Knowledge and practices on consumption of free-range chickens in selected rural communities of KwaZulu-Natal, South Africa, with focus on zoonotic transmission of *Toxoplasma gondii* and *Toxocara* spp.

Adejumoke Oluwatosin Omonijo1 2 ⋆ - Samson Mukaratirwa2 3

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Abstract

Chickens are a host to a variety of pathogens of zoonotic importance and this depends more on the husbandry system practiced. *Toxoplasma gondii* and *Toxocara* spp which are more prevalent in free-range chickens (FRC) can be acquired by humans via the ingestion of raw or undercooked meat (muscle) and/or viscera contaminated with infective stages of *T. gondii* and *Toxocara* spp. This study aimed to assess knowledge and practices on the household consumption of FRC meat and viscera by rural communities in KwaZulu-Natal (KZN) province, South Africa, as a risk factor in the transmission of zoonotic pathogens with special emphasis on *T. gondii* and *Toxocara* spp. A cross-sectional study was conducted on twenty (20) randomly selected households in four selected communities located on the northern coast (Gingindlovu and Ozwathini) and southern coast (uMzinto and Shongweni) of KZN province using a semi-structured questionnaire. To determine the presence of selected zoonotic pathogens in FRC, birds were purchased from randomly selected households in the study localities for sacrifice. Brain tissues were collected and subjected to molecular detection of *T. gondii* using TOX4 and TOX5 primers while other tissues and organs that were collected were subjected to molecular detection of *Toxocara* spp using Nem 18S primers. Questionnaire data were analyzed using the statistical package for social sciences (SPSS) version 25.0. Descriptive and chi-square statistics were used to assess knowledge and practices related to FRC consumption and zoonosis transmission. Molecular results showed four positive samples for *T. canis* from Gingindlovu (n = 1), uMzinto (n = 1), and Shongweni (n = 2). The role of FRC consumption in zoonosis transmission is discussed.

Keywords *Toxoplasma gondii* · *Toxocara* spp · Knowledge · Practices · Free-range chickens · Zoonoses · KwaZulu-Natal · South Africa

Introduction

Chickens are major agents in parasite transmission due to their exposure to infective stages of parasites existing in a contaminated environment (Javaregowda et al. 2016; De Vries et al. 2018). Commonly reported zoonotic parasites from chickens include but are not limited to *Toxoplasma gondii* and *Toxocara* spp (Zibaei et al. 2017; dos Santos Silva et al. 2020). *Toxoplasma gondii* is an apicomplexan parasite that causes toxoplasmosis while *Toxocara* spp are responsible for toxocariasis in animals and humans globally (Dubey 2020). The main definitive hosts of *Toxoplasma gondii* are felids, while birds and a wide range of animals serve as intermediate hosts (Gaulin et al. 2020). Toxoplasmosis and toxocariasis are neglected infections of zoonotic significance in
animals and humans that are underreported due to lack of surveillance (Schurer et al. 2016; Etter et al. 2019). Toxoplasmosis causes production losses in animals resulting in huge economic losses in livestock industry. It can result in abortions mainly in primiparous women infected during pregnancy and hydrocephalus in infants as well as causing fatal diseases such asencephalitis in immunocompromised people (Etter et al. 2019). Approximately one-third of humanity has been exposed to Toxoplasma gondii (Dubey 2020). Although Toxoplasma infections are reportedly mild in South Africa due to the low virulence of the local strains (Jacobs, 1977), toxoplasmosis has been reported in Johannesburg, the Free State, the Western and Eastern Cape, and KwaZulu-Natal (Sonnenberg et al. 1998; Khabisi 2001).

Toxocara canis and T. cati are helminth parasites of canids and felids; they also are responsible for human toxocariasis worldwide (Yoshida et al. 2016), and a variety of paratenic hosts are involved which include chickens and especially free-range chickens (FRC) due to the husbandry practices which allow them to have contact with contaminated environments (Zibaei et al. 2017). According to the extrapolation of the world population in 2016, about 1.4 (1.2–1.5) billion individuals worldwide were estimated to be exposed to Toxocara (Rostami et al. 2019). The public health and economic importance of toxocariasis are greatest in the tropics and subtropics, where it is estimated that more than 1 billion people are infected with parasitic helminths (Ziegler and Macpherson 2019). Previous studies have reported the prevalence of Toxocara spp in animals and the seroprevalence of T. gondii in animals and humans in South Africa; however, studies on molecular identification of these parasites in food animals are limited in South Africa. Hence, this study aimed at molecular identification of T. gondii and Toxocara spp in free-range chickens in South Africa.

