

## Molecular characterization and phylogenetic analysis of *Toxocara* species in dogs, cattle and buffalo in Egypt

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### Summary

*Toxocara canis* of dogs and *Toxocara vitulorum* of cattle and buffalo are nematode parasites that cause serious economic and public health problems all over the world. This study aims to provide molecular data to identify and distinguish between *Toxocara* spp. from dogs, cattle and buffalo in Egypt. Moreover, constructing a phylogeny and phylogenetic relationships among these *Toxocara* spp. were performed through an analytic study of ATPase-6, a mitochondrial gene; 12S, small subunit ribosomal RNA gene and ITS-2, the second internal transcribed spacer nuclear ribosomal gene. *T. vitulorum* from cattle and buffalo were found to be almost identical. The ATPase-6 and 12S regions showed 87.78 % and 90.38 % nucleotide similarity between *T. canis* and *T. vitulorum*, while for the ITS-2 region, only 78.38 % was found. Analysis of the three studied genes revealed that each *Toxocara* spp. has distinct molecular characteristics. Moreover, it was revealed that these genes, especially the ITS-2 gene, are useful and sensitive molecular markers for classifying and studying the phylogenetic analysis and relationships among closely related *Toxocara* spp. All sequences obtained in this study were registered in the GenBank under the accession numbers: MG214149 -MG214157.

**Keywords:** *Toxocara vitulorum*; *T. canis*; molecular characterization; PCR-mitochondrial gene- ribosomal gene; dogs; cattle; buffalo; Egypt

### Introduction

*Toxocara vitulorum* is a nematode parasite of the small intestine of cattle and buffalo, particularly young calves that causes high morbidity and mortality Dormy *et al.* (2015). These calves are infected when they suckle colostrum/milk contaminated with infective larvae from infected adult buffalo, Starke-Buzetti and Ferreira (2006). Moreover, *T. canis* is a zoonotic parasite that can cause serious diseases in dogs and humans. *T. canis* is usually found in 2 weeks to 2 months old puppies, which can be infected via the placenta (major route) or milk of the mother (minor route), Rahbarm *et al.* (2013). Migration of larvae through tissues produces severe

inflammatory reactions and, consequently, a wide range of pathological and clinical manifestations, Joy *et al.* (2017).

The low economic status and lack of knowledge of farmers in tropical and subtropical countries that drink raw cattle or buffaloes' milk (Rast *et al.*, 2013) are likely to accelerate the transmission of the parasite, which causes visceral larval migrans (VLM) in the human body. Additionally, poor hygiene, poor sanitation and close contact with dogs lead to the transmission of *T. canis*, Borecka *et al.* (2010). Coprological examination techniques fail to detect prepatent infections in adult buffalo and mild infections in young calves, Jyoti *et al.* (2011). Generally, there are limitations for diagnosing of *Toxocara* infections depending on morphological characters of its

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eggs or by using traditional serological methods. Nowadays, PCR considered being a valuable molecular diagnostic method that could be used for accurate identification of infection in different hosts. Therefore, it is critical to develop specific identification using molecular characterization methods, such as PCR-based techniques utilizing a range of genetic markers in the nuclear and mitochondrial genomes; these techniques are useful approaches to determine the genetic composition and phylogenetic relationships with high sensitivity, specificity, rapidity and utility, Chen *et al.* (2012). This aimed to assist ordinary diagnosis, understanding epidemiology and control of parasitic infections in the medical and veterinary sciences, Li *et al.* (2016). VLM diagnosed in Egypt still an open question as it caused by *Toxocara* species of canine or bovine origin, with the intimate morphological similarity between *T. vitulorum* extracted from cattle and buffalo, the disease is more common and dangerous in buffalo calves than in cattle one. According to the Egyptian conditions, the probability of infection by VLM in humans by *Toxocara* species from dogs or bovine sources is equal to the level of the rural area; the genomic study still required to investigate the source of infection to human. In this respect several authors, such as Zhu *et al.* (2000) in Australia, Wickramasinghe *et al.* (2009) in Sri Lanka, Jeeva *et al.* (2014) in India, and Oguz *et al.* (2018) in Turkey have investigated the value of using the sequences of ITS-1, ITS-2 and ATPase-6, 12S, 28S genes in the identification of *Toxocara* spp.

