



First phylogenetic evidence of *Trichuris spp.* affecting Hamadryas baboon (*Papio Hamadryas*) in Egypt.

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Trichuris spp., known as whipworms, are recognized to infect numerous mammalian species including humans and non-human primates. In the present study, forty five fecal samples collected from Hamadryas baboon at Alexandria Zoo, Egypt were examined microscopically using concentration sedimentation and flotation techniques to detect prevalence of *Trichuris spp.*

Furthermore, the genetic variation of *Trichuris spp.* recovered from naturally infected Hamadryas baboon, was analyzed using the ribosomal DNA (ITS) as molecular marker by PCR and sequencing. By combining this dataset with Genbank records for *Trichuris* isolated from other humans, non-human primates and pigs and phylogenetic analysis, we proved the presence of two distinct *Trichuris* genotypes that infect baboons, *T. trichura* and *T. suis.*, which is important for the ongoing treatment of *Trichuris* that estimated to infect 600 million people worldwide. To our knowledge this is the first study in Egypt genetically characterized *Trichuris spp.* in Hamadryas baboon, with future prospective to detect genetic characterization of *Trichuris* in other non-human primates.

Key words:

Trichuris, Hamadryas baboon, Phylogeny, non-human primates

Introduction

Parasitic infestation is considered as a great menace for captive animals in Zoos, where intensity in restricted locations enables and enhances their transmission, particularly in parasites of direct life cycle (Gracenea et al. 2002). Likewise several parasites in Zoos are considered zoonotic parasites which perform public health dilemma (Berrilli et al. 2011). *Trichiuris spp.* infection represents one of three main groups of soil transmitted helminthes, with hook worms and *Ascaris spp.* infection. Even though, it is a neglected tropical disease particularly in developing countries (Hotez et al. 2012). Genus *Trichiuris* contains about one hundred species (Yamaguti, 1961), while simply three species are zoonotic *Trichiuris trichiura*, *Trichiuris suis* and *Trichiuris vulpis* (Ravasi et al. 2012)

Trichiuris trichiura has a world-wide distribution. It causes an important public health problem in human and non-human primates in developing countries (Pawlowski, 1984), exhibited as diarrhea, growth retardation, anemia and malnutrition, while, light infections are usually asymptomatic (Bundy and Cooper, 1989). *T. trichiura* is found in humans especially in regions with deprived hygiene and is responsible for approximately 460 million infections. Mainly in tropical and subtropical areas where the temperature and humidity conditions are favorable for embryonation of the eggs with the maximum prevalence in Sub-Saharan Africa and South East Asia (Pullan et al. 2014). As presence of two distinct *Trichiuris species* in murid and arvicolid rodents (Cutillas et al. 2002). This sequence has also been used to separate between *T. vulpis* isolated from dogs and *T. suis* isolated from swine (Cutillas et al. 2007)

civilization and human population progress imposes humans and wild animals into frequent contact, there is a mounting regard that cross infection of parasites among different primates can produce a new diseases in non-human primates and humans (Jones-Engel and Engel, 2006)

The taxonomic position of *Trichiuris spp.* in non-human primates is unclear but they are appointed as *T. trichiura*. Infection has been detected in several species including New World species (e.g. woolly monkeys and howler monkeys), and Old World species (e.g. baboons, rhesus monkeys and Japanese macaques) and apes (chimpanzees and gibbons) (Abee et al. 2012). Morphological characters are not enough to differentiate between *Trichiuris spp.* in human and non-human primate, Moreover, it is unclear if *T. trichiura* identified in non-human primates are of one or more different species (Ravasi et al. 2012). So molecular techniques are progressively used as the master gadget in the identification of species (McManus and Bowles, 1996). Internal transcribed spacers (ITS) of the ribosomal DNA used as a genetic marker to differentiate between *T. trichiura* isolated from the non-human primates *Colobus guerezakikuyensis* and *Nomascus gabriellae* (Cutillas et al. 2009), and to ascertain the The main objective of this study was to detect the presence of *Trichiuris spp.* in Hamadryas baboon in Alexandria Zoo in addition to detect its genetic characterization based on the ribosomal DNA (ITS) as a molecular marker.

