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Research Article

DIAGNOSIS OF TOXOCARA CANIS IN DOGS IN BAGHDAD BY PCR TECHNIQUE

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ABSTRACT

The study was determined the total infection rate of *Toxocara canis* 52% in the domestic and stray dogs(42.4% , 65.5%) respectively; at 215 fecal samples were collected from five different areas of Baghdad city that distributed as north, south, west, east and center, from March to October 2015.

Statistical analyses appeared that no significant differences p>0.01 among the areas of Baghdad city, no significant differences p>0.01 between sexes of dogs, there are significant differences p <0.01 between the ages of dogs that little puppies more sensitive for infection; and no significant differences p>0.05 among stray dogs.

Solid tissue and protocol procedure was applied to *T. canis* adult worm due to isolate a pure DNA. The result of DNA extraction showed that fresh tissue of adult worm yielded enough DNA concentration for PCR amplification that 100 ng with purity 1.6. The PCR was used for amplify the target fragment by using specific primers were designed manually in the *T. canis* specific part of ITS-2 Sequences. This study reported the development of sensitive and specific PCR assay allowing rapid and reliable identification of *T. canis* by the fragment size amplified was 380 bp in ITS-2 gene.

INTRODUCTION

Pet dogs and cats can play an important role in transmission of zoonotic nematodes such as *Toxocara canis* and *Toxocara cati*, by excreting eggs directly into the human environment, without the involvement of vectors or intermediate hosts. Human toxocariosis remains a hazard despite the availability of highly effective anthelmintics for dogs and cats. A closer collaboration between veterinary and public health professionals within the 'One Health' concept is also required.[1]The close relationship of people with their companion animals, known as the human–animal bond, provides benefits with regard to socialisation, mental health and even physical wellbeing[2]. *Toxocara canis*, have an oral–fecal transmission cycle and humans can be infected by ingestion of infective eggs from contaminated soil (gardens, sandpits and play-grounds); from unwashed hands or raw vegetables, or by direct contact with pets [3,4].*T.canis*is originally a parasitic nematode of canine, bitches and their puppies [5]. However, *T. canis*eggs can also hatch in a large number of non-canid species, including human beings [6]. Larvae in non-canid species hatch and distribute themselves throughout the viscera; most often these larvae become encapsulated in a granulomatous response [7] or accumulated in the brain, where they elicit little or no histological reaction[8].The environmental contamination with *Toxocara canis* in a dog feces, especially in public parks, children playgrounds and streets [9,10,11,12].

The aim of present study is to determine the prevalence of *T. canis* in domestic and stray dogs with different ages and both sexes, in Baghdad city the capital of Iraq, include five regions: north, south, east, west and center. And Molecular diagnosis of infection of *T. canis* among dogs by PCR techniques.

MATERIALS AND METHODS

Samples collection: 215 fecal samples were collected from stray and domestic dogs randomly, in Baghdad city, which were divided into five areas: north area include Al-Shaab and Al-Rashdia; south area include Al-Ciadia and Al- Doraa; west area include Al- Kadhimiya and Al- Shulla; east area include Al-Ghadeer and Al-Sadder; center area include Al- Adamiyah and Bab Al-Muadum; from March to October 2015.

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Concentration technique

Sheather s solution preparation

Sugar solution that was prepared as fellow: 454 gm sucrose was dissolved in 355 ml distilled water [13]. This solution will grow mold if left out, so it would be added 6.5 gm phenol. Flotation method was done for all the fecal samples as previously described by[14].

DNA extraction and PCR

DNA extraction was performed according to manufacture's instruction kit using gSYNC DNA Extraction Kit (Geneaid), as the following:

Sample preparation: Adult worms of *T. canis* were obtained from infected dogs by treatment with anthelminthic drugs. Individual parasites were repeatedly washed in physiological saline, ph 7.3;A bout one centimeter was cut from *T. canis* worm tissue and put in centrifuge tube. 200 µl of GST Buffer was added and 20 µl of proteinase K, vertex thoroughly as suggested by [15].