Furthermore, studies by Mikaeili *et al.* (2015) in Iran and Oguz (2018) in Turkey demonstrate the value of the mitochondrial cytochrome c oxidase subunit I (cox1) gene in the identification of *T. vitulorum* from cattle calves. Regarding Oguz *et al.* (2018), existing keys and PCR sequencing of the ITS-2 fragment were used to identify the phylogenetic tree of *T. canis* from stray dogs in Turkey. With significant variations in pathogenicity and destitution of *T. vitulorum* infection between cattle and buffalo calves in Egypt, no previous studies in this respect concerning the genomic difference between *T. vitulorum* extracted from two different hosts; cattle and buffalo as well as its computability or differences with that extracted from dogs. In Egypt, Sultan *et al.* (2015) in the mid-Delta region in Egypt demonstrate the amplified sequenced of *T. vitulorum* (cattle origin only) used ITS-1 and 18S genes of ribosomal DNA and constructed the phylogenetic trees of the obtained results without register to their sequenced products in GenBank.

Therefore, the objective of this study was to perform molecular characterization to identify and distinguish between the two closely related *Toxocara* spp. (*T. canis* and *T. vitulorum*) obtained from different definitive hosts (dogs, cattle and buffalo-calves) in Egypt. Through this study, ATPase-6, a mitochondrial gene, 12S, small subunit ribosomal RNA gene and ITS-2, the second internal transcribed spacer nuclear ribosomal gene were used. Moreover, phylogenetic analysis constructed the phylogenetic trees and compared them with nucleotide sequences for obtained *Toxocara* spp. The results of this study will provide new molecular data to support the study of the evolution and genetic diversity of *Toxocara* spp.

concerning their different definitive hosts. The first published from Egypt of obtained sequences has been registered in the GenBank for future use by the scientific community.

## Materials and Methods

All study steps and experimental procedures were approved by the Institutional Animal Care and Use Committee (CU-IACUC number II AS1516) of Cairo University.

### Collection and preparation of samples

Intact, active 20 *Toxocara vitulorum* adult worms were collected from the intestine of freshly slaughtered buffalo and cattle calves from Cairo abattoir. While, seven adult *T. canis* worms were collected from naturally infected stray puppies (3weeks – 3months age), from Cairo governorate harbor eggs in their feces. The isolated ascarids nematodes specimens were washed extensively in phosphate buffered saline (PBS) to remove any debris and then were fixed in 70 % ethanol, cleared in lacto-phenol, permanent mount in glycerol and identified by microscopy as *T. canis* and *T. vitulorum* according to existing keys Soulsby (2012). After that, the parasites were fixed in 70 % ethanol. Other adults *Toxocara* species worms (n=3) from each *T. vitulorum* (cattle, buffalo) and *T. canis* of dogs origin were frozen at -20 °C until extraction of genomic DNA.

### DNA extraction

DNA was extracted from a small portion of specimens of adult *T. vitulorum* and *T. canis* samples using QIA amp DNA Mini Kit (Qia-gen, GmbH, Hilden, Germany) following the procedures recommended by the manufacturer.

### DNA amplification

Amplification was performed using conventional PCR assays, according to the methods of Casiraghi *et al.* (2001).

The analysis was performed according to Wickramasinghe *et al.* (2009) using the listed primers' sequences in standard PCR procedures for amplification of the mitochondrial gene (ATPase-6, including the partial sequence of NAD1 and NAD2 genes), 12S gene (the small subunit ribosomal RNA) and ITS-2 nuclear ribosomal gene.

