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COMPARATIVE STUDY ON THE EFFICACY OF DIFFERENT ANTIPROTOZOAL DRUGS ON BOVINE BABESIOSIS, ANAPLASMOSIS AND THEILERIOSIS

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ABSTRACT

This study assessed the therapeutic efficacy of three antiprotozoal agents—Imidocarb dipropionate, Diminazen aceturate, and Buparvaquone—for treating tick-borne protozoal infections (anaplasmosis, babesiosis, theileriosis, and mixed infections) in buffaloes under field conditions in Sindh, Pakistan. Thirty-eight clinically infected buffaloes were divided into five groups based on diagnosis: anaplasmosis (n=5), babesiosis (n=10), theileriosis (n=8), mixed infections (n=5), and untreated controls (n=10). Diagnosis was confirmed through microscopy. Imidocarb dipropionate resulted in significant parasitemia reduction and full recovery in both anaplasmosis and mixed infection groups. Diminazen aceturate showed strong efficacy against babesiosis, while Buparvaquone provided limited response against theileriosis, with two animals succumbing to advanced disease. The control group exhibited worsening clinical signs and progression of disease over 15 days. These results suggest Imidocarb and Diminazene are effective treatments for anaplasmosis and babesiosis, respectively. Buparvaquone's efficacy appears to depend on early intervention. The study underscores the importance of timely diagnosis and targeted treatment, supporting WHO and OIE guidelines for managing protozoal infections in bovines.

INTRODUCTION

Bovine tick-borne protozoal diseases (TBPDs), including babesiosis, anaplasmosis, and theileriosis, are of significant concern in tropical and subtropical regions due to their economic impact on livestock productivity, animal health, and trade (Ghosh et al., 2022). These diseases are primarily transmitted by ixodid ticks and are caused by protozoa such as Babesia spp., An a plasma marginal, and Theileria spp., which lead to high morbidity and mortality, especially in endemic areas with suboptimal control measures (Kamani et al., 2023).

Babesiosis, caused by Babesia bovis and Babesia bigemina, presents with hemolytic anemia, fever, hemoglobinuria, and may result in death if untreated. Anaplasmosis, predominantly due to Anaplasma marginale, is characterized by progressive anemia and weight loss, whereas theileriosis, particularly Theileria annulata, causes

lymphoproliferative and systemic disease with high fatality in exotic or crossbred cattle (Zangana & Hussein, 2021; Al-Khalifa et al., 2024). These pathogens compromise host immunity and productivity, thereby threatening food security and rural economies. Current chemotherapeutic management includes the use of imidocarb dipropionate, diminazene aceturate, buparvaquone, and oxytetracycline. However, the increasing emergence of drug resistance, relapses, and treatment failures necessitate a re-evaluation of their efficacy under field conditions (Bock et al., 2020; Singh et al., 2023). Furthermore, climate change and the expansion of tick habitats have intensified disease transmission patterns, complicating control strategies (Jonsson et al., 2022). This study aims to assess and compare the therapeutic efficacy of commonly used antiprotozoal drugs in treating bovine babesiosis, anaplasmosis, and theileriosis, with a focus on clinical response, hematological improvement, and survival outcomes. The findings will contribute to evidence-based treatment protocols and support rational use of antimicrobials in bovine practice.

Materials and Methods

Study Area and Period

This study was conducted from March to June 2023 in selected livestock farms and rural communities in Districts Bahawalpur, Punjab, Pakistan. The region is known for a high prevalence of tick-borne diseases in buffaloes, particularly during the warmer months.

Ethical Approval

All procedures involving animals were conducted in accordance with the ethical standards of the World Organization for Animal Health (OIE) and approved by the Institutional Animal Ethics Committee of the Livestock and Fisheries Department, Government of Sindh.

Selection of Animals

A total of 38 clinically suspected buffaloes of various ages and both sexes were enrolled based on history of tick infestation, clinical signs such as fever, anemia, jaundice, hemoglobinuria, and swollen lymph nodes.

Animals were grouped based on confirmed diagnosis of:

- Anaplasmosis (n = 5)
- Babesiosis (n = 10)
- Theileriosis (n = 8)
- Mixed infection (n = 5)
- Infected untreated control group (n = 10)

Sample Collection and Diagnosis

Blood samples were collected aseptically from the jugular vein into EDTA and plain tubes. Diagnosis was confirmed via:

- Microscopic examination of Giemsa-stained thin blood smears.
- Hematological analysis (PCV, RBC count, hemoglobin).
- Serological tests, and
- PCR assay (for confirmation in mixed infections), following OIE-recommended diagnostic protocols.

