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## Molecular detection of *Rhipicephalus (Boophilus)* sp recovered from wild goat in Mizoram, India

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### Abstract

The aim of the present study is to describe morphological structures by light microscopy (LM) and molecular confirmation of *Rhipicephalus (Boophilus)* spp which were recovered from wild goat. Light microscopic study revealed both male and female ticks with hexagonal basis capituli, shorter mouthparts and posterior border without festoons and other similar *Rhipicephalus (Boophilus)* morphological features. Polymerase chain reaction (PCR) selectively amplified a fragment length of about 400bp. It seems that this is the first report of *Rhipicephalus (Boophilus)* sp from wild goats in India.

**Keywords:** *Rhipicephalus (Boophilus)*; Wild goat; LM study; PCR; Mizoram; India

### 1. Introduction

Various species of ticks are often found in the wild animals including wild goats but the intensity of infestation and the effects of ticks on their hosts are poorly studied [1]. Studies concerning ticks were of considerable importance to evaluate the distribution and composition of species affecting animals [2]. The comparative lists of ixodid ticks of different host are described by several authors [3-10]. Hoogstral *et al* [11-12] reported 17 species of ixodid and argasid ticks from wild sheep (*Ovis orientalis*) and wild goats (*Capra hircus aegagrus*) in Iran. Rahbari and Nabian [13] described *Rhipicephalus (Boophilus) kholsi* from Iran.

Although the history of the dissemination of *Rhipicephalus (Boophilus)* sp is not recognized, the species is known to have originated from India [14]. It is claimed that species *Rhipicephalus (Boophilus)* originated in the southern and south-eastern regions of Asia and subsequently spread to other countries across globe [15]. Taxonomy of ticks is based on morphology and molecular techniques. Mitochondrial and ribosomal RNA transcribed spacer sequence 2 are increasingly being used for molecular identification of species.

The objective of this study is to describe *Rhipicephalus (Boophilus)* sp which were recovered from wild goats on the basis of morphology and molecular techniques.

### 2. Materials and Methods

#### 2.1 Collection of ticks

A wild goat (*Capra hircus aegagrus*) was killed by locals near Aizawl forest on 12<sup>th</sup> June, 2017. The outer surface of the animal was examined. On examination, 4 (three) ticks were recovered and those were brought to the Department of Parasitology, college of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram, India. They were washed thoroughly with distilled water and then transferred to 70% alcohol for further identification on the basis of key provided by Hoogstral *et al* (1981) [16].

#### 2.2 Morphological study

When seen under stereo zoom microscope (Euromex, Holland) all three ticks were found females and one male. One male and one female tick s were processed and permanent slide were made after proper staining. Male ticks are distinguished on the basis of smaller in size, extended scutum on the whole dorsal surface of the body and presence of ventral plates on the ventral surface of the body. Preliminary morphological study revealed all four ticks belong to the genus *Rhipicephalus (Boophilus)*. Further speciation was done on the basis of keys provided by previous workers [16]. PCR was done after extracting DNA from whole tick.

### 2.3 DNA extraction

DNA was extracted from one female tick by using a commercial kit (DNeasy® Blood and Tissue kit, Qiagen) following manufacturer's protocol with slight modification. Briefly, one female tick was triturated with buffer and Proteinase K was added to the mixture and incubated at 56°C for complete lysis. After adding ethanol (96-100%) the mixture was transferred into a spin column and centrifuged. After washing with buffer twice, the elution buffer was added to the column membrane and incubated for 2 min at room temperature. Finally the column was centrifuged at filtrate was collected and kept in -20°C.

Polymerase chain reaction (PCR) was performed following the standard time-temperature protocol using the oligonucleotide primers, F – 5' AAA CTA GGA TTA GAT ACC CT 3' and R – 5' AAT GAG AGC GAC GGG CGA TGT 3' to amplify the mitochondrial 16S rRNA gene fragment.

### 3. Results

#### Male

Males were found to be much smaller than female (Figure 1). Hypostome is toothed with dental formula of 4/4 (Figure 2). Basis capituli is hexagonal dorsally. The whole dorsal surface is covered with scutum. Coxa I bears a median external spur and a short internal spur. The ventral surface is with accessory adanal ventral plates and the posterior part posses an appendage without any festoons (Figure 3).

#### Female

Both dorsal and ventral surface of the female tick was examined microscopically. The position of basis capitulum is sub-dorsal and the posterior border is found devoid of any festoons. Left palp showed two teeth like protuberances and the right one showed one similar structure (Fig. 4). Each last segment of the tarsus showed a pair of crab like claw (Fig. 5). The dorsal surface of basis capitulum bears two porose areas which are pyriform in shape. The scutum covers one third of the total body surface.

