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Full Length Research Article

Prevalence and molecular characterization of *Toxocara canis* in domestic dogs in Ekiti State

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Abstract

Dogs offer good sentinel surveillance for human disease infections, such as *Toxocara canis* infection. The involvement of domestic dogs as hosts of *T. canis* had been observed, subsequently resulting in higher health concerns due to the closeness between domestic dogs and their owners. This study assessed the prevalence of *T. canis* and its molecular identification in domestic dogs in Ado Ekiti, Local Government Areas (LGA) Ekiti State. Fifty (50) fecal samples were randomly taken from domestic dogs and were examined using Sheather's sugar flotation technique. Samples that were positive were pooled together for polymerase chain reaction (PCR) and deoxyribonucleic acid (DNA) sequencing. Overall, a *T. canis* prevalence of 46.0% was detected, which was further validated by utilising Nem18S to amplify an internal portion of the 18S gene. This is the first study that adopted the use of molecular technique to characterize *T. canis* in domestic dogs in Ekiti State. This study underscores the role of domestic dogs in environmental contamination and the need for domestic dog owners to maintain good hygiene. There is a need to use a One Health approach in establishing successful control programmes for *T. canis* and other dog-borne infections in the state.

Keywords: Domestic dogs, *T. canis*, pcr, public health, zoonotic, One Health

Introduction

Toxocara canis is a gastrointestinal nematode often found in dogs globally

(Phoosangwalthong *et al*., 2022). The parasite is of great public health importance, considering that domestic dogs serve as important hosts in the spread of pathogens to people, especially in Africa (Kroeger *et al*., 2019). The proximity of dogs to humans, especially as companion animals, heightens the risk of transmission by direct contact or the fecal-oral route (WHO, 2019). Shared resource utilisation, specifically residential zones, emerge as significant sites for disease transmission. This risk is intensified in environments with insufficient sanitation and poor canine vaccination rates, facilitating the transmission of the disease (Savioli *et al*., 2018).

Infection in dogs occurs through the ingestion of infective eggs present in contaminated soil, faeces, or prey (Xu and Han, 2024). Upon hatching in the dog's small intestine, the larvae are released, infiltrating the intestinal wall and migrating to the liver, lungs, and other tissues, resulting in injury and inflammation (Phoosangwalthong *et al*., 2022). The migration of *T. canis* in juvenile dogs occurs through hepatopulmonary pathways, whereas in older dogs, the migration is halted in tissues, and larvae are suppressed (Hon *et al*., 2022). Larvae ultimately develop into adult worms within the dog's small intestine. Adult worms attach to the gut wall, consuming nutrients and reproducing (Schwartz *et al*., 2022). Adult female worms produce eggs that are excreted in the dog's faeces. The eggs contaminate the environment and infect other dogs thereby perpetuating the cycle (Schwartz *et al*., 2022).

Clinical signs associated with young pups include coughing, anorexia, emaciation, abdominal distension, diarrhoea or vomiting, while infections in adult dogs are usually asymptomatic (Hon *et al*., 2022). Humans get infected via ingestion of larvae from paratenic hosts or eggs from the environment. *T. canis* infection in humans manifests in clinical syndromes such as ocular larva migrans (OLM) or ocular toxocariasis, covert toxocariasis (CT), neurotoxocariasis (NT), and visceral larva migrans (VLM) or visceral toxocariasis (VT) (Schwartz *et al*., 2022).

Diagnosis of *T. canis* in dogs involves a combination of clinical evaluation, laboratory tests, and fecal examinations (Al-Ghabban, 2003). This includes identification of *T. canis* eggs in the fecal samples of definitive hosts (Al-Ghabban, 2003), morphological characterization of adult worms, serology tests to detect antibodies against *Toxocara* in the blood through ELISA or molecular techniques (Hon *et al*., 2022).

The application of molecular techniques in the diagnosis of *Toxocara* species has been described (Dias-Correia *et al*., 2023), mainly through the analysis of the internal transcribed spacer region (ITS) (Jacobs *et al*., 1997), and mitochondrial genes (Li *et al*., 2008), however, studies on molecular data on *T. canis* from Ekiti State are scarce.

Earlier findings have identified the prevalence of *T. canis* in stray dogs in the study area, using fecal floatation technique to be 12.0% (n = 6) (Omonijo *et al*., 2024), but limited studies exist on the application

of molecular techniques in the diagnosis of *T. canis* from domestic dogs in Ekiti State, hence the objective of this work was to determine the prevalence of *T. canis* in domestic dogs in Ekiti State using molecular approach. The findings from this study provide insight into the role of domestic dogs in the transmission of toxocariasis in the study area.

Materials and Methods

Guideline ethics for animal handling in Research: The study was done in accordance with the guide for handling vertebrate animals in research with permission of ethics committee of research in the Federal University Oye Ekiti, Nigeria. The study was ethically approved by the FUOYE Faculty of Science Research Ethics Committee (RECOM) and conducted under ethical number (FUOYEFSC 201122 – REC2024/027).