Material and methods

A total number of 45 fresh fecal samples were collected from hamadryas baboon at age from 7- 30 years old from Alexandria Zoo in clean labeled plastic cups. Samples were transferred in a cooling ice box to the laboratory of parasitology at Faculty of Veterinary Medicine, Alexandria University for parasitological examination

Fecal examination:

Feces were examined by naked eye for presence of any adult worms or strange objects. Direct smears were prepared from each sample and stained with Geimsa stain. About two grams of each fecal sample was separated and tested using concentrated flotation and sedimentation techniques, examined by light microscope at x10, x40. The remaining feces were frozen at -20°C until DNA extraction and molecular examination.

Molecular examination:

DNA extraction:

DNA was extracted from fecal samples using TriFast method (Peqlab, VWR company) (Isolation of RNA, DNA and Protein simultaneously). Molecular studies were done at Parasitology department, Faculty of veterinary medicine, Alexandria University.

PCR amplification:

Specific DNA was amplified through GeneAmp Polymerase Chain Reaction (Creacon, Thermo cycler, Holand) system cycler. PCR for amplified genomic DNA was carried out via internal transcribed spacer (ITS) (~1200 bp) primers (Listed in table 2). PCR reactions (50 µl) were performed under the following cycling conditions: initial denaturation at 94 ° C for 5 min, 35 cycles of 94 ° C for 30 s, 45.2 ° C for 30 s and 72 ° C for 45 s; followed by a final step at 72 ° C for 10 min.

Agarose gel electrophoresis and detection of the amplification products:

Agarose of 1.5% was used to visualize DNA bands. Specific DNA bands were eluted from agarose gel. Resultant PCR

products were purified with E.Z.N.A.® Gel Extraction Kit, (D2500-01, Omega BIO-TEK, USA). Sequence analysis was employed using the ABI PRISM® 3100 Genetic Analyzer (Micron-Corp.Korea).

Data analysis:

Gel documentation system (Geldoc-it, UVP, England), was applied for data analysis using Total lab analysis software, www.totallab.com, (Ver.1.0.1). Aligned sequences were analyzed on NCBI website (<http://www.ncbi.nlm.nih.gov/webcite>) using BLAST to confirm their identity. Genetic distances and Multi Alignments were computed by Pairwise Distance method using ClusteralW software analysis (www.ClusteralW.com). The nucleotide sequences were also compared with *Trichuris* isolates sequences available in the GenBank.

Results

Examination of fecal samples revealed the presence of *Trichuris spp.* eggs in 25 out of 45 fecal samples representing 55.5 % (Table, 1). Molecular examination of the infected samples using primers showed in Table (2) detected the presence of *Trichuris* DNA fragment of 1200 pb in 13 out of 25 sample representing 52%.

Sequencing analysis was performed on selected positive samples. Results of sequencing for the detected band were showed in (Table, 3). BLAST analysis of the detected band showed that the representative sample belongs to both *T.trichura* and *T.suis* based on identity percent (Table, 4). Blast results showed 100% identity to *Trichuris suis* ITS1 (partial), 5.8S rRNA gene and ITS2 (partial), isolate TsZJ2b; *Trichuris suis* ITS1 (partial), 5.8S rRNA gene and ITS2 (partial), isolate TsZJ1c; *Trichuris suis* ITS1 (partial), 5.8S rRNA gene and ITS2 (partial), isolate TsZJ2c; *Trichuris suis* ITS1 (partial), 5.8S rRNA gene and ITS2 (partial), isolate TsZJ1b; and 99.64% identity to *Trichuris suis* ITS1 (partial), 5.8S rRNA gene and ITS2 (partial), isolate TsZJ3b. Moreover, identity was 99.59% to

