Evaluation of medicinal turpentine used for the prevention of bovine babesiosis in southern KwaZulu-Natal and the eastern Free State

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Medicinal turpentine has been used extensively in the eastern Free State and KwaZulu-Natal provinces of South Africa with reportedly excellent results. It is believed that it is able to prevent and treat babesiosis (redwater) in cattle. Redwater is an often-fatal disease in cattle and results in losses of large numbers every year in South Africa. This study was initiated in an attempt to investigate the validity of the use of the turpentine as a medicinal agent. Using a semi *in vitro* screening assay, *Babesia caballi* grown in primary equine erythrocytes was exposed to various concentrations of turpentine in comparison to diminazene and imidocarb. The turpentine had no parasiticidal effect following direct exposure. During the recovery phase, the previously exposed parasites appeared to grow more slowly than the controls. In comparison, diminazene and imidocarb were 100% effective in killing the parasites. In a subsequent tolerance study in adult cattle (n = 6) at 1x (2 mL), 3x and 5x the recommended dose, the product was non-toxic. Irritation was noted at the injection site with the higher dose. The only major finding on clinical pathology was a general increase in globulins, without a concurrent change in native babesia antibody titres. It was concluded that it is unlikely that medicinal turpentine is an effective treatment against babesiosis.

Introduction

The genus *Babesia* belongs to the phylum Apicomplexa, class Sporozoasida, order Eucoccidiorida, suborder Piroplasmorina and family Babesiidae (De Vos & Potgieter 2004). The two species of economic importance in southern Africa are *Babesia bigemina* (African redwater) and *Babesia bovis* (Asiatic or European redwater). Babesiosis, the disease caused by the parasite, is principally a vector-borne disease transmitted by rhipicephalid ticks (Du Plessis, De Waal & Stoltsz 1994). Once the host is infected, the parasite has an incubation period of approximately 14 days for *B. bigemina*, and shorter for *B. bovis*, at 9 days. Clinical signs that develop include: pyrexia, listlessness, anorexia, a decrease in or cessation of rumen movements, decreased milk production, and for *B. bovis* especially, neurological signs. Due to the erythrolysis induced by parasites exiting the erythrocytes, animals are also anaemic. After being infected and surviving, animals develop what is known as premunity. In this state, the animals appear to be resistant to further infection due to some parasites remaining latent in the blood (De Vos & Potgieter 2004). If the disease remains untreated, the mortality rate may reach 90%.

Treatment of babesiosis in cattle usually involves the use of diminazene and imidocarb, both of which are diamidines (De Vos & Potgieter 2004). Whilst both drugs are effective, they have limited prophylactic effects. Another method commonly used is vaccination, as cattle develop a durable immunity after a single infection with *B. bigemina* and *B. bovis* (Carrington, Du Plessis & Naidoo 2011). Whilst many of the farmers have relied on vaccination, as recommended by the manufacturer (Onderstepoort Biological Products, South Africa), losses still continue to occur. This has resulted in the local farmers using medicinal turpentine, both as a therapeutic and preventative agent. From informal discussions with members of this farming community, ten farmers were able to confirm that they used the product, based on recommendations from a neighbour; therefore, the origin of treatment was unknown. From these discussions, the standard dose used was seen to be 2 mL, administered subcutaneously every three months. The farmers were also of the belief that treatment resulted in an overall reduction in the total incidence of babesiosis on their farms, even though they were unable to confirm conclusively that their cattle were even infected with *Babesia*.