Primer	Sequence
nema ATPase-6F	5'-TWYCCWCGTTWTCGTTATGA-3'
nema ATPase-6R	5'-CTTAAACAAATRCAYTTMT-3'
12SF	5'-GTTCCAGAATAATCGGCTA-3'
12SR	5'-ATTGACGGATGAGTTTGTACC-3'
Forward primer 3S	5'-CGGTGGATCACTCGGCTCGT-3'
Reverse primer 28A	5'- CCTGGTTAGTTTCTTTTCTCCGC-3'

PCR was carried out in a final reaction volume of 25 µl. The amplifications were performed with 5 µl of DNA extract, 12.5 µl Taq® Green Master Mix, 5.5 µl of nuclease-free water and 1 µl of each forward and reverse primer. The amplification program was carried

out in MyCycler™ thermal cycler (BioRad, USA) with 3 min of initial denaturation at 94 °C; 31 cycles of 30 s at 94 °C; 30 s at 46 °C and 1 min at 72 °C; followed by a final 5 min extension at 72 °C for all primers except the 12S and ITS-2 primers, for which annealing was performed at 50 °C and 53 °C, respectively. The nine amplified PCR products were visualized via agarose gel electrophoresis and the molecular weight (M.W.) of each band was calculated using a 100 – 1500 bp DNA ladder. The obtained gel was examined under a transilluminator and photographed.

#### DNA sequencing

The PCR products of the three genes were purified with a QIA quick PCR purification column (Qiagen, GmbH, Hilden, Germany), and then the purified products were sequenced with an ABI 3730XL DNA sequencer using a Sequencing Clean-Up kit.

#### Sequence alignments and phylogenetic analysis

The obtained sequences were introduced into the NCBI Basic Local Alignment Search Tool (BLAST) for searching of sequence similarity for each one then; all sequences were aligned using BIOEDIT software and CLUSTAL W application. Phylogenetic analysis was performed using the MEGA program and the neighbor-joining method was performed to obtain phylogenetic trees which estimated by performing 1000 bootstrap replicates. The sequences were submitted to the GenBank following the provided instructions.

## Results

The ascarids of both species *Toxocara* nematodes; *T. canis* and *T. vitulorum* were identified microscopically based on morphological features. *Toxocara vitulorum* is a large, robust worm up to 25 cm long with three large, prominent lips. The body was soft and translucent with clearly cuticle (Fig.1A – B). Male and females were ranged from 17.51 (15.54 – 19.00 cm) and 22.93 (19.32 – 29.11 cm) in average length, and 0.5 – 0.65 mm (mean 0.6 mm) in width, respectively. The three well defined lips; two subventral and one dorsal lip were determined from the worms (Fig.1D). The male worms had a posterior end curved ventrally (Fig.1B). The male posterior end exhibited two spicules, post cloacal papillae and the bell\_ shaped projection were observed (Fig.1G). While, in female posterior end was distinguishable a straight-tailed (Fig.1F). *Toxocara canis* revealed a cephalic alae in the anterior region in both sex. The male worms had a posterior end coiled. The tail was conical and reached 0.89 – 1.3 mm (mean 1.1 mm) long. The spicules were equal, and reached 2.9 – 3.3 mm (mean 3.1 mm) long. In females, the cervical alae measured 3.2 – 3.9 mm in length and 0.22 – 0.32 mm (mean 0.34 mm) in width. In female worms, the uterus occupied 2/3 of the body, and contained many eggs. The eggs were measured 70 – 80 μm (mean 75.5 μm, n=10) in diameter, rounded in shape and brown in color. It contained a single-cell embryo, and had a thin shell with an aluminous surface which is finely mammillated.

