

**Determination of the Prevalence of Babesia DNA in the Blood Samples of Goat from Aurangabad by PCR.**

**SECTION: ANIMAL, VETERINARY AND FISHERY SCIENCES.**

**TITLE: DETERMINATION OF BABESIA DNA IN BLOOD SAMPLES OF GOAT IN AURANGABAD BY PCR.**

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**ABSTRACT:**

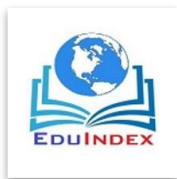
Babesiosis is a zoonotic disease caused by Babesia species. Babesia species are protozoan parasites that parasitize the red blood cells of domestic animals and humans causing anemia in the host. The purpose of current study was to determine the presence of Babesia DNA in the blood samples obtained from goats from Aurangabad, Maharashtra, India. Blood samples were collected from 100 Goats from randomly selected herds located in the main livestock production regions of Aurangabad. PCR was used to detect babesia DNA in the blood samples whereby an amplified band size of 428 bp was considered positive for Babesia. The results indicate that 2% of the blood samples were positive for Babesia species.

**Keywords: Babesia spp, Goat, PCR, Zoonotic.**

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**INTRODUCTION:**

Caprine babesiosis is a tick borne haemoprotozoan disease causing significant production loss among Goat herds (Alessandra and Santo 2012). Babesiosis is a highly prevalent tick borne



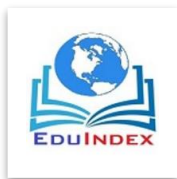
disease affecting a wide range of host species worldwide especially ruminants of tropical and subtropical countries where an abundant tick population is present (Demessie and Derso, 2015). It can cause severe damage to the health and production of domestic animals; hence has huge potential impacts on the economics of livestock farming (Kivaria, 2006). Tick infestation and the resulting transmission of serious pathogens in ruminants is one of the most important problems of the livestock industry in developing countries. (Ghirbi et al., 2008; Adham et al., 2009; Atkas et al., 2012).

The causative agents of Babesiosis are intra-erythrocytic protozoan parasites from the phylum Apicomplexa and the order Piropasmida. There are two major species involved in caprine babesiosis: *Babesia motasi*, the large piriform shaped organisms found in pairs at acute angle inside erythrocytes causing severe haemolytic anaemia and fever and *Babesia ovis*, the small round shaped form usually found towards the margin of erythrocytes causing mild signs of anaemia, jaundice and haemoglobinuria (Taylor, Coop & Wall, 2007).

In India, the most pathogenic species causing severe forms of the disease in Goats is *motasi* (Friedhoff, 1997). The major vectors involved in the transmission of *B. motasi* and *B. ovis* are *haemophysalis* species and *Rhicephalus bursa* respectively (Fakhar et. al., 2012). Clinically, Babesiosis occur as variable forms ranging from mild to severe in relation with the species of babesia involved, age and immune status of the animal (Zintl, Gray, Skerreltt & Muleahu 2005).

Carrier hosts infected with babesia are difficult to detect due to the low number of parasites that occur in peripheral blood. However the diagnosis of low level infections by the parasite is important for control and epidemiological studies (Fahrimal et al 1992).

Goats infected with babesia showed clinical signs including in appetite, weakness, coughing, nasal and ocular discharges, diarrhea, pyrexia, abortion, anaemia, thrombocytopenia and leucocytosis with neutrophilia (Muthuramalingam et al., 2014). Severe cases of caprine babesiosis were associated with jaundice, diarrhea, haemoglobinuria, microcytic hypochromic anaemia, hypoproteinemia and increased erythrocyte sedimentation rate (ESR), AST activity, ALT activity, BUN and Icterus index (Sulaiman, Arslan, Al-obaidi & Daham, 2010). Babesiosis due to *B. ovis* infection is usually associated with a mild chronic form of disease accompanied by jaundice and haemoglobinuria (Taylor et al; 2007). Experimental infection of sheep by transfusing *B. ovis* infected blood resulted in development of mild to moderate haemolytic anaemia and jaundice (Rahbari, Nabian, Khaki, Alidadi & Ashratihelan, 2008)



In India, the overall prevalence of tick population is highest (83.74 %) during monsoon season and is highly associated with the incidence of babesiosis (Nagar, Raizada, & Saxena 1977; Singh & Rath 2013). The pathology of babesiosis is related to the haemolytic anaemia as a consequences of the intravascular destruction of erythrocytes either by the immune system or by the direct damage caused by the parasite itself and with associated symptoms (Furlanello, Fiorio Caldin, Labes & Solana- Gallego, 2005)