Genotyping: PCR reaction was performed using specific primers were designed for the ITS-2 genes, primers were designed depending on their nucleotide sequenceby Alpha DNA Company as a lyophilized product of different picomolsconcentrations. Lyophilized primers have been dissolved in a free DNase/RNase water to give a final concentration of (100 pmol/µl) (as stock solution), to prepare 10µM concentration as work primer resuspended 10 pmol/µl in 90 µl of deionized water to reach a final concentration 10µM.The sequences of these primers have been listed in (table 1) as [16].

Table 1 Sequences of primers used in this study.

Gene marker	Primer name	Primer (5′ → 3′)	Primer length	Acc No. (NCBI)
ITS-2	Tcan1	(F): 5′-AGTATGATGGGCGCGCCAAT-3′	20	AB110034
	NC2	(R): 5′-TAGTTTCTTTTCCTCCGCT-3′	19	AB110034

*Primer from ALPHA Canadian company.

PCR Programs

The sample was put in the Thermocycler device for amplification of DNA and set the device program as fellow: Denature template94C at 30 sec.; Initial denaturation94C at 60 sec.; Annealing58C at 30 sec.; Extension72C at 30 sec. that 35 cycle; Final Extension72C at 10 min.

Analysis of PCR Products

The PCR products and the ladder marker have been resolved by electrophoresis. 3 µl of loading buffer was loaded on 1 % agarose gel (1g agarose/100 ml 1X TBE buffer) and run at 75 volt for 3-4 hours. The gel was stained with ethidium bromide for 15 minutes. In addition, bands were visualized on Gel documentation system with specific camera. Molecular marker ladder (100 bp) made by Bionner company has been used to estimate the molecular size of the bands.

RESULTS

The current study recorded high rate of infection52% with *T. canis* in domestic and stray dogs. Out of 215 fecal samples examined, 112 samples were positive by using sucrose flotation method.

High rate of infection with *T. canis* in dogs was recorded from different areas of Baghdad (north, south, west, east and center), out of 125 fecal samples were collected from domestic dogs, the rate of infection was 42.4%, and from 90 fecal samples of stray dogs, 65.5% was the rate of infection, (Table 2).

Table 2 Total rate of infection with *T.canis* in dogs in Baghdad.

Canids	No. of fecal samples examined	No. of positive samples	%
Domestic dogs	**125**	**53**	**42.4**
Stray dogs	**90**	**59**	**65.5**
Total	**215**	**112**	**52**

All the study areas in Baghdad city showed high rates of infection with *T. canis* in domestic dogs, the high rate 47.6% in west of Baghdad, and the low rate 32% in the east of Baghdad.(Table 3).

Table 3 Rates of infection in domestic dogs with *T. canis* in Baghdad according to areas.

Areas	No. of samples examined	No. of positive samples	%
North	**24**	**11**	**45.8**
South	**23**	**9**	**39.1**
Center	**32**	**15**	**46.8**
West	**21**	**10**	**47.6**
East	**25**	**8**	**32**
Total	**125**	**53**	**42.2**

Domestic dogs showed that total rate of infection in male with *T. canis* was more than female infection (46.2%, 38.6%) respectively. However, statistical analysis showed that there were no significant differences p>0.01 between sexes of dogs (Table 4).

Table 4 Rates of infection in domestic dogs with *T. canis* in Baghdad according to sexes.

Areas	No. of male samples	No. of male positive	No. of female samples	No. of female positive
North	**8**	**4**	**13**	**5**
South	**13**	**5**	**7**	**3**
Center	**12**	**6**	**10**	**4**
West	**8**	**5**	**8**	**3**
East	**13**	**5**	**13**	**5**
Total	**54**	**25 (46.2%)**	**51**	**20 (38.6%)**

x^2 2.6* No significant differences p>0.01 between sexes of dogs.

Table 5 Rates of infection in domestic dogs with *T. canis* in Baghdad city according to ages.

