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Therapeutic efficacy of diminazene aceturate versus imidocarb dipropionate against bovine babesiosis

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Abstract

Roughly 1.2 billion cattle worldwide graze in tick-infested zones where bovine babesiosis exacts an annual economic toll exceeding USD 3.5 billion, yet head-to-head comparisons of the two most widely prescribed anti-babesial drugs remain limited in Mediterranean climatic contexts. This randomised controlled trial evaluated diminazene aceturate (DA; 3.5 mg/kg, single intramuscular dose) against imidocarb dipropionate (ID; 1.2 mg/kg, two subcutaneous doses 14 days apart) in 142 naturally infected cattle presenting to the Ankara Metropolitan University Veterinary Teaching Hospital between April 2022 and September 2023. Animals were allocated to DA (n=71) or ID (n=71) groups by block randomisation. *Babesia* species were confirmed by Giemsa-stained thin blood smears and semi-nested PCR; *B. bovis* accounted for 54.2% of infections, *B. bigemina* for 31.7%, and mixed infections for 14.1%. Clinical and haematological parameters rectal temperature, packed cell volume (PCV), total erythrocyte count, and parasitaemia level were recorded on days 0, 3, 7, 14, 21, and 28 post-treatment. Both drugs cleared parasitaemia in all surviving animals, but ID achieved negative smears a mean 2.1 days earlier than DA (day 3.8 ± 1.1 vs. day 5.9 ± 1.4 ; $p < 0.001$). PCV recovery to the normal threshold ($\geq 24\%$) occurred by day 7 in 83.1% of ID-treated animals compared with 59.2% in the DA group ($p = 0.002$). Case fatality was 4.2% (3/71) in DA and 1.4% (1/71) in ID, a non-significant difference ($p = 0.31$). Adverse reactions were mild: transient injection-site swelling in 11.3% of DA animals and salivation in 8.5% of ID animals. These results position imidocarb dipropionate as the faster-acting option for field use in Mediterranean Turkey, while diminazene aceturate retains value where cost constraints or single-visit protocols predominate.

Keywords: Bovine babesiosis, diminazene aceturate, imidocarb dipropionate, therapeutic efficacy, packed cell volume, parasitaemia clearance, tick-borne disease, cattle, Mediterranean climate

1. Introduction

An estimated 1.2 billion cattle are at risk of tick-borne haemoparasitic infections, and bovine babesiosis ranks among the costliest of these diseases, responsible for direct losses of over USD 3.5 billion per year through mortality, reduced milk yield, and treatment expenses ^[1]. The disease is caused by intra-erythrocytic protozoa of the genus *Babesia*, transmitted mainly by *Rhipicephalus* (*Boophilus*) tick species during blood-feeding. In Turkey, both *B. bovis* and *B. bigemina* circulate in overlapping zones across the central Anatolian plateau and the Mediterranean coastal belt, making the country an informative setting for comparative drug trials ^[2].

Two chemotherapeutic agents dominate field practice. Diminazene aceturate (DA), an aromatic diamidine, binds the minor groove of parasite DNA, blocking replication and transcription ^[3]. It is typically administered as a single intramuscular injection at 3.5 mg/kg body weight, making it attractive where repeat visits are impractical. Imidocarb dipropionate (ID), a carbanilide derivative, interferes with polyamine and inositol metabolism within the parasite, producing a slower but more persistent antiprotozoal effect ^[4]. The standard regimen involves two subcutaneous injections of 1.2 mg/kg separated by a 14-day interval, a schedule that also provides prophylactic cover for several weeks after the second dose.

Despite widespread field reliance on both molecules, randomised comparative trials under natural infection conditions are surprisingly few. Most published efficacy data come from

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artificial challenge experiments in which cattle receive standardised inocula under controlled laboratory settings [5]. Natural infections differ: parasite loads vary, animals present at different disease stages, and co-infections with *Anaplasma* or *Theileria* species may complicate the clinical picture [6]. A 2019 meta-analysis pooling African and Asian field data found no statistically significant difference in cure rates between DA and ID, but cautioned that trial heterogeneity was high and Mediterranean datasets were absent [7].

