

# Parasitological examination for *Trypanosoma theileri* infection of cattle from Quirino Province, Philippines

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**Abstract:** *Trypanosoma theileri* is one of the protozoan parasites reported in cattle and carabaos in the Philippines. The distribution of *T. theileri* infection in livestock in this country is not well established. To date, more than two decades have passed without any new *T. theileri* prevalence reported in the country. The present study endeavored to determine whether the parasite is endogenously present in cattle from the remote areas of Region II (Cagayan Valley), particularly in the province of Quirino, Philippines and to establish the current parasite prevalence of *T. theileri* in this province using the blood parasite examination method. A total of 246 field blood samples of cattle was collected from 5/6 (83%) of the municipalities of Quirino Province, Philippines. Blood parasite examination of Giemsa-stained smears revealed that all samples were negative for the presence of the *T. theileri* parasite. Given that the mainstay of parasitological examination in the field is the classic blood parasite examination method, the data suggest that the negative results of these examinations can probably be attributed to very low parasitemia.

**Keywords:** *Trypanosoma theileri*, blood parasite examination, Quirino Province, Philippines

## Introduction

Livestock are hosts for several trypanosomatid species. The reported trypanosomes in small and large animals include *Trypanosoma congolense*, *T. vivax*, *T. brucei*, *T. evansi*, and *T. theileri*.<sup>1-3</sup> Among the different species, *T. theileri* is the least studied. This is probably attributable to the described nonpathogenic nature of the parasite.<sup>2</sup>

*T. evansi* (salivarian) and *T. theileri* (stercorarian) were the two ubiquitous livestock trypanosome species in cattle and water buffalos reported in the Philippines in past studies.<sup>4-7</sup> *T. evansi* is well-studied and known to be pathogenic to horses, whereas *T. theileri* were considered nonpathogenic.<sup>2</sup> However, it was previously reported that adverse conditions may prime the animal to succumb to trypanosomosis due to *T. theileri*.<sup>2,8</sup> Several studies implicated *T. theileri* as the etiologic agent of trypanosomosis in cattle.<sup>9-12</sup> In the report of Pietersma et al,<sup>13</sup> *T. theileri* parasitemia in a neonatal bison calf was observed. Clinical signs upon presentation included severe dehydration accompanied by hypernatremia, hyperalbuminemia, azotemia, and hyperphosphatemia. Complete blood counts revealed a degenerative left shift, toxic neutrophils, lymphocytosis, mild monocytosis, and severe thrombocytopenia. Since the transmission of *T. theileri* by tabanid flies was unlikely at this young age, it was deduced that the animal was transplacentally infected.

The reports of parasite prevalence in the field, as with the succeeding data, resulted from the blood parasite examination (BPE) method. *T. theileri* infection has likewise

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been reported in the European continent. In Zaragoza, Spain a sample prevalence of 33.3% (4/12) *T. theileri* infection was obtained from a Spanish bullfighting farm located in the Seville Province.<sup>14</sup> A prevalence of 3.2% (31/970) was also recorded in cattle from the African continent, particularly Mafia Island, Tanzania.<sup>15</sup> According to Kennedy,<sup>16</sup> the infection had also reached North America and surveys showed that the prevalence in the United States and parts of Canada ranged from 10% to 80%. The study also reported the first documented trypanosomes in adult cattle in Alberta, Canada.

On the Asian continent, difficulty of microscopic detection of the *T. theileri* parasite in blood smears is commonly encountered and affects the prevalence data. In Iran, the hemoflagellate parasite was not detected when using blood parasite examination method in any of the 238 cattle in the survey.<sup>17</sup> Conversely, in the Philippines the latest published report of sample prevalence of *T. theileri* infection in cattle, from the National Capital Region, was 12% (12/100).<sup>5</sup> However, the distribution of *T. theileri* infection in livestock in this country is not well established; to date, more than two decades have passed without any new *T. theileri* prevalence reported in the Philippines. The current study endeavored to determine whether the parasite is endogenously present in cattle from the remote areas of Region II (Cagayan Valley), particularly in the province of Quirino, Philippines and to establish the current parasite prevalence of *T. theileri* in this province using the BPE method.

A total of 246 field blood samples of cattle from five out of the six municipalities of Quirino province were found to be negative for the *T. theileri* hemoparasite. Based on the corroborating findings of repeated BPE of the samples, the study suggests that the negative results are probably attributed to very low parasitemia.

## Materials and methods

### Animals and sampling site

A total of 246 cattle (*Bos taurus*) was randomly sampled from five out of six municipalities of Quirino. The chosen municipalities included Diffun, Cabarroguis, Saguday, Aglipay, and Maddela. The sample size was determined using a formula previously described<sup>18</sup> (Figure 1).

### Blood collection and blood parasite examination

Using 5 mL ethylenediaminetetraacetic acid (EDTA)-impregnated vacutainer tubes, 3–5 mL of blood were

collected from the jugular vein of cattle. Blood smears were immediately prepared and the rest of the blood samples were stored at  $-40^{\circ}\text{C}$  until required for molecular processing.