Areas	No. of examined samples for (3-5) months puppies	No. of positive samples for (3-5) months puppies	No. of examined samples for (6-12) months dogs	No. of positive samples for (6-12) months dogs
North	**2**	**2**	**1**	**-**
South	**2**	**-**	**1**	**1**
Center	**6**	**3**	**4**	**2**
West	**3**	**-**	**2**	**2**
East	**3**	**3**	**3**	**-**
Total	**16**	**8 (50%)**	**11**	**5 (45.4%)**

x^2 9.4* There are significant differences p <0.01 between the ages of dogs.

Furthermore, little puppies (3-5) months showed that total infection rate with *T. canis* more than total infection rate of young dogs (6-12) months (50%, 45.4%) respectively. Thus, statistical analysis showed that there are significant differences p <0.01 between the ages of dogs (Table 5).

Stray dogs showed high total infection rate 65.5%, and center of Baghdad showed the highest rate 100% while south of Baghdad showed the lower rate 52.5%. However, statistical analysis showed that there were no significant differences p>0.05 among stray dogs (Table 6).

Table 6 Rates of infection in stray dogs with *T. canis* in Baghdad city.

Areas	No. of examined samples	No. of positive samples	%
North	**16**	**9**	**56.25**
South	**19**	**10**	**52.6**
Center	**17**	**17**	**100**
West	**22**	**12**	**54.5**
East	**16**	**11**	**68.7**
Total	**90**	**59**	**65.5**

x^2 0.8 *No significant differences p>0.05 among stray dogs.

All the study areas of Baghdad city showed high rates of infection, the highest rate was 65.3% in center area, and the lowest rate was 45.2% in the south area for both domestic and stray dogs. Statistical analysis showed that there were no significant differences p>0.01 in the rates of infection among areas (Table 8).

Table 8 Total rates of infection in domestic and stray dogs with *T. canis* in Baghdad city.

Areas	No. of examined samples	No. of positive samples	%
North	**40**	**20**	**50**
South	**42**	**19**	**45.2**
Center	**49**	**32**	**65.3**
West	**43**	**22**	**51.1**
East	**41**	**19**	**46.3**
Total	**215**	**112**	**52**

x^2 1.7 *No significant differences p>0.01 among the areas.

Genomic DNA extraction from worm

DNA extraction was done by using gSYNC DNA Extraction Kit (Geneaid) from a piece of *T. canis* worm which obtained 100 ng a pure DNA that purity was 1.6 for PCR amplification.

PCR technique

The region spanning the ITS-2 was amplified from the gDNA (100ng) by PCR using. The amplicon sizes for *T. canis* were 380bp, (fig.1) primers were purchase from Alpha DNA, Canadian company.

DISCUSSION

Prevalence of Toxocaracanis in dogs in Baghdad city

T. canis infection in dogs is a public health problem in most countries, although it has been poorly documented in many of them, even Iraq. The current study recorded to high rate of infection with *T. canis* in dogs, the total rate of infection was 52%, in Baghdad city capital of Iraq, indicates that there is high risk for human toxocariasis and there should be a prompt evaluation of the significance of *T. canis* for public health. The prevalence of *T.canis* depend on many factors that can influence in the rate of infection including age, sex , source of dogs, aggregation of stray dogs, high offspring and distribution puppies.

The current study determined the prevalence of *T. canis* in dogs in Baghdad city that include five areas: north, south, west, east and center, the high rate of infection was 65.3% in the center area of Baghdad, and the low rate 45.2% in the south area of Baghdad for both domestic and stray dogs, that may be due to the difference in density stock and the number of dogs, however, statistical analysis showed that there were no significant differences in the rates of infection among areas, that similar to [17] who denied the differences between Urban and rural areas in Basarh city. Furthermore, [18] reported to the number of dogs infected with *T. canis* that varied at three main stations in Basrah city, the highest was shown in the north station and there were no significant differences among three stations in Basrah city.

