21 to 58 in the adult reindeer, and from 5.4 to 12 in the calves.

3.5. *Interspecies allometric scaling of estimated extravascular eprinomectin clearances in ruminants retrieved from literature references*

The pharmacokinetic characteristics of eprinomectin after topical or s.c. application have been explored in a number of ruminant species. The results of the literature review for eprinomectin pharmacokinetic parameters in ruminants are shown in Table 2 (plasma) and Table 3 (faeces). Comparative studies have shown that similar or even higher C_{max} and AUC were reached by s.c. application of doses lower than a factor of 2.5 as topical applied doses (Zhang et al., 2015; Aksit et al., 2016). Moreover, dose linearity was less pronounced after topical (Dupuy et al., 2001; Lifschitz et al., 2008; Hodoščeк et al., 2008; Hamel et al., 2017; Ballent et al., 2022) than after s.c. application (Briqué-Pellet et al., 2017).

The data for buffalo and camel appeared to differ considerably from other ruminant species, reaching only low C_{max} and AUC with the same pour-on dose (Dupuy et al., 2008; Bengoumi et al., 2007). This was also seen in alpaca, even though the respective study was performed with a higher dose, 5 mg/kg b.w. (Pollock et al., 2017). In contrast, the reindeer calves and adults in the present trial both reached C_{max} and AUC in

Table 2
Overview of reported eprinomectin pharmacokinetic parameters in plasma after topical or s.c. application in different ruminant species.

