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Original Article

Prevalence of *Echinococcus* infection in dogs in Akola district of Maharashtra (India) by Copro- PCR

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ABSTRACT

A study on prevalence of *Echinococcus* infection in dogs was carried out in Akola district of Maharashtra state in India. Total 289 fresh fecal samples of dogs (stray and domesticated) from seven tahsils of Akola district were collected and were analyzed by conventional floatation method as well as by PCR. The overall prevalence of *E. granulosus* infection in dogs was found to be 6.57%. Numerically higher prevalence was observed by PCR method (6.57%) compared to conventional floatation methods (2.77%) indicating PCR is more specific techniques for *Echinococcus* infection in dog's fecal samples. The numerically higher prevalence was recorded in Akola tahsil (9.84%) followed by Akot (9.30%), Patur (8.82%), Telhara (6.25%), Barshi Takli (4%), Balapur (3.85%) and Murtijapur (2.38%). It was observed those dogs around the slaughter houses and in rural areas was more prevalent compared to domesticated and in resided in town. It is thus concluded that Echinococcal infection is prevalent in Akola district of Maharashtra.

1. Introduction

Echinococcosis is a worldwide zoonotic parasitic disease caused by the metacestode of the dog tapeworm *Echinococcus granulosus* having major medical and socio-economic importance for humans and threatens livestock industry (Budke et al., 2006). *E. granulosus* has an indirect life cycle where dogs and other canids act as a definitive host while many herbivorus and omnivorus species including wildlife act as an intermediate host. Ingestion of infected cyst organs containing protoscolices act as a source of infection for dogs and fecal sample of such dog may act as a source of infection for animals as well as humans. Human are considered as an accidental intermediate host and contamination may occurs either through ingestion of taeniid eggs, contaminated food, vegetables and water (Adanir and Tasci, 2013) or by direct contact with contaminated dogs that retain eggs on their coat (Eckert and Deplazes, 2004). This leads to the development of Cystic *Echinococcosis* (CE). It is the second most important helminthic disease of significance (Sangaran et al., 2014) and recognized it as one of the seventeen neglected diseases in the world with more than one million human cases at any given time (WHO, 2011).

Echinococcosis in dog is well recognized and has been studied in developed countries, but in India canine *Echinococcosis* poses a lowly prioritized public health importance (Moon and Khemalpure, 2017).

The slaughterhouse based prevalence studies in cattle, buffalo, sheep, goat and pigs were ranged in between 3.02% to 28.26%, on the contrary the actual status of *E. granulosus* infection in the definitive host is not well studied although a few attempts were made to note the prevalence of gastrointestinal parasites in dogs (Prathiush et al., 2008). The overall *Echinococcus* infection rate in Assam was found to be 17.02%, while in Meghalaya and Mizorum the rate of infection was 27.77% and 18.18% respectively, in stray dogs around the abattoir (Deka et al., 2008). Prathiush et al. (2008) reported 4.35% prevalence in stray dogs in urban parts of Bangalore districts by coproantigen detection. In most of reports the prevalence of *E. granulosus* infection in dogs was based on either detection of coproantigen (Benito and Carmena, 2005), worm at necropsy examination (Barnes et al., 2012) or direct fecal examination under microscope (Eckert, 2003). The direct examination of dog feces is relatively a strong diagnostic tool for *Echinococcus* infection in dogs but routine coprological techniques cannot differentiate the eggs of *Echinococcus* from other Taenia species due to extreme morphological similarity (Dinkel et al., 1998). However, molecular tools like PCR permit the specific identification of *E. granulosus* (Mathis and Deplazes, 2006). Reliable epidemiological data is required to design an effective control program for any of the zoonotic disease. Considering this the present study was planned to provide a baseline data for prevalence of *Echinococcus* infection in dogs for developing epidemiologically control strategies for control and prevention of *Echinococcus* infection in definitive host and intermediate host in

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Akola district of Maharashtra.

2. Materials and methods

2.1. Study area

A cross-sectional pilot study on prevalence of *E. granulosus* infection in dogs by copro PCR was conducted during March 2012 to February 2015 throughout the year in the Akola districts of Maharashtra State (India). Geographically, Akola district is located at 20.17 to 21.16 North altitudes and 76.7 to 77.4 East altitudes covering an area of 5428 km^2. The Northern parts of the district consisting of hills and mountains that are raised to about 950 to 1000 m. The summers are extremely hot having temperature of 44–47 °C while the winters are dry and very cold and the temperature may drop below 2 °C. The annual rainfall averages 800 mm. Most of the rainfall occurs in the monsoon season between June and September, but some rain does fall during January and February (https://en.wikipedia.org/wiki/Akola_district). As per report on livestock census-2012, Maharashtra state, Akola district contributes total livestock population as 465,913 which includes Cattle (269343), Buffalo (49455), Sheep (4657), Goat (134981), Pig (6160), Horses & ponies (438), Donkeys (1177) and Camels (2).