Although FRC contributes significantly as an affordable source of animal protein, information on their role in the transmission of zoonotic pathogens is scanty (Rodrigues et al. 2019). They are normally infected during scavenging when they ingest the infective stage of various species of parasites from a contaminated environment (Sasse et al. 2020). Humans may acquire infection indirectly via the consumption of raw or undercooked infected chicken viscera or meat containing, for example, tissue cysts of T. gondii or larvae of Toxocara spp from canids and felids (Fan et al. 2015; Gaulin et al. 2020). Once ingested by humans, the parasites depending on the species migrate through the viscera and are deposited in various organs where they cause varying degrees of symptoms ranging from fever, headache, sore throat, arthralgia, myalgia, and blindness depending on the affected organs, infection intensity and duration, host age, and immunity status of the infected host (Holland and Hamilton 2013; Fan et al. 2015; Gaulin et al. 2020).

Consumption of FRC viscera or meat is a dietary habit common in different resource-poor rural communities worldwide and dependent on socio-cultural practices and culinary habits where it can either be eaten raw or undercooked (Broglia and Kapel 2011). The practice of eating raw or undercooked viscera or meat is associated with zoonosis transmission (Trevisan et al. 2019), for instance, cases of toxocariasis transmission have been reported after ingesting raw chicken liver (Nagakura et al. 1989; Morimatsu et al. 2006; Campos-da-Silva et al. 2015). Similarly, human toxoplasmosis outbreaks have been reported among individuals who consumed raw or undercooked meat (Dawson 2005; Choi et al. 1997).

In South Africa, the greater population lives in rural areas and rears chickens following a free-range system (Mwale and Masika 2009; Mukaratirwa and Khumalo 2010; Malatji et al. 2016). In the KwaZulu-Natal (KZN) province of South Africa, the majority of the population are rural livestock farmers and rear FRC for consumption, marketing, and socio-cultural purposes (Naidoo 2005). The farming practice allows chickens to scavenge freely in the environment during the daytime and use trees for shelter at night or be confined to rustic chicken runs (Naidoo 2005).

Furthermore, there is an increased possibility of FRC ingesting infective oocysts and/or eggs of T. gondii and Toxocara spp respectively in the environment frequented by stray cats and dogs during scavenging in the KwaZulu-Natal (KZN) province (Tannent et al. 2010; Mukaratirwa and Singh 2010). Cats are the definitive hosts of T. gondii while cats and dogs are definitive hosts of Toxocara cati and Toxocara canis respectively. The occurrence of these stray definitive hosts in the province may lead to persistent environmental contamination with these parasites (Mukaratirwa and Singh 2010; Szwabe and Błaszkowska 2017).

Moreover, change in globalization has led to the adoption of a variety of culinary and consumption patterns regarding raw or undercooked food as delicacies (Broglia and Kapel 2011). Besides, due to the high poverty level in rural areas of KZN, there is a high level of household food insecurity thereby leading to alternative foods and various ways of food preparation (Tarwireyi and Fanadzo 2013). Understanding the consumption pattern of the much available FRC viscera or meat is imperative in these communities as a basis for guaranteeing food security as well as identifying possible transmission routes of food-borne diseases such as toxoplasmosis and toxocariasis.

Considering the poor socio-economic status and food insecurity of the rural communities in the KZN province of South Africa, this study aimed to determine the presence of selected zoonotic parasites in FRC and the factors related to the transmission of zoonotic pathogens through household consumption patterns of FRC viscera or meat and preparation practices in the study area.
Methodology