Reference	Species (n)	Dose [mg/kg b.w.]	C_{max} [ng/mL]	T_{max} [d]	AUC_{0-t} [ng*d/mL]	MRT [d]	b.w. [kg]
Alvinerie et al. (1999a)	cattle (5)	0.5; pour-on	43.8 ± 18.2	2.1 ± 0.3	239 ± 77.2	4.2 ± 0.6	746 ± 98
Baoliang et al. (2006)	cattle (4)	0.2; s.c.	44.0 ± 24.2	1.6 ± 0.8	306 ± 77.5	8.8 ± 2.3	600–750
Lumaret et al. (2005)	cattle (5)	0.5; pour-on	12.2	2.0	–	–	589
Wen et al. (2010)	cattle (5)	0.5; pour-on	16.2 ± 6.0	3.2 ± 1.3	91.0 ± 25.3	5.0 ± 1.0	580 ± 95
Rehbein et al. (2012)	cattle (8)	0.5; pour-on	9.7 ± 2.2	5.2 ± 0.9	124 ± 24.0	–	206–256
Aksit et al. (2016)	cattle (5)	0.5; pour-on	20.7 ± 4.0	4.4 ± 0.9	168 ± 15.7	8.2 ± 0.5	450–575
Aksit et al. (2016)	cattle (5)	0.2; s.c.	59.7 ± 12.9	1.3 ± 0.3	296 ± 61.5	4.7 ± 1.0	450–575
Ballent et al. (2022)	cattle (6)	0.5; pour-on	14.1 ± 7.5	2.7 ± 1.9	80.8 ± 15.3	5.9 ± 1.4	622 ± 68
Ballent et al. (2022)	cattle (6)	1.0; pour-on	24.6 ± 9.6	3.0 ± 1.1	136 ± 50.9	4.6 ± 0.4	622 ± 68
Ballent et al. (2022)	cattle (6)	1.5; pour-on	30.7 ± 13.9	2.2 ± 1.6	165 ± 44.7	4.8 ± 0.7	622 ± 68
Bengone-Nd. et al. (2006)	zebu (5)	0.5; pour-on	8.8 ± 2.2	1.3 ± 0.3	30.6 ± 5.6	3.4 ± 0.6	220–270
Dupuy et al. (2008)	buffalo (5)	0.5; pour-on	2.7 ± 0.9	1.4 ± 0.2	11.4 ± 4.0	3.2 ± 0.4	500–600
Zhang et al. (2015)	yak (6)	0.5; pour-on	15.3 ± 3.7	3.1 ± 1.2	194 ± 26.3	10.7 ± 1.4	420 ± 95
Zhang et al. (2015)	yak (6)	0.2; s.c.	35.8 ± 10.5	0.9 ± 0.4	134 ± 32.5	3.1 ± 1.5	420 ± 95
Sutra et al. (1998)	goat (1)	0.5; pour-on	7.4	4.0	–	–	–
Alvinerie et al. (1999b)	goat (6)	0.5; pour-on	5.6 ± 1.0	2.6 ± 0.9	72.3 ± 11.2	9.4 ± 0.4	41–90
Dupuy et al. (2001)	goat (5)	0.5; pour-on	2.2 ± 0.5	0.8 ± 0.1	8.2 ± 3.5	2.7 ± 0.6	54–78
Dupuy et al. (2001)	goat (5)	1.0; pour-on	3.0 ± 1.4	1.0 ± 0.5	15.7 ± 8.8	3.7 ± 0.9	54–78
Lespine et al. (2003)	goat (6)	0.2; s.c.	10.0 ± 4.5	0.9 ± 0.7	68.5 ± 23.2	6.6 ± 1.3	46–73
Lifschitz et al. (2008)	goat (5)	0.5; pour-on	5.0 ± 0.6	1.8 ± 1.3	16.5 ± 2.8	2.5 ± 0.3	33–45
Lifschitz et al. (2008)	goat (5)	1.0; pour-on	13.1 ± 5.0	1.6 ± 0.6	39.1 ± 15.3	2.3 ± 0.3	33–45
Lifschitz et al. (2008)	goat (5)	1.5; pour-on	16.2 ± 6.1	2.0 ± 0.7	57.3 ± 24.6	2.6 ± 0.2	33–45
Rehbein et al. (2014)	goat (8)	1.0; pour-on	5.9 ± 1.9	1.1 ± 0.4	31.0 ± 9.4	–	23–37
Hamel et al. (2015)	goat (8)	1.0; pour-on	3.7 ± 1.1	1.8 ± 0.5	23.5 ± 5.2	–	28–35
Hamel et al. (2015)	goat (8)	1.0; pour-on	5.3 ± 1.4	1.5 ± 0.5	36.0 ± 8.7	–	34–52
Briqué-Pellet et al. (2017)	goat (8)	0.2; s.c.	20.7 ± 12.9	1.5 ± 0.0	83.5 ± 34.8	4.2 ± 1.8	33–60
Briqué-Pellet et al. (2017)	goat (8)	0.4; s.c.	39.8 ± 17.3	1.3 ± 0.4	169 ± 43.4	4.0 ± 0.9	33–60
Hamel et al. (2021)	goat (10)	1.0; pour-on	5.4 ± 2.3	0.7 ± 0.5	23.8 ± 9.7	–	44–66
Shi et al. (2003)	sheep (5)	0.2; s.c.	20.0 ± 10.0	0.6 ± 0.1	49.5 ± 15.4	–	25–30
Hoste et al. (2004)	sheep (6)	0.5; pour-on	–	–	56.0 ± 26.2	5.3 ± 1.0	–
Imperiale et al. (2006)	sheep (12)	0.5; pour-on	2.3 ± 0.5	3.1 ± 0.4	15.8 ± 3.7	–	86
Hodoščeк et al. (2008)	sheep (6)	0.5; pour-on	2.2 ± 0.9	1.2 ± 0.4	13.6 ± 4.8	7.7 ± 1.2	–
Hodoščeк et al. (2008)	sheep (6)	1.0; pour-on	5.3 ± 2.7	1.5 ± 0.5	33.7 ± 22.5	9.0 ± 2.1	–
Hamel et al. (2017)	sheep (8)	1.0; pour-on	6.2 ± 1.7	3.1 ± 3.0	48.8 ± 19.2	–	67–102
Hamel et al. (2017)	sheep (6)	0.5; pour-on	2.2 ± 0.9	1.2 ± 0.4	13.6 ± 4.8	–	–
Bengoumi et al. (2007)	camel (5)	0.5; pour-on	1.8 ± 1.4	1.5 ± 0.4	6.3 ± 4.8	5.3 ± 2.4	490–550
Pollock et al. (2017)	alpaca (6)	5.0; s.c.	5.7 ± 3.3	3.9 ± 5.2	443 ± 150	62.4 ± 9.2	70.7
			6.1 ± 2.5	77 ± 12.5			
Davidson et al., this study	reindeer (3)	1.0; s.c.	32.2 ± 6.6	5.7 ± 5.9	889 ± 163	63.6 ± 0.3	99–130
Davidson et al., this study	r. calves (2)	1.0; s.c.	63.6 ± 11.8	0.6 ± 0.1	746 ± 62.9	18.4 ± 3.1	54–59

