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K Jeeva
National Research Centre on Yak, Dirang, West Kameng, Arunachal Pradesh, India

N Chatterjee
National Research Centre on Yak, Dirang, West Kameng, Arunachal Pradesh, India

AK Bera
National Research Centre on Yak, Dirang, West Kameng, Arunachal Pradesh, India

S Maiti
National Research Centre on Yak, Dirang, West Kameng, Arunachal Pradesh, India

Hira Ram
Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

PS Banerjee
Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

TK Goswami
Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

PJ Das
National Research Centre on Yak, Dirang, West Kameng, Arunachal Pradesh, India

JK Chamua
National Research Centre on Mithun, Jharnapani, Medziphema, Nagaland, India

SM Deb
National Research Centre on Yak, Dirang, West Kameng, Arunachal Pradesh, India

D Bhattacharya
Principal Scientist, NRC on Yak, Dirang, National Research Centre on Yak, Dirang, West Kameng, Arunachal Pradesh, India

Corresponding Author:
D Bhattacharya
Principal Scientist, NRC on Yak, Dirang, National Research Centre on Yak, Dirang, West Kameng, Arunachal Pradesh, India

Genetic structure of *Toxocara* of canine, feline and ruminant origin: An evaluation with RAPD marker

K Jeeva, N Chatterjee, AK Bera, S Maiti, Hira Ram, PS Banerjee, TK Goswami, PJ Das, JK Chamua, SM Deb and D Bhattacharya

Abstract
T. canis was collected from dogs in yak tracts of Arunachal Pradesh and Tamil Nadu. *T. cati* was isolated from cats of Arunachal Pradesh; *T. vitulorum* was collected from yaks of Arunachal Pradesh, mithun of Nagaland and cattle of West Bengal. DNA extraction was done by following conventional liquid-liquid extraction method, purity of genomic DNA and quantification of DNA was done by spectrophotometric analysis and for conducting RAPD-polymerase chain reaction (RAPD-PCR), total of 25 arbitrary primers were used in appropriate cycling condition. RAPD fingerprinting was conducted using 25 arbitrary primers and only 9 primers could yield scorable bands which differentiate three species of *Toxocara* which could be appreciated on the basis of simple matching co-efficient (SMC) and distance matrix after third clustering step based on single lineage. *T. canis* collected from two different geographical locations exhibited indistinguishable RAPD profile. Further, *T. vitulorum* collected from yak, mithun and cattle of three different geographical locations had similar RAPD profile which indicated genetic similarity of parasites irrespective of host assemblages and geographical locations. This study seems to be the seminal Indian effort on comparative genetic profile of three *Toxocara* Spp., of different host species and varied geographical locations.

Keywords: *Toxocara*, RAPD-PCR, yak, mithun, dog, cat

Introduction
Toxocara Spp. is an important ascaridoid nematode of *Toxocara* genus containing species of animal and human health significance (Magnaval *et al.*, 2010, Rubinsky-Elefant *et al.*, 2010) [2]. Three species of *Toxocara* is very common in dog, cat and large ruminants (Yak, Mithun and Cattle). Out of three species, *T. canis* and T. cati infection are not only infectious to their respective final host but also infective to human being due to very well-known human-animal bond (Paul *et al.*, 2010) [3]. *T. vitulorum* is a parasitic ascarid of Bubalus and Bos (Warren, 1971) [4]. This parasitic infection is mainly prevalent in tropical and subtropical climates. A perusal of available literature suggests that *T. canis* and *T. cati* infection are cosmopolitan in distribution (Fisher, 2003, Lee *et al.*, 2010, Overgaauw and Knapen, 2013) [5, 6, 7]. Likewise T. vitulorum is prevalent worldwide, except in North America, where this species is rarely available (Zajac and Conboy, 2006) [8].
Previous studies indicated widespread existence of genetic variation among parasite populations, which has been translated in terms of systemic, population structure and epidemiology of parasite (Zhu *et al.* 2001, Gasser, 2006) [1, 11]. Earlier reports suggested that genetic variation could be evidenced among same species of *Toxocara* which were isolated from varied geographical distribution (Zhu *et al.*, 1998) [10].
Considering the importance of molecular characterization, genetic variation has been dealt during the present study on *T. canis*, *T. cati* and *T. vitulorum* which were isolated from different host assemblages such as dog, cat, cattle, yak and mithun from various geographical locations of India.

Materials and Methods
Isolation and processing of parasites
For isolation of parasites, faecal samples of the animals were initially screened by concentration method. The positive animals were treated with piperazine (Antepar®) at the dose rate 200 mg/kg body weight to collect the intact adult worms. *T. canis* was collected from dogs of Tamil Nadu and Arunachal Pradesh. *T. cati* was collected from cats of Arunachal Pradesh. *T. vitulorum* was collected from yak of Arunachal Pradesh, mithun of Nagaland, and cattle of West Bengal.

