



SHORT COMMUNICATION

## Molecular Prevalence of *Hepatozoon canis* Infection in Dog Tick, *Rhipicephalus sanguineus*, from Punjab, India

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### ABSTRACT

Canine hepatozoonosis, caused by *Hepatozoon canis*, is transmitted by the ingestion of brown dog tick, *Rhipicephalus sanguineus*. In the present study *R. sanguineus* ticks (n=60) collected from dogs presented at Small Animal Clinics, Teaching Veterinary Clinical Complex, GADVASU, Ludhiana, Punjab, India were screened by PCR based assay for the detection of *H. canis* specific 18S ribosomal RNA gene fragment. Results of the PCR assay revealed an overall prevalence of 8.33% *H. canis* infection in the tested ticks. Furthermore, the male ticks showed higher prevalence (11.53%) than the female counterparts (5.88%) but the data was statistically non-significant (P=0.6439).

**Keywords:** Dog tick, *Hepatozoon canis*, PCR, Punjab, *Rhipicephalus sanguineus*

Canine hepatozoonosis caused by *Hepatozoon canis* is a widespread vector borne disease, transmitted by the ingestion of brown dog tick, *Rhipicephalus sanguineus* (Baneth *et al.*, 2001; Otranto and Dantas-Torres, 2010). In majority of the cases, *H. canis* causes chronic type infection with relatively mild or no clinical alterations to its host. However, severe and fatal cases of the disease have also been recorded with symptoms like fever, paralysis, inappetence, anaemia, ocular discharge, hind limb weakness and emaciation (Gondim *et al.*, 1998). Regarding the diagnostic approaches, infection can be suspected in canines based on the clinical symptoms, microscopic detection of the parasite in stained blood smears, serology (Karagenc *et al.*, 2006) and molecular diagnostic assays (El-Dakhly *et al.*, 2013).

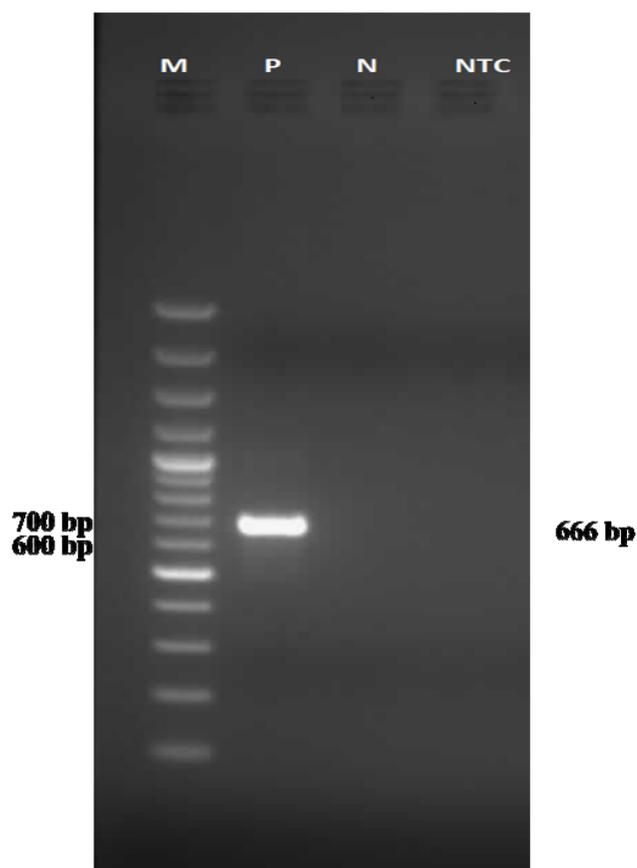
Though data has been on records regarding the prevalence of *H. canis* infection in dogs, worldwide, including India (Abd Rani *et al.*, 2011; Singh *et al.*, 2012a, b), still meagre information is available on the prevalence of infection in dog ticks (Dantas-Torres *et*

*al.*, 2012; Latrofa *et al.*, 2014). Therefore, the present study was carried out with the objective of detection of *H. canis* infection in *R. sanguineus* ticks from Ludhiana district, Punjab by PCR based assay.

Canines of both sexes and all age groups presented at the Small Animal Clinics (SAC), Teaching Veterinary Clinical Complex (TVCC), GADVASU, Ludhiana were screened for the presence of tick infestation. The dog owners were requested to manually remove the adult ticks and submit the same for detection of *H. canis*. Collected ticks were transferred to the glass vials, marked properly, brought to the Entomology Laboratory of department and processed and mounted as per standard protocols. The permanent mounts were then examined microscopically and characterized morphologically according to Dantas-Torres (2008).