Table 3
Overview of reported eprinomectin pharmacokinetic parameters in faeces after topical or s.c. application in different ruminant species.

Reference	Species (n)	Dose [mg/kg b.w.]	$C_{max,faeces}$ [ng/g]	$T_{max,faeces}$ [d]	$AUC_{0-t,faeces}$ [ng*d/g]	$MRT_{,faeces}$ [d]
Kožuh Eržen et al. (2007)	sheep (6)	0.5; pour-on	471	3.0	–	–
Jiang et al. (2007)	cattle (3)	0.5; s.c.	505–797	1.0	–	–
Lumaret et al. (2005)	cattle (5)	0.5; pour-on	350	3.0	–	–
Aksit et al. (2016)	cattle (5)	0.5; pour-on	99.5 ± 43.2	3.2 ± 0.8	779 ± 117	9.4 ± 2.5
Aksit et al. (2016)	cattle (5)	0.2; s.c.	223 ± 64.0	2.9 ± 0.9	1189 ± 492	5.9 ± 1.7
Nieman et al. (2018)	cattle (10)	1.0; s.c.	184 188	14 84	17,759	–
Davidson et al., this study	reindeer (3)	1.0; s.c.	3040 ± 1064	4.7 ± 1.7	30,878 ± 13,414	25.5 ± 8.7
Davidson et al., this study	r. calves (2)	1.0; s.c.	1312 ± 162	2.5 ± 0.5	6164 ± 1756	18.2 ± 12.2

the same range as most other species treated s.c. with eprinomectin. This became particularly clear, when we carried out allometric scaling of the reported AUC, doses and b.w. of different ruminant species to estimate the apparent plasma clearance CL/F = D/AUC independent of b.w. (Fig. 5).

The allometric scaling had different levels of success for data obtained after s.c. (Fig. 5a) and topical (Fig. 5b) application. Whereas $R^2 = 0.8$ indicated good correlation for the s.c. data, after exclusion of the alpaca study with the extraordinary high dose, only a trend could be assumed for the topical data ($R^2 = 0.28$). Even the removal of apparent outliers, such as the camel and buffalo data, did not improve the correlation, but further decreased R^2, showing the great variation in the data set.

Nevertheless, the allometric scaling showed also a greater distance of the reindeer calf data from the centre of the dataset as compared to the adult reindeer (Fig. 5a). Although the sample number was small, it was obvious that C_{max} was considerably higher and the MRT shorter in the calves (Table 1). The resulting AUC was, however, in the same range as for the adults, leading to the good correlation in the upscaling of the derived apparent CL.

Data on eprinomectin concentrations in the faeces of treated animals are scarce and have been acquired in different species (Table 3). It is noticeable that $C_{max,faeces}$ and AUC_{faeces} are generally higher than the plasma $C_{max,}$ and AUC in the same animal (Table 1) (Lumaret et al., 2005; Aksit et al., 2016). Moreover, eprinomectin in faeces is detectable for an extended time period, and the concentrations are higher after s.c. than after topical application, if the dose-normalised data are compared. In the present study on reindeer, the major part of the total faecal eprinomectin was excreted in the calves within the first 7 days after the application, and in the adults within 28 days, adequate to what has been observed for other species. Taking the different b.w. and the supposedly proportional amount of produced faeces into consideration ($A \sim AUC_{faeces}$ * b.w./dose), the estimated total amount of faecally excreted eprinomectin appeared to be similar between species, but smaller in the reindeer calves. The initially high concentrations of eprinomectin in the faeces of reindeer post treatment could be of concern and might pose an environmental risk. The LD_{50}/EC_{50} for freshwater fish, invertebrates and plants range from 25 ng/L to > 5 g/L (MSDS, 2015), and was for non-target dung beetles about 7 ng/g (Lumaret et al., 2005).