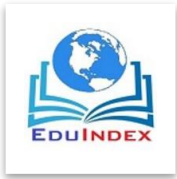
In 1957, the demonstrated case of human babesiosis in the world was reported in a Yugoslavian farmer (Skrabalo and Deavoni, 1957). In india the first case of human babesiosis was reported by Maratha et al; from Baroda (Gujarat) in 2005. Peripheral smears of babesiosis can be easily confused with plasmodium species so careful examination of peripheral smears of babesiosis can be easily confused with plasmodium smears and surveillance studies may be necessary to know the actual prevalence of human babesiosis in India.

Serological methods are frequently employed in determining subclinical infections. However, serology for detecting carrier state lack specificity and sensitivity, especially for infection status (Passos et al., 1995). Furthermore, false positive and negative results are commonly observed in these tests. Therefore, DNA amplification methods which are more sensitive and specific than other conventional method may facilitate and are a powerful tool for the diagnosis of babesiosis and theileriosis (Aktas et al, 2005; Altay et al 2005; Nagore et al 2004; Schnittger et al 2004)

The purpose of current study was to determine the presence of Babesia DNA in the blood samples of Goat from Aurangabad to establish a reliable and sensitive molecular tool (PCR) for the detection of Babesia DNA for rapid, accurate and early diagnosis of Babesiosis. To overcome the economic losses early and proper diagnosis of babesiosis is important in livestock which could be achieved only through highly sensitive techniques like PCR. The present work was aimed at standardizing the molecular diagnostic PCR technique for the early and accurate diagnosis of babesiosis in ruminant; livestock region of Aurangabad.

## **MATERIALS AND METHODS:**

Animals were randomly selected from herds located in different regions of Aurangabad during Oct to March 2017. Blood was collected from jugular vein and immediately preserved by adding EDTA. Samples were sent to the research lab , Bioera Pune in cool icebox . Samples stored at - 20°C for further use. DNA was extracted from samples using DNA extraction kit according to



the manufacturers protocol. The concentration of the DNA was measured at 260 nm. Optical density according to the method described by Sambrook & Russell 2001). The extracted DNA of blood sample was kept frozen at -20°C until its use.

PCR was performed on the genomic DNA of babesia species to amplify a 428bp small 18s rRNA gene fragment using the following set of primers: Bab-sp-F: 5'-GTTTCTGCCCCATCAGCTTGAC-3' AND Bab-sp-R: 5'CAAGACAAAAGTCTGCTTGAAAC-3' (Hilpert shauser et al 2006). The amplification of DNA was done using thermocycler (Eppendorf, Hamburg, Germany). PCR reaction of 45 cycles carried out as follows: Denaturation of 94°C for 30S, annealing at 61°C for 30S, extension at 72°C for 45S and lastly incubation at 72°C for 10 min. The amplicons were stained with ethidium bromide and electrophoresed in 1.5% agarose gel at 80V for 30 min. The PCR products were compared against a 100 bp DNA marker.

In present study, PCR was used to detect the presence of Babesia DNA in blood samples of 100 Goats. The samples were considered positive if a 428bp small 18s rRNA gene fragment of Babesia species was amplified during PCR. The Babesia DNA was present in Goats.

## **OBSERVATION:**

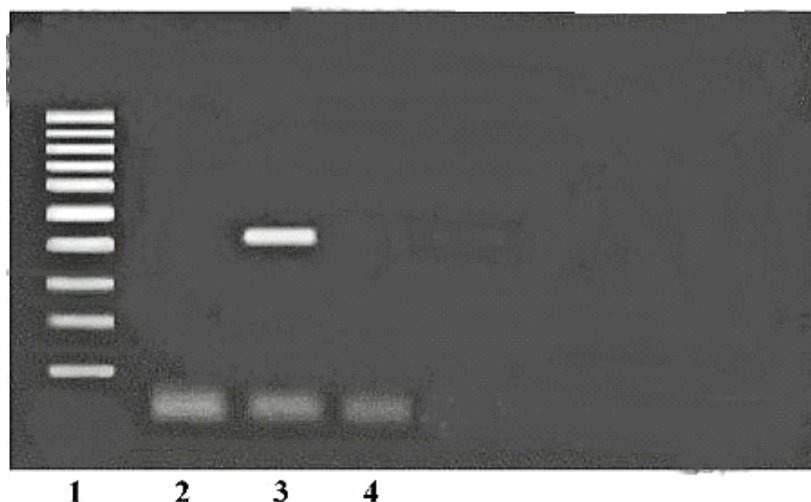
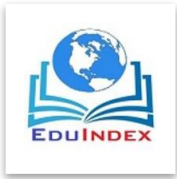