Turpentine oil is naturally derived and is mainly composed of the monoterpenes alpha-pinene and beta-pinene (Mercier, Prost & Prost 2009). The oil is obtained as a by-product from the distillation of resin obtained from pine trees (*Pinus* spp.) during the Kraft process and is widely used as a complementary medicine, with a history of use extending from ancient times (Burt 2004;

Kleppe 1970; Mercier *et al.* 2009). Whilst the predominant use of the medicine is in chest rubs and for the management of respiratory conditions, it has also been reported to be effective in the treatment of both external and internal parasites (Daubney 1930; Sheather 1923). Turpentine oil has been evaluated in controlled research studies for its effect against internal parasites (Krause *et al.* 2007; Vreden *et al.* 1992). In a rodent model of malaria, subcutaneous administration of turpentine oil 24 h or 5 min before inoculation of sporozites resulted in 80% and 35% reduction of schizont development, respectively (Vreden *et al.* 1992). To date, the effects of turpentine on *in-vivo* or *in vitro Babesia* infections have not been evaluated. However, with the similarity in pathogenesis between malaria and babesiosis, the use of the product may be valid (Krause *et al.* 2007).

In the opinion of the authors, if effective against babesia, turpentine could control babesia either by inhibiting parasite growth or by somehow acting as an adjuvant to pre-existing premunity. As a first step in validating the use of turpentine, the *in vitro* effect of turpentine on babesia was evaluated and the safety in a bovine model of overdose was established. As a secondary objective, changes in humoral immunity and lymphocyte counts were also evaluated. The treatment response of infected cattle was not evaluated in the present study, as it was believed to be unethical to proceed with an *in-vivo* study prior to obtaining any information on *in vitro* efficacy.

Material and methods

In vitro anti-babesial effect

Babesia caballi was grown in primary equine red blood cells, using a cell free medium, as previously described (Naidoo *et al.* 2005). This species was used for the study, because, whilst attempts were also made to culture *B. bigemina* in primary bovine red blood cells, the parasite repeatedly failed to grow. Following a period of 48 h to allow for parasite establishment (Day 0), the cultures were exposed to turpentine (20.0 µg/mL, 10.0 µg/mL, 5.0 µg/mL, 2.5 µg/mL) (n = 6) in the media for 48 h (Day 3). The turpentine was bought in South Africa and was supplied with a certificate of analysis by the company (0.880 g/mL). Thereafter, the media with turpentine were removed and the cultures were allowed an additional 48 h to re-establish (Day 5). The positive controls were diminazene (n = 6, 10 µg/well) and imidocarb dipropionate (n = 3, 10 µg/well), whilst untreated wells served as the negative control. A thin blood smear using a slide-on-slide technique was made from the cultures on Days 0, 3 and 5, and stained with Giemsa (Sigma Aldrich, South Africa). Parasitaemia was determined by the number of infected cells in an area of 5000 cells under 100x magnification. Results are presented as the percentage parasitaemia.

Toxicity and clinical pathology study

Twenty-four cattle (18–24-month-old replacement heifers) without prior vaccination against babesia were used in the study. The animals (n = 6) were treated subcutaneously at the recommended dose (2 mL or 4.25 mg/kg), 3x the recommended dose (6 mL or 12.75 mg/kg), 5x the recommended dose (10 mL or 21.25 mg/kg) or saline (2 mL). The doses were selected to obtain the best representation of toxicity and changes in clinical pathology. The animals were housed under natural field conditions, in order to simulate natural exposure to parasites, as per normal farm practice (see ethical clearance).

Blood samples (EDTA and serum) were collected prior to treatment and at 18 h, 48 h and 120 h post injection. As part of the evaluation, the clinical pathology laboratory also evaluated thin blood smears of all samples. The blood was analysed immediately using an automated counter (Dr Bouwer and Partners Inc, South Africa) for changes in haematology before and 48 h after treatment. Frozen serum samples from all time points were analysed 2 weeks later for specific clinical chemistry parameters (AST, GGT, urea, creatinine, creatine kinase, TSP, albumin and globulin) (Onderstepoort Clinical Pathology laboratory, South Africa). Serum samples were also analysed by Indirect Fluorescent Antibody Test for changes in *B. bovis* or *B. bigemina* antibodies pre-treatment and post-treatment (Day 5) (Onderstepoort Veterinary Institute, South Africa) (Tønnesen *et al.* 2006).

Clinical monitoring took place from 0–120 h post turpentine or saline injection, and 37 days later. Parameters monitored included presence of salivation, restlessness, head shaking, licking of the injection site, looking at the injection site and swelling at the injection site. Swellings were measured with callipers. Changes in body temperature were also monitored. On Day 37, the animals' additional evaluation included measuring skin thickness and pregnancy evaluation by rectal palpation.