3.6. *Occurrence of parasite eggs and L1 larvae in faeces of eprinomectin-treated reindeer*

All animals had low GIN levels in faeces at study start (day 0), which were classified as Strongylida. The FEC did not exceed 200 EPG (Fig. 6). After the treatment with LongRange®, GIN above the detection limit (20 EPG) were no longer detectable in the adult reindeer at any time point. Moreover, eggs of other nematode species were not identified either. In contrast, the untreated adult control animal had low to moderate levels of Strongylida eggs (up to 900 EPG) in the faeces at all sampling points. The faeces of the two calves contained low levels of Strongylida eggs on day 0, and additionally on day 7 (only in the male calf) and day 42 (both calves) (Fig. 6). The quality of the eggs on days 7 and 42, however, was poor. Strongylida eggs were not detected at any other time point in the study including day 98, before the Valbazen treatment of the calves.

Apart from Strongylida eggs, the calf faeces contained *Capillaria* sp. (60–100 EPG) and *Trichuris* sp. (20 EPG) eggs at day 0. Moreover, *Moniezia sp.* were detected in the calf faecal samples from day 28 to day 98. Finally, oocysts of protozoan *Eimeria* sp. (subclass Coccidia) were detected at low levels (20–400 oocysts per gram) in the calf faeces at

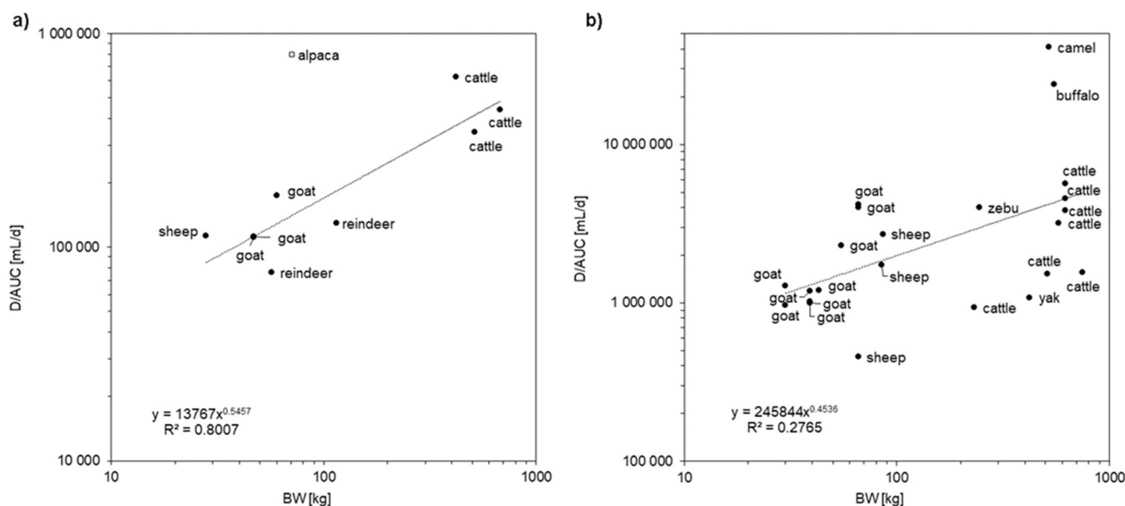

Fig. 5. Allometric scaling using published data on AUC, dose and b.w. for different ruminant species (Table 3) after a) s.c. application; or b) pour-on topical application of eprinomectin. R^2: correlation factor. Data points shown as unfilled squares were not considered.

