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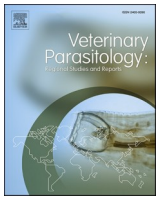

Original Article

Pharmacokinetics of a topical application of moxidectin in bare-nosed wombats (*Vombatus ursinus*)

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ABSTRACT

Sarcoptic mange is a debilitating disease that affects bare-nosed wombats (*Vombatus ursinus*). One of the drugs currently used for treatment is moxidectin, as it has a relatively high efficacy against endo and ectoparasites and side effects are uncommon in domestic species, thus it is considered a relatively safe drug to use at the recommended doses. Developing further understanding of the pharmacokinetics of moxidectin will aid in developing treatment regimens for sarcoptic mange in wombats. Here we analyzed the pharmacokinetic parameters of using 100 ml of moxidectin (5 g/l) applied topically. We found that mean peak plasma concentration was 0.50 ng/ml and half-life was 8 days. Moxidectin was excreted in scats with the mean peak concentration of 2461.43 ng/g (on a dry matter basis). Our study has provided the pharmacokinetic parameters of a commonly used treatment for sarcoptic mange in wombats. There were no adverse side effects recorded in the wombats after applying moxidectin topically. This study replicated real-world conditions using topical application on free-living wombats. The relatively low plasma concentration suggests the drug is not accumulating in the blood stream and is excreted via scats.

1. Introduction

Moxidectin is a potent macrocyclic lactone endectocide (*endo*- and ectoparasiticide) (Prichard et al., 2012; Rock et al., 2002) which affects chloride ion channel activity in the nervous system of parasites, disrupting electrical activity in nerve and muscles cells, causing paralysis and death (Plumb, 1999). Moxidectin has a high efficacy for treating parasitic infections, and it is generally considered safe as side effects are uncommon when given at recommended doses (Prichard et al., 2012; Schraven et al., 2021). It is widely used in veterinary practice for the treatment of *Sarcoptes scabiei* mite infestations in livestock, companion animals and wildlife (Schraven et al., 2021).

Sarcoptic mange is a highly infectious disease that affects a range of species including wombats (Old et al., 2018; Pence and Ueckermann, 2002), and is caused by an astigmatid ectoparasite, *Sarcoptes scabiei*, a sub-macroscopic mite that burrows into the stratum corneum of the epidermis (Arlian, 1989). It is thought the mites feed on intercellular fluid (lymph) that seeps into the burrow they make in the skin (Arlian and Morgan, 2017). *Sarcoptes scabiei* causes an inflammatory response due to significant amounts of waste products produced by the mites, causing irritation, inflammation, hyperkeratosis, alopecia, pruritus, dermatitis, skin lesions (often self-inflicted due to the pruritus), along with secondary infections (Pence and Ueckermann, 2002). The bare-nosed wombat (*Vombatus ursinus*) is particularly affected by sarcoptic mange and has incurred population declines in New South Wales and Tasmania (Gray, 1937; Martin et al., 2018). Sarcoptic mange in wombats can progress to a debilitating and multisystemic disease that causes death (Skerratt, 2001).

Moxidectin is one of the approved chemicals for treating sarcoptic mange in wombats under the Australian Pesticides and Veterinary Medicines Authority. One allows up to 4 ml per kg body weight, and a maximum of dose of 80 to 100 ml on an adult wombat per single treatment (PER90094). A second permit allows for 0.8 mL/kg with a maximum dose of 20 ml on an adult wombat, once weekly for 15 weeks

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(PER89040). The recommended pour on dose for eutherian animals set by the manufacturer is 1 ml/10 kg (0.5 mg/kg) generally recommended monthly. Efficacy of moxidectin to treat sarcoptic mange in wombats has been established using 5 ml doses (0.96–1.79 mg/kg) in burrow flaps over 12 weeks, however number of doses administered was not stated and pharmacokinetics was not studied (Martin et al., 2019). Wildlife carers treating wombats in the field are currently using a wide variety of dose rates (4–100 ml) within the permit and in some cases doses are exceeding the permitted dose (Old et al., 2021).

Previous investigations of pharmacokinetics of moxidectin have been undertaken in the southern hairy-nosed wombat (*Lasiorhinus latifrons*) (Death et al., 2011). Although subcutaneous administration is effective for treating sarcoptic mange, wombats are generally treated in the field applying moxidectin topically with a pole and scoop or in burrow flaps (Old et al., 2021). Administering the same drug using differing methods (e.g. oral, topical, subcutaneous) influences absorption, distribution and excretion through the body, for example, in giraffe (*Giraffa camelopardalis*) differences in the pharmacokinetic parameters of moxidectin have been determined for topical versus oral doses (West et al., 2017). Oral administration produced a higher maximum plasma concentration compared to topical administration (West et al., 2017), hence there is a need to understand how administration method effects drug kinetics.

Determination of the concentration in plasma and half-life (time taken for drug concentration to reduce by half) are important for determining the drug's efficacy and dosing intervals (Prichard et al., 2012). To ensure a drug's effectiveness, concentration in plasma should be maintained near the half-life concentration (Smith et al., 2018; Toutain and Bousquet-Mélou, 2004). Pharmacokinetics of moxidectin in bare-nosed wombats has yet to be assessed (Mounsey et al., 2022), and topical application has yet to be assessed in any wombat species, hence we aimed to determine the plasma pharmacokinetics, fur drug deposition and fecal excretion of moxidectin in bare-nosed wombats following topical administration. Although moxidectin is approved for use to treat sarcoptic mange in wombats, little is known about the short and long-term effects that may have on wombat health (Mounsey et al., 2022). In addition, the study aimed to determine if a single high dose has any short-term adverse effects on wombat health.

