Int.J.Curr.Microbiol.App.Sci (2018) Special Issue-7: 5167-5174

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Special Issue-7 pp. 5167-5174**
Journal homepage: http://www.ijcmas.com

Original Research Article

Prevalence of Babesiosis in Cattle in Patna Region, India

Bipin Kumar[1*], D. B. Mondal[2] and M. V. Jithin[1]

[1]BVC, Patna -14, India
[2]Division of Medicine, IVRI, Izatnagar, Bareilly, India
**Corresponding author*

A B S T R A C T

The present study was undertaken to know the status of Babesiosis in cattle in the study region. The retrospective study on the basis of past clinical data and incidence pattern of Babesia infections in cattle through microscopic examination (MO) and PCR were done from the Patna region, India. The comparative sensitivity of the MO and PCR were also done. Since the research was based on clinical cases and retrospective data, thus there was no need of taking ethical approval from the committee. The PCR assays based on the *Babesia bigemina* rhoptry associated protein gene (RAP) was done which revealed the overall percentage of *B. bigemina* were 36% (180/500) and by microscopic observation 3% (22/500). Age wise results revealed that animals of >3yrs are most susceptible (45.42%) followed by 1-3yrs (32.37%) and least <1yr (16.88%). The season wise prevalence was highest in rainy (58.55%), then summer (34.73%) and lowest in winter (6.71%). The HF cross cattle were found most susceptible (47.03%) followed by Jersey cross (40.62%) and least in Nondescript (21.42%). The sex wise results shows female were more affected (29.70%) than Males (27.71%). The lactating cattle were more susceptible (53.77%) than the nonlactating (16.02%). Thus the season, age, sex, breed and lactation were identified as potential risk factors. This is the first report on *B. bigemina* in the study area, providing useful information for the management and control of the disease.

Keywords

Babesia bigemina, Piroplasm, Prevalence, Hemoglobinuria, PCR

Introduction

Babesiosis is an important tick-borne disease of cattle caused by infection with hemoprotozoan parasites of the genus *Babesia*. Among the genus Babesia parasites, *B. bigemina* and *B. bovis* are the main species in cattle, with high prevalence in tropical and subtropical regions of the world (Bock *et al.*, 2004; Uilenberg, 1995). Clinical signs of babesiosis include anorexia, depression, dyspnea, anemia, fever, hemoglobinuria, and in many cases death (Brown *et al.*, 2006). However, cattle may remain persistently infected without showing any clinical symptoms, and thus plays an important role in parasite transmission. Therefore, for the detection of asymptomatic carrier animals (Bock *et al.*, 2004) effective control strategies must be required. The microscopic detection of Babesia parasites in blood smears has always been considered the gold standard for the diagnosis of acute babesiosis, but detection is difficult in carrier animals with low parasitemia levels (Almeria *et al.*, 2001). Molecular diagnosis by PCR-based techniques has been developed for the detection of Babesia DNA and used for epidemiological surveys in livestock

populations (Figueroa *et al.*, 1992, 1993). Recently an improved PCR method targeting the rhoptry associated protein-1 gene (RAP-1) was demonstrated for specific detection of *B. bigemina* in many countries, including Egypt, Mongolia, Sri Lanka, Vietnam and the Philippines (Elsify *et al.*, 2015; Sivakumar *et al.*, 2012, 2013; Yba˜nez *et al.*, 2013). In India, the molecular prevalence of *Babesia bigemina* and *Trypanosoma evansi* infection in Punjab has been reported recently (Sharma *et al.*, 2013) suggesting tick-borne pathogens were also distributed in India. Since no previous data referring to these parasites are available so far in the region of study.

In the present study, infection of cattle with *B. bigemina* was investigated by PCR in the Patna district of Bihar state. Patna is located on the southern bank of the river Ganges in Eastern India. The exact cartographic co-ordinates of Patna are 25.6°N 85.1°E. It has an average elevation of 53 m (174 ft).