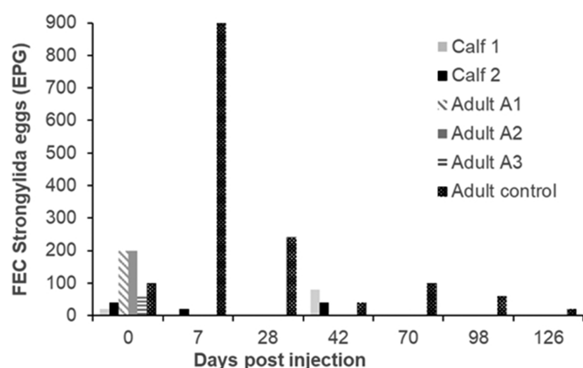

Fig. 6. Gastrointestinal nematode levels (Strongylida; faecal egg counts (FEC) measured in eggs per gram (EPG)) in the faeces of reindeer calves and adult females (A1 to A3) before (day 0) and after a single s.c. application with LongRange® (days 7–126; samples of A1 to A3 on day 98 were not available). The efficacy of the drug is evident by comparison with the untreated adult control animal.

days 98 and 126.

The presence of L1 stage larvae of *E. rangiferi* in the reindeer faeces was investigated using the Baermann method on day 0 (n = 6), day 7 (n = 6), day 98 (n = 1) and day 126 (n = 3). No larvae were detected.

4. Discussion

There is an immediate need to improve prophylaxis and treatment of brainworm infections in semi-domesticated reindeer in Norway due to increased infection pressure (Davidson et al., 2020; Idland et al., 2021; Closset, 2021). In this present pilot study, we have explored the anthelmintic potential of the avermectin derivate eprinomectin as a single-dose therapeutic in reindeer, using the recommended dose of 1 mg/kg b.w. (Yazwinski et al., 2016; European Medicines Agency, 2018).

Eprinomectin is considerably lipophilic and widely distributed to different body tissues, transferring also into the milk of lactating animals (Bengone-Ndong et al., 2006; Bengoumi et al., 2007). A number of methods for the analysis of eprinomectin in different matrices such as plasma, liver, milk and faeces, but also soil and surface water, have therefore been developed (Ballard et al., 1997; Durden, 2007; Thompson et al., 2009; Litskas et al., 2010). The reported sample preparation and clean-up procedures include liquid/liquid extraction, solid phase extraction and protein precipitation, and the detection methods used are liquid chromatography (LC) or LC–mass spectrometry (LC–MS), with or without analyte derivatisation with fluorescence markers and/or fragmentation of ionised molecules (MS/MS) (Danaher et al., 2006). In our study, we took advantage of high-resolution MS for the unambiguous identification of eprinomectin. We were thus able to streamline sample preparation to a single step, applying protein precipitation/phospholipid removal cartridges for plasma samples and SPE extraction for faecal samples, and still achieve satisfactory method performance. Linearity, sensitivity (LOD), uncertainty and recovery of the novel UHPLC-HRMS method for eprinomectin in plasma and faeces were in the range of comparable methods (Aksit et al., 2006; Litskas et al., 2010).

The novel method was applied to measure eprinomectin in the plasma and faeces of reindeer adults and calves after a single s.c. application of 1 mg/kg b.w. LongRange®. Concentrations dropped below the LOD in plasma between 70 and 98 days, and in faeces between 98 and 126 days post injection. The inter-individual differences, however, were considerable, likely due to the low sample number, the skin reaction in one adult reindeer, and a possible disparity in the metabolism capacity of young and old animals. Skin irritation and alopecia in connection with eprinomectin as we observed in one female adult reindeer have not been reported before. The animal appeared to be otherwise unaffected and presented no symptoms of impaired welfare, supporting the general good tolerability of s.c. eprinomectin injections documented in other species (European Medicines Agency, 2018). Nevertheless, we assume that the changes in the injection site were responsible for notable changes in the eprinomectin absorption kinetics, leading to differences in the plasma concentration-time profile of reindeer A1 with a delayed C_{max} as compared to the other animals in the study. It can also not be excluded that some of the injection went intradermal rather than subcutaneous, resulting in different absorption kinetics. Previously, it has been shown that eprinomectin residue concentrations at the application site can vary considerably and remain high for an extended period, especially when using a sustained release formulation such as LongRange®. Changes in the pharmacokinetic profiles have also been observed in cattle depending on the injection site, e.g. in the shoulder or the base of the ear (European Medicines Agency, 2018), and the total body fat (Wen et al., 2010), so that apparently multiple factors can influence the initial absorption and distribution phase of eprinomectin. Regarding the results for the male calf and the females in the study, a sex-related impact could neither be excluded, although the available data were too few to draw a conclusion.