Study design and sample size determination

A cross-sectional study was conducted in four rural communities in the KwaZulu-Natal province to assess knowledge and practices of consumption of FRC viscera or meat with a focus on *T. gondii* and *Toxocara* spp transmission from March to July 2019. Localities where the study was conducted and their population sizes are as follows: Gingindlovu (GI) (1109) and Ozwathini (OZ) (1979) on the northern coast and uMzinto (MZ) (16,205) and Shongweni (SH) (427,613) in the southern coast of KZN (Fig. 1) (http://www.durban.gov.za/). These localities have sugar cane farming as their main livelihood followed by livestock farming which includes rearing of FRC. The study population comprised 80 participants selected using simple random sampling where all households in each locality were given numbers which were subjected to a random selection (lottery method) of 20 households per locality. The sampling frame consisted of the number of households in each study locality and each participant selected represented a household for each locality. The sample size was calculated using the following equation with a 95% confidence level and 11% error margin; \( n = \frac{1.96^2 \times p \times q}{L^2} \), where \( n \) = sample size, \( p \) = prevalence (0.5), \( q \) = 1-p, and \( L \) = limits of error on the prevalence.

Study procedure

After briefing the community leaders regarding the objectives of the study, 20 household representatives were randomly selected from each locality, and consent was obtained regarding their willingness to participate in the study. Questionnaires were translated from English to isiZulu, which is the local language in all the study localities, and were administered to the randomly selected participants following an interview-guided approach. Before administration, a pilot study was done to validate the tool. The questionnaire administration process took approximately 20 min for each participant. Before completion of the questionnaires, all participants gave their written informed consent to take part in the study. They were also reassured of the confidentiality of all disclosed information and that only anonymised findings will be disclosed during feedback and in written reports.

Data collected from the interview included socio-demographic information, knowledge, and practice of participants related to the preparation and consumption of FRC meat and viscera. Questions were asked specifically on habits related to the consumption of chicken meat and viscera, the preferred method of preparation, and the designated members of the family who eat each type of viscera. The demographic information of the participants interviewed included age, gender, household size, educational qualifications, and occupation of respondents. Information on ownership of FRC including the number of FRC owned per household was also collected.

Collection of samples from free-range chickens

Forty-two FRC were randomly purchased from households owning chickens on a willing seller basis in four selected rural communities in the Northern [Gingindlovu (GI), Ozwathini (OZ)], and Southern Coasts [uMzinto (MZ), Shongweni (SH)], of the KZN province. Chickens selected for the study were euthanized by decapitation according to guidelines approved by Animal Ethics of the University of KwaZulu-Natal South Africa. Tissue from various parts of each chicken such as the brain, heart, spleen, lungs, liver, kidney, crop, duodenum, intestines, thigh, breast, and pectoral were collected, digested, and examined for *Toxocara* larvae using the modified acid/pepsin digestion (Zibaei et al. 2017). The digests were washed and filtered through
a sieve with 200/125/20-µm apertures. Collected *Toxocara* larvae were kept in 70% ethanol until DNA extraction. Brain samples for molecular detection of *T. gondii* were collected as previously described by Carlos and Jack 1998. Briefly, after euthanization, the skull was opened with a pair of small, curved scissors to expose the brain. The brain was then removed with forceps and carefully preserved in 70% ethanol.

**Molecular identification**

The retrieved nematode larvae from each of the chicken samples were subjected to molecular analysis using a QIAamp DNA Mini Kit (Qiagen Inc.) and used in subsequent PCR reactions. PCR reactions for the amplification of 18S rRNA were performed with nematode-specific primers Nem_18S_F (CGC GAATRGCTATTACACAGC (23 bases) and Nem_18S_R (GGGCGGTATCTGATGC (18 bases). A standard reaction volume was 20 µL, comprising the following: NEB One Taq 2X MasterMix with Standard Buffer at 10 µl, primers (10 µM) at 1 µl each, and nuclease-free water at 7 µl were added. To each reaction, 1 µl of extracted nematode DNA template was added, typically containing around 10–30 ng/µl of genomic DNA. The PCR conditions for the amplification of 18S rRNA were as follows: initial denaturation at 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 30 s, primer annealing at 50 °C for 30 s, and extension at 68 °C for 1 min. The final extension was at 68 °C for 10 min. The integrity of the PCR amplicons was visualized on a 1% agarose gel.