The whole genomic DNA was isolated from single tick (n=60) of either sex by using QIAamp® DNA

mini kit (QIAGEN, GmbH, Germany) following the manufacturer's recommendations with minor modifications in terms of increasing the centrifugation time by 30 sec at every step. The PCR protocol was standardized and optimized targeting a portion of the 18S rRNA gene of *Hepatozoon* spp. using the primers as per Rubini *et al.* (2005) [cited by Abd Rani *et al.*, 2011] in a 25 uL final reaction volume. Appropriate negative and positive controls were also run alongside. The PCR cycling conditions adopted in the present study are as under:



**Fig. 1:** Standardization of PCR assay

**Lane M:** Generuler™ 100 bp Ladder plus

**Lane P:** Positive Control (*H. canis* DNA from positive dog)

**Lane N:** Negative Control (Leucocyte DNA from healthy dog)

**Lane NTC:** Non-template Control

Initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing

at 58°C for 30 sec, and extension at 72°C for 1.5 min, and thereafter final extension at 72°C for 5 min.

The PCR products were checked for amplification, if any, by performing horizontal gel electrophoresis on a 1.5% agarose gel at 70 V and visualized using gel documentation system (Syngene, UK). Prevalence of *H. canis* infection in the collected ticks was calculated as the number of ticks positive for *H. canis* by PCR assay divided by the total number of tested ticks with 95% confidence intervals (95% CI) being also calculated. The effect of prevalence of *H. canis* infection with regards to sex (male and female) of the tick was also determined. The data was compared using the Fisher's exact test with a 5% level of significance by SAS Software (SAS 9.3 version).

The ticks were identified as *Rhipicephalus sanguineus* as per the characteristic morphological features described elsewhere. It has been observed that geo-climatic conditions, in terms of moisture and temperature, prevailing in Ludhiana district provide favourable and conducive conditions for the survival and propagation of ticks. Furthermore, in the past also *R. sanguineus* has been reported to be the major tick infesting canines (Gill and Gill, 1977).

The PCR assay standardized for detection of *H. canis* revealed a desired amplicon of 666 bp in positive control sample (Fig. 1).

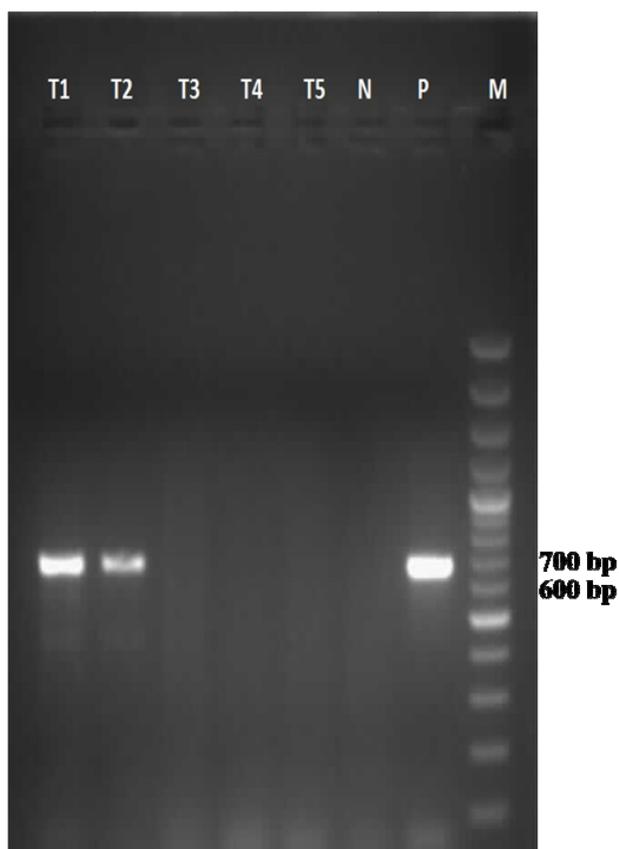
When employed on the isolated field tick DNA samples, an overall positivity rate of 8.33% (95% CI, 1.35–15.30) was revealed by the PCR. The positive field samples revealed the presence of an amplicon of 666 bp while no amplification was observed in negative samples (Fig. 2).

Furthermore, male ticks recorded higher percentage of samples (11.53%) to be positive for *H. canis* than the female counterparts (5.88%), however, the data was non-significant ( $P=0.6439$ ) (details in Table 1).