Fig.1 Ethidium bromide stained 1.5% Aagrose gel electrophoresis of PCR products. Bab sp-F and Bab sp-R were used to amplify a 428 bp small 18s tRNA gene fragment of Babesia spp. Lane 1 containing 100 bp DNA marker, Lane 2 & 4 negative control. Lane 3 contained PCR products from Goat, which is positive for Babesia DNA

***Distinction of Babesia spp infection in Goats from Aurangabad***

<i>Examined Subject</i>	<i>No of Animals</i>	<i>Babesia spp</i>
<i>Goats</i>	<i>100 (100%)</i>	<i>2 (2%)</i>

**RESULTS:** In this study, PCR was used to detect the presence of Babesia DNA in the blood samples of 100 Goats from the livestock regions of Aurangabad, Maharashtra. The tested samples were considered positive if a 428bp small 18s, tRNA gene fragment of Babesia species was amplified using PCR. The Babesia DNA was present in 2% of the Goat population.



Prevalence of babesiosis was noticed in well-defined breeds (Sirohi, Barbari and Jamnapari ) followed by non-descript goats.

**DISCUSSION:** The economic losses due to babesiosis in sheep and goat production are significant in tropical and subtropical areas (Mehlhorn & Schein, 1984; Bai et al.,2002) and remain an important impediment to meat and milk production because infected animals exhibit high parasitemia and mortality (Caracappa, 1999).

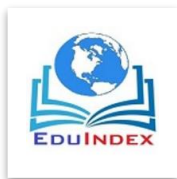
The aim of the present study was to optimize a specific, reliable and sensitive molecular tool, the polymerase chain reaction (PCR), for the detection of Babesiosis sp. In blood of small ruminants as parasite cannot easily be diagnosed by examination of stained blood film and negative microscopic examination does not exclude the possibility of infection.

The current study indicates that the Babesia species were present in the study area. This type of work is carried out mostly in sheep where the infection rate is much higher than in Goats. In small ruminants disease caused by protozoan (Theileria and Babesia) have been reported to cause high economic losses worldwide (Shayan et al, 2008) . chaudhary et al. (2010) reported an overall prevalence of 29% for Babesia species using PCR where 11% were positive for *B. ovis* and 18% for *B. bigemina* (Calder et al 1996) reported the average sensitivities of three PCR based tests for *B. ovis* to range from 58 to 70% for a single determination, while that of complement fixation test was 6%. In general, Babesiosis has the highest mortality in domestic animals in many areas of Iran (Ziapour et al., 2011). The animal reservoirs increase the risk of the potential spread of disease to other animals and especially humans and this deserves special attention. The study has indicated that the use of PCR in the surveillance of babesiosis will enable the detection of asymptomatic carrier animals that could not be detected using conventional methods.

The objective of this pilot study is to provide baseline data regarding the prevalence of Babesia species and risk factors involved in the spread of Babesiosis in Aurangabad.

A major reason for prevalence of parasite in Aurangabad could be the poor hygienic conditions and poverty especially in villages. In many cases, veterinarians are not available for the help and guidance of livestock owners. By generating the public awareness regarding the risk factor, the prevalence of piroplasms can be significantly decreased resulting in better health and output of sheep and goats.

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ISSN: 0971-1260 Vol-22, Special Issue-31

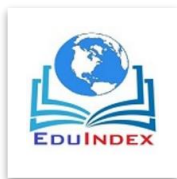
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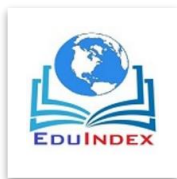
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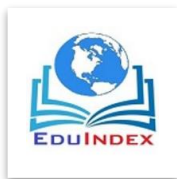
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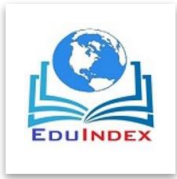
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**Think India Journal**  
*ISSN: 0971-1260 Vol-22, Special Issue-31*  
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