

# **Molecular epidemiology of tick-borne bovine blood protozoa deciphering the emerging and new variants in Bangladesh**

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## **Objective**

Tick-borne blood protozoan parasitic diseases have significant impacts on livestock and dairy production. The global warming and geo-climatic condition of Bangladesh is very conducive to a wide variety of ticks which increases the incidence of the diseases. The present study was aimed to determine the seroprevalence of *Anaplasma* infection and molecular epidemiology of major tick-borne blood protozoan parasites of cattle from selected districts.

## **Materials and Methods**

A total of 384 blood and sera samples were randomly collected from different commercial cattle farms as well as small stakeholder farms of producer groups (PG) of Livestock and Dairy Development project (LDDP) of the selected regions of Bangladesh. All sera samples (384) were analyzed using a commercially available competitive enzyme linked immunosorbent assay (cELISA) kit. For molecular epidemiology, after DNA extraction of the blood samples, initially multiplex PCR was performed for the presence of the infections. Then PCR was done by specific genes of the parasites and subsequently sequenced for bioinformatics and phylogenetic analysis.

## **Results**

The cELISA tests reveals high seroprevalence of *Anaplasma* species in dairy cattle (46.39%) followed by calves (41.08%) and breeding bulls (40.0%). Significantly ( $p < 0.05$ ) high seroprevalence (52.76%) was found in cattle (aged >1 year) than cattle (aged <1 year) (32.97%); females (47.87%) than the males (30.39%) and crossbreed cattle (46.05%) than indigenous cattle breed (34.04%), respectively. The study reveals that calves are also susceptible to *Anaplasma* infections due to transplacental transmission. The multivariable logistic regression analysis identified the age (>1 year), sex (female) and breed (crossbreed) of cattle as potential risk factors for bovine anaplasmosis. The molecular prevalence of *Theileria*, *Anaplasma* and *Babesia* species revealed 40.35%, 17.5% and 0.35% respectively. The highest prevalence of The co-infections of *Anaplasma* and *Theileria* species (8.92%) and *Babesia* and *Theileria* species (0.35%) have been found. The sequenced analysis revealed, *A. marginale* (44.5%) followed by *A. bovis* (33.3%), *A. centrale* (11.1%) and *Candidatus A. cinensis* (11.1%) and *T. orientalis* (40.35%) indicate an emerging bovine anaplasmosis and theileriosis in Bangladesh. Further bioinformatics analysis is

going on and interestingly, the combined phylogenetic analysis (ML) of 16S rRNA and *groEL* sequences demonstrated that *Anaplasma* sp. (Mymensingh proposed) is closely related to *A. platys* and 18S rRNA sequences revealed that *Babesia* sp. (Mymensingh proposed) is closely related to *B. bigemina* and *Theileria orientalis* BR-BDH3 clustered together with *T. orientalis* type buffeli from Australia and *Theileria buffeli* from China, respectively.

## **Conclusions**

Genetic characterization and whole genome sequencing of *T. orientalis* and unknown *Anaplasma* sp (Mymensingh) and *Babesia* (Mymensingh) will help to deciphering the genetic variants and new species relevant to virulence as well as vaccine or new drug development.

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