Data analysis

All results were analysed using the SPSS 20 (IBM, South Africa). Normality was tested with a Shapiro-Wilk's test. A paired t-test was used to assess for changes to pre-treatment values per treatment group, whilst an ANOVA was used to test for differences between groups at selected time points. When significance was evident on ANOVA, a Dunnett *post-hoc* test was used to ascertain if any of the groups were significantly different to the control. Biological significance was evaluated against the reference range provided by the evaluating laboratories. The serology results for *B. bovis* and *B. bigemina* before and after treatment were evaluated by means of a Pearson's Chi-square test.

Ethical approval

This study was approved by the Animal Use and Care Committee of the University of Pretoria.

Results

Babesia cultures

Results are reported only for *B. caballi*, as *B. bigemina* failed to grow. No reasons could be found for the latter, as both

parasites were placed under the same conditions, except for the source of the red cells. No change was evident in *B. caballi* growth in the turpentine-treated and negative control wells (Figure 1), whilst those with diminazene and imidocarb showed negligible growth (0.01% vs 0%). Following subculture of the treated well, the parasite numbers of the turpentine treatment cultures further increased, albeit at a lower percentage than the control group. Imidocarb-treated and diminazene-treated wells showed no recovery of the parasites on subculture. No significant difference was present between the treated and control wells for any of the evaluated time points.

Animal phase study

The animals remained healthy for the duration of the study, despite the turpentine-treated animals all showing a mild febrile reaction (39.08 ± 0.19, 39.78 ± 0.70, 39.78 ± 0.33 and 39.58 ± 0.23 for the control and three turpentine groups in ascending order, respectively). The mild febrile reaction occurred concurrently with an injection site reaction characterised as palpable masses under the skin. Reactions were most severe 2 days and 3 days post treatment, with 0, 1, 2, and 2 animals in the saline, 2 mL, 6 mL and 10 mL turpentine groups, respectively showing severe reactions. Despite the febrile reactions, most of the animals showed an increase in mass by Day 37 post treatment. For no apparent reason, three animals in the 10 mL turpentine group developed corneal opacity 2 days after administration. The opaque corneas did not, however, interfere with the animals' mass, feed intake or behaviour. The corneal opacity in two of these animals had resolved by Day 5 of the study, whilst the third had resolved by Day 37. Whilst not specifically evaluated in the study, at least two animals per group were confirmed pregnant at dosing. No abortions were recorded for the period of monitoring. Subsequent telephonic discussion with the farm owner confirmed that all animals calved successfully.

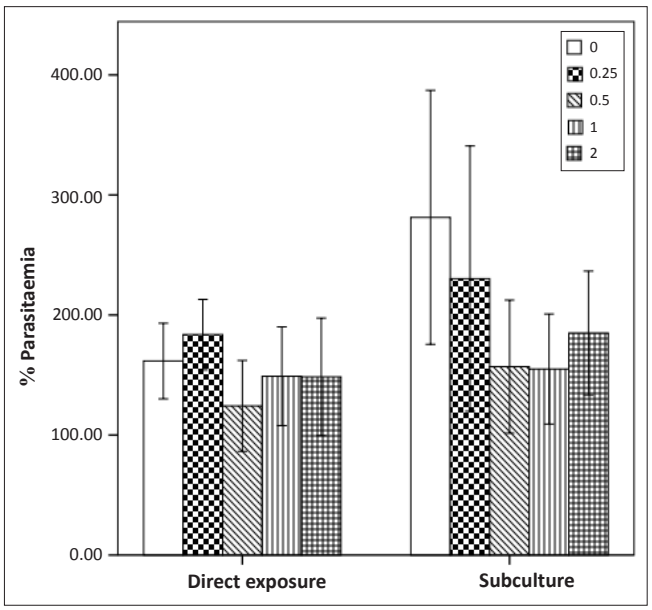

FIGURE 1: Percentage parasitaemia following direct exposure of *Babesia caballi* cultures to turpentine and percentage parasitaemia following subculture in the absence of turpentine.

