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NOTE

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Molecular detection and internal transcribed spacer-1 sequence diversity of *Trypanosoma evansi* in goats from Cavite, Philippines

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ABSTRACT. Goat production is an important source of livelihood and food. Goats may serve as reservoir of surra affecting livestock production. Here, forty-two free-roaming goats from Cavite, Philippines were screened using two primer sets, *Trypanosoma brucei* minisatellite chromosome for initial detection and the internal transcribed spacer 1 (ITS-1) to determine phylogeny. Initial PCR detection showed that 19/42 (45%) goats were positive, much higher than the rate previously reported in goats from Cebu (34%). The infectivity rate was higher in male (56%) than in female (42%) and the rate was higher in young ≤1 year old (100%) than in adult >1 year old (43%). Phylogenetic analysis of the ITS-1 sequences between *T. evansi* goat samples and other isolates indicate potential interspecies transmission.

KEYWORDS: Cavite, goat, internal transcribed spacer-1, PCR, *Trypanosoma evansi*

Surra, caused by *Trypanosoma evansi* is endemic in Central and South America, Northern Africa and Southeast Asia including the Philippines [17]. The infection causes anemia, lethargy and wasting that result in decreased production and impair performance in the animal which can lead to severe economic losses. This hemoflagellate parasite can infect a wide range of animal species including horses, cattle and goats [3, 9, 14]. In the Philippines, goat is considered to be an important livestock animal, valued at $210.7 million nationally and contributes to about 3.4% of the total livestock industry of the country. While annual economic losses attributed to trypanosomosis amounts to $770,000 [13].

Surra is mainly transmitted by tabanid flies. These flies are intermittent feeders and can contribute to the efficient transmission of surra across different animal species [2, 14]. Although goats are generally considered trypanotolerant, their role and function as reservoir of infection has not been clearly substantiated especially in the Philippines [4, 5, 8].

Blood samples from 42 free-roaming goats were collected from Naic, Cavite, Philippines. Briefly, 2 mL of blood was collected via venipuncture of the jugular vein. The blood was placed in a 3 mL tube containing ethylenediaminetetraacetic acid (EDTA) as the anticoagulant (Surgitech, Quezon city, Philippines). Sample collection was conducted in accordance with Philippines' law on animal welfare and by virtue of permit No. 2019-001 issued by the Institutional Animal Care and Use Committee (IACUC) of Cavite State University. Total genomic DNA from the samples were extracted using QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) following manufacturers protocol. All DNA samples were stored in −30°C freezer prior to PCR.

Two rounds of PCR were done in this study, The first PCR amplified the *Trypanosoma brucei* minisatellite chromosome repeat sequence a ~164 bp repeat of minisatellite chromosome and the internal transcribed spacer 1 (ITS-1) region approximately ~480 bp in size. These primers have previously been used successfully for the molecular detection and characterization of *T. evansi* [5, 11, 16, 19]. The primers used for the first PCR (TBR1 and 2) were as follows; forward (5′-CGA ATG AAT ATT AAA CAA TGC GCA G-3′) and reverse (5′-AGA ACC ATT TAT TAG CTT TGT TGC-3′), respectively [10]. The optimized PCR conditions used for the amplification of this gene was done as previously described [18]. Positive samples from the initial PCR were then subjected to the

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Table 1. Prevalence of *Trypanosoma evansi* in goats in Cavite, Philippines based on PCR with *Trypanosoma brucei* minisatellite chromosome

Variable	Total (n)	Detection Rate (%)	Positives (n)	*P-value*
Sex				
Male	9	56	5	0.71
Female	33	42	14	
Age group				
≤1 year	2	100	2	0.20
>1 year	40	43	17	

second PCR for amplifying ITS-1 sequence. The primers used were as follows; forward (5′-CCG GAA GTT CAC CGA TAT TG-3′) and reverse (5′-TTG CTG CGT TCT TCA ACG AA-3′), respectively [12].

The second PCR amplifying the ITS-1 region were cloned into TOPO vector (Invitrogen, Carlsbad, CA, USA) and sequenced using BigDye™ Terminator V3.1 Sequencing Kit (Thermo Fisher Scientific, Tokyo, Japan) with ABI Prism 3130 sequencing machine as previously described [8]. The M13 forward (5′-CTG GCC GTC GTT TTA C-3′) and reverse (5′-CAG GAA ACA GCT ATG AC-3′) primers were used to assemble the forward and reverse sequences of the insert. The ITS-1 sequence showing the highest identity with the sequence obtained from *T. evansi* in goats was retrieved from National Center for Biotechnology Information (NCBI) following a Basic Local Alignment Search Tool (BLAST). In addition, identities between ITS-1 sequences from *T. evansi* in goat samples were determined by generating an identity matrix using Genetyx software (Genetyx Corp., Tokyo, Japan). A phylogenetic tree was assembled using MEGA X software [7]. Statistical analysis using Fisher's exact test to compare the differences between the infection rate found in males and females were done using GraphPad QuickCalcs (Dotmatics, San Diego, CA, USA).

This study, showed that 19 out of the 42 (45%) goats were PCR-positive in the first PCR for TBR1 and TBR2 sequence as shown in Table 1. This rate was much higher than the prevalence (34%) reported in a recent study for goats in Cebu, Philippines [5]. The result indicates high prevalence of *T. evansi* infection in goats in Cavite since almost half of the samples were PCR positive. Therefore, it is suggestive that increased infectivity rate in goats, that are generally considered trypanotolerant [11], may perpetuate the parasite infection. Action toward control of the infection in goats must be taken in order to prevent cross infection between goats and other animals [6]. We likewise observed a higher detection rate in males at 56% than in females 42% although the difference is not statistically significant. It may be attributed to the small sample size used in this study. The infection rate between goats ≤1 year old was higher as compared to goats >1 year old (Table 1). Similar to male goats, the sample size of young goats is few such that it may not be able to capture the actual prevalence rate in the population [15].