Stray dogs showed high rate of infection with *T. canis* 65.5% that acceptance with [19] who recorded 67.5% in Baghdad city. While, in Sulaimani province, Kalar city, [20] found the rate of infection with *T. canis* was 36% in stray dogs. In Mosul province [21] Pointed out that the percentage of infection of 35 stray dogs with *T. canis* was 25.7%, while,[22] reported that 19 dogs from 58 examined dogs (32.6%) were infected with *T. canis* in Mosul city, and in Najaf province [23] showed that 46% of dissection stray dogs were infected with *T. canis*. [17] During his survey on intestinal nematodes found that the percentage of infection with *T. canis* of 51 dissected stray dogs in Basrah was 23.5%. [24] Recorded 6% rate of infection rate with *T. canis* between stray dogs in Konya province/ Turkey. While, [25] reported that 66.6% was found to be infected with *T. canis* from 21 stray dogs examined in Izmir/ Turkey. These differences in rates of infection were due to the difference of climates (months of study) and number of samples collected.

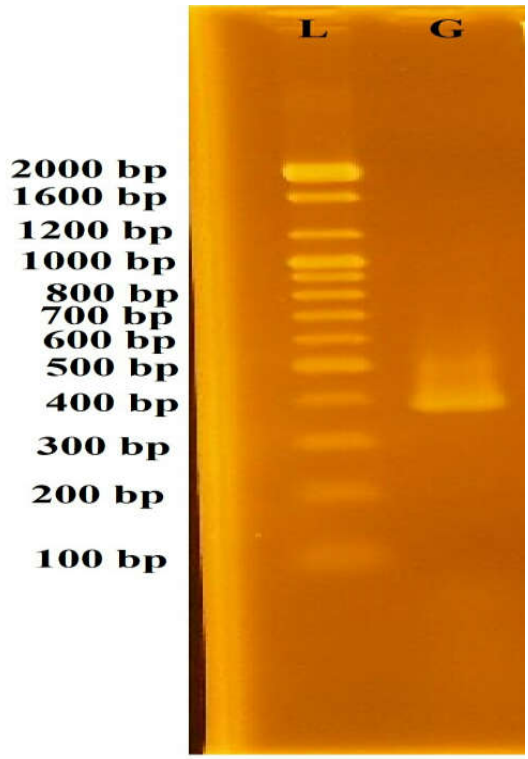

Fig.1 PCR product (380 bp) of fragment of ITS-2 gen of *T. canis* adult worm.

The infection rate in stray dogs was 65.5% higher than the infection rate in domestic dogs 42.4% that was similar to [26] in New Zealand, who showed that higher rate of infection with *T.canis* among stray and unwanted pets than in well cared dogs. 42.4% rate of infection in domestic dogs that recorded in current study disagreement with [27] who showed that the percentage of infection with *T. canis* eggs was 6.55% when they examined 305 fecal samples from pet dogs in Tehran/ Iran; that may be due to varied from one country to another depending on the distribution of veterinary hospitals, monthly examination of dogs and dog's treatments.

No significant differences were recorded in the rates of infection between male and female dogs(46.2%, 39.2%) respectively in current study, this result dislike with [17] results, who revealed that female dogs were more infected than male in Basrah this was due to the differences in samples that collected. The results were similar to [28] in California, who reported that sex of the host had no significant influence on the degree of parasitism with *T. canis* infection. Also, [29] showed that the prevalence of *T. canis* infection was similar in both sexes in Kinshasa, Zaire. Furthermore, [30] showed that male's dogs were more often infected than females and no significant differences between them in Calabar, Nigeria.

According to age our study recorded that *T. canis* infection in puppies (3-5) months of age was higher than older puppies (6) months of age and up, that because the close contact between small puppies and their mothers that transport the infection either by: transplacental, transmomary, or direct contact. This result was similar to [31] who examined 76 puppies aged between 2-4 months in Japan and showed that 68.4% were infected with *T. canis*. While, [32] pointed out that *T. canis* was the major parasite in all age groups of dogs after they examined 1126 dogs in Japan.