All of the evaluated clinical pathology parameters measured prior to the start of the study (0 h) were not statistically different (Table 1), with the exception of eosinophil counts (p-value = 0.02), with the 2 mL turpentine dose being higher than the control group on *post hoc* analysis (p-value = 0.009). This difference was not considered to be of biological significance, as it was within the reference interval (0.00×10^9/L – 2.4×10^9/L) specified by the laboratory. At 18 hours, only the neutrophil counts were found to be significantly different on ANOVA ($p = 0.015$), with the 2 mL (normal dose) (p-value = 0.009) and the 10 mL (5x normal dose) (p-value = 0.035) dosing groups being significantly different from the control group. However, with the evident change being within the reference interval, this change is not considered to be of biological significance. The laboratory reported no babesia parasites for any of the blood smears evaluated. No differences in the clinical chemistry were present in the 18 h and 48 h samples (Table 2). For the 120 h time point, a significant difference was present for globulins ($p = 0.46$) (Table 3), with the 6 mL (p-value = 0.035) and the 10 mL (p-value = 0.049) groups being significantly different from the control group. For the 6 mL group, all six animals, and five animals for the 10 mL group, were outside the globulin reference interval (28 g/L – 42 g/L). Total serum proteins (TSP) demonstrated significant difference in the 6 mL group ($p = 0.038$).

All animals tested serologically positive for exposure to either *B. bigemina* or *B. bovis*, despite having no prior history of vaccination. When the animals were evaluated for the presence or absence of positive titres (> 1/80) to *B. bovis* (22 positive before and 18 after treatment) or *B. bigemina* (18 before and 7 after), no changes in titres were evident before or after treatment. Due to the unexpected decrease in the number of positive animals for the latter, the contract laboratory reanalysed all samples and was able to reproduce this peculiar result.

Discussion

In the management of babesiosis, the methods available to induce immunity are to decrease the parasite burden through the use of drugs such as diminazene or imidocarb (Vial & Gorenflot 2006). The alternative method would be to vaccinate the animal, either with the live parasite in combination with treatment or to use a sub-unit protein vaccine that stimulates an immune response directly (Timms *et al.* 1984). For the present study, both of these effects were evaluated indirectly. For the former, the direct effect of turpentine on the parasite was evaluated in blood cultures, at various concentrations, for death or altered capacity to divide. The immune stimulatory effect was evaluated through *in vivo* changes in babesia-specific antibody titres and circulating leucocyte counts. In both cases, higher concentrations than clinically recommended were used.

For the cell culture experiment, the cultures were exposed to four different concentrations of turpentine oil. These concentrations were calculated based on the dose of 2 mL

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TABLE 1: Haematological parameters obtained for the treatment groups before (0 h) and after (18 h) treatment with turpentine in comparison to the control groups.