Trichuris suis ITS1 (partial), 5.8S rRNA gene and ITS2 (partial), isolate TsZJ3a, and 99.31% to *Trichuris trichura* ITS1 (partial), 5.8S rRNA gene and ITS2 (partial), isolate Ttr6b ; *Trichuris trichura* ITS1 (partial), 5.8S rRNA gene and ITS2 s(partial), isolate Ttr6a; *Trichuris trichura* ITS1 (partial), 5.8S rRNA gene and ITS2 (partial), isolate Ttr6c. Results also showed 98.86% identity to *Trichuris trichura* clone H11j internal transcribed spacer 2, complete sequence; *Trichuris trichura* clone H8j internal transcribed spacer 2, complete sequence; *Trichuris trichura* clone H6h internal transcribed spacer 2, complete sequence and 98.61% to *Trichuris spp.* ex Papiohamadryas JP-2011 isolate T2 5.8S ribosomal RNA gene and internal transcribed spacer 2, partial sequence ; *Trichuris trichura* ITS1 (partial), 5.8S rRNA gene and ITS2 (partial), isolate Ttr7a ; *Trichuris spp.* Ph2 internal transcribed spacer 1, partial sequence;5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence; *Trichuris trichura* ITS1 (partial), 5.8S rRNA gene and ITS2 (partial), isolate Ttr7c. Identity was 98.48% to *Trichuris trichiura* clone H4b internal transcribed spacer2, complete sequence and *Trichuris trichura* clone H7a internal transcribed spacer 2, complete sequence, while it was 98.10% to *Trichuris trichura* clone H2d internal transcribed spacer2,complete sequence; *Trichuris trichura* clone H11j internal transcribed spacer 2,complete sequence. In addition to that identity was 97.72% to *Trichuris trichura* clone H4a internal transcribed spacer 2,complete

sequence; *Trichuris trichura* clone H2b internal transcribed spacer 2,Complete sequence and *Trichuris trichura* clone H1h internal transcribed spacer 2, complete sequence. The lowest identity was 97.57% to *Trichuris spp.* SC4 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence.

Phylogenetic analysis using Neighbor-Joining (NJ) method (Fig. 1) revealed that the detected band (from the current study) placed in a cluster with *Trichuris suis* ITS1 (partial), 5.8S rRNA gene and ITS2 (partial),isolate TsZJ3a.; *Trichuris suis* ITS1 (partial), 5.8S rRNA gene and ITS2 (partial), isolate TsZJ1b; *Trichuris suis* ITS1 (partial), 5.8S rRNA gene and ITS2 (partial), isolate TsZJ2c; *Trichuris suis* ITS1 (partial), 5.8S rRNA gene and ITS2 (partial), isolate TsZJ1c; *Trichuris trichura* clone H11j internal transcribed spacer 2, complete sequence and *Trichuris trichura* clone H11j internal transcribed spacer 2, complete sequence with accession numbers AM993014, AM993009, AM993013, AM993010, JN181860 and JN181833 respectively, which confirmed the risk of infection of hamadryasis baboon with both *Trichuris trichura* and *Trichuris suis*.

Table 1: The total prevalence of *Trichuris spp.* in Hamadryas baboon.

| Diagnostic technique | No. examined | Positive | |
|----------------------|--------------|----------|------|
| | | No. | % |
| Fecal examination | 45 | 25 | 55.5 |
| PCR | 25 | 13 | 52 |

Table 2: Specific Primer sequence.

| ITS Primers | Sequences | Target fragment | Reference |
|-------------|---|-----------------------|--|
| | Forward (5'-ATC AGA ACACAG CAA CAG-3') | Approximately 1200 bp | Xie <i>et al.</i> Parasites & Vectors (2018) 11:516 |
| | Reverse (5'-AAC ATC GAGGAG ACG TAC-3') | | |

