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Molecular identification of Ixodid ticks of mithun from Nagaland

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Ticks cause huge economic losses as a result of injury, tick pyaemia and tick paralysis, besides transmitting various pathogens including bacteria, virus and protozoan parasites to the host animals. Prevalence of several tick species from domestic ruminants has been reported by different workers (Saravanan *et al*. 2008, Ronghang and Roy 2014, Patel *et al*. 2013 and Patel *et al*. 2014) but very limited reports are available on the prevalence of ticks on the mithun (Rajkhowa *et al*. 2005, Chamuah *et al.* 2012, Chamuah *et al.* 2015). During a study in two villages of Kohima district of Nagaland, 2 ixodid tick species were collected from the mithun. Identification of the ticks was based on the morphological features but the species were identified using internal transcribed spacer region-2 as the molecular probe.

Live specimens of gross ticks were collected from the dewlap and inner aspects of thighs of the mithun from Jotsoma and Khonoma villages with the help of thumb forceps in plastic vials containing 70% alcohol and thereafter processed in the laboratory by standard procedures using 10% potassium hydroxide solution (HMSO, 1979; Soulsby, 1986). Based on the available morphological keys like anal grooves anteriorly, long palpi and absence of festoons, these were identified as Ixodes ticks. For the accurate identification of the ticks to the species level, internal transcribed spacer-2 region of some of the tick specimens was PCR amplified using the following primers 5'-CTGCGAGACTTGGTGTGAAT–3' and 5'-TATGCTTAAGTTCAGCGGGT–3' flanking 5.8S and 28S rRNA genes (Poucher *et al*. 1999). Genomic DNA of the 6 tick specimens was isolated with a commercial genomic DNA isolation kit and was used as a template for PCR amplification of the ITS-2 region of these ticks. The PCR master mix (25 µl) was composed of 14µl nuclease free water, 4 µl of dream Taq buffer, 2 µl of 10mM dNTP, 1 µl of 25Mm $MgCl_2$, 1 µl of each forward and reverse primer (10pm), Taq DNA 0.4 µl and 1.6 µl genomic DNA.

The PCR conditions followed for the amplification of the ITS-2 were initial denaturation at 95°C for 5 min; 35 cycles at 95°C for 1 min, 60°C for 1 min, 72°C for 2 min and one cycle at 72°C for 5 min. The PCR products were purified using gel extraction kit and custom sequenced at University of Delhi. The sequence analysis and similarity searches were performed with the basic local alignment search tool (BLAST), NCBI. Specific identification of the ticks was made by sequence comparison with available sequences of the corresponding species in the GenBank database.

ITS-2 region of the ticks collected from Khonoma village on PCR amplification resulted in a PCR product of 900 bp whereas *Ixodes* ticks collected from Jotsoma village amplified a product of 1000 bp due to species difference. Sequence analysis of the ITS-2 of the *Ixodes* tick from Khonoma region showed 100% similarity with *Ixodes ovatus* (acession no. AB280550) whereas ticks from Jotsoma region showed 95% similarity with *I. acutitarsus* (accession no. AB105168). Therefore, ticks collected from Khonoma village were assigned to *I. ovatus* and those from the Jotsoma village to *I. acutitarsus*. Based on the morphological features of each tick specimen and subsequent sequence analysis, it was thus confirmed that both *I. ovatus* and *I. acutitarsus* were infesting the mithun in these areas.

Ticks and tick borne diseases are a major constraint for improved production of the animals. Moreover, infestation of different ticks has been reviewed by different authors from time to time. In India, *I. acutitarsus* was reported from domestic cattle and yak in Arunachal Pradesh, Asom and Sikkim (Ghosh *et al*. 2007, Sarvanan *et al*. 2008 and Chamuah *et al.* 2015). *I. acutitarsus* was reported from the mithun of Arunachal Pradesh by Ronghang and Roy (2014) for the first time based on the morphological features of

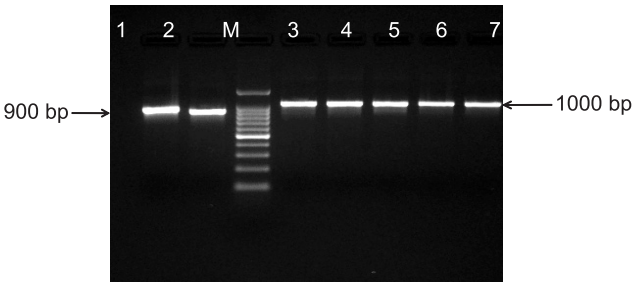

Fig. 1. PCR amplification of ITS-2 region of *Ixodes ovatus* (lanes 1,2) and *I. acutitarsus* (lanes 3–7). Lane M 100 bp DNA ladder marker.

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these ticks. Human biting activity of *I. acutitarsus* was also recorded in Taiwan (Chao and Shih 2012). The occurrence of *I .ovatus* in Japan was reported by Megumi *et al.*(2004). In India, occurrence of Ixodid ticks and seasonal prevalence in cattle and buffalo was also reported from Mathura district of Uttar Pradesh (Patel *et al*. 2013, Patel *et al.* 2014). However, the present communication is the first report of *I. ovatus* from the mithun of Nagaland. Based on PCR-RFLP of the internal transcribed spacer-2 17 species of *Ixodes* were identified in the United States of America (Poucher *et al*. 1999). The species validity was also proved for *Boophilus annulatus*, *Hyalomma dromedarii* and *H. anatolicum* by PCR-RFLP of the ITS-2 (Kawther *et al.* 2005). The present finding is the first report on *I. ovatus* infestation on mithun, along with the infestation of *I. acuitarsus* being recorded by ITS-2 sequencing.

SUMMARY

An animal health check-up and treatment camp during the year 2015 for the free ranging mithun (*Bos frontalis*) organized at Khonoma and Jotsoma villages of Kohima district, Nagaland showed Ixodid tick infestation on these animals, with the ticks attached to the dewlap and inner aspects of the thighs. Based on their morphological features, the ticks were identified to belong to the genus *Ixodes*. However, identification of these ticks to *Ixodes ovatus* and *I. acutitarsus* was made by the sequence analysis of the internal transcribed spacer-2 region of these ticks. The present study showed both *I. ovatus* and *I. acutitatarsus* are prevalent on the mithun in this area and is the first report on the occurrence of *I. ovatus* on the mithun

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