Parameter	Units	RI	0 h								18 h							
			0		2		6		10		0		2		6		10	
			n	s.d.	*n*	s.d.	*n*	s.d.	*n*	s.d.	*n*	s.d.	*n*	s.d.	*n*	s.d.	*n*	s.d.
Hb	g/dL	18–15	12.2	1.57	13.2	1.38	12.9	0.72	12.8	1.25	11.8	1.53	11.7	0.75	11.9	1.04	11.8	0.57
Ht	%	24–45	32.6	3.93	34.8	3.93	34.1	2.29	34.2	3.32	32.1	4.18	31.2	2.00	32.2	3.34	31.4	1.28
MCH	pg	11–17	15.5	1.54	15.9	1.26	15.2	0.40	16.5	1.74	15.2	1.43	15.9	1.19	15.0	0.49	16.4	1.70
MCHC	g/dL	31–37	37.4	0.67	37.8	0.83	37.8	0.53	37.6	0.74	37.0	0.70	37.6	0.24	37.1	1.02	37.6	0.65
MCV	fL	40–60	41.5	3.85	42.2	3.27	40.2	1.46	43.8	4.25	41.2	3.86	42.3	3.25	40.5	1.45	43.5	4.20
RDW	%	12.0–14.5	20.0	0.78	20.4	1.19	19.5	0.71	19.1	1.44	19.9	0.78	20.5	1.21	19.5	0.75	19.5	1.04
RCC	$x10^{12}$/L	5–10	7.9	0.97	8.3	0.72	8.5	0.36	7.8	0.61	7.8	0.96	7.4	0.60	7.9	0.68	7.3	0.55
Reticulocyte	$x10^{9}$/L	50–100	3.0	0.80	5.3	2.55	3.1	1.29	3.7	1.88	3.7	1.15	4.8	2.75	4.7	0.83	4.9	2.65
WCC	$x10^{9}$/L	4–12	14.4	3.17	11.1	2.29	15.1	2.71	12.9	2.77	12.5	1.97	10.5	1.47	10.9	1.83	10.4	1.59
Neutrophil	$x10^{9}$/L	0.64–4.00	4.5	2.16	2.1	0.59	4.4	2.13	2.1	1.37	4.7	1.57	2.75*	0.37	3.6	0.49	2.91*	0.66
Lymphocyte	$x10^{9}$/L	2.5–7.5	8.5	2.01	7.1	1.42	9.0	1.74	7.2	3.77	6.4	0.96	6.2	1.47	6.4	1.45	6.3	1.13
Monocyte	$x10^{9}$/L	0.25–0.85	0.57	0.20	0.64	0.37	0.53	0.17	0.39	0.29	0.41	0.10	0.44	0.24	0.32	0.07	0.23	0.07
Eosinophil	$x10^{9}$/L	0.0–2.4	0.68	0.27	1.18*	0.37	0.96	0.39	0.95	0.69	0.86	0.76	1.04	0.22	0.56	0.24	0.83	0.47
Basophil	$x10^{9}$/L	0.0–0.2	0.12	0.02	0.10	0.02	0.13	0.02	0.10	0.05	0.10	0.02	0.09	0.02	0.07	0.02	0.07	0.02
Platelet	$x10^{9}$/L	100–800	313.7	52.79	283.7	122.94	339.2	110.89	293.8	93.93	283.5	54.12	247.2	117.46	288.7	130.56	232.8	68.99

RI, Reference Interval; Hb, haemoglobin; Ht, haematocrit; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; RDW, red cell distribution weight; RCC, red corpuscular counts; WCC, white cell count; *n*, number; s.d., standard deviation.
*, $p < 0.05$ (on *post-hoc* testing for the same time point)

TABLE 2: Clinical chemistry parameters obtained for the treatment groups before (0 h) and after (18 h) treatment with turpentine in comparison to the control groups.

Parameter	Unit	RI	0 h								18 h							
			0		2		6		10		0		2		6		10	
			n	s.d.	*n*	s.d.	*n*	s.d.	*n*	s.d.	*n*	s.d.	*n*	s.d.	*n*	s.d.	*n*	s.d.
TSP	g/L	65–78	70.9	2.62	72.6	5.92	74.4	5.39	75.0	4.34	74.5	6.02	69.6	2.75	67.2	5.32	68.3	6.03
Albumin	g/L	28–37	30.8	2.48	29.6	1.98	31.2	2.00	30.6	1.76	29.7	1.95	29.5	1.41	29.1	2.23	27.9	1.60
Globulin	g/L	28–42	40.0	3.34	43.1	5.51	43.2	5.46	44.4	4.79	44.8	5.09	40.1	3.39	38.2	4.88	40.4	5.72
A/G	-	0.9–1.4	0.78	0.12	0.69	0.10	0.73	0.11	0.70	0.09	0.67	0.07	0.74	0.09	0.77	0.10	0.70	0.10
Urea	mmol/L	3.6–10.7	1.6	0.57	2.0	0.64	1.9	1.03	1.8	0.56	3.5	1.57	3.8	1.13	4.5	1.15	4.8	0.63
Creat.	µmol/L	10–133	142.7	12.11	139.8	22.36	133.2	22.92	144.2	18.43	145.7	5.89	149.0	15.79	143.8	23.48	152.3	22.99
AST	U/L	21–167	82.3	13.09	110.0	38.38	83.5	17.69	74.7	7.74	132.7	82.48	100.2	65.83	95.7	15.19	87.8	12.92
GGT	U/L	0–45	25.2	7.33	26.3	3.78	24.5	2.66	23.8	1.17	23.0	7.29	23.3	6.28	18.2	2.32	18.2	1.17
CK	U/L	12–146	642.7	432.49	2591.7	2734.25	925.0	949.28	197.5	56.19	2266.7	2040.99	1546.0	2652.09	716.8	346.98	570.8	388.17