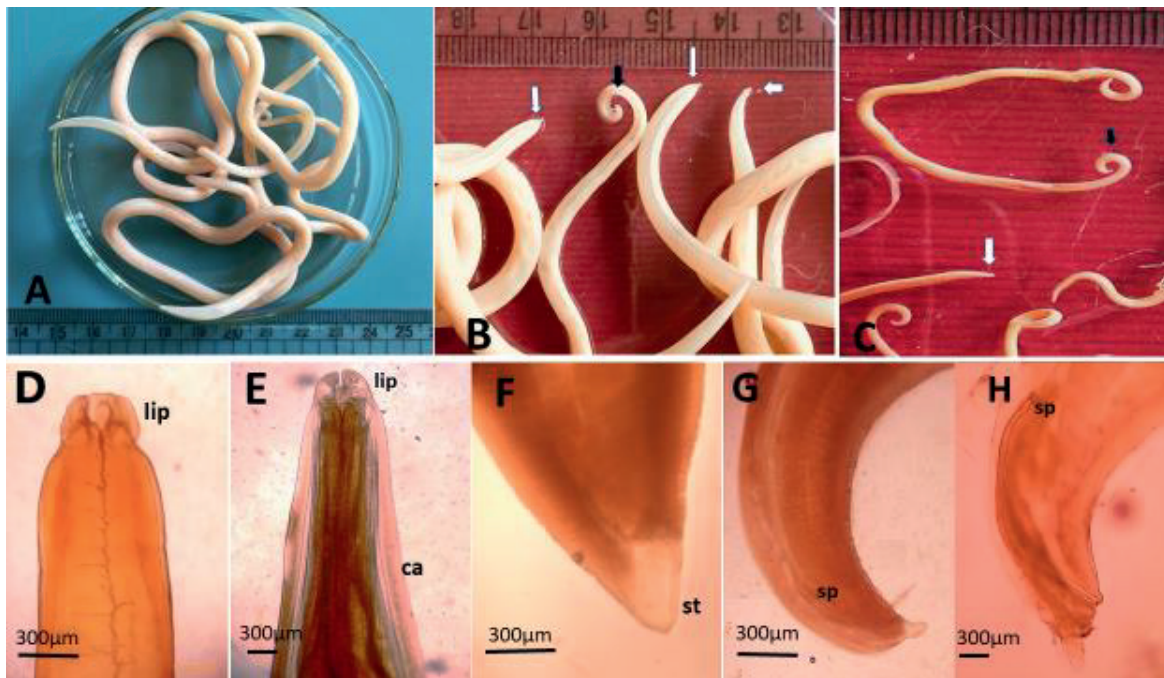


Fig. 1. A – B. *Toxocara vitulorum* collected from calves. B) Anterior end of worm showed lips (short white arrow), posterior end of male showed coiled tail (black arrow), and posterior end of female worm showed a straight tail end (long white arrow). D) Anterior end of *T. vitulorum* showed three lips (lip), F) posterior end of female showed short tail (st), G) posterior end of male showed two spicules (sp). C) *Toxocara canis* collected from street dogs; showed the coiled tail-end of male (black arrow), the straight tail-end of female (white arrow), E) Anterior end of *T. canis* showed the cephalic alae and lips, F) posterior end of male showed spicules.

Table 1. Characterization of the PCR products of the selected genes in the tested *Toxocara* spp. showing the M. W. of the bands for each gene.

Gene	M.W. (bp)		
	<i>T. canis</i>	<i>T. vitulorum</i> from cattle	<i>T. vitulorum</i> from buffalo
ATPase-6	804	828	828
12S	572	576	572
ITS-2	592	625	625

The data in Table 1 and Plate 1 show the results of agarose gel electrophoresis of the nine PCR products of the three worms from each tested *Toxocara* spp.; *T. canis* (dogs) and *T. vitulorum* (cattle and buffalo-calves). The ATPase-6 gene of *T. canis* produced a 804 bp band, while that of *T. vitulorum* from both cattle and buffalo calves produced 828 band bp. Electrophoretic analysis of the 12S gene showed bands of M.W.572, 576 and 572 bp for *T. canis*, *T. vitulorum* from cattle and *T. vitulorum* from buffalo respectively. Moreover, analysis of the ITS-2 gene revealed a band of M.W. 592 bp for *T. canis*. While a band of 625 bp was detected for *T. vitulorum* from both cattle and buffalo-calves.

#### ATPase-6 gene

Analysis of the ATPase-6 gene revealed that the sequences of this gene in *T. canis* of dogs and *T. vitulorum* from cattle and buffalo-calves were G/T rich. Moreover, the length of the nucleotide sequence in *T. canis* was 600 bp, while that in *T. vitulorum* from both cattle and buffalo-calves was 598 bp.