2. Methods

2.1. Experimental animals and treatment

Nine free-ranging wombats (eight male and one female) from a private property near Nimmitabel NSW (−36.511, 149.284), Australia were used for the study. Wombats were considered clinically healthy based on comparing blood parameters to published data (Booth, 1999; Bryant and Reiss, 2009; Hartley and English, 2005; Presidente, 1982), and had no observable signs of sarcoptic mange. Skin was checked for redness, scratches, bite marks, ticks, lice and leeches. The wombats live in or around the study site and occasionally come onto the property to graze. Whilst a wombat was grazing it was anesthetized with an intramuscular injection to the lateral thigh of Zoletil® (5 mg/kg). The observer walked up to the wombat calmly with a needle and syringe to deliver the injection by hand. Some animals in the study were hand raised and accustomed to humans. If a burrow entrance was nearby a net was placed over the burrow to prevent the wombat from entering. Wombats were weighed on an electronic scale (Adams Equipment, Connecticut, United States) and a sample of blood was collected using a 21G scalp vein/butterfly needle from the saphenous vein. Blood was then placed in EDTA (for hematology) and Lithium Heparin (for blood chemistry) tubes (BD Vacutainer®, Canada). While sedated pulse rate, pulse strength and oxygen saturation were monitored using a pulse oximeter (UT100V, Utech Medical Device Pty Ltd., Magill SA) attached to the ear, and respiration rate was measured manually by counting breaths over 30 s and multiplying by 2 to determine breaths per minute. Body condition was scored (Jackson, 2003), and fecal egg floats were performed on scats to check for endoparasites. Wombats were also monitored for signs of neurotoxicity by observing behavior (Table 1). A small section of fur was clipped from the shoulder area of each wombat in a pattern to allow identification on infrared cameras and from a distance. Wombats were allowed to recover from anesthesia and returned to the site where they were sedated.

Five to 14 days after the initial capture wombats were treated once with 100 ml of Cydectin (Moxidectin 5 mg/ml, Virbac, NSW) poured onto their backline using a pole and scoop, as per Australian Pesticides and Veterinary Medicines Authority permit: PER90094. Wombats were subsequently sedated, and a blood sample was collected on day 1, 7, 14, 21 and 28 after treatment with moxidectin.

Scat samples were opportunistically collected pre- and post-treatment where possible. Animals were observed and scats collected off the ground after defecation using gloves and a sterile plastic sample bag. Fur samples from shaving identification patterns (mentioned earlier) and shaving for blood sampling were collected. Fur and scat samples were frozen (−20 °C) until analysis. The project was approved by the Charles Sturt University ACEC, approval number: A21410.

2.2. Sample analysis

Blood samples were split equally for assessing basic health parameters and concentration of moxidectin. Hematology parameters were measured using a Vetscan HM5 Hematology analyzer (Abaxis, Union City CA) and blood chemistry measured using a Vetscan VS2 blood chemistry analyzer (Abaxis, Union City CA), using whole blood within 15 min of the sample being taken. A subsample of blood was centrifuged for 10 min at 3400 rpm (MXU Centrifuge, LW Scientific, Lawrenceville, GA), and plasma was separated and stored frozen (−20 °C) until analysis for moxidectin concentration.

In brief, plasma, scats (2 g subsample), fur (cut into 3 mm lengths) were spiked with moxidectin-D3, mixed and then proteins were precipitated with acetone. After centrifugation, the aqueous supernatant was twice extracted with hexane, evaporated under nitrogen at 30 °C and resuspended in 5 mM ammonium acetate in acetonitrile for analysis by LC-MS/MS. A gradient of acetonitrile/water containing 5 mM ammonium acetate was used on a Phenomenex Kinetex C8 (75 × 4.6 mm × 2.6 μm) column, with an injection volume of 20 μL (Doran et al., 2024).

2.3. Statistical analysis

To determine if there were any significant changes to blood parameters over the trial, hematology and blood chemistry values were analyzed using a repeated measures ANOVA in SPSS (IBM Corp., 2020). A linear regression was used to determine if there was a significant relationship between plasma concentration and dose rate. Concentrations of moxidectin in the plasma, scats and fur of individual animals were evaluated by non-compartmental linear trapezoidal analysis using Microsoft Excel add-in PKSolver (Zhang et al., 2010). Parameters calculated included maximum concentration (C_{max}), the time C_{max} occurred (T_{max}), area under the curve (AUC), mean residence time (MRT) and elimination half-life ($T_{1/2}$).

Table 1
Behavioral ethogram of neurological signs of moxidectin toxicity.

Behavior	Description
Twitching	Involuntary muscle contraction; occurs while inactive
Lethargy	Sleepiness, unresponsive or inactive
Shaking	Instantaneous shuddering, lateral whole-body movements
Ataxia	Loss of coordination, loss of balance, stumbling, diminished muscle control
Respiration rate changes	Labored breathing, increased rate/depth of ventilation compared with baseline

3. Results

Wombats were dosed with 12.8 to 19.6 mg/kg of moxidectin depending on body mass, which ranged from 24.5 to 39.0 kg. Change in body mass from initial to final weighing ranged between −6.3% to +5.3% for the male wombats and was +16.3% for the female, however this was not statistically significant ($F_{5,40} = 0.75$ $P = 0.48$; Supplementary Fig. 1). All wombats were given a body condition score of 5, excellent/fat condition. While sedated respiration rates were 28–40 breaths per minute and pulse rates were 50–147 beats per minute. No abnormal behaviors (Table 1) were observed during the times when animals were being sedated and monitored. Only one animal showed signs of aggression towards other wombats on day 7 (Supplementary Table 4).