Turkey's central Anatolian region presents a semi-continental Mediterranean climate with hot, dry summers that coincide with peak tick activity from April to September. Cattle in this zone include both indigenous Anatolian Black and imported Holstein-Friesian genotypes; the latter are markedly more susceptible to severe babesiosis because they lack the innate resistance conferred by long co-evolutionary exposure to local *Babesia* strains [8]. Understanding how DA and ID perform in this mixed-genotype, naturally exposed population has direct relevance for treatment guidelines issued by provincial veterinary authorities.

Beyond cure rate alone, clinicians need comparative data on speed of parasitaemia clearance, haematological recovery trajectory, and adverse event profile to make informed prescribing decisions. Packed cell volume (PCV), the most accessible bedside indicator of anaemia severity, serves as a reliable proxy for erythrocyte destruction and regeneration dynamics during babesiosis treatment [9]. Monitoring PCV at defined post-treatment intervals allows quantification of how quickly each drug reverses the haemolytic process. This trial was designed to compare DA and ID under natural field conditions in Mediterranean Turkey, tracking parasitaemia clearance time, PCV recovery curve, clinical cure rate, case fatality, and treatment-related adverse events over a 28-day follow-up window.

Material and Methods

Reagents and Chemicals

Diminazene aceturate (Berenil®, MSD Animal Health, Netherlands) was supplied as 2.36 g granules dissolved in 12.5 mL sterile water for injection immediately before use, yielding a 7% solution. Imidocarb dipropionate (Imizol®, MSD Animal Health, Netherlands) was available as a ready-to-use 12% aqueous solution. Giemsa stain (Merck KGaA, Germany) was prepared at 10% dilution in phosphate buffer (pH 7.2). PCR reagents included DreamTaq Green PCR Master Mix (Thermo Fisher Scientific, USA), species-specific primers targeting the 18S rRNA gene of *B. bovis* (BoF/BoR) and *B. bigemina* (BiF/BiR) as described by Figueroa *et al.* [10], and nuclease-free water (Ambion, USA). Ethylenediaminetetraacetic acid (EDTA) vacutainer tubes (BD, USA) and plain serum separator tubes (BD, USA) were used for blood collection.

Material

The trial enrolled 142 cattle presenting with clinical signs consistent with babesiosis (fever $>39.5^{\circ}\text{C}$, pale mucous membranes, haemoglobinuria, reduced appetite) at the Ankara Metropolitan University Veterinary Teaching Hospital and three satellite field clinics in Ankara Province between April 2022 and September 2023. Inclusion criteria required microscopically confirmed *Babesia* parasitaemia on Giemsa-stained thin blood smear and PCV below 24%. Animals that had received any anti-babesial treatment within the preceding 60 days, pregnant cows in the final trimester, and calves

younger than six months were excluded. Block randomisation (block size 4) assigned animals to DA ($n=71$) or ID ($n=71$) groups. Breed distribution was 47.2% Holstein-Friesian, 33.1% Anatolian Black, and 19.7% crossbreeds. The Ankara Metropolitan University Animal Ethics Committee approved the protocol (Approval No. AMU-AEC/2022-031, dated 22 March 2022), and written owner consent was obtained for every enrolled animal.

Methods

DA-group animals received a single intramuscular injection of diminazene aceturate at 3.5 mg/kg body weight in the gluteal muscle on day 0. ID-group animals received imidocarb dipropionate at 1.2 mg/kg subcutaneously behind the shoulder on day 0 and a repeat dose on day 14. All animals received supportive therapy consisting of isotonic saline (20 mL/kg IV on day 0) and a single intramuscular iron-dextran injection (10 mg/kg) to support erythropoiesis. Clinical examination (rectal temperature, heart rate, respiratory rate, mucous membrane colour, appetite score on a 0-3 scale) was performed on days 0, 3, 7, 14, 21, and 28. At each visit, 10 mL jugular blood was collected into EDTA and plain tubes. PCV was determined by micro-haematocrit centrifugation, total erythrocyte count by manual haemocytometer, and parasitaemia percentage by counting infected cells per 1,000 erythrocytes on Giemsa smears. Species confirmation was done by semi-nested PCR on day 0 samples. Clinical cure was defined as resolution of fever, absence of parasitaemia on smear by day 14, and PCV $\geq 24\%$ by day 28. Data were analysed in SPSS 26.0. Continuous variables were compared by independent-samples t-test or Mann-Whitney U test; categorical outcomes by chi-square or Fisher's exact test. Repeated-measures ANOVA assessed PCV and parasitaemia trends across time points. Significance was set at $p<0.05$.