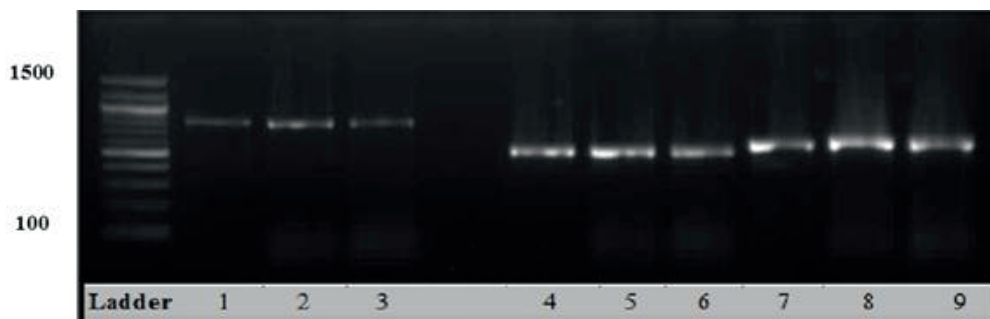
A phylogenetic tree constructed by the neighbor-joining method for the ATPase-6 region. There was 99.16 % identity in this gene between the tested *T. vitulorum* specimens from cattle and buffalo-calves in the present study, while 87.78 % identity was detected between the tested *T. canis* and *T. vitulorum* specimens from Egypt. Additionally, it was revealed that the *T. vitulorum* from buffalo-cattle calves (MG214154.1) in the current study was closely related to

*vitulorum* from in India (KJ777176.1), with 99.50 % identity to that in Sri Lanka (FJ418793.1) with 99.33 % identity. Moreover, the sequence for *T. canis* in the current study was 100 % identity to that *T. canis* isolated in India (KJ777174.1) While, with 99.67 % identity to *T. canis* isolated in Sri Lanka (FJ418787.1), Plate 2.

#### 12S rRNA gene

The data showed that the lengths of the 12S gene sequences obtained from *T. canis* (dogs), *T. vitulorum* (cattle-calves) and *T. vitulorum* (buffalo-calves) were 493, 464 and 465 bp, respectively. Moreover, the sequences of this gene in the tested *Toxocara* spp. were found to be A/T rich.

Concerning the phylogenetic tree constructed for the 12S gene, 98.01 % identity was observed between the tested *T. vitulorum* from buffalo origin and cattle origin in the present study, while 90.38 % identity was detected between the tested *T. canis* and *T. vitulorum* isolated in Egypt. Also, *T. vitulorum* from buffalo (MG214157.1) in Egypt was found to be closely related to *T. vitulorum* isolated in Sri Lanka (FJ418789.1), with 97.22 % identity. The gene sequence in *T. vitulorum* from cattle (MG214156.1) in the current study was 99.35 % identity to *T. vitulorum* isolated in India (KJ777171.1 – KJ777172.1). Moreover, the sequence in *T. canis* (MG214155) from Egypt in the current study was closely related 100 % identity to that from Sri Lanka (FJ418782.1), China (JN256964.1) and in India (KJ777168.1) and Plate 3.



- ATPase-6 primer:

- 1) *T. canis* from dogs
- 2) *T. vitulorum* from cattle
- 3) *T. vitulorum* from buffalo

- 12S primer:

- 4) *T. canis* from dogs
- 5) *T. vitulorum* from cattle
- 6) *T. vitulorum* from buffalo

- ITS-2 primer:

- 7) *T. canis* from dogs
- 8) *T. vitulorum* from cattle
- 9) *T. vitulorum* from buffalo

Plate 1. Results of agarose gel electrophoresis of the PCR products of the selected genes in *Toxocara* spp. showing the M. W. of each gene band.





Plate 2. Phylogenetic tree constructed in MEGA by neighbor-joining method for the ATPase-6 region of the tested *Toxocara* spp.

*ITS-2 gene*

Analysis of the ITS-2 gene showed that the lengths of nucleotide sequences were 503, 532 and 537bp for *T. canis*, *T. vitulorum* cattle and buffalo, respectively. Moreover, the sequences of this gene in the tested *Toxocara* spp. were found to be A/T rich. Regarding the phylogenetic tree constructed for the ITS-2 region, cleared that *T. vitulorum* of cattle origin and buffalo origin calves was 95.50 % identity. While they were detected 78.38.0 % identity between *T. canis* and *T. vitulorum* buffalo origin and 80.79 % identity between *T. canis* and *T. vitulorum* from cattle origin in Egypt. This result indicates that the ITS-2 gene appeared to be a good sensitive tool for

the identification of adult worms of *Toxocara* spp. The phylogenetic tree distinctly separated the ITS-2 sequences gene of *T. vitulorum* group from that of *T. canis* into two major lineages.