RI, Reference Interval; TSP, total serum protein; A/G, albumin to globulin ratio; Creat., creatinine; AST, aspartate aminotransferase; GGT, gamma glutamyltransferase; CK, creatine kinase; *n*, number; s.d., standard deviation.
*, $p < 0.05$ (on *post-hoc* testing for the same time point)

TABLE 3: Clinical chemistry parameters obtained for the treatment groups after treatment (48 h and 120 h) with turpentine in comparison to the control groups.

Parameter	Unit	RI	48 h								120 h							
			0		2		6		10		0		2		6		10	
			n	s.d.	*n*	s.d.	*n*	s.d.	*n*	s.d.	*n*	s.d.	*n*	s.d.	*n*	s.d.	*n*	s.d.
TSP	g/L	65–78	74.4	5.14	72.6	7.43	72.7	4.88	69.8	4.74	69.5	4.96	75.4	6.16	77.1*	2.28	76.1	5.32
Albumin	g/L	28–37	29.5	1.55	27.4	1.94	27.4	2.15	26.5	2.88	27.5	2.79	27.7	1.46	28.0	2.03	27.5	1.52
Globulin	g/L	28–42	44.8	4.92	45.2	6.91	45.3	3.48	43.3	2.89	42.0	2.91	47.7	6.46	49.1*	2.39	48.7*	5.04
A/G	-	0.9–1.4	0.67	0.08	0.62	0.10	0.61	0.05	0.61	0.07	0.66	0.06	0.59	0.09	0.57	0.06	0.57	0.07
Urea	mmol/L	3.6–10.7	2.8	0.52	3.9	0.75	3.5	1.76	3.3	1.12	2.1	1.00	3.1	0.82	2.8	0.95	2.7	0.83
Creat.	µmol/L	10–133	132.8	8.52	123.7	22.38	117.3	19.35	127.3	21.72	121.2	8.77	121.7	17.50	108.3	18.29	120.3	15.74
AST	U/L	21–167	102.2	35.39	67.3	19.17	127.8	121.79	71.8	8.61	81.8	13.70	72.3	13.82	82.0	55.86	68.0	9.44
GGT	U/L	0–45	22.5	6.38	21.7	4.68	20.8	3.82	19.2	2.93	21.0	4.94	23.7	3.61	20.8	2.23	21.8	2.79
CK	U/L	12–146	543.8	387.29	221.5	181.51	2717.2	6086.63	212.5	68.59	405.2	510.10	847.2	1113.91	463.5	773.50	185.5	103.13

RI, Reference Interval; TSP, total serum protein; A/G, albumin to globulin ratio; Creat., creatinine; AST, aspartate aminotransferase; GGT, gamma glutamyltransferase; CK, creatine kinase; *n*, number; s.d., standard deviation.
*, $p < 0.05$ (on *post-hoc* testing for the same time point)