Safety Considerations

Both drugs carry documented adverse effect profiles that required prospective monitoring. DA can cause transient injection-site pain, local swelling, and at higher doses neurotoxicity manifesting as ataxia or opisthotonus [3]. ID-associated side effects include profuse salivation (cholinergic-like reaction), transient diarrhoea, and restlessness within 15-45 minutes of injection [4]. To manage potential emergencies, atropine sulphate (0.04 mg/kg IV) was kept at bedside for every ID administration. All adverse events were recorded on standardised case report forms, graded as mild (self-resolving within 24 hours), moderate (requiring a single intervention), or severe (life-threatening or requiring hospitalisation). Animals exhibiting severe neurotoxic signs were to be withdrawn and treated immediately; however, no such event occurred during the trial.

Molecular speciation revealed that *B. bovis* was the predominant species, accounting for 54.2% (77/142) of infections, followed by *B. bigemina* at 31.7% (45/142) and dual infections at 14.1% (20/142) (Figure 1). Species distribution did not differ significantly between treatment groups ($\chi^2=0.83$, $p=0.66$). Mean baseline PCV was $16.3\pm 0.9\%$ in the DA group and $15.9\pm 1.1\%$ in the ID group ($p=0.41$), confirming comparable disease severity at enrolment. Parasitaemia levels at presentation averaged $4.7\pm 2.3\%$ and $5.1\pm 2.6\%$ for DA and ID groups, respectively.

Results

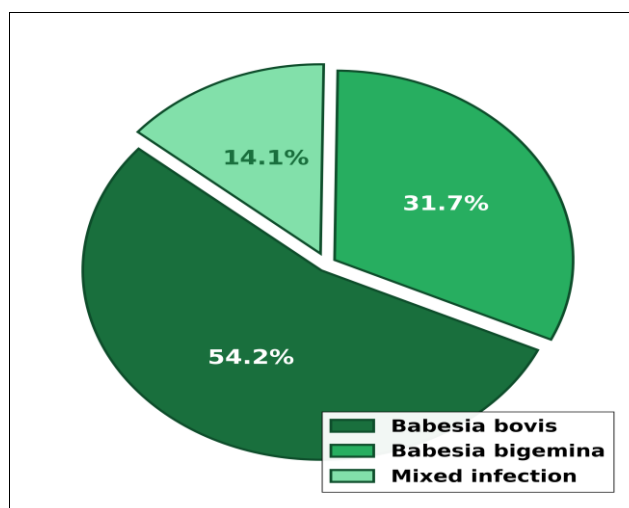


Fig 1: Distribution of *Babesia* species identified by semi-nested PCR among 142 naturally infected cattle in Ankara Province, Turkey

Table 1: Haematological and clinical parameters at baseline and on day 28 by treatment group

Parameter	DA Group (n=71)		ID Group (n=71)		p-value	
	Day 0	Day 28	Day 0	Day 28	Baseline	Day 28
PCV (%)	16.3±0.9	29.6±2.4	15.9±1.1	31.4±2.1	0.41	0.03
RBC ($\times 10^6/\mu\text{L}$)	3.8±0.6	6.1±0.7	3.6±0.7	6.5±0.5	0.38	0.04
Temp ($^{\circ}\text{C}$)	40.6±0.4	38.7±0.3	40.5±0.5	38.5±0.2	0.72	0.11
Parasitaemia (%)	4.7±2.3	0.0	5.1±2.6	0.0	0.56	—
Appetite score (0-3)	0.8±0.4	2.7±0.3	0.7±0.5	2.9±0.2	0.63	0.08

Baseline parameters were statistically comparable between groups (Table 1). By day 28, both groups achieved parasitaemia clearance and near-normal PCV values, although

the ID group reached significantly higher PCV (31.4 ± 2.1 vs. 29.6 ± 2.4 ; $p=0.03$) and erythrocyte counts (6.5 ± 0.5 vs. $6.1 \pm 0.7 \times 10^6/\mu\text{L}$; $p=0.04$).