Collected parasites were washed 5 times in PBS (pH 7.2) to remove fecal debris. Anterior and posterior end of the parasites were cut off and cleared in lactophenol for morphological identification (Soulsby, 1982). Rest of the samples parasite samples were preserved in 70% alcohol (v/v) at -20 °C for extraction of genomic DNA.

Genomic DNA extraction

Genomic DNA was isolated from individual worm by conventional liquid-liquid extraction methodology after minor modification (McManus *et al.*, 1985) [13]. Purity and quantification of genomic DNA was evaluated by spectrophotometric method. The ratio of absorbance at 260 nm and 280 nm (A260/280) was used to assess the purity of DNA sample and for pure DNA; the A260/280 was ~ 1.8. For quantification of parasitic DNA, OD value of 1 at 260nm was considered as 50 µg/ mL of DNA.

RAPD Fingerprinting

To conduct RAPD PCR, 25 arbitrary primers of 10 mers were used for the study (Table 1). The amplification reaction carried out in 25 µL volume containing 100 mM dNTPs each, 15-20 ng of primer (Sigma, USA), 25 ng of DNA and 1.5 unit of Taq DNA polymerase (Fermentas, Canada). PCR reaction with initial denaturation at 94 °C for 5 min followed by 40 cycles of denaturation (94 °C for 45 sec), annealing (36 °C for 45 sec) and elongation (72 °C for 1 min). Final elongation was done for 10 min.

To check the repeatability of RAPD fingerprinting, same sample was screened for three times. PCR products (15 µL) were analysed in 1.5% agarose gel and photographed upon trans-illumination, along with standard DNA marker (100 bp and 1 kb DNA ladder) (Fermentas, Canada).

Table 1: Details of arbitrary primers used in the present study

Code	Sequence (5'….3')
OP-01	5'CGGCCCACGT3'
OP-02	5'GTCCTCGTAG3'
OP-03	5'AGCGGCTAGG3'
OP-04	5'ACTTCCTCCA3'
OP-05	5'CCTCACCTGT3'
OP-06	5'GCCGCTACTA3'
OP-07	5'TCCCGAACCG3'
OP-08	5'GCGTGACCCG3'
OP-09	5'CACGGCTGCG3'
OP-10	5'AATCGGGCTG3'
OP-11	5'GGGTAACGCC3'
OP-12	5'AGCCAGCGAA3'
OP-13	5'GACCGCTTGT3'
OP-14	5'AGGTGACCGT3'
OP-15	5'GAGCCCTCCA3'
OP-16	5'CTGAGACGGA3'
OP-17	5'CTGACGTCAC3'
OP-18	5'CTCTCCGCCA3'
OP-19	5'GCGAACCTCG3'
OP-20	5'GTGACGTAGG3'
OP-21	5'TGCCGAGCTG3'
OP-22	5'GCGAGCGTCC3'
OP-23	5'ACCTGGACAC3'
OP-24	5'CAGCGACAA3'
OP-25	5'AGAGGGCACA3'

Analysis of RAPD fingerprinting

RAPD bands were assessed by observing the presence or absence of amplified fragments in at least two reactions. One band was considered as one locus. Based on the presence or absence of a band, SMC and distance analysis were calculated based on the following formula, which has been described by Sokal and Michere (1958).

Sij = p+s/t

Sij = simple matching coefficient

p = number of variables those are positive for both the isolates (loci/band)

s = number of variables those are negative for both the isolates (loci/band)

t = total number of variables (loci/bands)

dij = 1-Sij

dij = distance matrix in between the isolates

Based on the distance matrix, clustering of the isolates was done by following single lineage procedure as described by Mooi and Sarstedt (2011) [15].

Results

Out of 25 primers (Table 1), 9 primers could yield scorable bands, which could differentiate the three species of *Toxocara*. Using OP-05 primer, it was revealed that, RAPD profile of *T. cati* was completely different from *T. canis* and *T. vitulorum*. RAPD profile of *T. canis* of Tamil Nadu and Arunachal Pradesh isolate were same but intensity of two bands (in between 250-500 bp and 900 bp) were more for *T. canis* isolated from Arunachal Pradesh compared to *T. canis* from Tamil Nadu (Fig. 1). Using the values of SMC and distance as well as distance based on single lineage, *T. canis*, *T. cati* and *T. vitulorum* could be clustered in three different groups (data not shown). While analysing RAPD profile of *T. vitulorum*, it has been found that, one homologous 250 bp fragment was prominent in *T. vitulorum* (West Bengal isolate) compared to other two isolates. Further, it has been found that, one homologous fragment of 700 bp was more prominent for *T. vitulorum* of Arunachal Pradesh and Nagaland isolates compared to West Bengal isolate (Fig. 1).