used by the famers, which are believed to be unlikely to achieve a plasma concentration above 20 µg/mL. The primary culture revealed no primary parasiticidal activity on the cultured organism. The subculture did show reduced growth of 30% in comparison to the controls, which may indicate a potential 'post-antibiotic' effect. In a study using the same parasite, Naidoo *et al.* (2005) speculated that this effect was due to the product's innate ability to interfere with cell division, with the result that the parasites could not divide at the same rate as the control group (static effect). Nonetheless, the effect seen is considered to be minor and is probably insufficient to offer protection, as both imidocarb and diminazene resulted in 100% parasite clearance. The failure of turpentine to produce a visible effect *in vitro* was also not unexpected, despite farmers reporting the successful management of treated animals with clinical signs of babesiosis. A similar negative result was reported in 1918 when turpentine was tried as a means of treating horses with *Theileria equi* (formerly *Nutella equi*, later *B. equi* and more recently *T. equi*) (De Kock 1918). According to this historic report, the treated horses showed no signs of improvement and died from classical equine anaemia. Whilst it may be argued that the absence of a positive result was due to this study making use of *B. caballi* and not *B. bovis* or *B. bigemina*, in reality both these species are sensitive to imidocarb dipropionate and diminazene under clinical conditions, making it likely that the turpentine would allow for the sufficient clearing of both these parasites.

With regard to the direct effect on the immune system, healthy animals under field conditions were exposed to a single intramuscular administration of turpentine. All treatments were well tolerated with a general increase in mass in all the treated animals on Day 37. The administered turpentine did, however, induce a mild to moderate inflammatory reaction at the injection site that was reversible with time. This reaction occurred in conjunction with a mild increase in body temperature and globulins, the latter most likely being a non-specific change resulting from the acute inflammatory response. The only other major clinical change evident was an increase in the occurrence of corneal opacity in the animals receiving the higher turpentine dose and a high incidence of injection site reactions. For the former, no explanation could be found and it was of minimal importance to the animals as they continued to feed without any discomfort.

Whilst the turpentine was not expected to increase the antibody response directly, it was considered to be theoretically possible, albeit very unlikely, that the turpentine could act as an adjuvant to a naturally acquired premunity, as these animals grazed on infected fields. To evaluate for this effect, animals were evaluated for changes in the anti-babesia antibody titres as well as changes in circulating leucocyte counts. Whilst changes were evident in serum globulin concentration, this change was not associated with changes in the globulin titres against both *B. bovis* and *B. bigemina*. Therefore, this increase would be an indication of an inflammatory response, especially when considered in conjunction with the injection site reaction, as opposed to a specific humoral immunity response. The absence of change in peripheral lymphocyte and monocyte counts was an unexpected finding. Previous studies in the mouse showed that turpentine oil increased plasma concentrations of interleukin-6 (IL-6) (Tsujinaka *et al.* 1997). With IL6 known to be an important stimulator of lymphocyte differentiation (Horn, Henze & Heidrich 2000), an increase in lymphocyte counts was expected. In addition, *in vitro* bovine lymphocyte cultures exposed to babesia antigens showed a direct proliferative response after 6 days of exposure (Tetzlaff *et al.* 1992). In the absence of any meaningful clinical pathological changes, it is believed that the maximum of 10 mL (± 200 µL/kg, 5x utilised dose) was insufficient to stimulate any meaningful response in cattle.

Conclusion

The lack of an *in vitro* parasiticidal effect and the poor static effect of medicated turpentine, in combination with the lack of a specific humoral response and a non-specific lymphocytic cellular immune response, suggest that medicinal turpentine is not a direct anti-babesial compound. However, to conclusively demonstrate this effect, an infectious model for post-turpentine exposure may be required to determine whether the product has an indirect effect. Based on the somewhat slower growth of babesia in the culture systems it may be possible, albeit doubtful, that the product slows down babesia growth, thereby allowing for the generation of protective immunity *in vivo* under field conditions.

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Competing interests

The authors declare that they have no financial or personal relationship(s) which may have inappropriately influenced them in writing this article.

Authors' contributions

L.J.B. (University of Pretoria) participated in the study design, carried out the laboratory and clinical research and drafted the manuscript as a post-graduate student; C.A.P.C. (University of Pretoria) acted as student supervisor, participated in the study design and edited the manuscript; V.N. (University of Pretoria) acted as student co-supervisor, participated in the study design, undertook statistical analysis and edited the manuscript.

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