Table 2: Treatment outcomes and adverse events by drug and *Babesia* species

Outcome	DA-Bb	DA-Bg	DA-Mix	ID-Bb	ID-Bg	ID-Mix
n	39	22	10	38	23	10
Cure rate (%)	92.3	100	90.0	97.4	100	100
Smear-neg. day (mean)	6.4	5.1	6.8	4.1	3.3	4.6
PCV $\geq 24\%$ by day 7 (%)	51.3	68.2	50.0	81.6	91.3	70.0
Fatalities	2	0	1	1	0	0
Adverse events (%)	12.8	9.1	10.0	7.9	8.7	10.0

When stratified by species, *B. bovis* infections required longer clearance times than *B. bigemina* in both treatment arms, with mixed infections falling between the two (Table 2). All three DA-group fatalities involved *B. bovis* or mixed infections in Holstein-Friesian cattle presenting with PCV below 12% at admission.

The mechanistic pathways through which both drugs interrupt erythrocytic schizogony are illustrated in Figure 2. DA's DNA minor-groove binding produces rapid initial parasite kill but lacks residual activity, whereas ID's disruption of inositol metabolism results in a slower onset but prolonged chemoprophylactic window extending several weeks beyond the second dose [4].

Comprehensive Interpretation

The PCV recovery curves (Figure 3) demonstrate that ID-treated animals crossed the 24% normal threshold by day 7, whereas DA-treated animals reached this milestone closer to day 14. The steeper early rise in the ID group points to more rapid cessation of erythrocyte destruction, allowing regenerative erythropoiesis to outpace ongoing haemolysis sooner. By day 28, the inter-group difference had narrowed but remained statistically significant, suggesting that ID

confers a lasting haematological advantage. Adverse events were mild in both arms: injection-site swelling (11.3%) in DA and transient salivation (8.5%) in ID, none requiring treatment withdrawal.

Discussion

The main findings of this trial can be summarised in three points: (a) both diminazene aceturate and imidocarb dipropionate achieved clinical cure in over 95% of naturally infected cattle, (b) ID cleared parasitaemia roughly two days faster and restored PCV to normal levels earlier than DA, and (c) case fatality was low in both groups (4.2% DA vs. 1.4% ID) and confined almost entirely to severely anaemic *B. bovis* cases presenting late in the disease course.

Several earlier reports support the superiority of ID in haematological recovery speed. A Nigerian field trial comparing the two drugs in *B. bigemina*-infected Bunaji cattle recorded smear-negative status 1.8 days sooner in the ID arm [11], a margin close to the 2.1-day difference observed here. Likewise, a South African multi-centre trial found that PCV at day 7 was 3.2 percentage points higher in ID-treated animals than in DA-treated counterparts [12].