Molecular study

Solid tissue and protocol procedure was applied to *Toxocaracan is* adult worm to isolate a pure DNA, for the first time in Iraq. The result of DNA extraction showed that fresh tissue of adult worm yielded enough DNA concentration for PCR amplification that 100 ng with purity 1.6 this result was similar to the quantification of DNA by spectrophotometer which revealed that the DNA concentration ranged between 6-10 ng / µl and purity rang was between 1.6 – 2 [33].

This study reported the development of sensitive and specific PCR assay allowing rapid and reliable identification of *T. canis* by the fragment size amplified was 380 bp in ITS-2 gene as shown in figure 1; by using specific primers, the targeted fragment was determined according to Gen bank, [AB110034]. This result was acceptant with [16] who revealed that the positive control of *T. canis* consist of 380bp. While, the result was not acceptance with [34] who represented PCR with 14ng of *T. canis* DNA, isolated from dog; and 10ng of *T. canis* DNA, isolated from fox with 587bp for both. The causes of these difference were either due to different amount of DNA or differences of primers. The second internal spacer (ITS-2) of gDNA had proven in current study was particularly valuable in this context for molecular studying of *T. canis*, that similar to [15, 34, 35, 36] whom extracted genomic DNA from adult worms that collected from dogs, foxes and cats. Furthermore, [37, 38] proved that the advantage of DNA-based methods was a high genetic variability within molecular markers such as ITS2 for the discrimination of *T. canis* and *T. cati* from their closely-related neighbors: *T. leonina*, *T. vitulorum* and *T. malaysiensis*. Previously, [39] compared between target gene ITS-1 and ITS-2; thus, they revealed that the higher degree of within – species variation in the ITS-1 sequences compared with the ITS-2 suggests that the later sequences may provide more reliable species markers for Ascaridoid nematodes. [15] Revealed that the PCR method has been a potential as a supporting technique for the diagnosis of human toxocariasis and infected liver of Mongolian gerbils experimentally infected by. Furthermore, [40] described a novel real-time PCR that, the availability of a validated direct molecular assay for *Toxocara* spp. DNA in BAL will improve diagnosis of human toxocarosis, particularly for patients with respiratory distress. Recently, [41] compared among the performance of three diagnostic tests, that was, histopathology, enzyme-linked immunosorbent assay (ELISA), and polymerase chain reaction (PCR), for the diagnosis infection of *Toxocaracati* in the orbital tissue of Mongolian gerbils and Wistar rats experimentally. That of the three evaluated methods, the PCR could be recommended for scientific and laboratory diagnoses of ocular toxocariasis in experimentally infected animals.

The current study revealed that Molecular techniques provide useful tools for the genetic characterization of *T. canis* in Iraq for first time, and can use for diagnosis of Toxocariasis in human tissues in the future.

References

1. Paul, M. and Frans, K. (2013). Veterinary and public health aspects of *Toxocara* spp. Vet. Parasit., 193(4):398-403.
2. Paul, M.; King, L. and Carlin, E.P. (2010). Zoonoses of people and their pets: a US perspective on significant pet-associated parasitic diseases. Trends Parasitol. 26:153–154.
3. Hill, S. L.; Cheney, J. M.; Taton-Allen, G. F.; Reif, J. S.; Bruns, C.; Lappin, M. R. (2000). Prevalence of enteric zoononotic organisms in cats. J. Am. Vet. Med. Asso. 216: 687–692.
4. Wolfe, A. and Wright, I. P.(2003). Human toxocarosis and direct contact with dogs. Vet. Rec. 152: 419–422.
5. Kuroda, E.; Yoshida, Y.; Shan, B. E. and Yamashita, U. (2001). Suppression of macrophage interleukin-12 and tumour necrosis factor-α production in mice infected with *Toxocara canis*. Parasite Immunol., 23: 305-311.
6. Kayes, S. G.; Omholt, P. E. and Grieve, R. B. (1985). Immune responses of CBA/J mice to graded infections with *Toxocara canis*. Inf. Immun., 48: 697-703.
7. Kayes, S. G. and Oaks, J. A. (1978).Development of the granulomatous response in murine toxocariasis. Am. *J. Pathol*., 93: 277-294.
8. Dunsmore, J. D.; Thompson, R. C. and Bates, I. A. (1983). The accumulation of *Toxocaracanis* larvae in the brains of mice. *Int. J. Parasitol*., 13: 517-521.
9. Holland, C.V.; OConnor, P.; Taylor, M.R.; Hughes, G.; Girdwood, R.W. and Smith, H. (1991).Families, parks, gardens and toxocariasis. *Scandinavian J. Infect. Dis*., 23, (2): 225-231.