Using OP-10 primer *T. canis*, *T. cati* and *T. vitulorum* could be differentiated into three different clusters (data not shown). When amplicons were analyzed in ethidium bromide stained gel, it was revealed that, homologous bands of *T. canis* varied in terms of intensity (lane 1 and 2, Fig. 2). Scorable bands of *T. cati* completely differed from *T. canis* and *T. vitulorum* isolates collected from different locations of the country. RAPD profile of *T. vitulorum* of Arunachal Pradesh isolate and Nagaland isolate were indistinguishable from each other in terms of homologous bands and as well as intensity. But in case of *T. vitulorum* from West Bengal isolate, it was observed that, intensity of homologous bands differed, compared to remaining isolates of *T. vitulorum* (Fig. 2).

Although number of scorable bands of amplified products of *T. canis* by OP-13 primer were same (n=5), but intensity of the homologous bands were more for *T. canis* of Tamil Nadu isolate compared to *T. canis* of Arunachal Pradesh isolate (lane 1 and 2, Fig. 3). Further analysis of the data revealed that, RAPD profile of *T. vitulorum* of Arunachal Pradesh isolate and Nagaland isolate was indistinguishable from each other based on band sharing index and intensity as well (lane 4 and 6, Fig. 3). But on the contrary, intensity of homologous bands were less for *T. vitulorum* of West Bengal isolate compared to Arunachal Pradesh and Nagaland isolates (lane 4, 5 and 6, Fig. 3).

T. canis, *T. cati* and *T. vitulorum* could be differentiated from each other by OP-17 primer (Fig. 4). One unique intense band could be observed in ethidium bromide stained agarose gel for

T. canis of Arunachal Pradesh isolate which was approximately 400 bp fragment compared to *T. canis* of Tamil Nadu isolate (lane 1 and 2, Fig. 4). RAPD profile of *T. vitulorum* for three isolates was indistinguishable from each other but intensity of amplified products were more for West Bengal isolate (lane 4, 5 and 6, Fig. 4).

Using OP-18 primer, *Toxocara* of different species collected from different geographical locations could be differentiated from each other, which was deduced on the basis of SMC and distance between the isolates and as well as distance based on single lineage (data not shown). From RAPD profile of *T. canis*, it was evidenced that, three prominent fragments were shared by both the isolates between 400 bp to 750 bp (lane1 and 2, Fig. 5). Amplification of genomic DNA of *T. cati* revealed unique banding pattern compared to *T. canis* and *T. vitulorum* (Fig. 5).

Amplicons generated after enzymatic amplification by OP-19 primer could distinguish *T. canis*, *T. cati* and *T. vitulorum* from each other and the isolates formed three distinct clusters (data not shown). On the contrary, intensity of the amplified products varied between the isolates of T. canis (lane 1 and 2, Fig. 6). Amplified product from *T. cati* showed characteristics difference in terms of presence of unique bands ranging from 250 bp to 750 bp (lane 3, Fig. 6). It was further observed that, *T. vitulorum* of Arunachal Pradesh isolate and *T. vitulorum* of Nagaland isolate was indistinguishable from each other, which could be appreciated on the basis of similar banding pattern. Although homologous bands could be observed after amplification of *T. vitulorum* of West Bengal isolate, but intensity of bands varied compared to Arunachal Pradesh and Nagaland isolates (Fig. 6).

Using OP-20 primer, three different species of *Toxocara* could be well differentiated (data not shown). Amplification of *T. cati* genomic DNA showed characteristics banding pattern of variable sizes which ranged between 100 bp to 1000 bp. Both the isolates of *T. canis* had also similar banding pattern (lane1 and 2, Fig. 7). Similar banding pattern could also be seen in the three isolates of *T. vitulorum*, isolated from Arunachal Pradesh, West Bengal and Nagaland (Fig. 7).

OP-23 primer was also able to differentiate three species of *Toxocara* collected from different locations and host assemblages (data not shown). By using this primer, it was evidenced that, *T. cati* had also unique banding pattern compared to *T. canis* and *T. vitulorum*. After observing the banding pattern of *T. canis* and *T. vitulorum*, it could be witnessed that, in both the species one 900 bp fragment was common which was more prominent and bright for *T. vitulorum* (Fig. 8).

OP-24 primer could also differentiate *T. canis*, *T. cati* and *T. vitulorum* (data not shown). Although RAPD profile of *T. canis* for the isolates (Tamil Nadu and Arunachal Pradesh) were same, but intensity of band varied between two isolates (lane1 and 2, Fig. 9). RAPD profile of *T. cati* was distinguishable from *T. canis* and *T. vitulorum*. Further it was also observed that, RAPD profile of *T. vitulorum* of Arunachal Pradesh and Nagaland isolates was same. West Bengal isolate of *T. vitulorum* shared homologous band when compared with Arunachal Pradesh and Nagaland isolates. But intensity of homologous bands was less in West Bengal isolate. A common band of 500 bp was also shared between three isolates of *T. vitulorum* but intensity of this fragment was more in case of West Bengal isolate of *T. vitulorum* (lane 4, 5 and 6, Fig. 9).