Mean C_{max} was 0.50 ± 0.40 ng/ml (range: 0.16–1.28 ng/ml) and T_{max} was 6 days (Fig. 1), MRT was 13.72 days and the mean AUC was 6.00 ng/ml/d (Table 2). Elimination half-life ($T_{1/2}$) of plasma ranged from 5 to 12 days, with a mean of 8 days (Table 2). The mean fecal C_{max} was 2461.43 ± 3807.65 ng/g T_{max} was 5 days on a dry matter basis (Fig. 2). There was a large variation in scats data due to the opportunistic nature of collection (SupplementaryTable 1). Fur samples mean C_{max} was 1 day and 19.99 ± 10.39 μg/g (Table 2; Fig. 3). The pattern of decline of moxidectin in fur was similar to that in the plasma, a peak followed by a gradual reduction. There was a significant positive correlation between the plasma C_{max} observed and the dose rate ($F_{1,8} = 22.43$, $P < 0.05$; Fig. 4).

Mean hematology and blood chemistry measures are shown in Table 3. Repeated measures ANOVAs on the hematology and blood chemistry parameters showed there were no significant changes to the values pre or post treatment for most parameters analyzed, with the exception of sodium and potassium (see Supplementary Table 2 and 3). Sodium was significantly lower on day 28 post treatment compared to pre-treatment and days 1, 7, and 14 post-treatment (Fig. 5a). Potassium was significantly higher on day 28 post-treatment compared to pre-treatment (Fig. 5b).

Table 2
Pharmacokinetics (mean ± SD) parameters for bare-nosed wombats treated topically with 100 ml moxidectin.

Kinetic parameters	Plasma (n = 9)	Scats (*n* = 9)	Fur (n = 5)
C_{max}	0.50 ± 0.40 ng/ml	2461.43 ± 3807.65 ng/g	19.99 ± 10.39 μg/g
T_{max}	6.44 ± 2.07 d	5.22 ± 3.31 d	1.00 ± 0 d
$T_{1/2}$	8.41 ± 2.46 d	8.82 ± 6.23 d	6.40 ± 2.95 d
AUC total	6.00 ± 3.95 ng/ml/d	16,768.10 ± 18,544.16 ng/g/d	125.61 ± 83.77 μg/g/d
MRT	13.72 ± 3.61 d	29.62 ± 27.78 d	6.22 ± 2.03 d

4. Discussion

Our study investigated the concentration of moxidectin in plasma over time, as reflected in the AUC, and $T_{1/2}$ for plasma, scats and fur following a single topical dose of moxidectin. There is no comparable study to ours in terms of drug administration method, dose rate and species. On comparison with the only other pharmacokinetics study of moxidectin in wombats, in which southern hairy-nosed wombats received a subcutaneous injection of moxidectin (Death et al., 2011), our C_{max} is lower (0.50 ng/ml verses 98.6 ng/ml) and $T_{1/2}$ higher (8.41 d verses 5.03 d). Thus, administering moxidectin at a high dose rate, topically on wombats has a longer residence time in the plasma but lower concentration peak. The lower C_{max} is due to a lower bioavailability of moxidectin when applied topically versus subcutaneously (Xiao et al., 2019). It should be noted however, there are differences in administration method and frequency of sample collection between our and Death et al. (2011) studies.

C_{max} data had a wide range across individual wombats, 0.16–1.28 ng/ml, and this variation was influenced by dose as there was a positive correlation between C_{max} and dose. It is also not unusual to have a large range in pharmacokinetic parameters as there is usually some random variation within a population, and individual factors such as age, sex, body mass, kidney function and liver function influence absorption and distribution of drugs within the body (Hedaya, 2024). Wide ranges in