The elimination phase of eprinomectin is strongly affected by the interaction of the drug with intestinal P-gp (Lespine et al., 2009; Kiki-Mvouaka et al., 2010). P-gp efflux pumps, situated on the apical side of epithelial cells, effectuate the transport of compounds from the blood into the gut lumen, reducing the systemic concentrations. Reabsorption from the gut can, however, lead to a repeated increase, as observable in the multi-phasic eprinomectin plasma concentration profiles that we found in the reindeer, and that have been reported in different ruminant species (Aksit et al., 2016; Pollock et al., 2017). Since biotransformation of eprinomectin occurs only to a very small extent, and major metabolites have not been identified, P-gp-supported efflux and excretion via the faeces is the main elimination pathway (Kiki-Mvouaka et al., 2010). Intestinal P-gp activity can be influenced by several factors such as the luminal pH, gut content or physiological determinants including the P-gp expression rate or blood contents. It can thus be assumed that the faecal excretion rate of eprinomectin in reindeer will vary depending on e.g. feed, age and health status.

Differences in the eprinomectin absorption kinetics between calves and adult animals are not unexpected. A similar age disparity has previously been reported for cattle (Rehbein et al., 2012), which also was demonstrated in the allometric scaling exercise for pour-on applications by featuring as the lowest data point for cattle (Fig. 5**b**). LongRange® is not recommended for use in animals under three months of age (European Medicinal Agency, 2018), and this pilot study confirms that disproportionally high and potentially harmful C_{max} could occur in calves, perhaps due to the undeveloped body fat and thereby different distribution and elimination kinetics. The resultant shorter eprinomectin plasma MRT and half-life ($t_{1/2}$) in the reindeer calves (Table 1) suggested a reduced long-term efficacy as compared to the adult animals. Moreover, the applicability of LongRange® for calves is also uncertain, because the synchronisation of the drug application with calf-marking in early summer means that not all animals are at least three months old. Since calves and yearlings have a particularly high risk of developing symptoms upon brainworm infection given their lack of immune protection from previous exposure, calf-marking could be delayed to allow treatment. In cases where this is not feasible, infected animals could be treated during the slaughtering round-up, so that at least the emergence of clinical symptoms in the winter could be avoided.

The reindeer in this study were regularly fed and in a good to very good body condition throughout the experiment. This, however, may not be the case in free-roaming herds. Usually, reindeer lose strength and fat reserves during winter and spring because of the restricted access to feed and the metabolic demands of pregnancy and nursing a calf. The fat reserves are then rebuilt throughout the summer and autumn by

extensive grazing. Since the anthelmintic treatment has to coincide with calf-marking, the body conditions will vary between reindeer, which can have an impact on the eprinomectin pharmacokinetics in each animal (Danaher et al., 2006). In this regard, this seasonal timing of the treatment in this pilot study can have impaired the comparability to in-field applications. The trial was started after the autumn equinox, where daylight hours are becoming considerably shorter. This was caused by the delayed delivery of the LongRange® preparation, but was also necessary to ensure that the calves were more than 3 months old. Notably, it has been shown that the photoperiod can influence the metabolic activity in reindeer. The animals have adapted to large changes in daylight periods, ranging from almost 24 h of light in the summer to the winter with nearly permanent darkness. Metabolic studies have shown how some metabolic pathways are closely linked to the photoperiod, whilst others remain unaffected (Meier, 2021). Considering this, further studies should ensure that the animals receive treatment during mid-summer, coinciding with the period of the year, in which protection of the semi-domesticated reindeer against brainworm infection is required.