In the present study, based on the presence or absence of the bands, SMC and distance based on single lineages, *Toxocara* Spp. was clustered into three groups. *T. canis* isolated from two geographical regions formed one cluster; *T. cati* collected from Arunachal Pradesh was in another cluster. *T. vitulorum* collected from three different provinces of India could be clustered in one group in all 9 primers which yielded scorable bands (data not shown). RAPD profile was similar for *T. canis* collected from two different geographical locations. It was further observed that RAPD profile of *T. vitulorum* isolated from three different host assemblages were indistinguishable from each other (Fig. 1-9). Distance matrix based on band sharing index revealed that distance between *T. canis* and *T. cati* varied between 0.667- 0.875 and between *T. canis* and *T. vitulorum* ranged from 0.688-0.875. The details of the data based on the results of 9 primers have been depicted (Table 2).

Table 2: Distance matrix of *T. canis*, *T. cati*, and *T. vitulorum* after third clustering step in respect of presence or absence of loci in *T. canis*.

Distance matrix			
Primers	***T. canis***	***T. cati***	***T. vitulorum***
OP-05	1.000	0.858	0.778
OP-10	1.000	0.750	0.800
OP-13	1.000	0.834	0.728
OP-17	1.000	0.875	0.778
OP-18	1.000	0.786	0.734
OP-19	1.000	0.693	0.688
OP-20	1.000	0.667	0.875
OP-23	1.000	0.700	0.800
OP-24	1.000	0.750	0.688

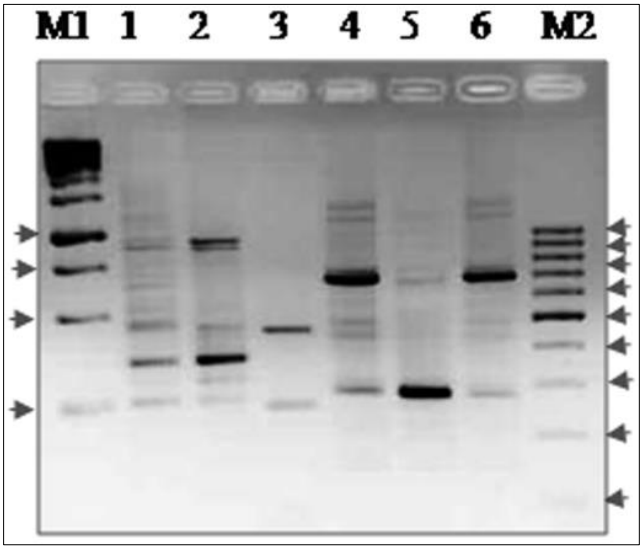

Fig 1: Showing RAPD profile of different species and isolates of *Toxocara* by OP- O5 primer. M1= 1 kb DNA ladder (arrows from the bottom: 250 bp, 500 bp, 750 bp, 1000 bp); lane1= *T.canis* (Tamil Nadu isolate); lane2= *T. canis*(Arunachal Pradesh isolate); lane3= *T. cati* (Arunachal Pradesh isolate); lane4= *T. vitulorum* (Arunachal Pradesh isolate); lane5= *T. vitulorum* (West Bengal isolate); lane6= *T. vitulorum* (Nagaland isolate); M2= 100 bp DNA ladder (arrows from the bottom: 100 bp, 200 bp, 300 bp, 400bp, 500 bp, 600 bp, 800bp, 900 bp).

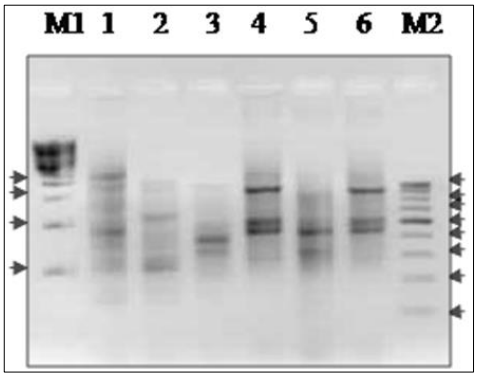

Fig 2: Showing RAPD profile of different species and isolates of *Toxocara* by OP- 1O primer. M1= 1 kb DNA ladder (arrows from the bottom: 250 bp, 500 bp, 750 bp, 1000 bp); lane1= *T. canis* (Tamil Nadu isolate); lane2= *T. canis* (Arunachal Pradesh isolate); lane3= *T. cati* (Arunachal Pradesh isolate); lane4= *T. vitulorum* (Arunachal Pradesh isolate); lane5= *T. vitulorum* (West Bengal isolate); lane6= *T. vitulorum* (Nagaland isolate); M2= 100 bp DNA ladder (arrows from the bottom: 100 bp, 200 bp, 300 bp, 400bp, 500 bp, 600 bp, 800bp, 1000 bp).