Materials and Methods

Retrospective epidemiological data for babesiosis were collected by multistage cluster random sampling technique from various veterinary hospitals in Patna for last 03 years. The data from following hospitals, namely teaching veterinary clinical complex (TVCC), Bihar Veterinary College (BVC) Patna, Bakhtiyarpur (Bazar), Fatuha (Station road), Masaurhi, Barh (kachahari) and Danapur (cant) were collected for prevalence study. Past data collected from Patna district statistically analyzed to get the prevalence status of the region. In addition to past report of Babesia infection in the study area, random blood sample from susceptible 500 tick infested animals, from Danapur, Punpun, Masaurhi, Bakhtiyarpur, Barh, Naubatpur, Patnacity, Fatuha, Daniyawan, Phulwarisharif, Mokama and Vikram blocks of Patna district, were collected to study the prevalence of babesiosis in the area. The on spot blood smears were made from tip of the ear to detect piroplasm in RBC if, any and DNA isolated from collected blood by using QIAamp® DNA blood mini kit (QIAGEN, GmbH, Germany) following the manufacturer's recommendations with minor modifications. The epidemiological studies consisted of various parameters viz, Age wise, sex wise, season wise, breed wise and production wise in terms of lactation status.

Age wise

All the animals were divided into three age groups for the prevalence study. Number of animals within the age group was taken as per available cases. Group-I consisted of animals of less than one year of age. Group-II consisted of animals of more than one year and less than three years of age. Group-III consisted of animals of more than three years of age.

Sex wise

Sex wise categorization of available cases made as male and female.

Lactation status

The affected animals were categorized as per stage of lactation or nonlactation.

Season wise

Impact of season on the vector population, infective stage of tick was recorded. The average temperature of the area in summer, rainy and winter seasons is 38^0C, 30^0C and 15^0C respectively. The average humidity of the area in summer, rainy and winter seasons are 45%, 85% and 60% respectively.

Breed wise

Prevalence of babesiosis in different available breeds of the area was recorded.

Collection of blood

Blood was collected from jugular vein in clean dry sterilize EDTA vials for DNA extraction and blood smear was prepared from the ear margin capillary bed and stained with Giemsa stain for microscopical examination of intracellular Babesia organisms, if any. Microscopic examination of Giemsa stained blood smears were examined for presence of *Babesia* organisms within the RBCs. The smears were examined for at least 100 optical fields before declaring negative for *Babesia* organisms and the results obtained were compared to that of PCR assay.

PCR assay

Genomic DNA extraction: For conducting the PCR, whole genomic DNA was extracted from whole blood using QIAamp® DNA blood mini kit (QIAGEN, GmbH, Germany) following the manufacturer's recommendations with minor modifications. In brief, 200µL of the blood sample was mixed with 20µL of Proteinase K and 200µL of lysis buffer and incubated at 56^0C for 10 min. The published primer Forward 5' CAT CTA ATT TCT CTC CAT ACC CCT CC-3' and Reverse 5'-CCT CGG CTT CAA CTC TGA TGC CAA AG-3' (Almeria *et al.,* 2001 and Figueroa *et al.,* 1992, 1993) for amplification of template DNA of Babesia were used for the confirmation of infection. DNA was extracted from isolated *Babesia bigemina* parasites from infected erythrocytes of clinically infected cattle were used as positive control. PCR products were analyzed by 1.5% agarose gel electrophoresis followed by ethidium bromide staining and photography.

Results and Discussion

Retrospective epidemiological data for babesiosis were collected by random sampling technique from various veterinary hospitals in Patna for last 03 years. Total number of suspected cases of babesiosis reported from July 2012 to July 2015 in various veterinary hospitals was found to be 2154. Out of them, 596 (27.66%) cases were confirmed positive for *Babesia bigemina by* Giemsa stained thin blood smear (GSTBS) technique. Overall percentage prevalence was highest in Danapur (35.51%) and lowest in Fatuha (26.21%) as shown in table 1. On the basis of data collected from various hospitals the season wise prevalence revealed the highest number of clinical babesiosis in rainy season (58.55%) followed by summer (34.73%) and least in winter (6.71%). Highly significant ($p<0.01$) difference in prevalence of babesiosis in cattle among winter, summer and rainy seasons have been observed (Table 2).