10. Uga, S.; Minami, T. and Nagata, K. (1996). Defecation habits of cats and dogs and contamination by *Toxocara* eggs in public park sandpits. *Amer. J. Trop. Med. Hyg.*, 54(2): 122-126.
11. Habluetzel, A.; Traldi, G.; Ruggieri, S.; Attili, A.R.; Scuppa P.; Marchetti, R.; Menghini, G. and Esposito, F. (2003). An estimation of *Toxocara canis* prevalence in dogs, environmental egg contamination and risk of human infection in the Marche region of Italy. Vet. Parasit. 113 (3-4): 243-252.
12. Avcioglu, H. and Balkaya, I. (2011).The relationship of public park accessibility to dogs to the presence of *Toxocara* species ova in the soil. Vector Borne and Zoonotic Diseases, 11 (2): 177-80.
13. Tom, N. (2006). Modified Wisconsin Sugar Flotation Method. VPTH 603 Veterinary parasitology. http://cal.vet.upenn.edu/projects/parasit06/website/modWis.htm
14. Thienpont, E.; Rochette, F. and Vanparijs, O. (1986). Diagnosing helminthiasis by coprological examination. Turnhoutsebaan 30, 2340 Beerse, Belgium). Available from: Vet. Lab. Services, UNIT 11.Station Road, South water, Sussex RH 13 7HQ.
15. Borecka, A.; Gawor, J.; Niedworok, M. and Sordyl, B. (2008). "Detection of *Toxocaracanis* larvae by PCR in the liver of experimentally infected Mongolian gerbils (*Merionesunguiculatus*)," *Helminthologia*, 45 (3): 147–149.
16. Khademvatan, Sh.; Fakher, R.; Mahdi, T.; Rahman, A. and Mahmoud, H. (2013). PCR-Based Molecular Characterization of *Toxocara* spp. Using Feces of Stray Cats: A Study from Southwest Iran. PLOS ONE | www.plosone.org|, 8 (6):1-6.
17. Al-Emara, G. Y. (1999). Epidemiology study of Nematode in digestive Tract of Dogs in Basrha area. MSc. Thesis. Coll. Vet. Med. Uni. Baghdad.
18. Awad, A. H. and Al-Azizz, S. (2006). Epidemiological study of *Toxocara canis* in Basrah city. Ph. D thesis. Univ. of Basrah. Coll. of Education, Dep. of Biology.
19. Hadi, A. M. and Faraj, A. A. (2016). Isolation and identification of intestinal parasites &protozoa from dogs in Baghdad. *Iraqi J. of Vet*. Med. 40(1): (in press).
20. Bajalan, M. M. (2010). Prevalence of intestinal helminths in stray dogs of Kalar city / Sulaimani province. *Iraqi J. Vet. Med*. 34 (1): 151-157.
21. Al-Kalidy, N. W.; Daoud, M.S.; Shubber, A. H. and Al-Alousi; T. I. (1988). A survey for internal and external parasites in dogs in Mosul (Iraq). *Iraq. J. Vet. Sc*. 1(2): 9-17.
22. Abul-Eis, S. E. (1983). Studies on parasites of public health importance from dogs in Mosul. M. Sc. Thesis, Coll. Vet. Med., Univ. Mosul: 128 pp.
23. Sultan, B. E. (1997). Epidemiology study of *Toxocara canis* in Najef area. PHD. Thesis. Coll. Vet. Med. Uni. Baghdad.
24. Aydenizoz, M. (1997). Helminthological investigations of dogs in Konya province. Acta. Parasitol. Turcica., 21: 429-434.
25. Korkmaz, M.; Yilmaz, M.; Uner, A. and Altintas, N. (2000). The prevalence of *Toxocaracanis* infection in stray dogs in Izmir, Turkey. Turk. Parazitol. Derg., 24: 211-213.