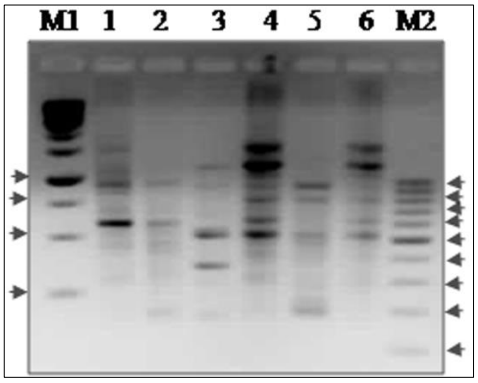

Fig 3: Showing RAPD profile of different species and isolates of *Toxocara* by OP- 13 primer. M1= 1 kb DNA ladder (arrows from the bottom: 250 bp, 500 bp, 750 bp, 1000 bp); lane1= *T. canis* (Tamil Nadu isolate); lane2= *T. canis* (Arunachal Pradesh isolate); lane3= *T. cati* (Arunachal Pradesh isolate); lane4= *T. vitulorum* (Arunachal Pradesh isolate); lane5= *T. vitulorum* (West Bengal isolate); lane6= *T. vitulorum* (Nagaland isolate); M2= 100 bp DNA ladder (arrows from the bottom: 100 bp, 200 bp, 300 bp, 400bp, 500 bp, 600 bp, 800bp, 1000 bp).

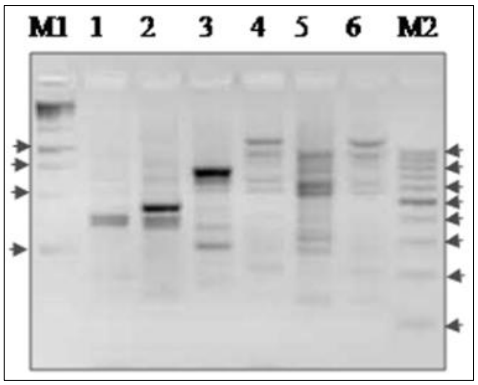

Fig 4: Showing RAPD profile of different species and isolates of *Toxocara* by OP- 17 primer. M1= 1 kb DNA ladder (arrows from the bottom: 250 bp, 500 bp, 750 bp, 1000 bp); lane1= *T. canis* (Tamil Nadu isolate); lane2= *T. canis* (Arunachal Pradesh isolate); lane3= *T. cati* (Arunachal Pradesh isolate); lane4= *T. vitulorum* (Arunachal Pradesh isolate); lane5= *T. vitulorum* (West Bengal isolate); lane6= *T. vitulorum* (Nagaland isolate); M2= 100 bp DNA ladder (arrows from the bottom: 100 bp, 200 bp, 300 bp, 400bp, 500 bp, 600 bp, 800bp, 1000bp).

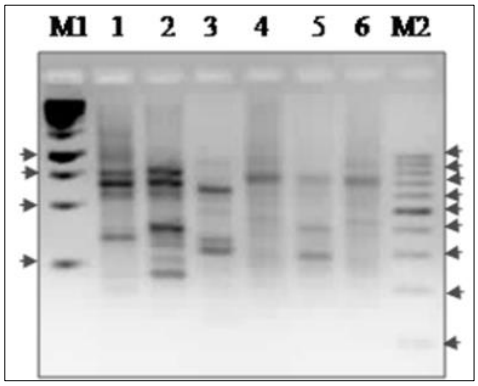

Fig 5: Showing RAPD profile of different species and isolates of *Toxocara* by OP- 18 primer. M1= 1 kb DNA ladder (arrows from the bottom: 250 bp, 500 bp, 750 bp, 1000 bp); lane1= *T. canis* (Tamil Nadu isolate); lane2= *T. canis* (Arunachal Pradesh isolate); lane3= *T. cati* (Arunachal Pradesh isolate); lane4= *T. vitulorum* (Arunachal Pradesh isolate); lane5= *T. vitulorum* (West Bengal isolate); lane6= *T. vitulorum* (Nagaland isolate); M2= 100 bp DNA ladder (arrows from the bottom: 100 bp, 200 bp, 300 bp, 400bp, 500 bp, 600 bp, 800bp, 1000 bp).