Table 3: Sequencing results of detected band of PCR product

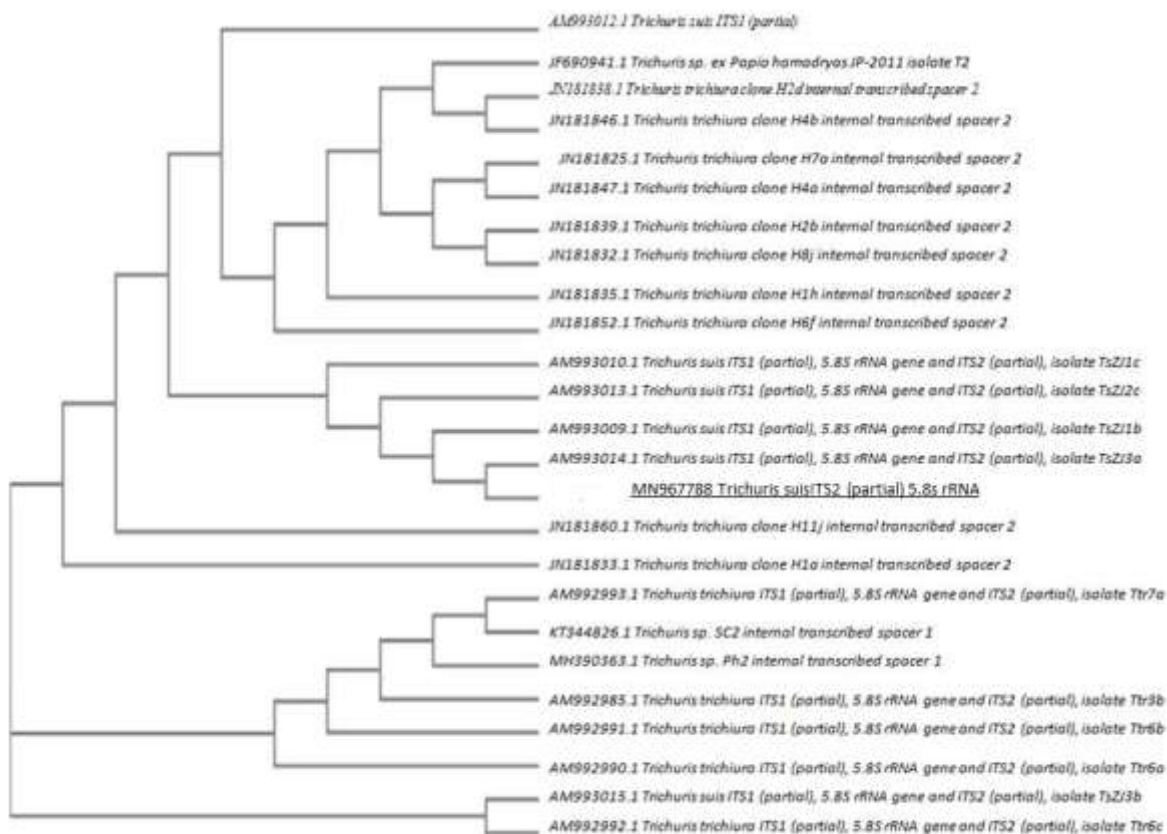
| Band | Sequence |
|---------------------------------------|--|
| <i>Trichuris spp.</i> 1200 pb band | AGGTCTGAAG AGCGCAGGCG GCGGCGGCTC GTTCCGCCCG GCGGGCGAGT CGTCGATCGT CGCCGTCACC GGCTCGATCA GGCAGCAGCA ATTTTCACTT GCTGCTCGCC GCCTGCGCGC AGTCACCGGT ACCTGTTCCG TTGTGGCTC CGTGGCTGCG CAGTTTGGGA TCTGGCTGTC GTTAGCGCCG CCGGTTGCAG TCGACCAGCA GCGGCTGGTG TGCGTTTGC TCGGTCCGAT GCGAGCAACG TCCGCCGGCC TACAGTTCCA GGTCTGAAGA GCGCAGGCGG CGGCTCGTTC CGCCGGCGG GCGAGTCGTC GATCGTCGCC GTCACCTA CCTGTCTGAG GGTCGTTAAG CATAATAGCG AATGCGCCGC TCAGGCTACA GGTTGAGGTT GGTGGCGAGC ACCGGACAAA CCTGCATCCG CGCGCAGCG AGCGTGACGC CGCAGCTCCG TTGCCAGCGA GCCGCGATGG CAACTGGTAG GCGGAGCAGC GGAGAGCGGC CAAGTCAGCG TAGGGCGAAG ACTACCCGAC TTGGCTACCG GCCGCGCCGT CGGCGTTCAG CAGCTGAGCA GGGAGCGGTG ACCGCACGCG CTCGTAGCAG CAAGTGGGCT CGATCAGGCA GCAGCGGTTT TCACTTGCTG CTCGCCGCT GCGCGCAGTC ACCGGTACCT GTTCCGTTGT GGCCGGCTGC AGACGGACGA CTGCGTGGCT GAGTCAGTCG GCATCGTCGC GGGACGTCCG TGCTTGCTCG CCTACGAAAC ATGTCGACGC GGACTTGCAC GGTCCGTGCA GCTAGCGGTA GCAAGCGGCG CGGATACGGC TACCCGAGGC TGTGAGCAGC CGCGACGCCG AGGCTCCTCC TGCTGCTAGC AGCGACGGCA GGTGCCCGTC ATCGTGGCA GGCAGCCGGA GCTGCGGAGA GCGGCTAACT CAGCGCAGTA CGGAAGCTGC CCGAGTTGGC TACGTCGCTA CATCTCCGTG GCTGCGGCAG TTTGGATCTG GCTGTCGTTA GCGCCGCCGG TTGCAGTCGA CCGTGCTGCC CCGCCCGTGC TGTCCCGCCA GTAGTTG |