Thirty brain samples (halves) were tested for *Toxoplasma gondii* DNA. The DNA was extracted from half of the brain tissue of chickens using a commercial kit. PCR on DNA extracts was done using the primer, TOX4 (5’-CGCTGACGG-GAG GAAGACGAAAGTTG-3’) and TOX5 (5’-CGCTGCAGA CACAGTGATCTGGAT-T-3’) targeting the 529 bp fragment. PCR was conducted according to the described protocol (Homan et al. 2000). The cycling protocol for the *T. gondii* DNA amplification included an initial cycle of 94 °C for 7 min (initial denaturation), followed by 35 cycles of 94 °C for 1 min (denaturation), 60 °C for 1 min (annealing), and 72 °C for 1 min (extension), followed by a final extension 72 °C for 10 min. PCR products were analyzed on 2% agarose gel by electrophoresis for 25 min at 120 V.

**Statistical analysis**

Data were processed and analyzed using the statistical package for social sciences (SPSS) version 25.0. Descriptive and chi-square statistics were used to assess knowledge and practices related to the consumption of FRC meat and viscera and awareness of the zoonotic transmission of *T. gondii* and *Toxocara* spp. A *p*-value < 0.05 was considered statistically significant.

**Results**

**Socio-demographic profile of participants**

Table 1 shows the demographic characteristics of participants in all four study localities. Participants interviewed in the four localities ranged in the category of father, mother, and household member greater than 18 years. Overall, the mean age of the respondents was (47.11 ± 18.02) (Table 1). Ozwathini (OZ) had the highest mean age (53.3 ± 16.82), followed by Shongweni (SH) (50.25 ± 17.12, GI (43 ± 18.98) and uMzinto (MZ) (41.9 ± 17.80). A significant difference was observed between the educational level of study respondents among study locations (*p* < 0.05). Most respondents (47.5%, 38/80) had a high school education while only (20%, 16/80) had completed tertiary education. The percentage of respondents who had tertiary education was highest in OZ (40%, 8/20), followed by GI (30%, 6/20), and SH (10%, 2/20), while none of the respondents in MZ had tertiary education.

The percentage of respondents that were unemployed was highest (90%, 18/20) in OZ, and (85%, 17/20) in GI and SH, followed by (70%, 14/20) in MZ. Overall, household sizes ranged from 1 to 16 with a mean of (6.40 ± 3.26). Household sizes ranged from 2 to 11 with a mean of 7.05 ± 2.65, 2–14 (6.65 ± 3.79), 2–13 (5.70 ± 2.89), and 1–16 (6.20 ± 3.67) in GI, MZ, OZ, and SH respectively (Table 1).

**Knowledge of zoonosis transmission associated with the consumption of FRC viscera**

Overall, knowledge of zoonotic disease (mainly related to toxoplasmosis and toxocariasis) transmission associated with the consumption of raw or undercooked FRC meat and viscera in the study localities was estimated at 31.3%. There were no significant associations found between knowledge and considered variables. Knowledge did not vary among the localities (35%, 7/20) in MZ and SH followed by GI (30%, 6/20) and OZ (25%, 5/20) (Table 2). The proportion of respondents knowing zoonosis transmission through consumption of raw/undercooked chicken viscera was high in the age group 41–50 years and highest in GI (20%, 4/20) followed by SH (15%, 3/20) and OZ (10%, 2/20), while in MZ, it was highest in the age group ≥ 61 (20%, 4/20). This difference was, however, not statistically significant (*p* > 0.05).

Based on the education level of participants, knowledge of zoonosis transmission (toxoplasmosis and toxocariasis) was highest among respondents with high school education (13.8%, 11/80), followed by tertiary education (8.8%, 7/80), primary education (7.5%, 6/80), and (1.3%, 1/80) among respondents with no formal education although this difference was not statistically significant (*p* > 0.05) (Table 2). The
Table 1 Socio-demographic characteristics of study respondents from four localities of KwaZulu-Natal province of South Africa (GI, Gingindlovu; OZ, Ozwathini; MZ, uMzinto; SH, Shongweni)