In addition, phylogenetic analysis revealed that the ITS-1 sequences were highly identical to the sequences from the parasite originating from a wide range of hosts including camel, water buffalo and horses as seen in Fig. 1. This further suggests that a potential active transmission between domestic animals is a common occurrence contributing to the genetic diversity of the parasite [1, 4, 8, 17, 18]. Further, complete ITS-1 sequences from 6 selected samples namely: S2, S6, S7, S9, S10 and S14 were analyzed from the total of 14 positive samples. The sequences obtained in this study were closely related to those previously described from different geographic locations in the Philippines [5, 19]. However, in contrast to the previous findings [5, 19], we found in this study that the ITS-1 sequences of the parasite obtained from goat samples were highly variable as seen in Fig. 1. It is possible that several genotypes may exist in a single host as these animals may be infected several times throughout their lifetime [4]. The sequence obtained from goat S6 and goat S2 samples showed the highest identity of 99.8% while those obtained from goat S14 and goat S9 samples showed the lowest identity of 33.8%. These results suggested a diversity in ITS-1 sequence of *T. evansi* in the goat population examined in this study. However, it should be noted that the ITS-1 sequence is not related to parasite virulence.

Considering the high prevalence rate of surra observed in goats in this study, and its proximity to equine, cattle and buffalo farms, it is highly likely that the transmission of this disease may be perpetuated by goats potentially serving as reservoir host as previously observed [4]. As the goat industry contributes greatly to the economy and food security of the Philippines, further studies are needed to determine the extent and impact of surra on goats [4, 14]. Likewise, additional studies on the infection dynamics between goats enrolling more samples and other domestic animals should be done in order to clarify parasite transmission. Therefore, careful consideration, revisit and redesign of control measures against surra in the Philippines incorporating goats as important livestock animals may help in the control of surra.

CONFLICT OF INTEREST. The authors declare no conflicts of interest in this study.

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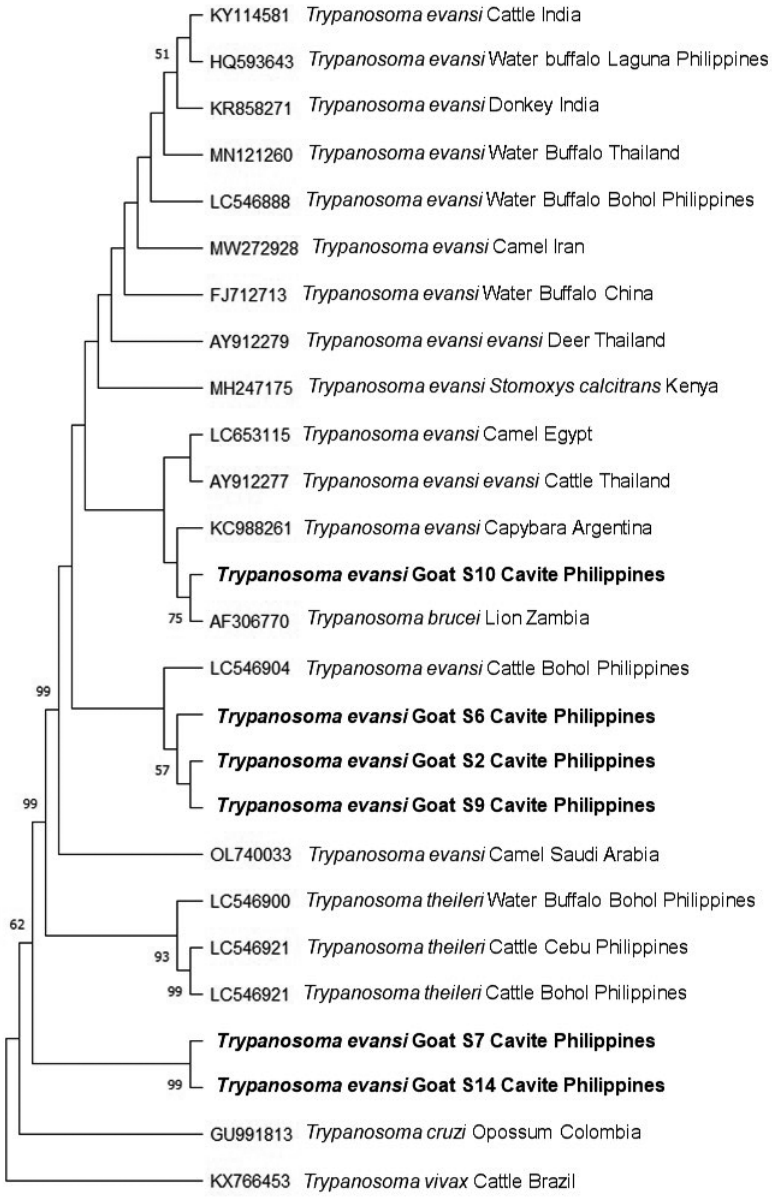

Fig. 1. Phylogenetic analysis of *Trypanosoma evansi* based on the internal transcribed spacer-1 sequences obtained from goat samples in this study indicate similarities with parasite isolated from a wide range of animal hosts and in different geographic locations. This further supports potential interspecies transmission of surra. The maximum likelihood tree was constructed using the Tamura-3 model. The phylogeny test was run using the bootstrap analysis with 1,000 iterations. The sequences obtained from the current study are shown in bold. *T. vivax* was designated the outgroup.

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