An approximately 400bp of the ITS2 region of 16s mitochondrial rRNA of *Rhipicephalus (Boophilus)* was amplified from the collected tick for molecular confirmation. Genomic DNA was amplified and the amplification of specific PCR product was checked by gel electrophoresis with 1.5 % agarose gel and viewed in UV trans illuminator system (Fig. 6).

### 4. Discussion

Basically, morphological features of *Rhipicephalus (Boophilus)* spp. from closely related ticks have been considered for identification and differentiation. However, it is difficult and sometimes requires expertise to identify correctly especially for some developmental stages of ticks such as larvae and nymphs. Hence, molecular markers may be a suitable alternative.

Wild sheep and goats occur throughout in Indian forests but goats prefer rocky, precipitous terrain. *Rhipicephalus (Boophilus)* spp parasitize domestic and wild animals in tropical and sub-tropical regions of the world. Ticks transmit a variety of microorganisms like bacteria, protozoa, viruses and even helminths than any other arthropod vectors. Hence, identification and characterization of ticks from a particular region is important as tick and tick borne diseases are responsible for significant loss of production. In this study, two teeth like articles on the left palps and one on the right

palp of *Rhipicephalus (Boophilus)* spp were observed under LM. These structures were not recorded in *Rhipicephalus (Boophilus)* spp by previous worker [13]. Another distinguishing morphological feature that was found under microscope was that beach leg bears a pair of crab-like claw at the last terminal segment. For systematic classification, all morphological features of parasites must be thoroughly studied under the light microscope. Some distinguishing morphological characters of tick includes mouth part structure, shape of basis capitulum, whether palpi and hypostomes are long or short, whether they are ornate or inornate and the presence or absence of festoons in the posterior region of the body. In the present investigation, the recovered tick from wild goat falls under the genus *Rhipicephalus (Boophilus)* on the basis of keys provided by Hoogstral *et al* (1981) [16].

Brahma *et al* (2014) [17] developed polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) to identify *Haemaphysalis bispinosa* and *Rh. (Boophilus) annulatus*. Ronaghi *et al* (2015) [18] molecularly characterized *Rhipicephalus (Boophilus) annulatus* using the ITS2 region of mitochondrial rRNA. In this study, the reliability of the morphological characters observed under light microscope was further reinforced by selectively amplifying 400bp by means of PCR. The positive PCR confirmed that the species belongs to the genus *Rhipicephalus (Boophilus)* group. However, it is worth mentioning that morphology and molecular confirmation are not sufficient enough to ascertain the correct species. The speciation requires complete sequencing of full length of the targeted ITS region.



Fig 1: Male *Rhipicephalus (Boophilus)* spp.

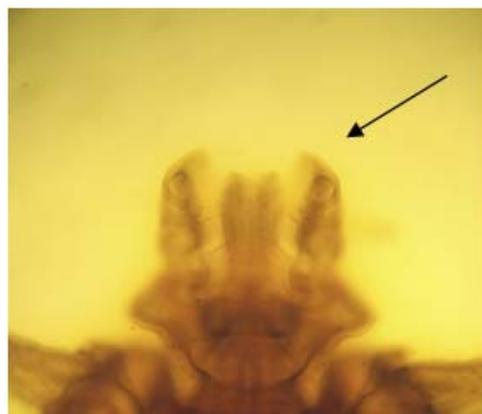


Fig 2: Mouth part structures of male *Rhipicephalus (Boophilus)* spp.



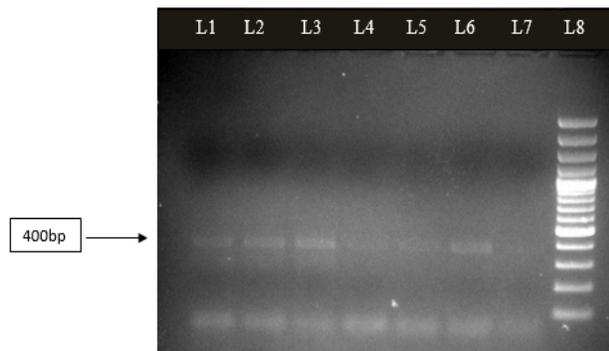
**Fig 3:** Ventral surface of Male *Rhipicephalus (Boophilus)* spp.



**Fig 4:** Anterior part female *Rhipicephalus (Boophilus)* spp.



**Fig 5:** Leg part of female *Rhipicephalus (Boophilus)* spp.



**Fig 6:** Conventional PCR selectively amplified 400bp DNA fragment L1 – L6 : showing positive amplification L7 : Negative control L8 : 100 bp DNA ladder

### Conclusion

This is the first report of *Rhipicephalus (Boophilus)* spp. infesting wild goats in Mizoram, India. However, further studies should be carried out to evaluate the ecological relationship of this tick involving domestic and wild ruminants in this area.

### Conflict of interest statement

We declare that we have no conflict of interest.

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