We performed an extensive literature search on eprinomectin pharmacokinetics in ruminant species to establish a data base for comparison with the parameters obtained in reindeer. Interspecies allometric scaling takes advantage of the finding that fundamental physiological mechanisms in mammals are size-related, including compound elimination reactions (Boxenbaum, 1982). In the present study, we explored the applicability of this technique for data obtained by extravascular applications of eprinomectin. Already from the outset, there was a considerable degree of uncertainty in this approach, since it was based on published data, which had been generated by differently conducted animal experiments and measured by various analytical methods. Results were often presented in ranges or showed considerable variabilities (SD) (Table 2). We decided to use mean values of both AUC and b.w., thus simplifying the data set, but losing information about the data distribution at the same time. A second significant factor adding to the result uncertainty was to base the upscaling on the apparent CL/F calculated for extravascular applications that contains the bioavailability F as unknown factor. As discussed, eprinomectin absorption kinetics are greatly influenced by the site of application and the body condition of the treated animal. Moreover, differences between species can have an impact on the result. It has been shown that T_{max} appeared later in sheep than in goats after the same pour-on eprinomectin dose, which was ascribed to differences in the skin structure and hair coat properties (Hamel et al., 2017). Clearances determined after intravenous application usually reach a correlation coefficient R^2 above 0.9 (Lindstedt and Schaeffer, 2002). Interestingly, R^2 for CL/F vs. b.w after s.c. exposure was close to this expected value in spite of the uncertainties of the approach, in contrast to R^2 after topical eprinomectin application. It thus appeared that the potential correlation of eprinomectin kinetics between species was overshadowed by the multiple uncertainty factors after pour-on application, whereas the s.c. applications showed good correlation across the species. The reindeer data fitted well into the data set. This is promising as it could allow the prediction of AUC for lower doses than the 1 mg/kg b.w. used in the present study, assuming comparable dose linearity as in the other species.

The differences in plasma T_{max} of s.c. and topical eprinomectin applications were remarkably low between the different species and studies (Table 2), reaching median values of, respectively, 1.3 d (0.6 – 3.9; n = 10) and 1.9 d (0.8 – 4.4; n = 28) after removal of the data for adult reindeer A1 (Table 1) and the second concentration maximum in the high-dose alpaca study. A similar congruency between most ruminant species was observable for the MRT, reaching medians of, respectively, 4.5 d (3.1–8.8; n = 6) and 4.8 d (2.3–10.7; n = 19) for s.c. and topical application. However, the MRTs of reindeer and alpaca were clearly different and were excluded from the determination of the median values. It is uncertain if the discrepancy was caused by species differences or the dosage considering the alpaca trial used higher s.c. eprinomectin doses than the other studies,

The persistence of eprinomectin in manure (time to 50 % degradation, DT_{50} = 312–3922 d; European Medicinal Agency, 2018) is of considerable concern in environmental risk assessment. Exposure to MLs is known to have non-targeted harmful effects on coprophagous insects and soil invertebrates like reduced biodiversity, inhibited development and reproduction as well as mortality, leading to the delayed decomposition of dung (Jacobs and Scholtz, 2015). There are only limited studies but it has been shown that eprinomectin in dung is larvicidal, increases juvenile mortality and suppresses brood ball production in some insect species. Interestingly, faeces containing eprinomectin was shown to have a tendency to repel insects in contrast to faeces with moxidectin and ivermectin residues (Floate, 2007). Modelling has suggested that reservoirs of untreated dung could considerably limit the impact of avermectin residues on invertebrates, independently of the actual residue levels in the dung of treated animals in the same area (Cooke et al., 2017). Thus, a targeted approach of treating a sufficient number of reindeer in a herd with the aim of keeping nematode numbers in the grazing area low to reduce the infection pressure, but of decreasing the environmental impact at the same time. This could be a suitable compromise to mitigate potential effects of eprinomectin on dung organisms. The ecotoxicological concerns were central to the European Union's refusal of LongRange® marketing authorisation (European Medicinal Agency, 2018).

