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Case report on an outbreak of theileriosis in a dairy herd: Could it be a co-infection of a virulent strain of Theileria mutans with Theileria parva?

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ABSTRACT

This case report aimed to alert veterinarians of the possibility of simultaneous infections with more than one *Theileria* species in cattle herds in Zimbabwe. Cows (N=7) and a postmortem specimen from a dairy farm located in Chivhu, Mashonaland East province, Zimbabwe were presented for clinical and postmortem examination, respectively. On history taking, the herd had a decrease in milk yield and high cow mortalities. The presented live animals had a heavy tick burden and were febrile and lethargic. Post-mortem findings revealed strong evidence suggestive of theileriosis. *Theileria* schizonts and piroplasms were identified in stained lymph node aspirate, impression, and blood smears. The herd was managed using tetracyclines and buparvaquone together with an intensive dipping protocol until deaths had stopped. Both the clinical and postmortem findings were strongly suggestive of the possibility of a simultaneous infection with more than one *Theileria* species in this herd. Thus, these finding highlight the need to characterise the *Theileria* species profile in Zimbabwe.

Contribution/Originality: This case report provides the first ever record of what could have been a coinfection of possibly *Theileria parva* and *Theileria mutans* in a dairy herd in Zimbabwe.

1. INTRODUCTION

Theileria species (and their tick vectors) which infect cattle in southern Africa include *Theileria parva* which is the most pathogenic (*Rhipicephalus appendiculatus*, *Rhipicephalus zambeziensis*), *Theileria taurotragi* (*Rhipicephalus appendiculatus*, *Rhipicephalus zambeziensis*), *Theileria mutans* (*Amblyomma* spp.) and *Theileria velifer*a (*Amblyomma* spp.) [1]. Theileriosis causes devastating production losses and limits the expansion of both the beef and dairy herds [2]. In Zimbabwe, most cases of *Theileria parva* theileriosis are in the form of January disease (cattle to cattle transmission) due to a widespread carrier status in cattle [3], with a few isolated cases of Corridor disease where there is transmission of the parasite from buffalo to cattle [4]. January disease is characterized by fever, a generalised lymphadenitis, and a notable brown ear tick infestation. *Theileria mutans* is generally known to cause a mild disease of cattle characterised by a moderate to severe anaemia, moderate lymphadenitis, particularly in cases with mixed infections with other tick-borne pathogens, resulting in production losses [2, 5].

In early 2018 and 2019, at the peak of the rainy season, Zimbabwe experienced an upsurge in cases of cattle theileriosis, with reports of up to 2,000 and an estimated 50,000 fatalities respectively [6, 7]. Most of these cases

were reported as January disease, however, *Theileria* co-infections in herds are not commonly reported. This case describes a herd with a history, symptoms, postmortem changes, and stained-impression smear findings suggestive of a mixed infection.

1.1. Case Presentation

1.1.1. History

During the period mid-November 2017 to January 2018, a Holstein-Friesian dairy farm (n = 250) experienced cow mortalities and decrease in milk yields. Twenty animals had been affected (7 live cows, 2 live bulls and 11 fatalities). Sick animals were reported as initially anorexic, with bloody diarrhoea. Fatalities came 3 to 4 days after manifestation of clinical signs and prior to death animals were dyspnoeic. Acaricide application was done using a knapsack sprayer and the last one dated as far back as mid-November 2017. A single sick cow had reduced milk yield on the day of the visit.

1.2. Ethical Considerations

Ethical considerations were taken into account during physical examination of the animals to minimise pain and suffering.

1.3. Clinical Investigation, Sample Collection, Laboratory Analysis, and Post-Mortem Findings

Animals tired quickly when driven from pasture to the milking parlour. Ticks were observed on the presented animals. Based on attachment sites, scutum colour, mouthpart length and festoons, most of these ticks were *Amblyomma* species, with some *Rhipicephalus appendiculatus/zambeziensis*. The seven cows presented had a fever (temperature: 39.8 - 40.2°C), an elevated heart rate (88 - 100bpm) and a rapid shallow respiratory rate (76 - 84bpm). Corneal opacity was noted on one heifer, pale mucous membranes on five of the seven cows presented for examination and, on palpation, only one of the live animals presented had swollen lymph nodes.