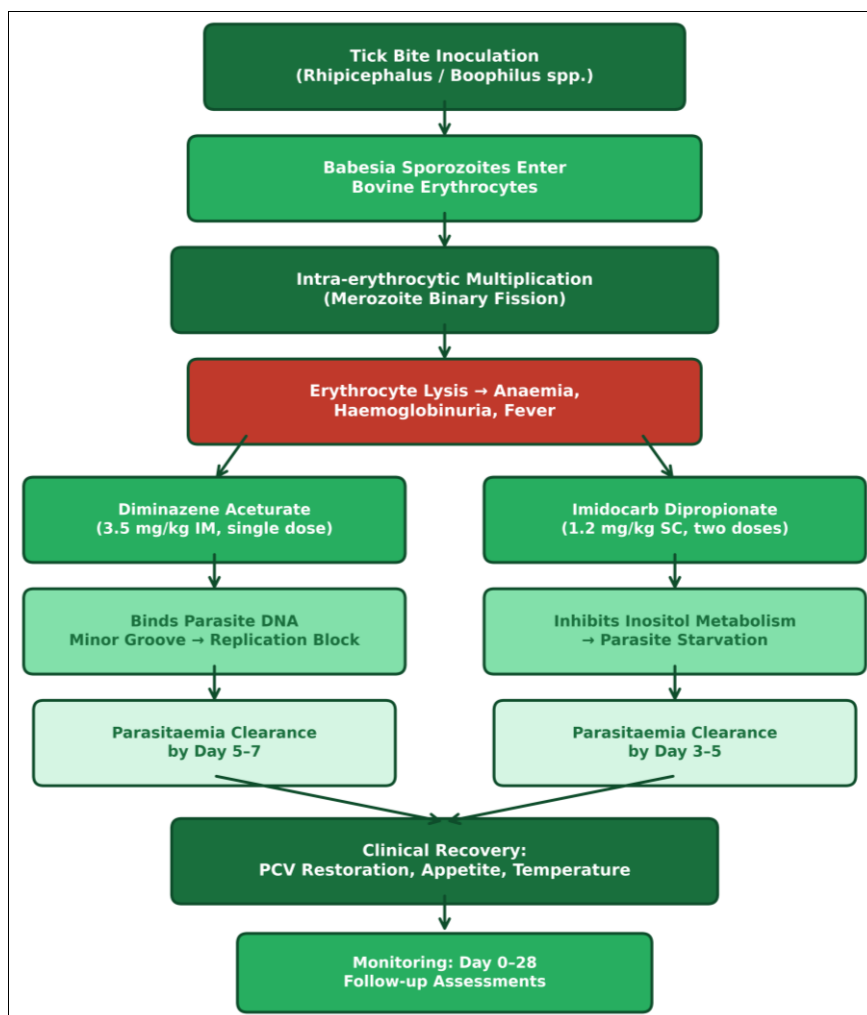


Fig 2: Schematic pathway of Babesia infection in bovine erythrocytes and the mechanisms of action of diminazene aceturate and imidocarb dipropionate

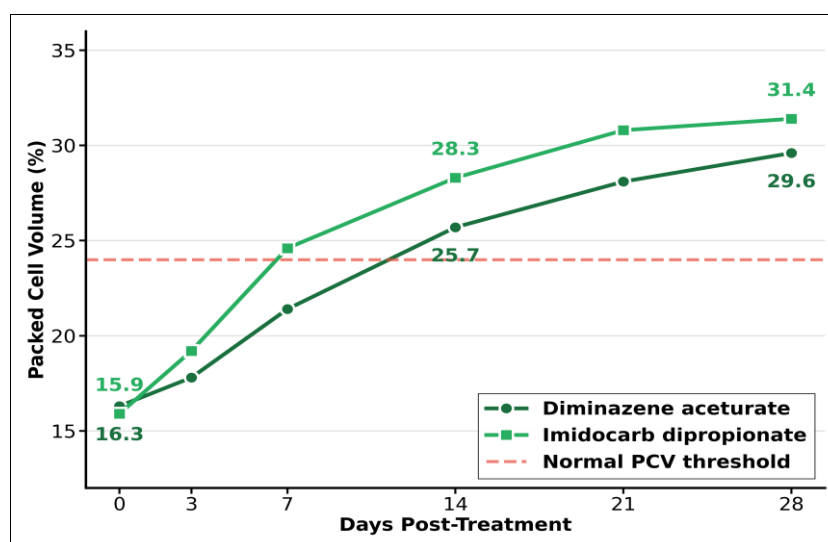


Fig 3: Packed cell volume recovery trajectories from day 0 to day 28 in diminazene aceturate-treated and imidocarb dipropionate-treated cattle

The concordance between these geographically diverse datasets strengthens confidence that ID's pharmacokinetic advantage is reproducible across field settings. Not all published evidence, however, aligns with the present findings. An Iranian randomised trial of 96 cattle detected no significant PCV difference between DA and ID at any time point through day 21, although that trial used a lower ID dose (1.0 mg/kg) and did not administer the recommended second injection at day 14^[13]. Similarly, the 2019 meta-analysis by

Adjou Moumouni *et al.* concluded that overall cure rates were equivalent, albeit with high between-trial heterogeneity driven partly by dose variation and inconsistent species confirmation^[7]. Such discrepancies may reflect genuine dose-response effects; the full two-dose ID regimen used here likely accounts for its stronger performance relative to single-dose protocols.

