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Dirofilaria repens in dogs from Assam, India

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ABSTRACT

Objective: To access the prevalence of *Dirofilaria repens* (*D. repens*) in dogs from Assam, India.

Methods: A total of 223 blood samples from local dogs were examined with conventional (wet film and Knott's concentration technique), serological (ELISA test using Snap4Dx kits) and molecular techniques (targeting internal transcribed spacer-2 region using panfilarial primers) in Guwahati, Assam, India.

Results: The study revealed 4 (1.79%) cases of asymptomatic canine dirofilariasis caused by *D. repens*. The blood samples were positive for *D. repens* with microfilaremia on wet blood film, at Giemsa stained smear and under Knott's concentration technique, but were negative at Snap®4Dx test (IDEXX Laboratory, Westbrook, USA) which is specific for *Dirofilaria immitis*. *D. repens* could be detected by molecular test. Further confirmation was obtained on the basis of DNA sequencing and homology searching by basic local alignment search tool. Sequence analysis revealed that the species prevalent in Guwahati was genetically distinct from the other *D. repens* reported from elsewhere.

Conclusions: Occurrence of *D. repens* in dogs from this part of India was recorded for the first time, confirming the presence of a autochthonous canine reservoir for the zoonotic filarial nematode in Assam, India, where three cases of human subcutaneous and ocular infection with *D. repens* (dirofilariasis) have been reported.

1. Introduction

Dirofilaria (*Nochtiella*) *repens* (*D. repens*) is a mosquito borne filarial nematode that parasitizes subcutaneous tissues of dogs and other carnivores. The parasite has been reported in many countries including India[1]. The highest prevalence is reported in Italy and Sri Lanka[2]. Erythema, papules, focal or multifocal alopecia, crusting and subcutaneous nodules containing adult worms are the most commonly observed clinical manifestations of the infection causing pruritic dermatitis in both dogs and cats[2]. Mosquitoes belonging to the genera of *Culex, Aedes, Armigeres* and *Anopheles* have been incriminated as suitable vectors[3]. *D. repens* accidentally affects humans and has been reported in about 410 cases over 30 different nations in the world[4]. Several human cases have been reported from India[5-7]. Canine prevalence ranging from 4.9% (Delhi) to 16.7% (Mumbai) have been recently reported from India on the basis of molecular findings[1]. This study aimed to report for the first time the presence of *D. repens* in dogs from North Eastern India diagnosed using both traditional and molecular techniques. The parasite is of zoonotic importance since dogs can be a source of infection to humans through interspecific mosquito bite.

2. Materials and methods

The study was done between August, 2011 and July, 2012 by screening canine heartworm infection in dogs from Guwahati

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city (26°19' N, 91°75' E) of Assam with average annual rainfall of 1 500-2 600 mm and average altitude of 52 m above sea level. A total of 223 blood samples were studied, including the hospital dogs blood samples as well as those blood samples referred to the Department of Parasitology for diagnosis of haemoparasitic infection. Blood sampling from hospital dogs was done at clinics of the Veterinary College. Approximately 5 mL of blood was drawn from the cephalic vein of the studied dogs, collected in vacuum tubes containing disodium salt of ethylene diamine tetra acetic acid and stored at 4 ˚C. Conventional, serological and molecular techniques were employed to detect the presence of *D. repens*. Conventional tests included wet blood film method, Giemsa staining of blood smear and Knott's concentration technique. Serological evidence was based on presence of heartworm antigen present in tested blood or serum samples using Snap® 4Dx kits (Idexx Laboratory, USA). For molecular techniques, genomic DNA was isolated from blood using the DNeasy blood and tissue kit (Quiagen® Kit, Catalogue No 51104) as per the handbook provided by the manufacturer. A single step multiplex PCR technique targeted to amplify the internal transcribed spacer-2 (ITS-2) region of ribosomal DNA developed by Rishniw *et al*. was followed for molecular screening for different canine filarial species in blood[8]. The primer utilized was referred to as panfilarial primers; forward: DIDR F1 5'-AGT GCG AAT TGC AGA CGC ATT GAG-3' and reverse: DIDR R1 5'-AGC GGG TAA TCA CGA CTG AGT TGA -3' were utilized to amplify and differentiate *Dirofilaria immitis* (*D. immitis*), *D. repens*, *Brugia malayi*, *Brugia pahangi*, *Acanthocheilonema* (*Dipetalonema*) *reconditum* and *Acanthocheilonema dracunculoides*. The amplified fragments were submitted to Xcelris Lab, Ahmedabad, India for sequencing and phylogenetic analysis of the *D. repens* on the basis of ITS-2 sequence comparison was performed. Alignment of ITS-2 sequences of Guwahati isolates with additional five sequences from the GenBank were analyzed by clustalW of DNAStar. Sequences from four isolates of India (JQ039744, JQ039743, JQ706073 and FJ717410) and one isolate from USA (AY693808) were included for the comparison.