Blood (4ml) from tail veins of the seven cows presented was collected into Ethylenediaminetetraacetic acid (EDTA) tubes for packed cell volume (PCV) analysis and peripheral blood smears were prepared using blood from the ear veins. Fine needle aspirates of the enlarged lymph nodes were used to prepare thin smears. A post-mortem was performed on one carcass and a lymph node impression smear was made from the cut surface of an enlarged pre-scapular lymph node (Figure 1B). All the blood and impression smears were fixed in 100% methanol and Giemsa-stained.

The carcass had diffused tick bite lesions on the skin surface indicative of a recent heavy tick burden, with ticks noted around the anal opening. There was a frothy nasal discharge (Figure 1A) and pulmonary oedema with copious fluid within the lungs, bronchi, and trachea (Figure 1B). Hepatomegaly, hydrothorax, lymphadenitis, and splenomegaly were also observed. PCVs of five of the seven presented animals were on the lower side (Table 1). Giemsa-stained lymph node and peripheral blood smears revealed presence of schizonts and a notable piroplasm parasitaemia, respectively (Figure 1C).

These findings were highly suggestive of theileriosis [8, 9], with a possible co-infection. The presence of *Amblyomma* ticks on the animals, pale mucous membranes, unpalpable lymph nodes in six of the seven animals examined, notable red blood cell piroplasm parasitaemia and the reduced PCV in five of seven cows are symptoms consistent with theileriosis *mutans* whilst presence of brown ear ticks, palpable enlarged lymph nodes (LN) and the bloody diarrhoea noted on history taking occur with January disease.

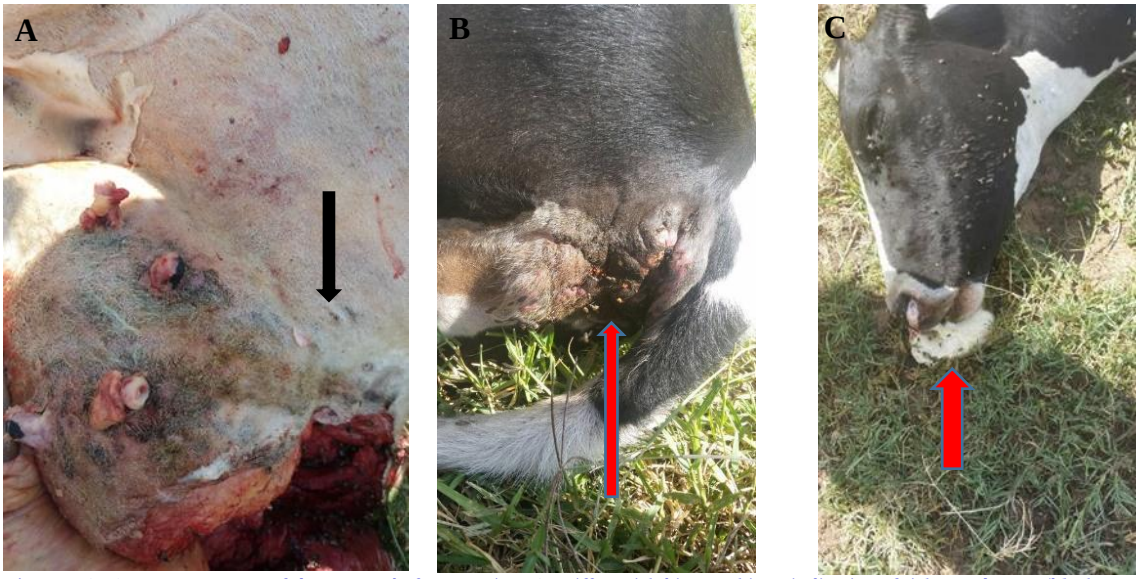

Figure 1A. Gross appearance of the carcass before opening. **A:** Diffuse tick bite markings indicative of tick attachment (black arrow); **B:** Ticks attached around the anus; **C:** Frothy nasal discharge.