It was revealed that *T. vitulorum* of cattle origin (MG214151.1) was found to be closely related to that in Canada (JQ083352.1) with 99.77 % identity. Concerning had 95.5 % identity with buffalo in Egypt (MG214152.1) and Sri Lanka (FJ418784.1). *Toxocara vitulorum* isolated from cattle in India (KJ777159.1) and Scotland (EU189085.1) with 97.38 % and 99.43 % identity. Moreover, *T. canis* in Egypt (MG214150.1) was found with 100 % identity to that in India (KJ777155.1 & MK728992.1), in Japan



Plate 3. Phylogenetic tree constructed in MEGA by neighbor-joining method for the 12S region of the tested *Toxocara* spp.

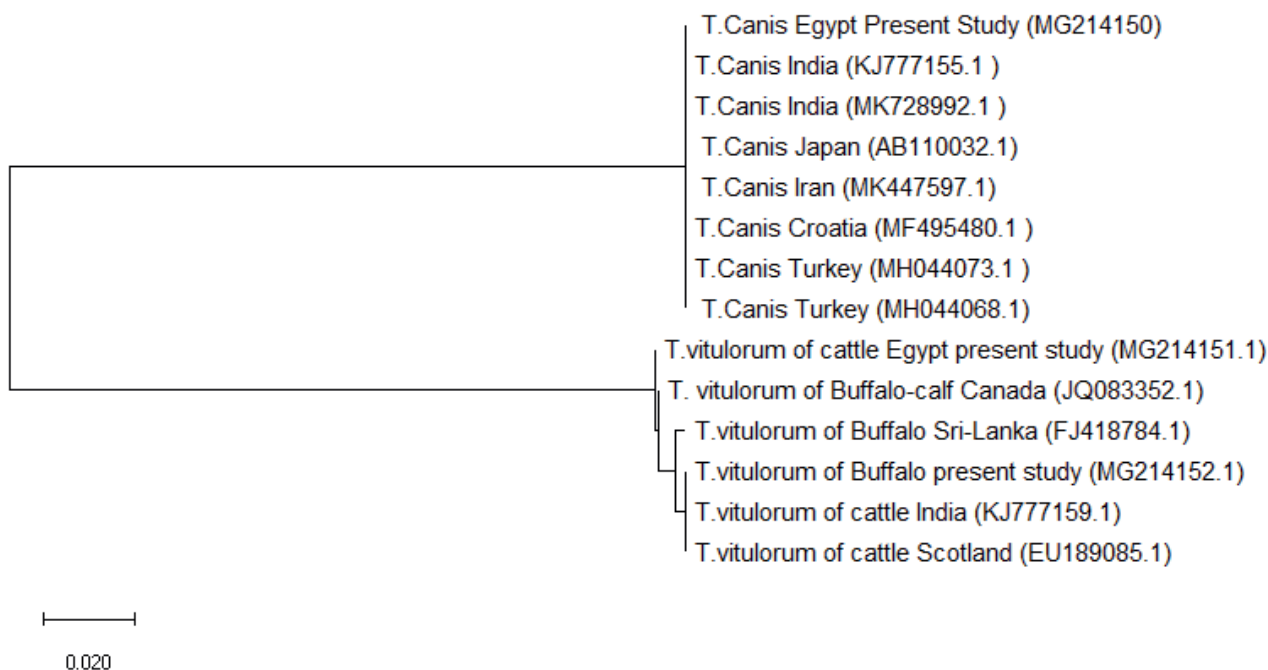


Plate 4. Phylogenetic tree constructed in MEGA by neighbor-joining method for the ITS-2 region of the tested *Toxocara* spp.