3. Results

Of the 223 samples screened for the presence of microfilaria, 4 (1.79%) blood samples were positive for the presence of *D. repens* with traditional diagnostic methods (Figure 1). These 4 samples were found negative at Snap® 4Dx test which is specific for *D. immitis* only. DNA samples extracted from these blood samples were screened for differentiating microfilarial identity using panfilarial primers. All 4 revealed an amplification size of 484 bp indicating positivity for *D. repens* (Figure 2).

Further confirmation was obtained after sequencing and comparison with the sequence by the basic local alignment search tool in NCBI web site. Thus only one sequence was submitted in NCBI and accession number was obtained (Accession No. JX 524743). Phylogenetic tree for ITS-2 region of *D. repens* and homology analysis of the sequence are shown in Figrues 3 and 4. Guwahati isolates were seen under the same group of Kuthiathode, Kadakkrapally and USA isolate and with same clad of Trichur isolate. Thiruvananthpuram isolate seems to be different from the other four.

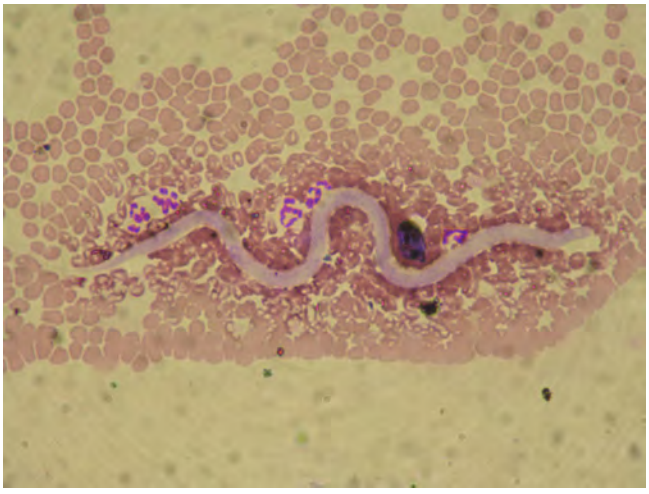

Figure 1. *D. repens* as seen under Giemsa stained blood smear (100×).

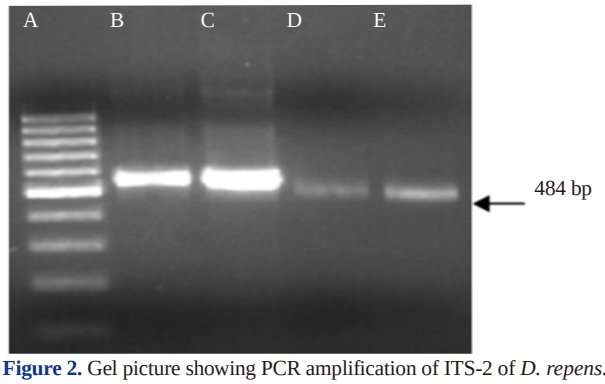

Figure 2. Gel picture showing PCR amplification of ITS-2 of *D. repens*. Lane A: 100 bp ladder; Lane B and C: *D. immitis*; Lane D and E: *D. repens*.