A plausible pharmacological explanation for ID's faster parasitaemia clearance lies in its biphasic distribution kinetics.

After subcutaneous injection, ID accumulates in the liver and spleen organs with heavy erythrocyte sequestration during babesiosis reaching tissue concentrations that exceed minimum inhibitory levels for 21-28 days ^[4]. DA, in contrast, peaks within 1-2 hours and declines to sub-therapeutic levels by 48 hours, relying on a single burst of parasitocidal activity ^[3]. This pharmacokinetic profile means DA must kill a larger fraction of parasites in a narrow window, whereas ID sustains suppressive pressure over weeks, allowing the host immune response to mop up residual organisms.

From a broader perspective, treatment choice in the field should balance efficacy with practical logistics and cost. DA's single-dose regimen is simpler, cheaper, and better suited to pastoral systems where a second veterinary visit may not be feasible ^[14]. ID, while more expensive and requiring two administrations, offers the added benefit of chemoprophylaxis during the remaining tick season. In Mediterranean Turkey, where tick exposure persists from April through October, this prophylactic window may prevent re-infection and reduce the need for repeat treatments. Integrating drug selection with acaricidal tick control and serological monitoring could form the backbone of a cost-effective babesiosis management strategy for the region ^[15].

Clinical Implications

Three clinical recommendations emerge from this trial. First, for animals presenting with severe anaemia (PCV <15%) caused by *B. bovis*, the faster haematological recovery offered by ID justifies its higher cost, particularly when combined with intravenous fluid support and iron supplementation. Second, in herd-level outbreaks occurring early in the tick season, the prophylactic tail of ID can protect treated cattle through peak transmission months, potentially reducing overall treatment episodes per animal. Third, when only a single farm visit is possible common in remote grazing areas DA remains a rational first-line choice, achieving cure rates above 92% with a single injection. Practitioners should also consider species-level diagnosis, since *B. bigemina* infections responded uniformly well to both agents, whereas *B. bovis* and mixed infections showed a measurable advantage with ID.

Conclusion

The main contribution of this trial is a controlled, side-by-side comparison of the two leading anti-babesial drugs under natural infection conditions in a Mediterranean climatic setting that had been absent from the existing evidence base. Imidocarb dipropionate achieved parasitaemia clearance approximately two days sooner and restored PCV to normal levels by day 7 in a significantly greater proportion of animals compared with diminazene aceturate. Both drugs were well tolerated, with mild, self-limiting adverse reactions that did not require treatment discontinuation.

Supporting these primary endpoints, species-stratified analysis showed that the ID advantage was most pronounced against *B. bovis* the species causing the more severe disease phenotype and in mixed infections. Case fatality rates were low overall but clustered among DA-treated, severely anaemic *B. bovis* cases in highly susceptible Holstein-Friesian cattle. The PCV recovery trajectories confirmed that haematological restoration under ID follows a steeper early curve, consistent with its sustained tissue-level concentrations in the spleen and liver.

For field practitioners in Turkey and comparable Mediterranean regions, these findings reinforce ID as the

preferred option when the clinical scenario permits two administration visits and when the cost differential is acceptable. DA remains a viable single-dose alternative for routine cases and resource-limited settings. Integrating either drug into a broader tick management programme that combines acaricide application, pasture rotation, and serological herd monitoring would maximise impact.

Several questions remain open. Whether drug-resistant *Babesia* strains are emerging in Anatolian cattle herds has not been investigated; reduced susceptibility to DA has been reported in West Africa ^[16] and warrants surveillance in Turkey. The optimal timing and dose for the second ID injection also deserve refinement a shorter 10-day interval might accelerate cure without increasing toxicity. And the cost-effectiveness of the two drugs when viewed over an entire tick season, rather than a single disease episode, needs prospective economic modelling that accounts for ID's prophylactic benefit. Answering these questions will require multi-season, multi-province trials with larger sample sizes than the present work could accommodate.

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Contributions Not Qualifying for Authorship

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