| Localities | GI (n = 20) | OZ (n = 20) | MZ (n = 20) | SH (n = 20) | Total (n = 80) |
|------------|------------|------------|------------|------------|--------------|
|            | Freq  | %     | Freq  | %     | Freq  | %     | Freq  | %     | Freq  | %     |
| Age groups (years) |       |       |       |       |       |       |       |       |       |       |
| < 21       | 1     | 5     | 1     | 5     | 0     | 0     | 1     | 5     | 3     | 3.75  |
| 21–30      | 7     | 35    | 6     | 30    | 3     | 15    | 2     | 10    | 18    | 22.50 |
| 31–40      | 1     | 5     | 4     | 20    | 1     | 5     | 2     | 10    | 8     | 10.00 |
| 41–50      | 5     | 25    | 4     | 20    | 5     | 25    | 5     | 25    | 19    | 23.75 |
| 51–60      | 2     | 10    | 2     | 10    | 4     | 20    | 4     | 20    | 12    | 15.00 |
| 61+        | 4     | 20    | 3     | 15    | 7     | 35    | 6     | 30    | 20    | 25.00 |
| Education level |       |       |       |       |       |       |       |       |       |       |
| None       | 2     | 10    | 2     | 10    | 1     | 5     | 1     | 5     | 6     | 7.50  |
| Primary    | 1     | 5     | 3     | 15    | 8     | 40    | 8     | 40    | 20    | 25.00 |
| High school| 11    | 55    | 7     | 35    | 11    | 55    | 9     | 45    | 38    | 47.50 |
| Tertiary   | 6     | 30    | 8     | 40    | 0     | 0     | 2     | 10    | 16    | 20.00 |
| Occupation status |       |       |       |       |       |       |       |       |       |       |
| Employed   | 0     | 0     | 4     | 20    | 1     | 5     | 2     | 10    | 7     | 8.75  |
| Unemployed | 17    | 85    | 14    | 70    | 18    | 90    | 17    | 85    | 66    | 82.50 |
| Self-employed | 3     | 15    | 2     | 10    | 1     | 5     | 1     | 5     | 7     | 8.75  |
| Respondents' categories |       |       |       |       |       |       |       |       |       |       |
| Father     | 1     | 5     | 2     | 10    | 1     | 5     | 5     | 25    | 9     | 11.25 |
| Mother     | 11    | 55    | 10    | 50    | 16    | 80    | 12    | 60    | 49    | 61.25 |
| Family members ≥ 18 years | 8     | 40    | 8     | 40    | 3     | 15    | 3     | 15    | 22    | 27.50 |
| Household size |       |       |       |       |       |       |       |       |       |       |
| 1–5        | 6     | 30    | 10    | 50    | 8     | 40    | 11    | 55    | 35    | 43.75 |
| 6–10       | 13    | 65    | 8     | 40    | 8     | 40    | 7     | 35    | 36    | 45.00 |
| 11–15      | 1     | 5     | 2     | 10    | 4     | 20    | 1     | 5     | 8     | 10.00 |
| 16–20      | 0     | 0     | 0     | 0     | 0     | 0     | 1     | 5     | 1     | 1.25  |

*p-value*
knowledge of zoonoses (toxoplasmosis and toxocariasis) due to consuming undercooked/raw FRC did not significantly vary among the localities (35%, 7/20) in MZ and OZ, followed by (30%, 6/20), and (25%, 5/20) in GI and OZ respectively (Table 2). Regarding occupation, although, knowledge was highest among the unemployed, (26.3%, 21/80), followed by (3.8%, 3/80) and (2.5%, 2/80) among the employed and self-employed participants, respectively, the observed difference was not statistically significant ($p > 0.05$). Furthermore, based on the household size, knowledge was highest (13.8%, 11/80) in household sizes 1–5 and 6–10, and decreased in household sizes 11–15 (2.5%, 2/80) and 16–20 (1.3%, 1/80); however, the difference was not statistically significant ($p > 0.05$) (Table 2).

Table 2 Responses on knowledge of zoonosis transmission from free-range chickens in four localities in the KwaZulu-Natal province of South Africa (GI, Gingindlovu; OZ, Ozwathini; MZ, uMzinto; SH, Shongweni)