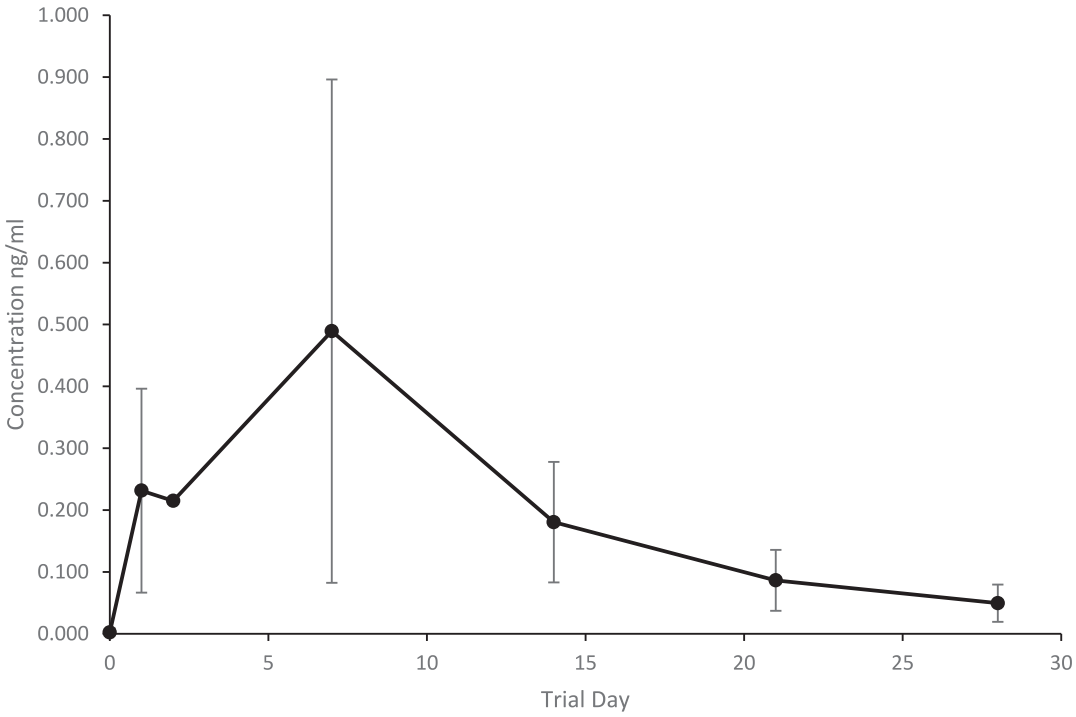

Fig. 1. Moxidectin concentration in plasma (mean ± SD) bare-nosed wombats (n = 9) pre and post topical dose with 100 ml of moxidectin. Day 1 n = 8 and day 2 *n* = 1.

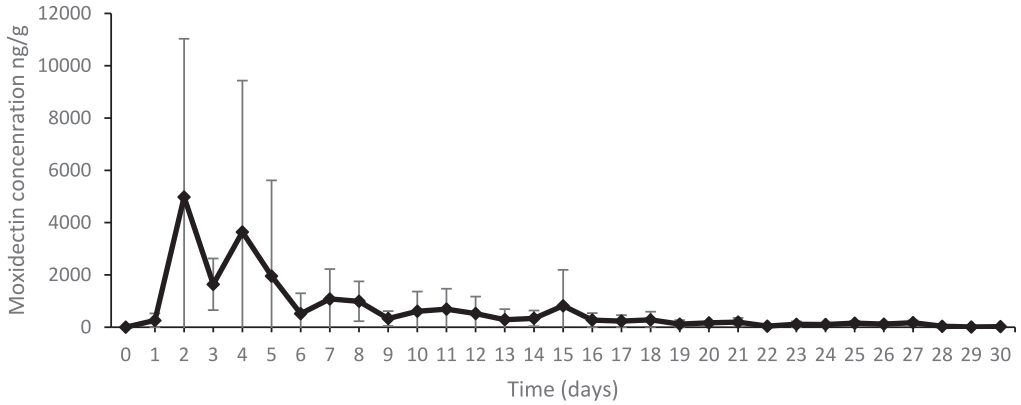

Fig. 2. Moxidectin concentration in scats bare-nosed wombats (n = 1–9) pre (day 0) and post topical dose with 100 ml of moxidectin.

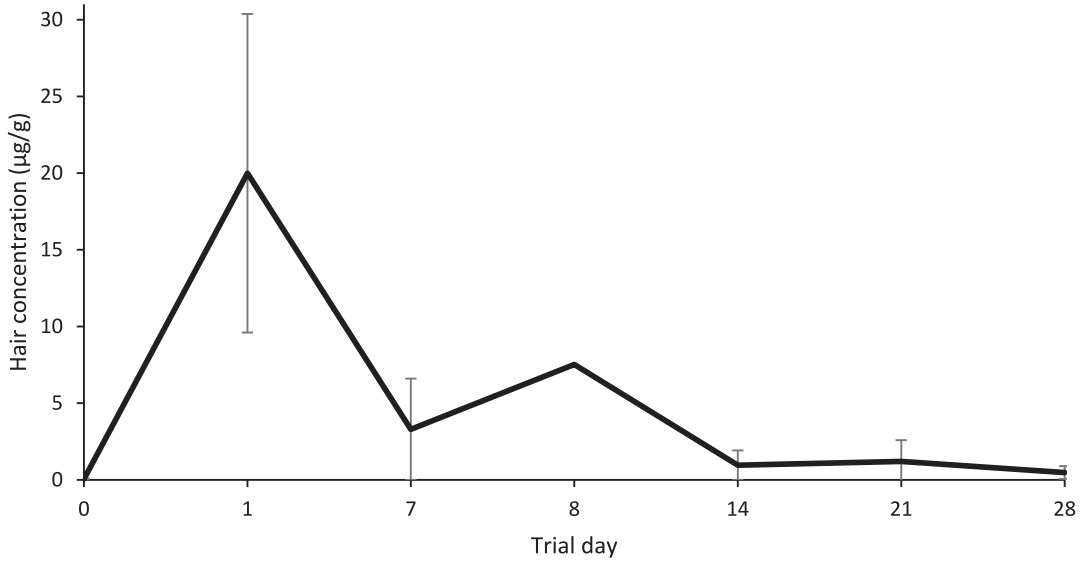

Fig. 3. Moxidectin concentration (Mean ± SD) in fur of bare-nosed wombats (n = 5) pre and post pour-on dose with 100 ml of moxidectin. Day 7 n = 4 and day 8 n = 1.