Table (4): Blast analysis of the detected band showing identity percent to each related isolate in the gene bank.

| Sequence | Accession Number | E-value | Identity | Reference |
|---|------------------|---------|----------|--------------------------------------|
| <i>Trichuris suis</i> ITS1 (partial), 5.8S rRNA gene and ITS2 (partial), isolate TsZJ2b | AM993012 | 8e-143 | 100% | Zhou et al, 2008 Unpublished |
| <i>Trichuris spp.</i> ex Papiohamadryas JP-2011 isolate T2 5.8S ribosomal RNA gene and internal transcribed spacer 2, partial sequence. | JF690941 | 2e-139 | 98.61% | Petrasova et al, 2011 Unpublished |
| <i>Trichuris trichiura</i> clone H2d internal transcribed spacer2, complete sequence. | JN181838 | 2e-124 | 98.10% | Nissen et al, 2011 |
| <i>Trichuris trichiura</i> clone H4b internal transcribed spacer2, complete sequence. | JN181846 | 4e-126 | 98.48% | Nissen et al, 2011 |
| <i>Trichuris trichiura</i> clone H7a internal transcribed spacer 2, complete sequence. | JN181825 | 4e-126 | 98.48% | Nissen et al, 2011 |
| <i>Trichuris trichiura</i> clone H4a internal transcribed spacer 2, complete sequence. | JN181847 | 8e-123 | 97.72% | Nissen et al, 2012 |