| Variable         | Localities   | GI (n = 20) | OZ (n = 20) | MZ (n = 20) | SH (n = 20) |
|------------------|--------------|-------------|-------------|-------------|-------------|
|                  | Yes (%)      | No (%)      | Yes (%)     | No (%)      | Yes (%)     | No (%)      |
| Age              |              |             |             |             |             |             |
| <21              | 0            | 1 (100)     | 0           | 1 (100)     | 0           | 1 (100)     |
| 21–30            | 2 (29)       | 5 (71)      | 1 (17)      | 5 (83)      | 1 (33)      | 2 (67)      |
| 31–40            | 0            | 1 (100)     | 1 (25)      | 3 (75)      | 0           | 1 (100)     |
| 41–50            | 4 (80)       | 1 (20)      | 2 (50)      | 2 (50)      | 0           | 5 (100)     |
| 51–60            | 0            | 2 (100)     | 1 (50)      | 1 (50)      | 2 (50)      | 0           |
| 61+              | 0            | 4 (100)     | 0           | 3 (100)     | 4 (57)      | 3 (43)      |
| Total            | 6 (30)       | 14 (70)     | 5 (25)      | 15 (75)     | 7 (35)      | 13 (65)     |
| $X^2, p$-value   | ($X^2 = 9.388, p = 0.095$) | ($X^2 = 3.556, p = 0.615$) | ($X^2 = 5.139, p = 0.273$) | ($X^2 = 8.864, p = 0.115$) |
| Educational level|              |             |             |             |             |             |
| None             | 0            | 2 (100)     | 0           | 2 (100)     | 1 (100)     |
| Primary          | 0            | 1 (100)     | 0           | 3 (100)     | 4 (50)      | 4 (50)      | 2 (25)      | 6 (75)      |
| High school      | 2 (18)       | 9 (82)      | 3 (43)      | 4 (57)      | 3 (27)      | 8 (73)      | 3 (33)      | 6 (67)      |
| Tertiary         | 4 (67)       | 2 (33)      | 2 (25)      | 6 (75)      | 0           | 1 (50)      | 3 (60)      | 2 (40)      |
| Total            | 6 (30)       | 14 (70)     | 5 (25)      | 15 (75)     | 7 (35)      | 13 (65)     | 7 (35)      | 13 (65)     |
| $X^2, p$-value   | ($X^2 = 5.859, p = 0.119$) | ($X^2 = 2.857, p = 0.414$) | ($X^2 = 1.618, p = 0.445$) | ($X^2 = 2.418, p = 0.490$) |
| Occupation       |              |             |             |             |             |             |
| Employed         | 0            | 0           | 1 (25)      | 3 (75)      | 0           | 1 (100)     | 1 (100)     | 1 (50)      |
| Unemployed       | 4 (24)       | 13 (76)     | 4 (29)      | 10 (71)     | 7 (39)      | 11 (61)     | 6 (35)      | 11 (65)     |
| Self-employed    | 2 (67)       | 1 (33)      | 2 (100)     | 0           | 1 (100)     | 0           | 1 (100)     | 1 (100)     |
| Total            | 6 (30)       | 14 (70)     | 5 (25)      | 15 (75)     | 7 (35)      | 13 (65)     | 7 (35)      | 13 (65)     |
| $X^2, p$-value   | ($X^2 = 2.260, p = 0.133$) | ($X^2 = 0.762, p = 0.683$) | ($X^2 = 1.197, p = 0.550$) | ($X^2 = 0.737, p = 0.692$) |
| Participant’s ID |              |             |             |             |             |             |
| Father           | 0            | 1 (100)     | 0           | 2 (100)     | 0           | 1 (100)     | 1 (20)      | 4 (80)      |
| Mother           | 3 (27)       | 8 (73)      | 3 (30)      | 7 (70)      | 6 (38)      | 10 (62)     | 4 (33)      | 8 (67)      |
| Children >18 years| 3 (38)    | 5 (62)      | 2 (25)      | 6 (75)      | 1 (33)      | 2 (67)      | 2 (67)      | 1 (33)      |
| Total            | 6 (30)       | 14 (70)     | 5 (25)      | 15 (75)     | 7 (35)      | 13 (65)     | 7 (35)      | 13 (65)     |
| $X^2, p$-value   | ($X^2 = 0.682, p = 0.711$) | ($X^2 = 0.800, p = 0.670$) | ($X^2 = 0.586, p = 0.746$) | ($X^2 = 1.832, p = 0.400$) |
| Household Size   |              |             |             |             |             |             |
| 1–5              | 1 (17)       | 5 (83)      | 3 (30)      | 7 (70)      | 2 (25)      | 6 (75)      | 5 (45)      | 6 (55)      |
| 6–10             | 4 (31)       | 9 (69)      | 2 (25)      | 6 (75)      | 4 (50)      | 4 (50)      | 1 (14)      | 6 (86)      |
| 11–15            | 1 (100)      | 0           | 0           | 2 (100)     | 1 (25)      | 3 (75)      | 0           | 1 (100)     |
| 16–20            | 0            | 0           | 0           | 0           | 1 (100)     | 0           |           |            |
| Total            | 6 (30)       | 14 (70)     | 5 (25)      | 15 (75)     | 7 (35)      | 13 (65)     | 7 (35)      | 13 (65)     |
| $X^2, p$-value   | ($X^2 = 2.845, p = 0.241$) | ($X^2 = 0.800, p = 0.670$) | ($X^2 = 1.319, p = 0.517$) | ($X^2 = 4.244, p = 0.236$) |
Ownership of FRC in study localities