Study Area

The study was carried out in Ado - Ekiti Local Government Area of Ekiti State. It is the largest town and the capital of Ekiti-State. Ado - Ekiti is located between latitude 7°34' and 7°41' North of the equator and longitudes 5°11' and 5°16' East of the Greenwich meridian. It has a tropical wet climate. The average annual temperature is 25.1°C. The rainy season (April-October) and the dry season (November-March) are the two prominent seasons. The city receives more than 1500mm of rain annually (Temitope *et al*., 2021). The city is characterized by civil servants, students, self-employed workers, and farmers. Most people rear animals, including dogs.

Sample size calculation

The sample size was calculated using a 95% confidence level, a standard deviation of 0.5, and a confidence interval (margin of error) of ±19.2% according to (Thrustfield, 2007).

Sample size = (Z-score)2 × Standard deviation × (1- Standard

Fecal sample collection

Fecal samples were collected between December, 2023 and January, 2024. Overall, fifty (50) fecal samples were collected randomly from domestic dogs into universal bottles following consent from dog owners. The samples were properly labelled and transported to the Animal and Environmental Biology Laboratory, Federal University Oye Ekiti and refrigerated at 4°C until ready for analysis.

Parasitological Analysis

Fecal samples were processed individually and examined microscopically using Sheather's sugar floatation technique. One gram (1g) of faeces was added to 15 ml of Sheather's sugar solution in a test tube. The content was thoroughly mixed and strained, collected into another test tube, and covered with a slip. The filtrate was left to stand for 15 – 30 minutes. The cover slip was then transferred to a microscopic slide to be examined under a light microscope. The samples were identified using the standard procedures (Duncan *et al*., 2020).

Isolation of DNA and PCR Analysis of *T. canis*

DNA Extraction: Samples that were positive for *T. canis* were subjected to

molecular analysis. Genomic DNA was extracted from the pooled samples of *T. canis* eggs using Genomic DNA Extraction kit provided by Inqaba (Quick-DNA™ Miniprep Kit) based on the guidelines. The DNA concentration and purity was checked by Nanodrop Spectrophotometer (Thermo Scientific Inqaba Biotec) in the Biotechnology Laboratory in Federal University Oye-Ekiti. The DNA was amplified through polymerase chain reaction (PCR), with PCR machine (MyGene™ Series Peltier Thermal Cycler Model MG96G, LongGene Scientific Instruments Co., Ltd.) using Nem 18S forward primer, CGCGAATRGCTCATTACAACAGC (23 bases) and the reverse primer, GGGCGGTATCTGATCGCC (18 bases) (Omonijo and Mukaratirwa, 2023).

A standard reaction volume was 25 µL, comprising: 5.5 µL distilled water, primers at 1.0 µL each and 12.5 µL OneTaq® 2X Master Mix. To each reaction, 5 µL of extracted nematode DNA template was added, typically containing around 2–10 ng of genomic DNA. The optimal thermocycling conditions were found to be: an initial denaturation at 94 °C for 5 min; 35 cycles of denaturation at 94 °C for 30 s; annealing at 54 °C for 30 s; and extension of 72 °C for 1 min; followed by a final extension at 72 °C for 10 min.

The amplified DNA fragments were submitted to Inqaba for DNA sequencing. The obtained sequences were cleaned up and aligned by BioEdit application. The nucleotide sequences obtained were compared using the BLAST algorithm against known sequences in GenBank.

Statistical Analysis

Data were analyzed using the statistical package for social sciences IBM-SPSS version 25.0. Descriptive statistic was used to determine the prevalence of *T. canis* by dividing the number of samples infected with the total number of samples collected and finding the percentage. Chi-squared test was used to determine the association between parasite prevalence and gender of domestic dog and p – value < 0.05 is considered significant.

Results

Prevalence of *T. canis* infections in domestic dogs in Ado Local Government Area, Ekiti State, Nigeria

Overall, the prevalence of *T. canis* infections was observed to be 46.0% (n = 23). Coprological analysis revealed that 18(36.0%) of domestic dogs showed single parasitism for *T. canis* while 5(10.0%) showed mixed infections comprising of 2(4.0%) *T. canis* + *Taenia* sp.; 1(2.0%) *T. canis* + *Ancylostoma* sp.; 1(2.0%) *T. canis* + *Taenia* sp. + *Trichuris* sp.; and lastly 1(2.0%) *T. canis* + *Fasciola* sp. + *Monieza* sp. (See Table 1).