The reindeer included in this study were not intentionally infected with parasites, but carried a natural burden at study start. In the faeces of the untreated adult control animal, nematode eggs were detectable during the whole study period. There was a considerable rise in Strongylida FEC from baseline until day 7, after which it decreased again to about baseline. This animal was kept indoors during the first study week and was therefore unlikely exposed to any additional infection pressure. Although none of the animals showed physical signs of stress or discomfort during this housing period, some stress from sedation and sampling might have led to raised stress hormones. This could in turn have led to a relaxation of host immunity, which in the untreated animal presented as increased FEC. In contrast, the single LongRange® treatment reduced the Strongylida FEC below the LOD in the treated adult reindeer for the whole study period, showing the efficacy of the drug. The presence of poorly preserved nematode eggs in the faeces of the calves at two study time points was more suggestive of transient passage of eggs through the digestive tract rather than an active infection. This suggests that the younger animals were also protected against Strongylida infections, but this requires further testing. Moreover, the FEC findings could also result from study bias. The samples collected from the mat placed behind the reindeer could have been cross-contaminated with material from previous days as the mats were brushed clean but not washed on a daily basis. In the outdoor pens, the calves could either have been re-infected and developed a patent infection, or more likely, the nematode eggs had been taken up during grazing and just passed through the gastrointestinal tract. The latter assumption fitted to the poor condition of the eggs. The presence of varying levels of *Moniezia* spp. and *Eimeria* spp. in the calves, however, was not unexpected since mL drugs are not effective against these parasites.

The dose of 1 mg/kg b.w. and the site for the s.c. injection were chosen by us based on the recommendations given for domestic ruminants, where the drug is approved as an anthelmintic, and with regard to published studies (Table 3). Anthelmintic effects are seen in other MLs when plasma mL concentrations are higher than 1–2 ng/mL (Alvinerie et al., 1995; Lanusse et al., 1997; Lifschitz et al., 1999). Assuming this also applies to reindeer and eprinomectin, the single dose of eprinomectin gave plasma concentrations above 2 ng/mL for more than 80 days in the reindeer adults and calves. This would provide sufficient protection against elaphostrongylosis during one grazing season for semi-domesticated herds in Norway. A field trial with free-grazing animals should be conducted for confirmation of the drug's applicability under natural conditions. Furthermore, a study for the determination of

the dose-response relationship of LongRange® in reindeer should be performed so that the lowest effect level could be found, with the aim of avoiding over-dosing and thus of reducing the environmental impact from residues in the faeces.

5. Conclusion

This pilot study investigated the pharmacokinetics and efficacy of the long- acting eprinomectin delivery form LongRange® after a single s.c. 1 mg/kg b.w. application in reindeer. It showed the drug's potential to provide prophylaxis against brainworm infection. According to our findings, plasma concentrations above the effective level can be expected to last for at least 80 days in both adult animals and calves. This would be long enough to bridge the period with the highest infection risk in July to August, and thus reduce the risk of brainworm disease outbreaks. The efficacy profile of the drug fits the limitations set by the timeframe of reindeer herding practices. A known draw-back of using eprinomectin is the drug's persistence in excrement. Under the conditions of this study, residues were detected in the reindeer faeces for almost 100 days at considerable concentrations, which could be of concern regarding environmental risks.

CRediT authorship contribution statement

Rebecca K. Davidson: Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition, Visualization. **Christiane Kruse Fæste:** Methodology, Resources, Formal analysis, Writing – original draft, Writing – review & editing, Visualization. **Silvio Uhlig:** Methodology, Validation, Investigation, Resources, Writing – original draft. **Feng-Ling Tukun:** Validation, Investigation, Writing – original draft. **Hans Lian:** Conceptualization, Investigation, Resources, Writing – original draft. **Hans Arne Solvang:** Investigation, Resources, Writing – original draft. **Renate Thorvaldsen:** Investigation, Resources, Writing – original draft. **Lars P. Folkow:** Conceptualization, Investigation, Resources, Writing – original draft, Funding acquisition. **Javier Sánchez Romano:** Investigation, Writing – original draft. **Marianne Vinje Kilvær:** Investigation, Writing – original draft. **Karin Elisabeth Holmgren:** Investigation, Writing – original draft. **Ingebjørg Helena Nymo:** Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing, Funding acquisition, Visualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Rebecca Davidson reports equipment, drugs, or supplies were provided by Boehringer Ingelheim GmbH.

Data Availability

Data will be made available on request.

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