2.2. Collection of fecal samples

Akola district is comprised of seven tahsils. For the present investigations villages in each tahsil were regularly visited for a period of three years (March 2012 to February 2015) for collection of dog fecal samples to record the prevalence of *Echinococcus* infection in dogs of Akola district of Maharashtra. The details of collection of fecal samples is presented in Table 1.

For the present investigation about 10 g of fresh fecal sample just after first defecation (early morning) was collected in a labeled sterile clinicol bottle (Himedia Laboratories Pvt. Ltd., Mumbai) without any preservative. Total 289 dogs fecal samples were collected from seven tehsils of Akola district which includes Akola (61), Balapur (52), Murtijapur (42), Akot (43), Patur (34), Barshi Takli (25) and Telhara (32). The fecal samples were collected from stray as well as from domesticated dogs having one year and above age irrespective of sex and breed. For safety reason, feces were decontaminated at 70 °C for 12 h and stored at −20 °C until molecular examination. All these fecal samples were processed for Zink sulfate centrifugal floatation method (1.18 specific gravity) as well as for Polymer Chain Reaction (PCR) using Mastercycler pro S & Control Panel (Eppendorf).

2.3. DNA extraction from fecal sample

The frozen fecal samples were first thawed at room temperature. Fecal genomic DNA was then retrieved from the each fecal sample (N = 289) using commercial specific QIAamp DNA Stool Mini Kit (cat.no.51504) (Quigen, Hilden, Germany) according to manufacturer instructions with minor modifications. Because of low concentration of Copro-DNA in extras, all fecal samples were concentrated by modified sedimentation technique prior to DNA extraction. Briefly, 2 g of fecal sample was suspended in 15 mL normal saline, filtered through strainer and gauze and centrifuged at 1500 rpm for 10 min until the supernatant came out clear. DNA extraction from the sediment was then performed as per manufacturer's instructions.

2.4. Polymerase chain reaction (PCR)

The primer pair for amplification of DNA of *E. granulosus* was chosen from the sequence of the mitochondrial 12S rRNA gene (GenBank accession no. AF297617; primer sequences Eg1f 5′-CATTAA TGTATTTTGTAAAGTTG-3′ and Eg1r 5′-CAC-ATC-ATC-TTA-CAA-TAA-CAC-C-3′) yielding an amplicon of 255 bp (Stefanic et al., 2004). Literature scanned revealed that these primers amplified DNA from almost all strains of *E. granulosus*, hence the primer pair was selected for the identification of segment of cox 1 gene intended to serve as the amplification target for copro-PCR diagnosis of *E. granulosus* in dog feces. The PCR reaction was carried out in a final concentration of 50 µL reaction mixture containing 25 µL of Dreamtaq green pcr master mix 2× (Thermo Scientific), 18 µL of nuclease free water, 1 µL (12.5 ppm) of each primer, 5 µL of template DNA. The PCR amplification was performed in Mastercycler pro S & Control Panel (Eppendorf) using following thermal condition: step 1 – one initial thermal cycle of 94 °C for 2 min, 53 °C for 1 min, 72 °C for 2 min. Followed by step 2 with 35 repeated thermal cycles of 94 °C for 30 s, 53 °C for 30 s and 72 °C for 30 s. Step 3 final elongation at 72 °C for 7 min and short term storage at 4 °C in a Mastercycler pro S & Control Panel (Eppendorf). After completion of PCR, amplified products were confirmed and analyzed by 1.5% Agarose gel electrophoresis. The electrophoresis was performed for 45 min which include 80 V for first 15 min and 120 V for next 30 min. Any non specific or difference in size of band was observed by running the 100 bp DNA ladder (Fermentas) along with PCR product.

2.5. Statistical analysis

Data was collected and recorded in Microsoft excel spread sheet and the prevalence of *Echinococcus* infection in dogs was calculated by dividing the number of positive dogs with total number of dogs examined. The prevalence rate in different tahsils was analyzed using Pearson's chi square method using online software, WASP ICAR Goa, Version 2. The probability (P) value less than 0.05 was set as statistically significant in all cases.

3. Results

3.1. Prevalence of E. granulosus infections by conventional methods

Out of 289 fecal samples observed by floatation method, only 14 (4.84%) samples were found positive for *E. granulosus* eggs on the basis

Table 1
Prevalence of *E. granulosus* infection in dogs by copro PCR during March 2012 to February 2015 in Akola district of Maharashtra in India.

Sr. No.	Name of Tehsil	No. of fecal sample examined	Type of dog		No. of positive samples	Prevalence	X^2 Cal	*P*-value
			Stray	Domestic				
1	Akola	61	50	11	6	9.84%	0.678	3.987
2	Akot	43	36	07	4	9.30%		
3	Murtijapur	42	37	05	1	2.38%		
4	Balapur	52	48	04	2	3.85%		
5	Barshi Takli	25	22	03	1	4.00%		
6	Telhara	32	29	03	2	6.25%		
7	Patur	34	30	04	3	8.82%		
Total		289	252	37	19	6.57%		

X^2 Cal = Chi square calculated.