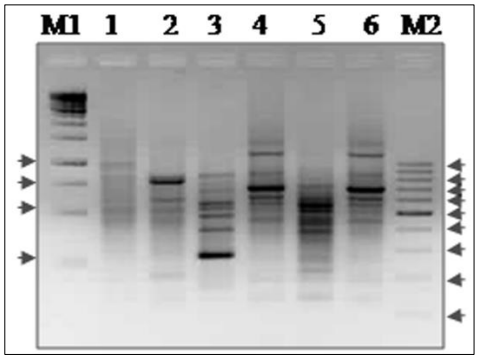

Fig 6: Showing RAPD profile of different species and isolates of *Toxocara* amplified by OP- 19 primer. M1= 1 kb DNA ladder (arrows from the bottom: 250 bp, 500 bp, 750 bp, 1000 bp); lane1= *T. canis* (Tamil Nadu isolate); lane2= *T. canis* (Arunachal Pradesh isolate); lane3= *T. cati* (Arunachal Pradesh isolate); lane4= *T. vitulorum* (Arunachal Pradesh isolate); lane5= *T. vitulorum* (West Bengal isolate); lane6= *T. vitulorum* (Nagaland isolate); M2= 100 bp DNA ladder (arrows from the bottom: 100 bp, 200 bp, 300 bp, 400bp, 500 bp, 600 bp, 800bp, 1000 bp).

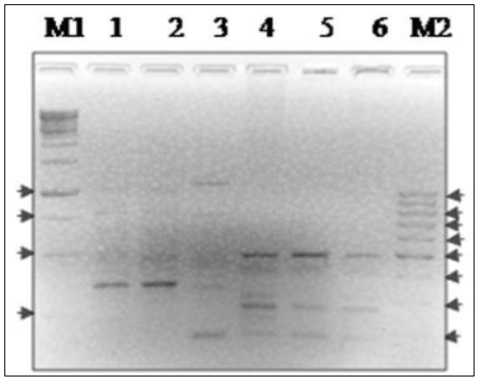

Fig 7: Showing RAPD profile of different species and isolates of *Toxocara* by OP- 20 primer. M1= 1 kb DNA ladder (arrows from the bottom: 250 bp, 500 bp, 750 bp, 1000 bp); lane1= *T. canis* (Tamil Nadu isolate); lane2= *T. canis* (Arunachal Pradesh isolate); lane3= *T. cati* (Arunachal Pradesh isolate); lane4= *T. vitulorum* (Arunachal Pradesh isolate); lane5= *T. vitulorum* (West Bengal isolate); lane6= *T. vitulorum* (Nagaland isolate); M2= 100 bp DNA ladder (arrows from the bottom: 100 bp, 200 bp, 300 bp, 400bp, 500 bp, 600 bp, 800bp, 1000 bp).

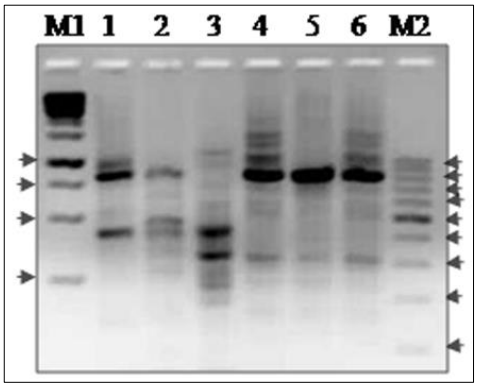

Fig 8: Showing RAPD profile of different species and isolates of *Toxocara* by OP- 23 primer. M1= 1 kb DNA ladder (arrows from the bottom: 250 bp, 500 bp, 750 bp, 1000 bp); lane1= *T. canis* (Tamil Nadu isolate); lane2= *T. canis* (Arunachal Pradesh isolate); lane3= *T. cati* (Arunachal Pradesh isolate); lane4= *T. vitulorum* (Arunachal Pradesh isolate); lane5= *T. vitulorum* (West Bengal isolate); lane6= *T. vitulorum* (Nagaland isolate); M2= 100 bp DNA ladder (arrows from the bottom: 100 bp, 200 bp, 300 bp, 400bp, 500 bp, 600 bp, 800bp, 1000 bp).

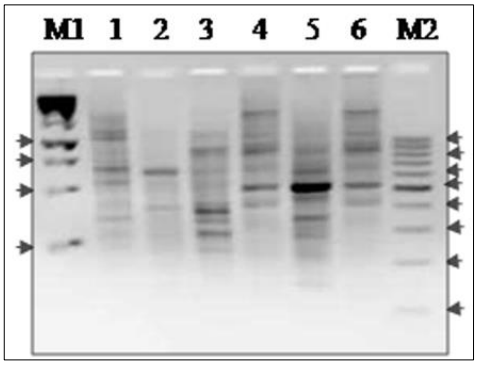

Fig 9: Showing RAPD profile of different species and isolates of *Toxocara* by OP- 24 primer. M1= 1 kb DNA ladder (arrows from the bottom: 250 bp, 500 bp, 750 bp, 1000 bp); lane1= *T. canis* (Tamil Nadu isolate); lane2= *T. canis* (Arunachal Pradesh isolate); lane3= *T. cati* (Arunachal Pradesh isolate); lane4= *T. vitulorum* (Arunachal Pradesh isolate); lane5= *T. vitulorum* (West Bengal isolate); lane6= *T. vitulorum* (Nagaland isolate); M2= 100 bp DNA ladder (arrows from the bottom: 100 bp, 200 bp, 300 bp, 400bp, 500 bp, 600 bp, 800bp, 1000 bp).