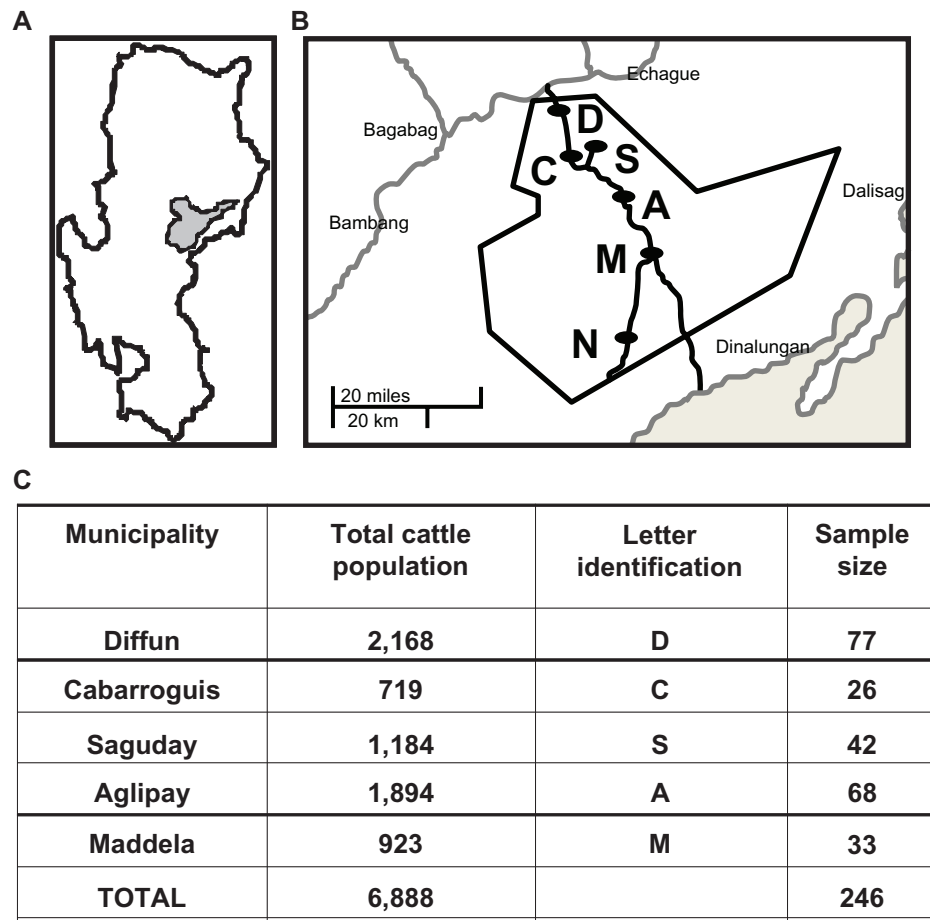
The smear samples were stained with Giemsa<sup>®</sup> (Medic Diagnostic Reagents, Quezon City, Philippines) and the whole slide examined following the method of Pritchard and Kruse<sup>19</sup> using a microscope (Olympus, Lehigh Valley, NY) at high power (40 $\times$ ) and oil immersion (100 $\times$ ) objectives. Slide examination was replicated three times.

## Results and discussion

Two reported species of trypanosomes are known to infect livestock in the Philippines. This study focused on *T. theileri*, which is the lesser studied species. Despite the scarcity of information pertaining to *T. theileri* infection in the country, reports of its opportunistic potential to cause morbidity and its importance in disease diagnosis against surra, warranted further investigation of the parasite.

The use of detection systems based on trypanosomal genomic DNA has revolutionized the diagnosis of trypanosomes. Polymerase chain reaction (PCR) has been proven to be more sensitive than BPE in detecting trypanosome genomic DNA in the animal or vector host.<sup>20</sup> According to Masiga et al<sup>21</sup> and Solano et al,<sup>20</sup> PCR is sensitive enough to be able to detect even one trypanosome genomic DNA per mL of blood. Furthermore, DNA-based methods for diagnosis have generally facilitated epidemiological studies of trypanosomes.<sup>22</sup> However, in the Philippines, the use of PCR technology is currently limited to academic and specialized government institutions. The possibility of field applications is currently being hampered by economic issues and questions, especially pertaining to its suitability as a diagnostic test in the field or countryside.

The predominant diagnostic tool for field diagnosis of trypanosomes in most municipalities in the country is still microscopic examination or BPE. It is probably the most economical and easiest technique for detection of trypanosomes. Similarly, in other countries, the diagnosis of trypanosomosis in mammalian hosts essentially relies on visualization of the parasites in the blood smears.<sup>23,24</sup> Results of the current study showed that all Giemsa-stained blood smears were negative for *T. theileri* blood stream forms. These findings align with previous data stating that although *T. theileri* are cosmopolitan blood parasites of cattle, they are rarely observed in direct blood smears.<sup>25</sup> Moreover, according to Nantulya,<sup>1</sup> standard techniques such as BPE are not sufficiently sensitive since they offer



**Figure 1** Randomly selected animals and sampling sites in Quirino Province, Philippines. **A)** Map of the northern part of the Philippines-Luzon. The gray area represents the location of Quirino Province. **B)** Map of Quirino Province. Black dots represent the sampled (D, Diffun; C, Cabarroguis; S, Saguday; A, Aglipay; and M, Maddela) and nonsampled (N, Nagtipunan) municipalities. **C)** Total population and sample size of cattle.

only 50% probability of positively identifying the parasite during microscopic examination of the blood smears. In addition, parasitological examinations are limited during chronic or subclinical cases wherein there is characteristically very low parasitemia.<sup>1,2</sup>

In summation, based on the corroborating results of repeated blood parasite examination of the samples, the study suggests that the negative results are probably attributable to very low parasitemia.

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## Disclosure

The authors report no conflicts of interest in this work.

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