Out of 77 animals of age group < 1yr screened for Babesiosis, 13(16.88%) were found positive for *Babesia bigemina*. 139 animals of 1-3 yrs of age, were screened of which 45(32.37%) were found positive. 284 animals of more than 3yrs of age were screened for Babesiosis, of which 129 (45.42%) were found positive by MO and PCR. Highly significant ($p<0.01$) difference among different age groups in the prevalence pattern have been observed. The highest prevalence (45.42%) was found in 3^{rd} age group followed by 2^{nd} and least in 1^{st} group (Table 3).

Available cases were divided into male and female (Table 4). Out of them, 20 males (27.77%) and 125 females (29.20%) were

found positive for Babesiosis by MO and PCR. However, there was no significant difference between males and female was observed.

The affected animals were categorized as per stage of lactation. Available cases were divided into lactating and non-lactating (Table 5). Out of them, 185 lactating (53.77%) and 25 non-lactating (16.02%) were found positive for Babesiosis by MO and PCR. Highly significant ($p<0.01$) difference between lactating and non-lactating animals in the prevalence pattern were observed.

Prevalence of babesiosis in different available breeds of the area was recorded (Table 6). Out of 500 animals screened in the study a total of 270 animals belongs to HF cross, of which 127(47.03%) was found positive for *Babesia bigemina*. 160 animals belongs to Jersey cross were screened for Babesiosis in the study, of which 65(40.62%) were found positive for *Babesia bigemina*.70 animals belongs to nondescript breed were screened for Babesiosis in the study, of which 15(21.42%) were found positive for *Babesia bigemina* by MO and PCR. Highly significant ($p<0.01$) difference among different breed in the prevalence pattern have been observed. The highest prevalence (47.03%) was found in HF cross followed by jersey cross (40.62%) and least in Nondescript breed (21.42%).

Comparison of microscopic observation (MO) and PCR Assay for the detection of Babesiosis in field sample

The entire 500 blood samples collected from various blocks of Patna were subjected to PCR and GSTBS to confirm the subclinical infection in animals as shown in table 7. A total of 180 animals (36%) were found positive for *Babesia bigemina* by PCR whereas only 22(3%) by GSTBS. The annealing temperature for the amplification of DNA was found 55^0C.The comparative results between MO and PCR shows the significant ($p< 0.01$) difference in sensitivity of diagnosis. The overall positivity of bovine babesiosis was recorded to be 36% (180/500) as revealed by the amplification of 278 bp amplicon. However, blood smear examination showed only 22 (3%) animals positive for the piroplasms of *B. bigemina* with low parasitaemia (never exceeding 1%). The sensitivity of PCR assay was recorded to be significantly higher ($p< 0.01$) than that of blood smear examination.

No prevalence data of bovine babesiosis has been reported from Patna (India) except some clinical case registered in different veterinary hospitals. In the present study it was found that total 2154 cases of bovine babesiosis suspected and brought for treatment in hospitals. Out of them, 596 (27.66%) cases were confirmed positive for *Babesia bigemina by* GSTBS technique. The season wise prevalence rate were chalked out from the data, which showed the 40 cases (6.71%) of babesiosis in winter (November to February), 207 cases (34.73%) in summer(March to June)and 349 cases(58.55%) in rainy season (July to October). Highest prevalence was observed in rainy season. The season has significant ($p<0.01$) effect on the occurrence of babesiosis.

This might be due to the fact that, during rainy season high temperature coupled with high humidity favours the development of ticks (Radostits *et al.,* 1994) and hence transmission of babesia occurs rapidly and easily to naïve animals subsequently results in clinical disease in the susceptible animals. The present findings are in agreement with (Ruprah, 1985; Bhikane *et al.,* 2001 and Kumar *et al.,* 2006) who have reported the

similar findings with regard to seasonal prevalence.