| | | | | |
|--|----------|--------|--------|----------------------------------|
| <i>Trichuris trichiura</i> clone H2b internal transcribed spacer 2, Complete sequence . | JN181839 | 8e-123 | 97.72% | Nissen et al,2012 |
| <i>Trichuris trichiura</i> clone H8j internal transcribed spacer 2,complete sequence. | JN181832 | 8e-128 | 98.86% | Nissen et al,2012 |
| <i>Trichuris trichiura</i> clone H1h internal transcribed spacer 2,complete sequence. | JN181835 | 8e-123 | 97.72% | Nissen et al,2012 |
| <i>Trichuris trichiura</i> clone H6h internal transcribed spacer 2,complete sequence. | JN181852 | 8e-128 | 98.86% | Nissen et al,2012 |
| <i>Trichuris suis</i> ITS1 (partial), 5.8S rRNA gene and ITS2 (partial), isolate TsZJ1c . | AM993010 | 8e-143 | 100% | Zhou et al,2008 Unpublished |
| <i>Trichuris suis</i> ITS1 (partial), 5.8S rRNA gene and ITS2 (partial), isolate TsZJ2c. | AM993013 | 2e-123 | 100% | Zhou et al,2008 Unpublished |
| <i>Trichuris suis</i> ITS1 (partial), 5.8S rRNA gene and ITS2 (partial), isolate TsZJ1b . | AM993009 | 2e-123 | 100% | Zhou et al,2008 Unpublished |
| <i>Trichuris suis</i> ITS1 (partial), 5.8S rRNA gene and ITS2 (partial),isolate TsZJ3a. | AM993014 | 1e-121 | 99.59% | Zhou et al,2008 Unpublished |
| <i>Trichuris trichiura</i> clone H11j internal transcribed spacer 2,complete sequence . | JN181860 | 2e-124 | 98.10% | Nissen et al,2012 |
| <i>Trichuris trichiura</i> clone H11j internal transcribed spacer 2,complete sequence. | JN181833 | 8e-128 | 98.86% | Nissen et al,2012 |
| <i>Trichuris trichiura</i> ITS1 (partial), 5.8S rRNA gene and ITS2 (partial), isolate Ttr7a. | AM992993 | 2e-139 | 98.61% | Zhou et al,2008 Unpublished |
| <i>Trichuris</i> spp. SC4 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence . | KT344826 | 2e-134 | 97.57% | Li et al,2015 Unpublished |
| <i>Trichuris</i> spp. Ph2 internal transcribed spacer 1, partial sequence;5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence. | MH390363 | 2e-139 | 98.61% | Xie et al,2018 |
| <i>Trichuris trichiura</i> ITS1 (partial), 5.8S rRNA gene and ITS2 (partial), isolate Ttr7c. | AM992985 | 2e-139 | 98.61% | Zhou,et al (2008) Unpublished |
| <i>Trichuris trichiura</i> ITS1 (partial), 5.8S rRNA gene and ITS2 (partial), isolate Ttr6b. | AM992991 | 8e-143 | 99.31% | Zhou,et al (2008) Unpublished |
| <i>Trichuris trichiura</i> ITS1 (partial), 5.8S rRNA gene and ITS2(partial), isolate Ttr6a. | AM992990 | 8e-143 | 99.31% | Zhou,et al (2008) Unpublished |
| <i>Trichuris suis</i> ITS1 (partial), 5.8S rRNA gene and ITS2 (partial), isolate TsZJ3b. | AM993015 | 4e-141 | 99.64% | Zhou,et al (2008) Unpublished |
| <i>Trichuris trichiura</i> ITS1 (partial), 5.8S rRNA gene and ITS2 (partial), isolate Ttr6c | AM992992 | 8e-143 | 99.31% | Zhou,et al (2008) Unpublished |

Figure (1): phylogenetic tree for whipworms (*Trichuris* spp.) isolates based on ITS sequence.



Discussion

Soil-transmitted *Trichuris spp.* are one of the most public parasitic nematodes in mammals, including humans and non-human primates, which usually considered as *T. trichura*. Nevertheless, latest molecular examinations, revealed that *T. trichura* is not the only species of *Trichuris* infecting humans and non-human primates, proposing the presence of a *Trichuris* species complex in primates. (Elliott,2007; Nissen et al. 2012). Moreover, there is an evidence of (ancestral) genetic exchange between *T. suis* and *T. trichura*, meriting surplus genetic sampling (Meekums et al. 2015). Molecular markers are very useful in the identification of taxa when morphology is not adequate to differentiate among very closely related species. For instance, the ITS of the nuclear ribosomal DNA is considered as a useful genetic marker for resolving nematode relationships at the species level (Zhu et al. 1998).

Our results showed that 25 fecal samples were positive for *Trichiuris spp.* but only 13 of them were PCR positive. Likewise, high infection rate of *Trichuris spp.* In Hamadryasis baboon may be returned to possible cross-infection between animals because of the close or sympatric housing conditions in Zoos.