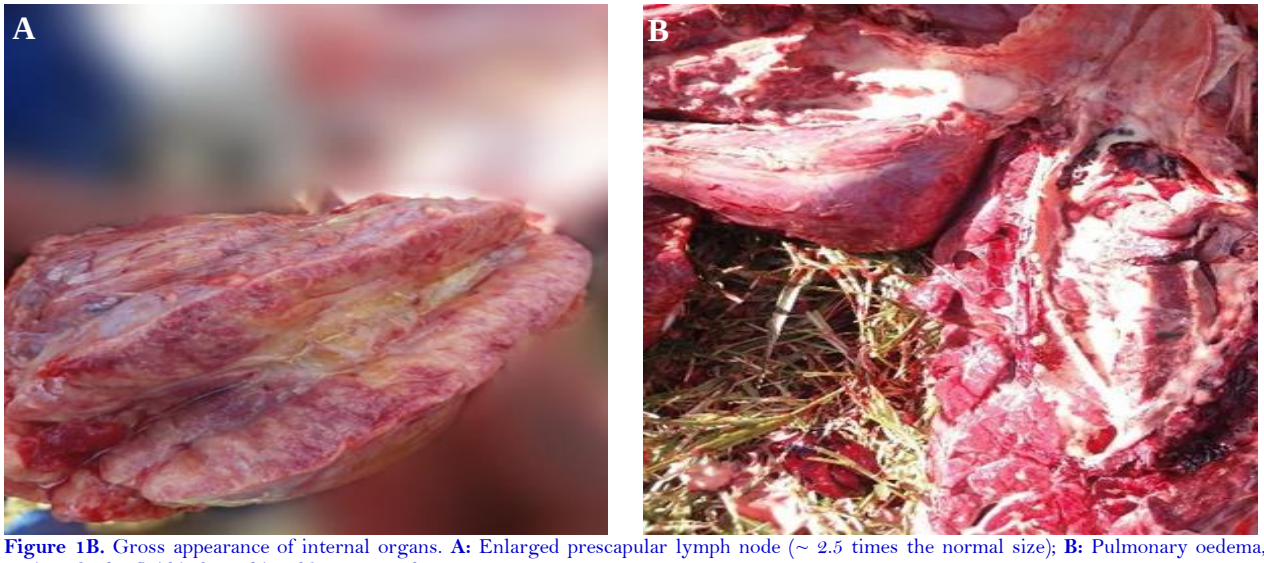

Figure 1B. Gross appearance of internal organs. **A:** Enlarged prescapular lymph node (~ 2.5 times the normal size); **B:** Pulmonary oedema, copious frothy fluid in bronchi and lung parenchyma.

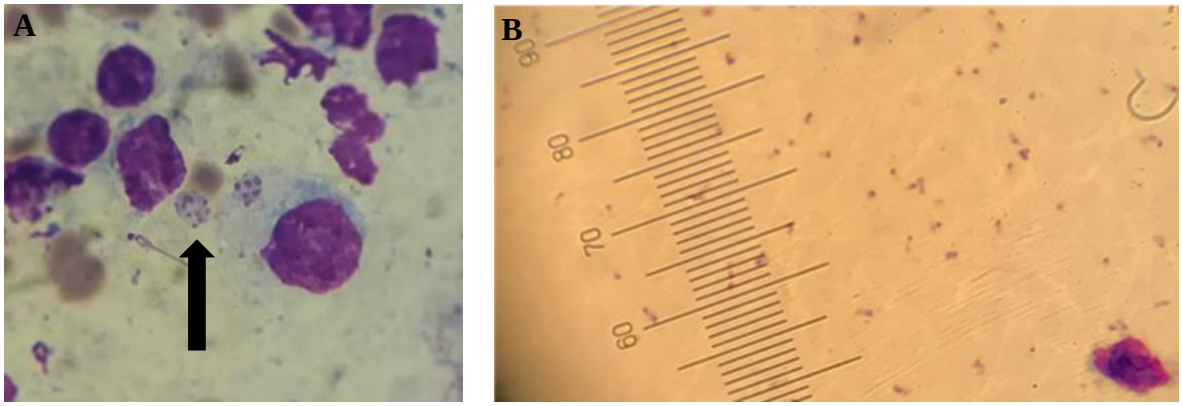

Figure 1C. Laboratory findings. **A:** *Theileria* schizonts (Koch's Blue Bodies); **B:** *Theileria* piroplasms in red blood cells.