26. Collins, G. H. (1981). A survey of gastro-intestinal helminthes of dogs in New Zealand. New Z. Vet. J., 29: 163-174.
27. Dalimi, A.; Mojarad, S. and Jamshidi, S. (2001). Intestinal parasites of pet dogs in Tehran and evaluation of knowledge of dog owners about zoonotic risk of parasites of dog. J. Fac. Vet. Med. Univ. Tehran., 4: 13-16.
28. Baker, D. G. and Strombeck, D. R. (1985).Intestinal parasitism in dogs from a placer country, California, animal control facility. Calif. Vet., 39: 32-36.
29. Schandevyl, P.; Mbundu, T. and Sumbu, W. (1987). Prevalence of intestinal parasites in dogs in Kinshasa, Zaire. Ann. Soc. Belg. Med. Trop., 67: 369- 374.
30. Umeche, N. and Hogan, U. E. (1989). A study of intestinal helminths of dog in Calabar, Nigeria. Arch. Vet. Ital., 40: 128-131.
31. Fukase, T. (1986). *Toxocara canis* infections of puppies in Kangawa prefecture. J. Jap. Vet. Med. Assoc., 39: 640-643.
32. Itoh, N. and Itoh, S. (1998). Survey of intestinal nematodes in young dogs. J. Vet. Med. Jap., 51: 983-986.
33. Sambrook, J. (1989). Molecular Cloning: A Laboratory manual. Second Edition. (Plainview, New York: Cold Spring Harbor Laboratory Press).
34. Borecka, A. (2004). Differentiation of *Txocara* spp. Eggs isolated from the soil by the PCR- linked RFLP method. Helminthologia, 41 (4):185-187.
35. Jacobs, D.E.; Zhu, X.; Gasser, R.B.; Chilton, N.B. (1997). PCR-based methods for identification of potentially zoonotic ascaridoid parasites of the dog fox and cat. Acta. Trop, 68:191- 200.
36. Zhu, X. Q.; Gasser, R.B.; Chilton, N.B. and Jacobs, D.E. (2001). Molecular approaches for studying ascaridoid nematodes with zoonotic potential, with an emphasis on *Toxocara* species. J. Helminthology, 75(2): 101-108.
37. Li, M.; Lin, R.; Chen, H.; Sani, R. and Song, H. (2007) PCR tools for the verification of the specific identity of ascaridoid nematodes from dogs and cats. Molecular and cellular probes 21: 349–354.
38. Chen, J.; Zhou, D.H.; Nisbet, A.J.; Xu, M.J.; Huang, S.Y.; Li, M.W.; Wang, C.R. and Zhu, X.Q.(2012). Advances in molecular identification, taxonomy, genetic variation and diagnosis of *Toxocara* spp. Infect. Genet. Evol. 12:1344–1348.
39. Zhu, X. Q.; Jacobs, D. E.; Chilton, N. B.; Sani, R. A.; Cheng, N. A. and Gasser, R.B. (1998). Molecular characterization of a *Toxocara* variant from cats in Kuala Lumpur, Malaysia. Parasitology, 117:155–164.
40. Pinelli, E.; Roelfsema, J. H.; Brandes, S. and Kortbeek, T.(2013). Detection and identification of *Toxocara canis* DNA in bronchoalveolar lavage of infected mice using a novel real-time PCR. Vet. Parasit.193: 337– 341.
41. Zibaei, M.; Sadjjadi, S.M.; Karamian, M.; Uga, S.; Oryan, A.; Jahadi-Hosseini, S.H.(2013).A comparative histopathology, serology and molecular study, on experimental ocular toxocariasis by *Toxocara cati* in Mongolian gerbils and Wistar rats. Bio Med. Res. Inter.,:1-5.

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