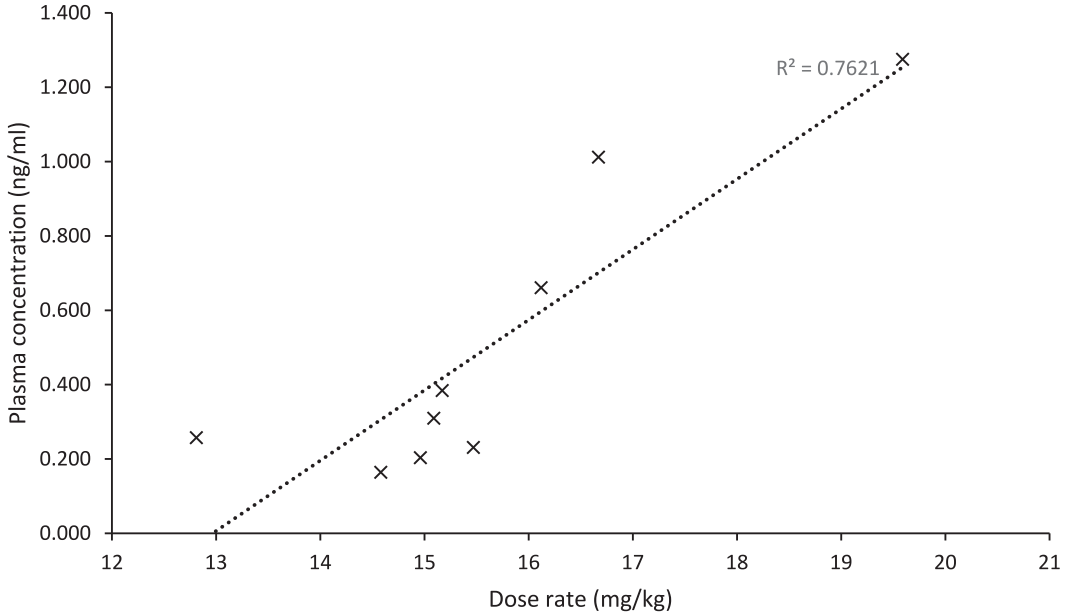

Fig. 4. Moxidectin concentration in plasma (C_{max}) and dose rate given to bare-nosed wombats.

Table 3
Hematology and blood chemistry (Mean ± SD) values from bare-nosed wombats treated with 100 ml of moxidectin compared to previously published studies.

Parameter		Unit	This study (n = 9)	Healthy wombats in captivity (n = 31) (Booth, 1999; Bryant and Reiss, 2009)	Healthy wombats in the wild (Hartley and English, 2005) (n = 10–14)	Healthy wombats in the wild wombats brought into captivity (Presidente, 1982) (n = 8–12)*
Hematology						
Total white blood cell count	WBC	10^9/l	13.5 ± 4.1	8.4 ± 2.9	12.72 ± 3.12	7.5–19.9
Lymphocyte count	LYM	10^9/l	4.5 ± 3.0	5.2 ± 3	4.19 ± 1.75	1.9–12.8
Monocyte count	MON	10^9/l	0.5 ± 0.4	0.5 ± 0.5	0.21 ± 0.22	0.1–1.0
Neutrophil count	NEU	10^9/l	5.9 ± 1.8	6.1 ± 3	6.74 ± 0.93	2.7–10.8
Eosinophil count	EOS	10^9/l	2.5 ± 3.0	0.15 ± 0.2	1 ± 0.35	0.0–0.6
Basophil count	BAS	10^9/l	0.2 ± 0.1	0.01 ± 0.05	–	0.0–0.5
Lymphocyte %	LY%	%	32.4 ± 15.6	–	–	50
Monocyte %	MO%	%	3.4 ± 2.5	–	–	5
Neutrophil %	NE%	%	46.6 ± 16.6	–	–	41
Eosinophil %	EO%	%	16.4 ± 14.8	–	–	3
Basophil %	BA%	%	1.2 ± 0.5	–	–	0.3
Red blood cell count	RBC	10^{12}/l	6.8 ± 0.9	5.2 ± 0.7	5.31 ± 0.63	–
Hemoglobin	HGB	g/dL	14.1 ± 1.8	11.9 ± 1.9	14.2 ± 1.37	–
Hematocrit (PCV) %	HCT	%	45.8 ± 5.1	36 ± 6	41 ± 4	–
Mean corpuscular volume	MCV	fl	67.8 ± 4.1	68 ± 4	70.77 ± 2.07	62–76
Mean corpuscular hemoglobin	MCH	pg/l	20.9 ± 2.6	22.7 ± 1.3	24.38 ± 1.17	20.0–25.8
Mean corpuscular hemoglobin concentration	MCHC	g/dl	30.7 ± 2.7	33.5 ± 1.7	34.3 ± 1.73	32.3–36.8
Red cell distribution width %	RDWc	%	16.6 ± 0.8	–	–	–
	RDWs	fl	43.4 ± 2.0	–	–	–
Platelet count	PLT	10^9/l	171.8 ± 70.3	–	220 ± 61.44	–
Platelet hematocrit %	PCT	%	0.2 ± 0.1	–	–	–
Mean platelet volume	MPV	fl	12.1 ± 1.0	–	–	–
Platelet distribution width	PDWc	%	42.2 ± 1.5	–	–	–
PDWs	PDWs	fl	23.7 ± 3.4	–	–	–
Blood chemistry						
Alanine aminotransferase	ALT	U/l	44.4 ± 17.6	23.7 ± 9.2	53 ± 55.9	7.0–60.0
Albumin	ALB	g/dl	3.1 ± 0.5	2.97 ± 0.6	2.921 ± 0.458	2.5–3.7
Alkaline phosphatase	ALP	U/l	237.7 ± 112.7	342 ± 214	192.3 ± 100.5	
Alpha-amylase	AMY	U/l	226.5 ± 95.6	–	–	–
Aspartate aminotransferase	AST	U/l	102.1 ± 51.1	156 ± 197	98.4 ± 45.1	–
Blood urea nitrogen	BUN	mg/dl	21.4 ± 5.7	10.4 ± 1.4	27.39 ± 7.9	18.9–56.8
Calcium	CA	mg/dl	10.0 ± 0.4	9.6 ± 1.6	9.46 ± 1	6.73–13.15
Carbon dioxide	TCO2	mmol/l	30.3 ± 2.2	33.6 ± 4.1	–	–
Creatine kinase	CK	U/l	109.5 ± 101.2	233 ± 454	1837.8 ± 1385.1	–
Creatinine	CRE	mg/dl	0.9 ± 0.2	0.9 ± 0.34	1.37 ± 0.3	–
Gamma-glutamyl transferase	GGT	U/l	43.0 ± 37.4	–	–	–
Globulin	GLOB	g/dl	4.7 ± 1.3	2.97 ± 0.75	3.79 ± 0.8	3.0–5.2
Glucose	GLU	mg/dl	99.1 ± 14.4	95 ± 25	73.3 ± 27.4	45.9–114.1
Phosphorus	PHOS	mg/dl	4.6 ± 1.1	5.01 ± 1.36	4.92 ± 1.7	2.6–7.9
Potassium	K	mmol/l	4.8 ± 0.4	4.7 ± 1.1	9.24 ± 4	3.8–5.2
Sodium	NA	mmol/l	134.3 ± 4.1	134 ± 3.2	137.19 ± 4.3	128–154
Total bilirubin	TBIL	mg/dl	0.5 ± 0.1	0.3 ± 0.18	0.15 ± 0.08	–
Total protein	TP	g/dl	7.9 ± 0.9	5.91 ± 1.08	6.63 ± 1.03	5.8–8.3