(AB110032.1), in Iran (MK447597.1), in Croatia (MF495480.1), in Turkey (MH044073.1 & MH044068.1), Plate 4. All generated sequences were registered in the GenBank database for the first time in Egypt with accession numbers including, MG214149 – MG214157.

## Discussion

*T. canis* and *T. vitulorum* are nematode parasites that cause serious problems in dogs, cattle and buffalo, Strub *et al.* (2013). With the great morphological difference between canine and bovine species, Soulsby (2012), the morphological characters of their diagnostic stages (their eggs) are similar. In a time where no clear morphological differences between *T. vitulorum* extracted from infected cattle or buffalo calves, the incidence and pathogenicity of them considered to be dangerous in buffalo than in cattle on the level of the same localities (Fagiolo *et al.* 2007). With the presence of recorded cases of VLM in humans who are in close contact with these animals, no accurate determination to genotypic data support the ability of *Toxocara* of bovine origin to cause problems similar to that previously caused by *Toxocara* of dog origin from the aspect of VLM in human. Due to the limitations of traditional (morphological and corpological) approaches for the identification of *Toxocara* spp. and the diagnosis of related infections, it was essential to develop specific identification criteria using molecular characterization techniques to determine genetic compositions and phylogenetic relationships with high sensitivity, specificity, rapidity and utility, Jyoti *et al.* (2011) and Chen *et al.* (2012).

Two common species of *Toxocara* present in Egypt (*T. canis* and

*T. vitulorum*) infect three widely distributed hosts (dogs, cattle & buffalo). Its eggs and larval stages were disseminated in feces and milk and considered to be easily transferred to humans. With the known data about the ability of *T. canis* larvae to cause VLM in humans, are the other species (*T. vitulorum*) that can do the same. With difficulty in proving this on the human level, the present study directed toward determine molecular and phylogenetic compatibility between them. The recent studies in Turkey by Oguz and Oguz *et al.* (2018) demonstrate the value of the mitochondrial cytochrome c oxidase subunit I (cox1) gene of *T. vitulorum* from cattle calves and ITS-2 fragment of *T. canis* from dogs were used to identify the morphological gene in identification, respectively. In this respect, DNA-based approaches have been applied to identify the nematode species, mainly using ITS fragments (rDNA) Li *et al.* (2006) and Fogt-Wyrwas *et al.* (2013). Sultan *et al.* (2015) in the mid-Delta region in Egypt demonstrate the amplified sequenced of *T. vitulorum* (cattle origin only) used ITS-1 and 18S genes of ribosomal DNA and constructed the phylogenetic trees of the obtained results without register to their sequenced products in GenBank. Therefore, in Egypt, the present study was extracted the first genomic DNA of *Toxocara* species from dogs, cattle and buffalo-calves using mitochondrial ATPase-6 and ribosomal gene 12S and the nuclear ribosomal gene ITS-2 regions were analyzed amplified and all obtained sequences were registered in GenBank. The mitochondrial ATPase-6, rather than single gene, to better account for genetic variability in these genomes across different regions of two closely related nematodes; *T. canis* of dog origin and *T. vitulorum* of cattle, buffalo-calves in Egypt. It is worth noting that the present study in Egypt, is the first to compare between

*T. vitulorum* from cattle and buffalo calves in addition to *T. canis* to search for any molecular differences between them that may occur due to the presence of the adult worm in the intestine of two different ruminant host.

The sequences of ATPase-6 gene of *T. canis* and *T. vitulorum* from cattle and buffalo were found to be G/T rich. While the sequences of the 12S gene and ITS-2 region were found to be rich with A/T. Similar results were reported for *Toxocara* spp. in Sri Lanka by Wickramasinghe *et al.* (2009). On the other hand, Hu and Gasser (2006) stated that all mitochondrial genes sequenced in *Toxocara* spp. are A/T rich.