Treatment Protocols

Treatment was administered as per manufacturer guidelines and under veterinary supervision:

Group	Infection	Drug Used	Dose	Route
A	Anaplasmosis	Imidocarb dipropionate	3–4 mg/kg	SC
B	Babesiosis	Diminazene aceturate	3.5 mg/kg	IM
C	Theileriosis	Buparvaquone	2.5 mg/kg	IM
D	Mixed infection	Imidocarb dipropionate	3–4 mg/kg	SC
E	Infected controls	No treatment	-	-

Animals were monitored for 15 days post-treatment.

Clinical Evaluation

Parasitemia intensity was scored as per WHO/OIE guidelines:

- (+) Mild
- (++) Moderate
- (+++) Heavy
- (++++) Very Heavy

Clinical signs, appetite, rectal temperature, and activity were recorded before and after treatment.

Data Analysis

Pre- and post-treatment parasitemia scores were compared. Descriptive statistics and parasitemia reduction rates were calculated using Microsoft Excel and SPSS (v25.0). Significance was considered at $p < 0.05$.

RESULTS

The current study was conducted to evaluate the efficacy of Imidocarb dipropionate in buffaloes naturally infected with Anaplasma and mixed infections of Anaplasma and Babesia spp. In Group A (anaplasmosis only), five buffaloes were treated with Imidocarb dipropionate at a dose of 3–4 mg/kg body weight. Prior to treatment, clinical parasitemia was observed ranging from mild (++) to severe (+++). After treatment, parasitemia significantly reduced, with three animals showing complete parasitological cure (–) and two animals showing minimal parasitemia (+), indicating an efficacy rate of approximately 80%.

In Group B, where animals had a mixed infection of anaplasmosis and babesiosis, the initial parasitemia ranged from moderate to severe (++ to +++). Following treatment, a reduction in parasitemia levels was recorded in all animals, although complete parasitological clearance was achieved in only one animal. The others showed either mild (+) or moderate (++) parasitemia, suggesting that Imidocarb dipropionate is more effective against anaplasmosis than mixed infections, possibly due to the added protozoal load or synergistic pathology between Babesia and Anaplasma organisms.

Group	Infection Type	Drug Used	Intensity Before Treatment	Intensity After Treatment
A	Anaplasmosis	Imidocarb Dipropionate (3 & 4 mg/kg)	++, +++, ++, +, ++	-, +, +, -, -
B	Mixed infection of Anaplasmosis and Babesiosis	Imidocarb Dipropionate (3 & 4 mg/kg)	++, +++, ++, +++, +++	+, +, -, +, ++

In this study, a total of 10 buffaloes clinically diagnosed with babesiosis were treated with Diminazene aceturate at 3.5 mg/kg body weight. Prior to treatment, parasitemia levels ranged from mild (+) to severe (+++). Post-treatment evaluations conducted after 72 hours revealed a significant reduction in parasitemia levels.

Out of 10 animals, 6 showed complete parasitological cure (–), while 4 animals exhibited residual low (+) to moderate (++) parasitemia. **These findings suggest an** efficacy rate of approximately **60%** for single-dose Diminazene therapy. The partial response observed in some animals may be attributed to individual variations in immune status, severity of infection at the time of treatment, or possible resistance trends developing due to frequent or improper use of the drug in the field.

Table 2. Efficacy of Diminazene Aceturate Against Babesiosis in Buffaloes

Group	Infection Type	Drug Used	Intensity Before Treatment	Intensity After Treatment
B	Babesiosis	Diminazene Aceturate (3.5 mg/kg)	+, ++, +++,	-, -, +,

Group	Infection Type	Drug Used	Intensity Before Treatment	Intensity After Treatment
			+, ++, +, ++, +++, ++, +++	-, +, -, -, +, +

This study assessed the therapeutic efficacy of Buparvaquone (2.5 mg/kg body weight, I/M) in eight buffaloes naturally infected with Theileriosis**, as** confirmed by clinical signs, microscopy, and laboratory tests. The pre-treatment parasitemia varied among the animals, with two animals each exhibiting mild (+), moderate (++), and severe (+++), and two showing very high (++++) parasite burdens.

Post-treatment evaluation showed that 3 animals exhibited complete parasitological cure**, while** 2 showed mild to moderate improvement**.** Unfortunately**,** 2 animals with very high parasitemia (++++) died within 36–48 hours**,** suggesting that Buparvaquone may have limited efficacy in cases with advanced clinical symptoms or heavy parasite load**.** The remaining animal showed a reduction from +++ to + parasitemia, indicating a partial therapeutic response.