Age wise prevalence was found to be highly significant ($p<0.01$) among the three age groups. 77 animals of < 1yr of age were screened for Babesiosis by MO and PCR. Out of them, 13(16.88%) were found positive for *Babesia bigemina*. 45 (32.37%) animals were found positive for *Babesia bigemina* from 139 animals of 1-3yrs of age were screened for babesiosis. Out of 284 animals in >3yrs of age, 129 (45.42%) were found positive for *Babesia bigemina*. The higher significant infection rate observed in animals of less than >3 years can be explained by fact that older animals can maintain the immunity and are less prone to recurrent infections. Acquired immunity has a very important role in maintenance of enzootic stability since animals become immune to infection giving rise to a fully protected adult herd (Awad *et al.,* 2011). The highest prevalence (45.42%) was found in 3rd age group followed by 2nd and least in 1st group. Truman *et al.,* (1987), reported that young calves possess a strong innate immunity against *Babesia bovis* infection that lasts for approximately 6 months after birth and is abrogated with the removal of the spleen. Findings of present study are in close agreement with the findings of (Levine, 1985; Ruprah., 1985; Bhikane., *et al.,* 2001; Kumar *et al.,* 2006).

There was no significant ($p<0.01$) difference between males and female were observed in the prevalence of the babesiosis. 20 males (27.77%) and 125 females (29.20%) were found positive for Babesiosis by MO and PCR. However, high prevalence (29.20%) was found in females than males (27.77%). The slightly higher rate of infection detected in female is attributed to the physiology of the female during pregnancy and lactation period, which are associated with hormonal and immunological changes (Khansari *et al.,* 1990).Many researchers reported similar findings regarding prevalence pattern, (Ruprah., 1985; Bhikane *et al.,* 2001; Kumar *et al.,* 2006). The highest prevalence (47.03%) was found in HF cross followed by jersey cross (40.62%) and least in Nondescript breed (21.42%). There is highly significant ($p<0.01$) difference among different breed in the prevalence pattern. High prevalence rate of babesiosis among the crossbreds as compared to nondescript could be explained on the basis of facts that the increased population of crossbred without adequate nutrition, proper managmental conditions, hygienic and health care services in the study area. These differences could also be explained by enhanced tick resistance or genetically based immunity to Babesia infection in the Zebu breed (Parker *et al.,* 1985; Uilenberg, 1995; Wambura *et al.,* 1998). The present observation is in agreement with (Radostits *et al.,* 1994; Ruprah., 1985; Kumar *et al.,* 2006). In contrast to present finding Bhikane (2001) observed the more prevalence of babesiosis in nondescript cattle and explained that it may be due to heavy work load and poor nutrition. The prevalence of babesiosis was found more (53.77%) in lactating than no lactating (16.02%) animals. The prevalence pattern was found significantly ($p<0.01$) higher in lactating than no lactating animals. Higher infection in lactating animals is most likely due to hormonal and immunological changes during the lactation period (Khansari *et al.,* 1990).

Comparison of microscopic observation (MO) and PCR Assay for the detection of Babesiosis in field sample

A total 180 animals (36%) were found positive for *Babesia bigemina* by PCR and only 22(3%) by GSTBS. The comparative

results between MO and PCR show the significant (P<0.01) difference in sensitivity of diagnosis. The overall positivity of bovine babesiosis through PCR was recorded to be 36% (180/500) as revealed by the amplification of 278 bp amplicon. PCR, due to its high sensitivity and specificity, is able to detect low level of infections in subclinical form and can be suitable for epidemiological study of *B. bigemina* infection in cattle. Sharma, *et al.,* (2013) reported the similar findings regarding PCR and MO tool for the study on prevalence of babesiosis in Punjab**.** The low level of piroplasms found in present study indicating the carrier status of animals. Thus, detection of infection in carrier animals becomes an important epidemiological parameter (d'Oliveira *et al.,* 1995). This would be helpful in designing better and effective control measures against the disease. Microscopic techniques are gold standard test to diagnose acute babesiosis but these are not ideally suited for detection of the parasite in carrier animals or recovered animals, having low parasitemia. Earlier reports by various workers have clearly indicated the higher sensitivity of PCR related techniques compared to other techniques, such as IFAT or blood smear observations in the diagnosis of *Babesia* species (Sparagano, 1999). PCR based techniques are more affective with regard to the sensitivity and specificity for detection and surveillance of hemoparasites (Nayel *et al.,* 2012; Hüe *et al.,* 2013; Ybañez *et al.,* 2013). A PCR based assay for detection of *B. bigemina* was originally described by Figueroa *et al.,* (1992), targeting 278 bp fragment specific of parasite DNA. They reported that the PCR product could be detected in DNA samples purified from 200 µl of blood with a parasitaemia as low as 1 in 108 cells containing an estimated 30 *B. bigemina* infected erythrocytes. In contrast to present finding, a comparatively higher prevalence of bovine babesiosis has been reported by microscopic (18%) and PCR analysis (29%) from Pakistan (Chaudhry *et al.,* 2010). From India, very few reports are available (Ravindran, 2002; Singh *et al.,* 2007) employing PCR based assay to know the status of the disease in other parts of the country.