Table 1: Prevalence of *Toxocara canis* infections in domestic dogs in Ado Local Government Area (LGA), Ekiti State, Nigeria

Toxocara canis infections	Number infected (N=50)	Prevalence (%)
Single *Toxocara canis* infections	18	36.0
Mixed infections	5	10.0
T. canis + *Taenia* sp.	2	4.0
T. canis + *Ancylostoma* sp.	1	2.0
T. canis + *Taenia* sp. + *Trichuris* sp.	1	2.0
T. canis + *Fasciola* sp. + *Monieza* sp.	1	2.0

Molecular identification of *T. canis* in domestic dogs in Ado Local Government Area, Ekiti State, Nigeria

Molecular analysis of pooled samples further confirmed *T. canis* diagnosis. The Blast analysis of 18S DNA showed homology to references obtained from GenBank sequences.

The obtained sequences for *T. canis* share 96.0% homology to USA isolates (U94382.1), 96.0% homology to UK isolates AF036608.1, 96.0% homology to isolates from Republic of Costa Rica (MZ714138) and 96.0% homology to isolates from China (JN256982). The sequences were then deposited in GenBank under accession numbers PV235268.

Discussion

Toxocara canis is a common helminth parasite of domestic and wild dogs with a global distribution (Varun *et al*., 2021). This study was conducted to determine the prevalence of *Toxocara* species in dogs in Ado Ekiti, Nigeria. This study reported a prevalence of 46.0% of *T. canis* infection in domestic dogs in Ado Ekiti LGA. This is consistent with the prevalence of 40.0%, 48.88%, and 51.2% reported in dogs from various Egyptian localities (Mubarak *et al*., 2023). However, the observed prevalence in this study is higher than the 33.33% (260/360) reported from domestic dogs in Egypt (Mubarak *et al*., 2023), 37.32% reported in Vietnam (Ng-Nguyen and Nguyen, 2024), 9.4% in Djelfa, Algeria (Laatamna *et al*., 2021), 6.75% from Rolim de Moura (Mendonça *et al*., 2024), 17.3% in Atlantic Forest and 18.1% in Amazon areas of Brazil (Dias-Correia *et al*., 2023), and this is higher than the prevalence of 12.0% (6/50) earlier reported among stray dogs in Ado Local Government Area, Ekiti State (Omonijo *et al*., 2024).

The reason for the higher prevalence in domestic dogs when compared to the earlier reported prevalence in stray dogs is however not known. However, literature has attributed variations in prevalence data of helminths to several factors such as, environmental factors like; area type (rural

or urban), hygienic conditions, as well as climatic conditions. Other contributing factors are, animal behavior and ownership (pet or stray dogs), host associated factors such as age of dogs, veterinary preventive measures, systematic anthelmintic treatment, deworming programs, sample types (faeces, soil or postmortem isolation of adults), samples size, and diagnostic techniques (Laatamna *et al*., 2021).

The prevalence of *T. canis* in domestic dogs observed in this study can be regarded as a good indicator of environmental contamination, especially around the home, which is imperative for the need to intensify surveillance and control of *T. canis* in the context of One Health (Delai *et al*., 2021; Dias-Correia *et al*., 2023; Souza *et al*., 2023). Furthermore, environmental contamination with parasitic agents is often associated with the prevalence of intestinal parasitic infections in animal populations (Phoosangwalthong *et al*., 2022).

The findings of this study confirmed the presence of *T. canis* in domestic dogs in Ado Ekiti (23/23 isolates). This is consistent with report from Vietnam where the DNA sequencing of all the (16/16 isolates) showed *T. canis* (Yen *et al*, 2023). This highlights a crucial need for a surveillance program in the state guided by a One Health framework, for the prevention of toxocariasis and other zoonotic agents transmitted by dogs.

To the best of our knowledge, our study is the first to carry out molecular identification of *T. canis* in domestic dogs providing molecular data through the use of Nem18S primers. While this study provides baseline information to public health officials towards the development of adequate prevention and control programs so as to reduce *T. canis* infections among the domestic dog population and the environment at large, it is imperative to further conduct more studies using sensitive molecular based methods for the purpose of understanding the *T. canis* infections and various coinfections cases in domestic dogs in various Local Government Areas in Ekiti State.

The findings from this study strongly advocate for a "One-Health" approach to ameliorate the zoonotic transmission of *T. canis* through increased hygiene, sanitation and regular deworming of domestic dogs in the study area.

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Data and Code Availability statement: The data have been deposited at NCBI Genbank.

Authorship contribution statement

Omonijo Adejumoke Oluwatosin, Omonijo Adetunji conceived and designed the research; Omonijo Adejumoke Oluwatosin and Gombedza Simba analyzed and interpreted the data. Adekale Romoke, Omolayo Ajibola, Bello Afeez, Adesunloro Olanike, Otakoya Zainab, Ajiferuke Bolanle, Akinola Maxwell, Adeola Mary, Oyedeji Victor: Conceived and designed the research; Omonijo Adejumoke Oluwatosin, Omonijo

Adetunji: Drafted the manuscript, Gombedza Simba critically revised the paper for intellectual content.

Declaration of conflicting interest

The authors declare that they have no conflicting interests that could have appeared to influence the report in this manuscript.\

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