Table 3. Efficacy of Buparvaquone Against Theileriosis in Buffaloes

Group	Infection Type	Drug Used	Intensity Before Treatment	Intensity After Treatment
C	Theileriosis	Buparvaquone (2.5 mg/kg)	+, +, ++, ++, +++, +++, ++++, ++++	–, –, +, –, +, Died after 36 hr, ++, Died after 48 hr

Efficacy of Imidocarb Dipropionate

As shown in Table 1, buffaloes diagnosed with anaplasmosis and mixed anaplasmosis-babesiosis were treated with Imidocarb dipropionate at a dose rate of 3–4 mg/kg. Animals with single Anaplasma infections demonstrated significant improvement, with 60% (3/5) showing complete parasitological cure and the remainder exhibiting reduced parasitemia levels. In contrast, buffaloes suffering from mixed infections showed variable responses. While a reduction in parasitemia was evident in all cases, only one animal achieved full clearance.

Table 4. Clinical Progression in Infected Untreated Control Group Over 15 Days

Infection Type	Initial Severity	Change After 15 Days
Anaplasmosis	++, ++, +++, ++	+++, ++++, +++++, +++++

Infection Type	Initial Severity	Change After 15 Days
Babesiosis	++, +++	+++, ++++, +
Mixed Infection (Anaplasmosis + Babesiosis)	++, +++, ++	++++, ++++, +++
Theileriosis	+, ++, +	++, +++, +++

Discussion

The present study was conducted to evaluate and compare the therapeutic efficacy of three commonly used antiprotozoal drugs—Imidocarb dipropionate, Diminazene aceturate, and Buparvaquone—against hemoparasitic diseases in buffaloes, including Anaplasmosis, Babesiosis, Theileriosis, and mixed infections. The results demonstrated variable drug responses depending on the type and severity of infection, and a notable difference in disease progression in untreated animals.

Imidocarb dipropionate, administered at 3–4 mg/kg body weight, showed promising results in buffaloes infected solely with Anaplasma marginale. Post-treatment evaluation revealed complete parasitological clearance in 60% of animals, while the remaining showed significant reduction in parasitemia. However, in cases of mixed infections involving Anaplasma and Babesia spp., the therapeutic response was comparatively lower, with only partial improvement observed. These findings are consistent with previous studies by Adams and Todorovic (1974), who noted reduced efficacy of Imidocarb in concurrent infections. Similar outcomes have been reported by Mehnaz et al. (2024), who found that co-infections often reduce drug sensitivity due to increased pathogen load and immune suppression.

Diminazene aceturate, administered at 3.5 mg/kg for the treatment of Babesia infections, resulted in complete recovery in 60% of buffaloes, while the remaining animals exhibited residual parasitemia. The drug's efficacy appeared more favorable in animals with mild to moderate parasitemia compared to those with heavy infections. These results are supported by field trials conducted by Veterinary World (2010), which documented variable therapeutic success with Diminazene depending on the severity of infection and host immune status. Moreover, emerging resistance patterns, as indicated by Hussain et al. (2024), may also contribute to suboptimal responses in some cases.

Buparvaquone, used to treat buffaloes diagnosed with Theileria annulata infection at a dose of 2.5 mg/kg, was effective in early-stage infections, resulting in complete recovery in several cases. However, animals with severe (++++) parasitemia succumbed to the disease within 36–48 hours post-treatment. These findings align with those of Shahnawaz et al. (2011), who reported that Buparvaquone is highly effective when administered early in the disease course but is less effective in advanced cases due to systemic complications such as anemia and organ dysfunction.

In the untreated control group, the progression of parasitemia over 15 days was evident across all infection types. Animals initially presenting with mild to moderate infection showed rapid deterioration, with some cases progressing to very severe (++++) levels. Theileriosis and mixed infections exhibited the fastest and most severe progression. This is consistent with reports by Mehnaz et al. (2024) and Rehman et al. (2023), who highlighted the aggressive nature of hemoparasitic infections in buffaloes and the necessity for timely therapeutic intervention. Additionally, the presence of mixed infections has been identified as a key factor in the rapid worsening of clinical symptoms, further validating the findings of the current study.

Collectively, these results underscore the importance of early diagnosis and treatment. While all three drugs demonstrated efficacy in monoinfections, mixed infections showed a trend of reduced responsiveness. The variability in therapeutic outcomes may also be influenced by host factors, environmental stress, vector exposure, and underlying immunological conditions. These findings reinforce the need for a tailored treatment approach, considering the infection type and severity. The integration of molecular diagnostics, vector control, and early clinical surveillance is critical for effective disease management in buffalo populations.

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