In conclusion, this is the first report of molecular detection of B. bigemina in cattle in Patna. The overall percentages of infection with parasites were 3% and 36% through GSTBS and PCR respectively. Thus outcome of this study revealed that the PCR method is a good tool for epidemiological study. Potential risk factors for the occurrence of babesiosis were identified as age 1-3 years, > 3years, Friesian cross breeds, lactating animals and rainy seasons, which may be useful information for the prevention and control of the babesiosis.

Acknowledgement

Authors are thankful to Director IVRI, Izatnagar, Bareilly, UP (India) for providing necessary facilities for completion of this research work.

References

Almeria, S., Castella, J., Ferrer, D., Ortuno, A., Estrada-Pe˜na, A., Gutierrez, J.F., 2001. Bovine piroplasms in Minorca (Balearic Islands, Spain): comparison of PCR-based and light microscopy detection. Vet. Parasitol. 99, 249–259.

Awad, H., Antunes, S., Galindo, R.C., do Rosario, V.E., de la Fuente, J., Domingos, A., El Hussein, A.M., 2011. Prevalence and genetic diversity of Babesia and Anaplasma species in cattle in Sudan. Vet. Parasitol. 181, 146–152.

Bhikane, A.U., Narladkar, B.W., Anantwar,

L.G., Bhokre, A.P. 2001. Epidemiology, clinicopathology and treatment of babesiosis in cattle. *Indian Vet. J*. August, 78: 726-729.

Bock, R., Jackson, L., de Vos, A. and Jorgensen, W. 2004. Babesiosis of cattle. Parasitology 129, S247–S269.

Brown, C. and Torres, A. 2008. USAHA Foreign Animal Diseases, Seventh Edition. Committee of Foreign and Emerging Diseases of the US Animal Health Association. Boca Publications Group, Inc.

Chaudhry, Z I., Suleman, M., Younus, M. and Aslim A. 2010. Molecular detection of *Babesia bigemina* and *Babesia bovis* in crossbred carrier cattle through PCR. *Pakistan J. Zool.*, 42: 201-204.

D'oliveira, C., van der Weide, M., Habela, M A., Jacquiet, P. and Jongejan, F. 1995. Detection of *Theileria annulata* in blood samples of carrier cattle by PCR. *J. Clin. Microbiol.*, 33: 2665–2669.

Elsify, A., Sivakumar, T., Nayel, M., Salama, A., Elkhtam, A., Rizk, M., Mosaab, O.,Sultan, K., Elsayed, S., Igarashi, I., Yokoyama, N., 2015. An epidemiologicalsurvey of bovine Babesia and Theileria parasites in cattle, buffaloes, and sheep in Egypt. Parasitol. Int. 64, 79–85.

Figueroa, J.V., Chieves, L.P., Johnson, G.S., Buening, G.M., 1992. Detection of *Babesia bigemina*-infected carriers by polymerase chain reaction amplification. J. Clin. Microbiol. 30, 2576–2582.

Figueroa, J.V., Chieves, L.P., Johnson, G.S., Buening, G.M., 1993. Multiplex polymerase chain reaction based assay for the detection of *Babesia bigemina*, *Babesia bovis* and *Anaplasma marginale* DNA in bovine blood. Vet. Parasitol. 50, 69–81

Hüe, T., Graille, M., Mortelecque, A., Desoutter, D., Delathière, J., Marchal, C., Teurlai, M. and Barré, N. 2013. Diagnostic methods used to monitor an outbreak of babesiosis (*Babesia bovis*) in a herd of feral cattle in New Caledonia. *Aust. Vet. J*. 91(6): 254–258

Igarashi, I., Inoue, N., Xuan, X., Yokoyama, N., 2013. PCR Detectionand genetic diversity of bovine hemoprotozoan parasites in Vietnam. J. Vet. Med. Sci. 75, 1455–1462.