* values are ranges. -value not reported.

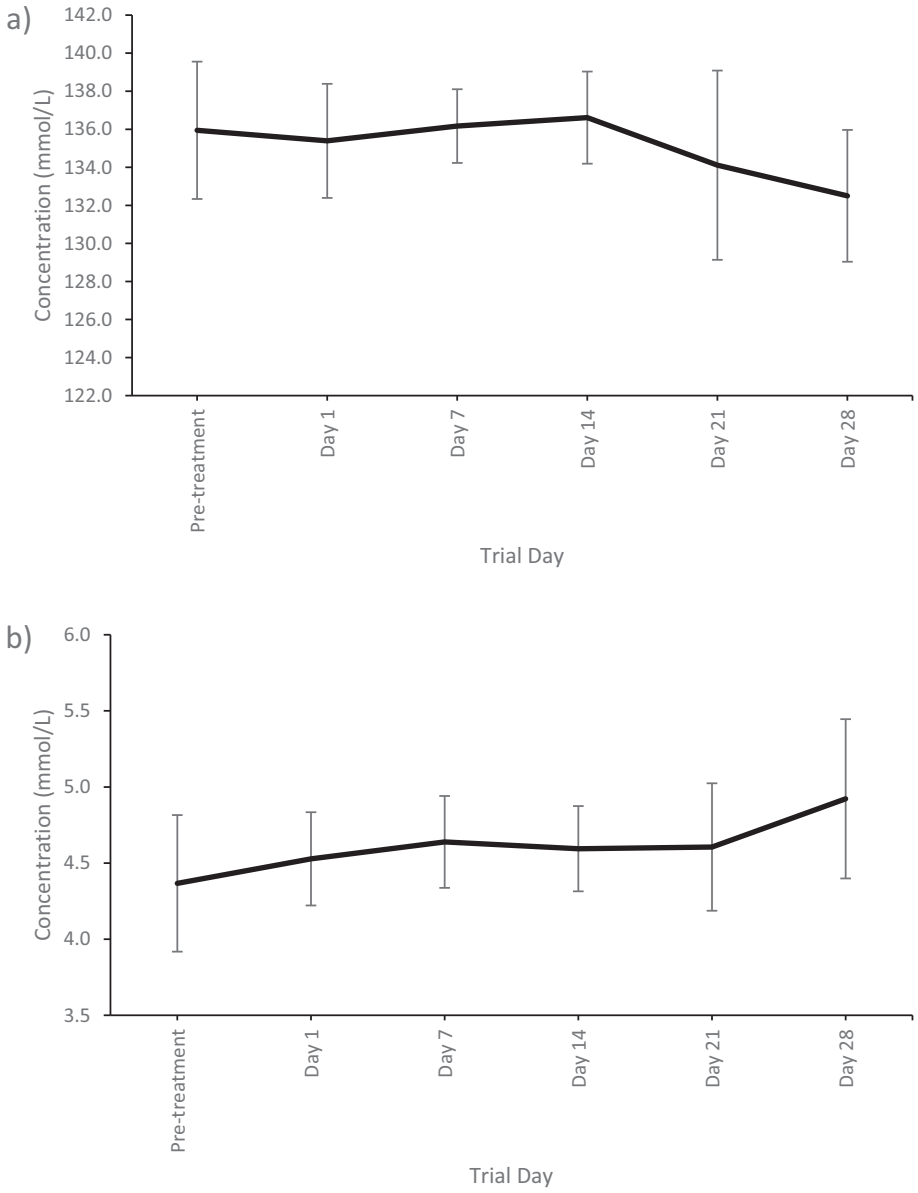

Fig. 5. Concentration of a) sodium and b) potassium in blood samples of bare-nosed wombats pre-treatment and day 1, 7, 14, 21, and 28 post-treatment with moxidectin.