In the present study, analyses of *Trichuris spp.* Ribosomal sequences from Hamadryasis baboon for phylogenetic estimations revealed the presence of *T.trichura* in addition to *T.suis*, which agree with (Xie et al. 2018) who proved that at least seven genetically separate subgroups of *Trichuris spp.* are present among human and non-human primates in China, supporting a previous proposal on eight non-human primate host species, including the golden snub-nosed monkey (*R. roxellana*), black snub-nosed monkey (*R. bieti*), vervet monkey (*Chlorocebusaethiops* Linnaeus, 1758), rhesus monkey (*M.mulatta*), northern white-cheeked gibbon (*N. leucogenys*),northern pig-tailed macaque

(*Macaca leonine* Blyth, 1863), anubis baboon (*P. anubis*) and hamadryas baboon (*P. hamadryas*) from the Chengdu Zoo (Sichuan, China) and Kunming Zoo (Yunnan, China)that a complex of *Trichuris* species other than *T. trichura* affecting these hosts.Our results agree also with those of Cavallero et al. (2015) who provided evidences for the existence of distinct clades of *Trichuris* from captive Japanese macaques (*Macacafuscata*) and grivets (*Chlorocebusaethiops*), using the ribosomal DNA (ITS) as molecular marker and to investigate the phylogeny and the extent of genetic variation following analysis of nuclear ribosomal marker (ITS). Moreover, Ravasi et al.(2012)confirmed the identification of two distinct *Trichuris* genotypes that infect primates in the Cape Peninsula, South Africa. In addition, Hawash (2014) suggested the presence of different *Trichuris spp.* infecting non-human primates. Nonetheless, Abee et al. (2012) and Cutillas et al. (2009) designated the *Trichuris* in non-human primates as *T. trichura*.

More additional studies on *Trichuris* from primates in natural habitat are necessary to have the ability to explore the transmission of *Trichuris* among the different primates, to detect zoonotic potential of *Trichuris spp.* from non-human primates. Particularly as a higher incidence of parasites of human in primates under captivity than wild ones (Mbaya and Udendeye, 2011; Messenger et al. 2014).

Conclusion:

Our phylogenetic analysis suggests that *Trichuris spp.* affecting Hamadryasis baboon in Egypt, is not a single species, but a species complex, it remains unclear whether there is genetic exchange between *T. trichura* and *T. suis* or many species of *trichuris* can infect Hamadryasis baboon warranting more studies and more genetically analyzed samples on species

identification and host affiliation to detect, cross-infection and hybridization that may complicate species boundaries which provide a good control measure in non-human primates.

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الملخص العربي

اول دليل تطوري للترايكورس فى الهامادرس بابون (القرد الحبشى) فى مصر

اميرة وحيد دوير

قسم الطفيليات

كلية الطب البيطرى- جامعه الإسكندرية

أبيس، الإسكندرية

الرمز البريدى: ٢١٩٤٤

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Correct the red colored words to be identical

تصيب ديدان الترايكورس والتي عرفت بالديدان السوطية عدد كبير من الثدييات , من ضمنها الانسان والرئيسيات غير البشرية. فى هذه الدراسة تم تجميع عدد ٤٥ عينة براز من حيوانات الهامادريس بابون (القرد الحبشى) الموجود فى حديقة الحيوان بالاسكندرية, مصر وتم فحصهم باستخدام اختبار الترسيب والتعويم المركز للكشف عن وجود طفيل الترايكورس. تم تحديد الاختلافات الجينية للحيوانات المصابة باستخدام اختبار تفاعل البلمرة المتسلسل اولاً ثم تحديد التسلسل مستخدمين جين (اى تى اس) كعلامة وراثية. وبمقارنة التسلسل لجين الترايكورس الموجود بالعينات مع بنك الجينات للعزلات المختلفة للترايكورس فى الانسان والرئيسيات غير البشرية وعمل التحليل الفايولوجي, تم اثبات وجود نوعين مختلفين من الترايكورس تصيب الهامادريس بابون وهى ترايكورس ترايكورا وترايكورس سيوس. مما يساعد فى علاج الترايكورس التى تصيب اكثر من ٦٠٠ مليون شخص فى العالم. وتعد هذه الدراسة اول دراسة فى مصر تقوم بالتوصيف الوراثةى للترايكورس فى الهامادريس بابون, مع وجود تطلعات مستقبلية لتحديد التوصيف الوراثةى للرئيسيات الغير البشرية الاخرى.