In the present study, the analysis of the ATPase-6, 12S and ITS-2 genes sequences revealed that the length of the nucleotide sequence in *T. canis* (600 bp, 493 bp, 503 bp., in *T. vitulorum* cattle origin; 598 bp, 464 bp, 532 bp. and in *T. vitulorum* buffalo origin, 598 bp., 465 bp, 537 bp. respectively. These results were nearly similar to those reported for specimens from Wickramasinghe *et al.* (2009), who stated that the length of the nucleotide sequence of ATPase-6 gene in *T. canis* and *T. vitulorum* was 597 bp. However, the present results are inconsistent with those of Zhu *et al.* (2000), who stated that the ITS-2 region ranged from 240 – 348 bp long in *T. canis* and *T. vitulorum* from Australia.

In the present study, after alignment of the sequences of the three analyzed genes/regions (ATPase-6, 12S and ITS-2) in *T. canis* and *T. vitulorum* from cattle and buffalo in Egypt and in *Toxocara* spp. from other countries as well as subsequent analysis and construction of a neighbor-joining phylogenetic tree, it was observed that *T. vitulorum* from both cattle and buffalo formed a single group, as both hosts are ruminants. In contrast, *T. canis* formed a separate group. Each group consisted of several sister clades for each species analyzed, each of which represented a different definitive host. These findings are consistent with those obtained by Li *et al.* (2006) in China. In addition, they present results stated that the ATPase-6, 12S and ITS-2 regions were more conserved 87.78 %, 90.38 % and 78.38 %, nucleotide similarity between *T. canis* and *T. vitulorum*. While, revealed that the previous regions was more conserved 99.16 %, 98.01 % and 95.5 % nucleotide similarity between *T. vitulorum* from cattle and buffalo-calves in Egypt. This result can provide a foundation for accurate identification of *T. canis* and *T. vitulorum* using PCR characterization of 12S and ITS-2 region with nucleotide similarity (90.0 % and 78.38 %).

This result agrees with Wickramasinghe *et al.* (2009) reported that the nucleotide identity between *T. canis* and *T. vitulorum* from Sri Lanka was 88.0 % and 85.0 % for the 12S and ITS-2 gene, respectively.

*T. vitulorum* were almost genetically identical to those isolated in other countries. These data are consistent with those of Sultan *et al.* (2015) who amplified, sequenced of *T. vitulorum* (cattle origin) using ITS- and 18S genes had 99.0 % identity with *T. vitulorum* (buffalo origin) registered by Wickramasinghe *et al.* (2009) in Sri Lanka.

Moreover, the obtained presently sequenced concluded that

*T. vitulorum* isolated from cattle, buffalo calves using the ITS-2 sequences and those other sequences of the same nematode species were previously registered in GenBank were genetically identical to those isolated from beef calf such as Wickramasinghe *et al.* (2009) in Sri Lanka, Woodbury *et al.* (2012) in Canada, Jeeva *et al.* (2014) in India and Redman *et al.* (2007) in Scotland. Furthermore, presently concluded genetically identical had 100 % identity of *T. canis* from dogs using the ITS-2 sequences and those other sequences of the same nematode species were previously register in GenBank such as Ishiwata *et al.* (2004) in Japan, Jeeva *et al.* (2014) in India, Valizadeh and Tahvildar (2019) in Iran, and Oguz *et al.* (2018) in Turkey.

Concerning, the ATPase-6 and 12S genes were found to be more conserved (87.9 % nucleotide similarity between *T. canis* and *T. vitulorum*) than the ITS-2 region (78.38 %). This result is consistent with that recorded by Durant *et al.* (2012), who stated that analysis of the ITS-2 gene appeared to be a sensitive tool for the identification of adult worms of *Toxocara* spp.

## Conclusion

This current study was comparing the molecular characterization and phylogenetic analysis of the relationship between closely related species of *Toxocara* species from three different hosts (dogs, cattle and buffalo) in Egypt. The characterization of the ITS-2 region can provide a foundation for accurate identification of *Toxocara* species using PCR than ATPase-6, 12S regions. Concerning, the ITS-2 gene appeared to be a sensitive tool for 78.38 % nucleotide similarity between *T. canis* and *T. vitulorum* when compared with ATPase-6, 12S regions were found to be more conserved (87.78 % and 90.38 % nucleotide similarity). In the present study, the first genomic DNA was extracted using three different genes amplified and register in GenBank for the first time in Egypt.

## Conflict of Interest

The authors have declared no conflict of interest

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