**Table 1:** Prevalence of *H. canis* in *R. sanguineus* ticks

Tick	Number examined	Number positive	% Prevalence
Male	26	3	11.53
Female	34	2	5.88
<b>Total</b>	<b>60</b>	<b>5</b>	<b>8.33</b>

With regards to detection of *H. canis* in ticks by PCR assay, Dantas-Torres *et al.* (2012) in southern Italy reported an overall positivity rate of 2.2% targeting the 18S rRNA gene of *H. canis* from *R. sanguineus* adult ticks, larvae and nymphs from the dogs as well as the shelter. High prevalence rates were reported from male ticks than female counterparts. The higher positivity rate detected in ticks collected from dogs in the present study may be due to the fact that the samples were collected in summer season predominantly, when the infestation of *R. sanguineus* ticks is more pronounced. However, the results of present study are in concordance to the results obtained by Latrofa *et al.* (2014), who recorded 7.4% positivity for *H. canis* in ticks.



**Fig. 2:** Field evaluation of PCR assay

**Lane M:** Generuler™ 100 bp Ladder plus

**Lane P:** Positive Control

**Lane N:** Negative Control

**Lane T1-T5 :** Field collected ticks

Further research is required for the better understanding of relationship between tick population dynamics and infection with *H. canis* in dogs along with seasonal prevalence of infection. The generated information will be useful for veterinary doctors and pet in terms of health care and management of hepatozoonosis.

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#### CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

#### REFERENCES

- Abd Rani, P.A.M., Irwin, P.J., Coleman, G.T., Gatne, M. and Traub, R.J. 2011. A survey of canine tick-borne diseases in India. *Parasit. Vectors.*, **4**: 141-148.
- Baneth, G., Samish, M., Alekseev, E., Aroch, I. and Shkap, V. 2001. Transmission of *Hepatozoon canis* to dogs by naturally-fed or percutaneously injected *Rhipicephalus sanguineus* ticks. *J. Parasitol.*, **87**: 606-611.
- Dantas-Torres, F., Latrofa, M.S., Weigl, S., Tarallo, V.D., Lia, R.P. and Otranto, D. 2012. *Hepatozoon canis* infection in ticks during spring and summer in Italy. *Parasitol. Res.*, **110**: 695-698.
- Dantas-Torres, F. 2008. The brown dog tick, *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae): From taxonomy to control. *Vet. Parasitol.*, **152**: 173-185.
- El-Dakhly, K.M., Goto, M., Noishiki, K., El-Nahass, el-S., Hirata, A., Sakai, H., Takashima, Y., El-Morse, A. and Yanai, T. 2013. Prevalence and diversity of *Hepatozoon canis* in naturally infected dogs in Japanese islands and peninsulas. *Parasitol. Res.*, **112**: 3267-3274.
- Gill, H.S. and Gill, B.S. 1977. Qualitative district-wise distribution of adult ixodid ticks in the Punjab state. Ixodid ticks of Domestic Animals in the Punjab State. PAU, Ludhiana, pp. 2-14.



- Gondim, L.F.P., Kohayagawa, A., Alencar, N.X., Biondo, A.W., Takahira, R.K. and Franco, S.R.V. 1998. Canine hepatozoonosis in Brazil: description of eight naturally occurring cases. *Vet. Parasitol.*, **74**: 319-323.
- Karagenc, T.I., Pasa, S., Kirli, G., Hosgor, M., Bilgic, H.B., Ozon, Y.H., Atasoy, A. and Eren, H. 2006. A parasitological, molecular and serological survey of *Hepatozoon canis* infection in dogs around the Aegean coast of Turkey. *Vet. Parasitol.*, **135**: 113–119.
- Latrofa, M.S., Dantas-Torres, F., Giannelli, A. and Otranto, D. 2014. Molecular detection of tick-borne pathogens in *Rhipicephalus sanguineus* group ticks. *Ticks Tick Borne Dis.*, **5**: 943-946.
- Otranto, D. and Dantas-Torres, F. 2010. Canine and feline vector-borne diseases in Italy: current situation and perspectives. *Parasit. Vectors.*, **3**: 2.
- Rubini, A.S., Paduan, Kd.S., Cavalcante, G.G., Ribolla, P.E.M. and O'Dwyer, L.H. 2005. Molecular identification and characterization of canine *Hepatozoon* species from Brazil. *Parasitol. Res.*, **97**: 91-93.
- Singh, H., Haque, M., Jyoti, Singh, N.K. and Rath, S.S. 2012a. Occurrence of parasitic infections in dogs in and around Ludhiana, Punjab. *Appl. Biol. Res.*, **14**: 108-110.
- Singh, H., Singh, N.K. and Rath, S.S. 2012b. Occurrence of canine hepatozoonosis in and around Ludhiana district (Punjab). *Indian J. Canine Pract.*, **4**: 64-66.