C_{max} have been recorded in other species, llamas (*Lama glama*) and alpacas (*Lama pacos*) given a topical dose of moxidectin (0.5 mg/kg) had a C_{max} range of 0.21–1.27 ng/ml (Hunter et al., 2004). The llamas and alpacas in Hunter et al. (2004) study had been shaved at the application site, thus absorption across the skin would be unencumbered by fur. In another wildlife species, the giraffe (*Giraffa camelopardalis*) given a topical dose of moxidectin (1 mg/kg) C_{max} range was 5–36 ng/ml (West et al., 2017). The C_{max} range was 55.7–142 ng/ml for southern hairy-nosed wombats given a subcutaneous dose of 0.2–0.3 mg/kg moxidectin (Death et al., 2011). Hence animals given a single dose rate still have a wide range in C_{max} values due to individual animal differences. It should be noted that our data collection occurred less frequently than these other studies and timing of data collection may have affected the C_{max} recorded as moxidectin has a relatively short half-life.

If drug concentration in plasma is too high, it may cause side-effects, and if it is too low it may be ineffective to treat the target parasite/illness, hence half-life is a measure used to determine frequency of drug reapplication (Smith et al., 2018; Toutain and Bousquet-Mélou, 2004). For treating sarcoptic mange in wombats Bryant and Reiss (2009) recommend weekly subcutaneous or pour-on doses of moxidectin (0.2–0.3 mg/kg) until two weeks after negative skin scapings. The plasma $T_{1/2}$ determined in our study suggests a reapplication of moxidectin should occur at approximately 8 days. *Sarcoptes* eggs can hatch after 2 days and it takes 10–13 days for mites to mature from the egg to an adult, dependent on humidity and temperature (Arlian and Vyszenski-Moher, 1988). Hence reapplication of moxidectin is important to ensure all mites are killed, as eggs may hatch after initial application to ensure any new mites that hatch are killed. There is also a chance of reinfection with mites from the environment as they can survive off the host for up to three weeks under optimal humidity and temperature conditions (Arlian et al., 1989). Reapplication of moxidectin would treat any potential reinfection from the environment. As suggested by Bryant and Reiss (2009) reapplication should occur until two weeks after a negative skin scrapping as this would encompass the mite life cycle.

Data collected on blood parameters, both hematology and chemistry, suggest that moxidectin had no adverse effects on the wombats treated. Most hematology analytes were similar to those reported previously for

bare-nosed wombats (Table 3), with only eosinophils and erythrocyte counts being higher than previously published values. There were no significant changes to these parameters during the study and hence the higher values are not an effect of the moxidectin. Eosinophils are usually elevated in the presence of parasites (Arruda Gimenes Nantes et al., 2019; Behm and Ovington, 2000), and although these wombats did not have sarcoptic mange they did have strongyloides, strongylidae and coccidia observed in their scats (data not shown).

Blood chemistry results were consistent with previously reported values for bare-nosed wombats, except total bilirubin which was slightly elevated compared to published values, and creatine kinase which was lower than previously published values for bare-nosed wombats (Table 3). Season may be the reason total bilirubin was elevated compared to published data and has been suggested previously for other marsupials (Stannard et al., 2013; Viggers and Lindenmayer, 1996). McKenzie et al. (2002) found season influenced bilirubin and hemoglobin, as bilirubin is a product of hemoglobin breakdown. Creatine kinase is used as an indicator of stress and capture myopathy, which has been observed in other marsupials (Green-Barber et al., 2018). Capture myopathy does not appear to occur in bare-nosed wombats (Berris et al., 2022), however it has possibly been observed in one northern hairy-nosed wombat (*Lasiorhinus krefftii*) (Reiss et al., 2008). The low values for creatine kinase in our study suggests the animals' stress levels were low at the time of sampling, which we believe is due to the unique in field sedation method involving no chasing, netting or trapping.

Although significant changes to sodium and potassium levels were recorded on day 28, it is unlikely these changes are related to treatment with moxidectin as it was largely eliminated by day 28. Our sodium range was slightly lower at 120–142 mmol/l, compared to the published range of 128–154 mmol/l (Presidente, 1982). Our potassium values had a slightly wider range, 3.3–5.5 mmol/l compared to previously published values, 3.8–5.2 mmol/l (Presidente, 1982), as illustrated in Table 3. It should be noted the previously published data are not considered 'normal baseline reference ranges' as they come from a small number of captive wombats. Blood chemistry analytes are affected by factors such as nutrition, hydration and season (Bradley, 1990; Stannard et al., 2016; Stannard et al., 2013; Wells et al., 2000) and it is likely changes in sodium and potassium reflect those changes rather than an effect of treatment with moxidectin.

There are limitations to using only blood parameters to draw conclusions about drug safety and animal health. This is because side effects recorded from animals treated with moxidectin are often neurological or behavioral (Davis et al., 2007; Schraven et al., 2021; Wagner and Wendlberger, 2000). For dogs given 0.4 mg/kg moxidectin orally daily for 42–120 days to treat *Demodex* mites a total of three showed side effects. One dog was lethargic and vomiting at day 10, and two had ataxia, one in the first week and the other after two weeks (Wagner and Wendlberger, 2000). When applied topically toxic side effects have been recorded in cats and dogs, however moxidectin was paired with imidacloprid in those studies (Arther et al., 2015; Davis et al., 2007; Heine et al., 2005; Krieger et al., 2005). In addition to hematology and blood chemistry, we performed physical examinations and observed behavior. During examinations cuts and wounds were noted on some wombats, and endoparasites (strongyloides, strongylidae, and coccidia) were observed in scats (Supplementary Table 4). No abnormal behaviors were seen while animals were under observation. Due to the wombats being free-living animals we could not monitor them continuously over the trial period. Clinical and observational monitoring occurred on trial days and wombats were observed opportunistically on other days while the trial was running. There were also no significant changes in body mass, except for the female that gained 16.9% of her initial body mass. The female is a young animal and still growing, thus her weight gain was expected.