Discussion

Nematodes are much conserved in gross morphology. For that reason nematodes are frequently identified and distinguished on the basis of morphological features, host specificity, pathological effects and geographical origin. Likewise, ascaridoid nematodes of the genus *Toxocara* can be identified based on their morphology and predilection sites. But accurate identification, detection, differentiation and taxonomy of *Toxocara* Spp. especially at the cryptic stages (larval and/or egg stages) are very difficult (Gasser *et al.*, 2006) [11]. Although immunological methods are in vogue but immunologically the species identification is not possible due to the presence of cross reactivity and stage specific antigens (Fisher, 2003) [5]. Therefore, different species of *Toxocara* can be identified by molecular mining. The approach of molecular characterization can only delineate the different species of *Toxocara* which vary on the basis of phenotypic characters like life cycle pattern, host specificity, development rate, pathogenicity, antigenicity, sensitivity to chemotherapeutic agents, and transmission dynamics.

In the present investigation a total of 25 random primers (10 mer) were used for amplification of the genomic DNA extracted from *T. canis*, *T. cati* and *T. vitulorum*. Out of 25 arbitrary primers, 9 primers could distinguish three species of *Toxocara* which were isolated from different geographical locations and host assemblages. This has been possible, because RAPD fingerprinting is mainly based upon the use of markers, which in turn help to study the pattern of inheritance. Phenotypic markers are sometimes easy to identify but in some cases failed to score accurately because of modulation by environment and microenvironment within different host species, stage of the parasite or by modifier genes. Therefore, during the present study 9 arbitrary primers could yield scorable differentiating bands which might be used to differentiate *T. canis*, *T. cati* and *T. vitulorum*.

Recently, RAPD markers has been described as DNA fingerprinting method by which complex and informative genomic DNA fingerprint could be readily produced. On the contrary RAPD banding pattern might be influenced by a number of factors which included DNA quality, primer specificity, template concentration, co-migration of non-homologous fragments and/or the use of different thermal cyclers which may result in a lack of reproducibility (Gasser, 2013) [16]. To overcome the limitation during the present investigation, DNA quality and template concentration has been checked by spectrophotometry and only reproducible bands in three different PCR reactions have been considered as scorable bands.

In several studies RAPD-PCR has been used for identification and differentiation of species and strains of a range of parasitic groups including protozoa and helminths (Bandi *et al.*, 1993; Gasser *et al.*, 1998; Sandoval *et al.*, 1999; Anou *et al.*, 2002; Pradeep *et al.*, 2002; Maravilla *et al.*, 2003; Sedinova *et al.*, 2003; Martin-Sanchez *et al.*, 2003; Daniel Mwambete *et al.*, 2004; Ferreira *et al.*, 2004; Kau *et al.*, 2004; Ramadan and Saber, 2004; Zemanova *et al.*, 2004) [17, 10, 22, 29, 20, 24, 23, 18, 25].

Further work on molecular characterization of *T. canis* revealed that, isolate collected from different host assemblages (red fox and dogs) exhibited a SMC of 0.95. This observation left the impression that, there is no genetic variability in between isolates of *T. canis* collected from two different host species (Epe *et al.*, 1999) [29]. In the present study *T. canis* collected from two different geographical locations exhibited similar RAPD profile which corroborated with the findings of Epe *et al.*, 1999 [29]. Moreover, single scorable band was observed in these two isolates after use of OP-5, OP-10, OP-13, OP-17, OP-18 OP-19, OP-20, OP-23 and OP-24 primers. Therefore, these primers may be recommended to have good discriminatory power, because the same may be used for RAPD-sequence confirmed amplified region (RAPD-SCAR) analysis.

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References

1. Magnaval JF, Glickman LT, Dorchies P, Morassin B. Highlights of human toxocariasis. Korean J Parasitol 2001;39:1-11.
2. Rubinsky-Elefant G, Hirata CE, Yamamoto JH, Ferreira MU. Human toxocariasis: diagnosis, worldwide seroprevalences and clinical expression of the systemic