Table 1. % Packed cell volumes (PCV) for the presented animals (n = 7).

Animal ID	1	2	3	4	5	6	7
PCV % (Normal range: 24 – 46)	24	36	23	25	24	26	34

1.4. Treatment and Management Plan

Animals that had shown clinical signs for > 24 hr were treated with buparvaquone (Butalex®) at 2.5 mg/kg q48h intramuscular. Those that started showing clinical signs for ≤ 24 hr were deemed to have had an early infection and were treated with oxytetracycline (Hi-TET®) at 10mg/kg intramuscular. The farmer was advised to carry out intensive dipping (with an amitraz dip at 250ppm), starting with the 3-3-5-day protocol followed by bi-weekly dipping until the end of the outbreak. Subsequently, he had to practise routine weekly dipping in the rainy season to prevent future outbreaks. He was also advised to use a plunge dip or spray race as they are more effective methods of acaricide applications on a large herd. If he opted to continue using a Knapsack sprayer, he had to ensure that each animal was thoroughly soaked using at least 10-15 litres per animal. He was also advised to complement the dipping with application of tick grease in hidden areas such as in the ears, on the perineum (under the tail) and on the tail switch. As prescribed by law, theileriosis is a notifiable and specified disease in Zimbabwe, hence the outbreak was reported to the Department of Livestock and Veterinary services (DLVS). A follow-up indicated that the herd had recovered and there were no more mortalities.

2. DISCUSSION AND CONCLUSION

The present case is the first in the country in recent years to report theileriosis in a herd that is most likely due to a simultaneous occurrence of more than one *Theileria* species and affected animals were adult exotic Holstein-Friesian dairy cattle. Cattle theileriosis caused by *Theileria parva* is associated with high mortalities in predominantly exotic and crossbred cattle [10]. Sporadically fatal infections of *Theileria mutans* characterised by a severe haemolytic anaemia, which can be fatal, have been reported in other parts of Africa [11, 12]. Older animals without a previous exposure often succumb to *Theileria mutans* [13] and co-infections with other pathogens (and stresses) [2, 5, 13] are thought to increase chances of the disease occurring [13].

Most of the animals examined here (5/7) had symptoms that were consistent with *Theileria mutans* whilst the post-mortem case pointed more to *Theileria parva*. In *Theileria parva* infection, the pathology is mainly due to lymphocytic merogony accompanied by pronounced enlargement of lymph nodes but limited erythrocytic merogony (absence of a haemolytic anaemia) whereas in *Theileria mutans* the main replication is in erythrocytes, with a high piroplasm parasitaemia hence the haemolytic anaemia but limited lymphocytic merogony (less prominent lymph nodes) [2, 5, 8, 11].

However, because of the greater pathogenicity *Theileria parva*, many are familiar with January and Corridor diseases and the brown ear tick vectors. In addition, many only associate *Amblyomma* species with the transmission of *Ehrlichia ruminantium*, the cause of heartwater but are not aware that the ticks could also transmit another *Theileria* species which is potentially pathogenic to cattle that are stressed and/or have intercurrent infections. On clinical diagnosis alone, the anaemic syndrome induced by *Theileria mutans* could easily be misdiagnosed as redwater or anaplasmosis.

A thorough investigation by a veterinarian which includes microscopic examination of stained blood and lymph node smears is hence necessary in coming up with a more accurate diagnosis that is more effective in the containment of an outbreak. In conclusion this case indicated the possibility of a simultaneous infection of a herd with more than one *Theileria* species, thus it is of paramount importance to perform molecular characterisation of *Theileria* species occurring in Zimbabwe.

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