Igarashi, I., Yokoyama, N., 2012b. A PCR-based survey of selected Babesiaand Theileria parasites in cattle in Sri Lanka. Vet. Parasitol. 190, 263–267.

Igarashi, I., Yokoyama, N., 2012b. A PCR-based survey of selected Babesia and Theileria parasites in cattle in Sri Lanka. Vet. Parasitol. 190, 263–267.

Khansari, D.N., Murgo, A.J. and Faith, R.E., 1990. Effects of stress on the immune system. Immunol. Today 11, 170–175.

Kumar, B., Verma, S.P., Sinha, B. and Shekhar, Sudhanshu. 2006. Epidemiological aspects of bovine babesiosis. *Indian J. Vet. Med*. Vol. 26, No.2, pp. 141-142.

Levine, N.D. 1988: The protozoan phylum Apicomplexa. Volume I. Volume II. CRC Press Inc., Boca Raton, FL 33431; USA, 203 + 154 p.

Nayel, M., El-Dakhly, KM., Aboulaila, M., Elsify, A., Hassan, H., Ibrahim, E., Salama, A. and Yanai, T. 2012.The use of different diagnostic tools for *Babesia* and *Theileria* parasites in cattle in Menofia, Egypt. Parasitol Res. 111(3):1019–1024.

Parker, R.J., Shepherd, R.K., Trueman, K.F., Jones, G.W., Kent, A.S., Polkinghorne, I.G., 1985. Susceptibility of *Bos indicus* and *Bos taurus* to *Anaplasma marginale* and

Babesia bigemina infections. Vet. Parasitol. 17, 205–213.

Radostits, OMC., Blood, CCG. and Hinchcliff, KW.1994. Veterinary Medicine. 9th Ed. WB. Saunders, London.

Ravindran, R. 2002. Polymerase chain reaction based diagnosis of *Babesia bigemina* infection in bovines. PhD thesis submitted to Indian Veterinary Research Institute, Izatnagar, India.

Ruprah, N.S. 1985. Text book at clinical protozoology oxanian press Pvt. Ltd. New Delhi, P. 286.

Sharma, A., Das, Singla L., Tuli, A., Kaur, P., Batth, BK.and Javed M. (2013). Molecular prevalence of *Babesia bigemina* and *Trypanosoma evansi* in dairy animals from Punjab, India, by duplex PCR: A step forward to the detection and management of concurrent latent infections. Biomed Res Int. 893862.

Singh, H., Mishra, A K., Rao, J R., Cheema, P S and Sankar, M. (2007). Polymerase chain reaction for diagnosis of *Babesia bigemina*. *Indian Vet. J.*, 84: 346-348.

Sivakumar, T., Altangerel, K., Battsetseg, B., Battur, B., Abou-Laila, M., Munkhjargal, T., Yoshinari, T., Yokoyama, N., Igarashi, I., 2012a. Genetic detection of Babesiabigemina from Mongolian cattle using apical membrane antigen-1 gene based PCR technique. Vet. Parasitol. 187, 17–22.

Sivakumar, T., Kothalawala, H., Abeyratne, S.A., Vimalakumar, S.C., Meewewa, A.S.,Hadirampela, D.T., Puvirajan, T., Sukumar, S., Kuleswarakumar, K., Chandrasiri, A.D., Sparagano, O. 1999. Molecular diagnosis of *Theileria* and *Babesia* species. *J. Vet. Parasitol.*, 13: 83–92.

Uilenberg, G., 1995. International collaborative research: significance of tick-bornehemoparasitic diseases to world animal health. Vet. Parasitol. 57, 19–41.

Wambura, P.N., Gwakisa, P.S., Silayo, R.S., Rugaimukamu, E.A., 1998.Breed-associated resistance to tick infestation in *Bos indicus* and their crosses with *Bos taurus*. Vet. Parasitol. 77, 63–70.