and ocular forms. Ann. Trop. Med. Parasitol 2010;104:3-23.
3. Paul M, King L, Carlin EP. Zoonoses of people and their pets: a US perspective on significant pet-associated parasitic diseases. Trends. Parasitol 2010;26:153-154.
4. Warren EG. Observations on the migration and development of *Toxocara vitulorum* in natural and experimental hosts. Int. J Parasitol 1971;1:85-99.
5. Fisher M. *Toxocara cati*: an underestimated zoonotic agent. Trends. Parasitol 2003;19:167-170.
6. Lee AC, Schantz PM, Kazacos KR, Montgomery SP, Bowman DD. Epidemiologic and zoonotic aspects of ascarid infections in dogs and cats. Trends Parasitol 2010;26:155-161.
7. Overgaauw PAM, Knapen FV. Veterinary and public health aspects of *Toxocara* spp. Vet. Parasitol 2013;193:398-403.
8. Zajac AM, Conboy GA. Veterinary Clinical Parasitology. 2nd ed. Ames, IA, Blackwell Publishing 2006, 82.
9. Zhu XQ, Gasser RB, Chilton NB, Jacobs DE. Molecular approaches for studying ascaridoid nematodes with zoonotic potential, with an emphasis on *Toxocara* species. J Helminthol 2001;75:101-108.
10. Zhu XQ, Jacobs DE, Chilton NB, Sani RA, Cheng NABY, Gasser RB. Molecular characterization of a *Toxocara* variant from cats in Kuala Lumpur, Malaysia. Parasitol 1998;117:155-164.
11. Gasser RB. Molecular tools- advances, opportunities and prospects. Vet. Parasitol 2006;136:69-89.
12. Soulsby EJL. Helminths, Arthropods and Protozoa of Domesticated Animals. 7thed. BailliereTindall, An Imprint of Elsevier 2006, 150-156.
13. McManus DP, Knight M, Simpson AG. Isolation and characterization of nucleic acids from the hydatid organisms, *Echinococcus* spp. (Cestoda). Mol. and Biochem. Parasitol 1985;16:251-266.
14. Sokal RR, Michener CD. A statistical method for evaluating systematic relationships. University of Kansas Scientific Bulletin 1958;38:1409-1438.
15. Mooi E, Sarstedt M. A Concise Guide to Market Research. Springer-Verlag Berlin Heidelberg 2011, 237-284.
16. Gasser RB. A perfect time to harness advanced molecular technologies to explore the fundamental biology of *Toxocara* species. Vet. Parasitol 2013;193:353-364.
17. Bandi C, La Rosa G, Comincini S, Damiani G, Pozio E. Random amplified polymorphic DNA technique for the identification of *Trichinella* species. Parasitol 1993;107:419-424.
18. Martin-Sanchez J, Diaz M, Artacho ME, Valero A. Molecular arguments for considering *Hysterothylacium fabri* (Nematoda: Anisakidae) a complex of sibling species. Parasitol. Res 2003;89:214-220.
19. Zemanova E, Jirku M, Mauricio IL, Miles MA, Lukes J. Genetic polymorphism within the *Leishmania donovani* complex: correlation with geographic origin. Am. J Trop. Med. Hyg 2004;70:613-617.
20. Pradeep KN, Patra KP, Hoti SL, Das PK. Genetic variability of the human filarial parasite Wuchereria bancrofti in South India. Acta Trop 2002;82:67-76.
21. Ramadan NI, Saber LM. Detection of genetic variabiltiy in nonhuman isolates of *Fasciola hepatica* and *Fasciola gigantica* by the RAPD-PCR technique. J Egypt. Soc. Parasitol 2004;34:679-689.
22. Sandoval H, Manga-Gonzalez Y, Campo R, Garcia P, Castro JM, Perez de la Vega M. Preliminary study on genetic variability of *Dicrocoelium dendriticum* determined by random amplified polymorphic DNA. Int. Parasitol 1999;48:21-26.
23. Sedinova J, Flegr J, Ey PL, Kulda J. Use of random amplified polymorphic DNA (RAPD) analysis for the identification of Giardia intestinalis subtypes and phylogenetic tree construction. J Eukaryot. Microbiol 2003;50:198-203.
24. Maravilla P, Souza V, Valera A, Romero-Valdovinos M, Lopez-Vidal Y, Dominguez-Alpizar JL *et al.* Detection of genetic variation in *Taenia solium*. J Parasitol 2003;89:1250-1254.
25. Daniel Mwambete K, Ponce-Gordo F, Cuesta-Bandera C. Genetic identification and host range of the Spanish strains of *Echinococcus granulosus*. Acta Trop 2004;91:87-93.
26. Kau P, Gupta I, Sehgal R, Malla N. *Trichomonas vaginalis*: random amplified polymorphic DNA analysis of isolates from symptomatic and asymptomatic women in India. Parasitol. Int 2004;53:255-262.
27. Ferreira AM, Vitor RW, Carneiro AC, Brandao GP, Melo MN. Genetic variability of Brazilian *Toxoplasma gondii* strains detected by random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) and simple sequence repeat anchored-PCR (SSR-PCR). Infect. Genet. Evol 2004;4:131-142.
28. Anou N, Yanwen X, Youren F. *Schistosoma japonicum* strains: differentiation by RAPD and SSR-PCR. Southeast Asian J Trop. Med. Public Health 2002;33:720-724.
29. Epe C, Meuwissen M, Stoye M, Schnieder T. Transmission trials, ITS2-PCR and RAPD-PCR show identity of *Toxocara canis* isolates from red fox and dog. Vet. Parasitol 1999;84:101-112.