Environmental factors such as rainfall are a limitation when assessing a topical application of moxidectin. However, by measuring pharmacokinetics in free-ranging wombats under usual environmental conditions replicates how moxidectin is used to treat sarcoptic mange in free-ranging wombats. Hence the data here are representative of real-world conditions. In addition, most pharmacokinetics studies use captive animals which may influence results as captivity can affect stress levels and cause changes in animal physiology (Baker et al., 1998; Geiser and Ferguson, 2001). A limitation of our study was a sex bias towards males with only one female used, which was also a young animal and still growing. She had the highest plasma concentration of moxidectin on most days of the trial and her plasma C_{max} was 1.275 ng/ml. Being the smallest animal in the trial she had the highest dose rate of 19.6 mg/kg which contributed to the higher levels recorded in her plasma, as there was significant positive correlation between dose and plasma concentration. Further kinetics data on young growing animals would be beneficial to understand how differences in physiology, related to growth, influence absorption and excretion of moxidectin. This would be of particular interest because moxidectin is lipophilic and tends to stay in body fat longer than in plasma (Zulalian et al., 1994) and younger growing animals tend to have higher body fat percentage compared to older animals.

Moxidectin is largely excreted through the feces (Rock et al., 2002). Wombats have long gastrointestinal retention times of approximately 62–75 h (Barboza, 1993), hence scat C_{max} occurred at five days post-treatment, followed by a steady decline. Excretion of moxidectin means there was a continuous reduction of the drug from the wombats, any excessive storage could lead to toxicity. We found moxidectin present in all scats collected post treatment, including one collected on day 217 (2.5 ng/g on dry mater basis) post-treatment. The prolonged excretion of moxidectin into the environment presents an ecotoxicology risk, particularly for coprophagic fauna. Moxidectin is considered less toxic than other avermectins and in some cases no negative side effects have been observed for dung beetles (Mackenzie et al., 2022; Wardhaugh et al., 2001). Dung beetles play an important role in dung processing particularly in agricultural systems and the impact of treating wombats on the environment should be monitored, with a particular focus coprophagic insects. Scats could not be collected consistently throughout the trial as wombats needed to be observed defecating to ensure scats were collected from wombats in the trial, therefore, the scat data has large variations across individuals.

Fur T_{max} was one day after application, which was earlier than observed in the plasma or scats, which is expected as the moxidectin was poured directly onto the fur. Distribution of moxidectin across the skin and fur would not be homogenous due to blood flow, sebaceous secretions (Monteiro-Riviere et al., 1990; Sallovitz et al., 2003) and hair structure (follicle density and length) (Gokbulut et al., 2011). These factors would account for the wide range of fur C_{max} values (5.37–33.17 μg/g) observed across individual wombats. Fur $T_{1/2}$ was six days suggesting that the moxidectin is at an active concentration at the site of application, which may contribute to controlling the mites at the site of infection for approximately six days post treatment.

Dose determination for this study was based on current treatment methods being used in the field and perceived effectiveness (Old et al., 2021) and the maximum allowable dose under the permit. Our doses were 12.8–19.6 mg/kg which is above any dose previously tested, with the highest topical dose used on cattle 12.5 mg/kg, and it showed no adverse effects (Rock et al., 2002). At these high doses there is potential for toxicity however, low absorption into the plasma and excretion in scats, paired with no observed abnormal behaviors, indicates toxicity did not occur in the wombats over the short-term. There is a need to understand the implications of using these high doses over the longer term if they continue to be used in the field. Often when treating wombats in the field an infected wombat may only be sighted once and therefore only treated once.

5. Conclusion

This study contributes to our understanding of pharmacokinetics of

topical moxidectin treatment in wombats. To determine the correct dosage and dosing regimens of antiparasitic drugs it is essential to establish pharmacokinetic data in wombats to avoid extrapolating potentially ineffective dosages from other species. Even though we used a variable dose, plasma concentration was substantially lower compared to giraffe, and similar to lamas and alpacas, given a consistent dose. This is likely due to differences in species anatomy and physiology; however, little is known about the skin anatomy of wombats. Relatively low concentrations of moxidectin were detected in plasma even though dose rate was relatively high. Our results show there is no bioaccumulation in plasma or fur and moxidectin is excreted via feces. Moxidectin was shown to be safe in the short term to use as a single high dose applied topically. This is the first study to use a real-world application of treatment to replicate how sarcoptic mange is currently treated in free-living wombats. Further studies should investigate the treatment regimens for mange-affected wombats to develop an effective treatment program at an individual and population level.

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Ethics approval

The project was approved by the Charles Sturt University Animal Ethics Committee, approval number: A21410.

Consent for publication and consent for publication

Not applicable.

CRediT authorship contribution statement

Hayley J. Stannard: Writing – original draft, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Marie B. Wynan:** Writing – review & editing, Methodology, Data curation, Conceptualization. **Ray J. Wynan:** Writing – review & editing, Methodology, Data curation. **Amanda Cox:** Writing – review & editing, Methodology, Conceptualization. **Howard Ralph:** Writing – review & editing, Resources, Conceptualization. **Gregory S. Doran:** Writing – review & editing, Methodology, Formal analysis.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Hayley Stannard reports financial support was provided by Wombat Protection Society of Australia. Marie Wynan reports a relationship with Wombat Protection Society of Australia that includes: board membership. Amanda Cox reports a relationship with Wombat Protection Society of Australia that includes: board membership. The other authors, declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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