The homology percentage among the ITS-2 of *D. repens* available in the GenBank is summarized in Figure 4. The percent identity among the *D. repens* of Guwahati isolate ranged from 46.7% (Thruvavavthpuram isolate) to 94.1% (Kadakkarapally isolate). There were very low divergence observed among USA, Kadakkarapally and Kuthiathode isolate when compared with

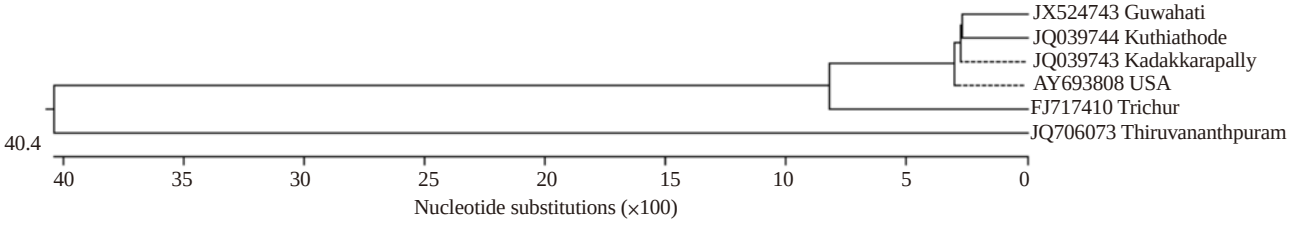

Figure 3. Phylogenetic tree of *D. repens* of Guwahati isolate (ITS-2).

Guwahati isolate of *D. repens*.

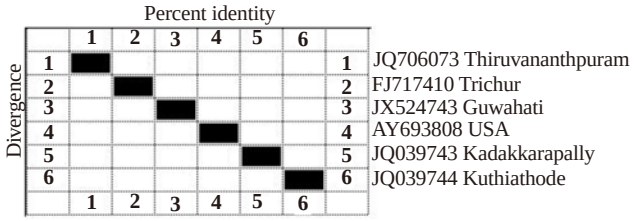

Figure 4. Homology analysis of ITS-2 of *D. repens*, Guwahati isolate with other isolates available in the GenBank using DNAStar software.

4. Discussion

So far, infection with *D. repens* has never been reported in animals from the north eastern states of India. However, this area is endemic for canine heartworm[9-11]. Mosquito vectors incriminated for transmitting both filarial nematodes are same. The finding of 4 (1.79%) canine cases infected with *D. repens* was the most significant result of this study, since few autochthonous human cases found in Northeast India so far lacked an animal reservoir. Wherever human filarial cases are detected, animal reservoirs are present. The present finding supports the previous reports that observed *D. repens* infection in humans from the study area[6], using PCR as molecular diagnostic tool, which is useful to differentiate *D. immitis* from other filarial parasites[8,12].

D. repens in dogs was earlier reported from India with a prevalence of 7%-24% from Kerala[13], and 38.09% from Mangalore[14]. More recently, based on PCR technique, 4.9% and 16.7% prevalence of *D. repens* were reported from Delhi and Mumbai, respectively[1]. The presence of *D. repens* in dogs from this part of the country confirms the sporadic occurrences of human ocular and subcutaneous dirofilariasis in Northeast India[6]. The present findings reflect an emerging zoonotic significance because dogs represent an animal reservoir of infection for humans. The present finding extends the zoogeography of the parasite to the north eastern part of India. In Italy, Genchi *et al*. discovered an immunological based interaction between *D. immitis* and *D. repens*[15]. They suggested that the superimposition of *D. immitis* on an existing *D. repens* infection is more difficult to treat than the superimposition of *D. repens* on one existing *D. immitis* infection. Based on their findings, it can be assumed that infection in this part of the country is either monospecific or dual showing concurrent infection with *D. immitis*. Differently, in Southern India, *D. repens* is the only dominant[14]. Phylogenetic analysis of *D. repens* from Northeast India indicated close resemblance with isolate of Southern Indian.

In conclusion, *D. repens*, a zoonotic nematode previously recorded in a number of human cases in Assam, was reported for the first time in dogs from this state of Northeast India.

Conflict of interest statement

We declare that we have no conflict of interest.

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