Overall, 65% (52/80) of the interviewed households in the study population owned FRC ranging from 1 to 51 (17.2 ± 1.4). There was no significant association recorded based on FRC ownership (p > 0.05). Twenty percent (16/80) of the households had FRC greater than 20, while (10%, 8/80) of the study population have ≤ 5. FRC ownership was highest in MZ (80%, 16/20), followed by OZ (70%, 14/20), SH (60%, 12/20), and GI (50%, 10/20) (Table 3).

Chicken viscera consumption

Overall, 76.3% (61/80) of respondents reported consumption of chicken viscera (Table 4). The proportion of

### Table 3 Ownership of free-range chicken in four localities in the KwaZulu-Natal province of South Africa (GI, Gingindlovu; OZ, Ozwathini; MZ, uMzinto; SH, Shongweni)

| Variable                        | GI (n = 20) | OZ (n = 20) | MZ (n = 20) | SH (n = 20) | Total (n = 80) | p-value |
|---------------------------------|-------------|-------------|-------------|-------------|---------------|---------|
| Ownership of FRC                |             |             |             |             |               |         |
| Yes                             | 10 (50%)    | 14 (70%)    | 16 (80%)    | 12 (60%)    | 52 (65.00%)   | 0.222   |
| No                              | 10 (50%)    | 6 (30%)     | 4 (20%)     | 8 (40%)     | 28 (35.00%)   |         |
| Number of FRC in the household  |             |             |             |             |               |         |
| 1–5                             | 1 (5%)      | 4 (20%)     | 1 (5%)      | 11 (55%)    | 8 (10.00%)    | 0.416   |
| 6–10                            | 1 (5%)      | 5 (25%)     | 2 (10%)     | 7 (35%)     | 14 (17.50%)   |         |
| 11–15                           | 2 (10%)     | 10 (50%)    | 2 (10%)     | 1 (5%)      | 4 (5.00%)     |         |
| 16–20                           | 1 (5%)      | 0 (0%)      | 1 (5%)      | 1 (5%)      | 1 (1.25%)     |         |
| 20+                             | 5 (25%)     | 3 (15%)     | 5 (25%)     | 0 (0%)      | 16 (20.00%)   |         |

### Table 4 Responses from participants on consumption and type of viscera from free-range chickens in four localities in KwaZulu-Natal province of South Africa

| Respondent category           | Viscera type consumed in Gingindlovu (n = 12) | Viscera type consumed in Ozwathini (n = 16) | Viscera type consumed in uMzinto (n = 15) | Viscera type consumed in Shongweni (n = 18) |
|-----------------------------|-----------------------------------------------|-------------------------------------------|------------------------------------------|-------------------------------------------|
|                             | 1, 3, 1, 2, 3, 1, 3, 4, 1, 2, 3, 6, 1, 2, 3, 4, 5, 1, 3, 4, 5, 6 | 1, 3, 1, 2, 3, 4, 5, 6, 1, 3, 4, 5, 6, 1, 2, 3, 4, 5, 6 | 1, 3, 1, 2, 3, 4, 5, 6, 1, 2, 3, 4, 5, 6 | 1, 3, 1, 2, 3, 4, 5, 6, 1, 2, 3, 4, 5, 6 |
| Father                      | 0, 0, 0, 0, 0, 0, 0, 0, 0 | 0, 0, 0, 0, 0, 0 | 0, 0, 0, 0, 0, 0, 0, 0, 0, 0 | 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0 |
| Mother                      | 0, 0, 0, 0, 0, 0, 0, 0, 0 | 1, 2 | 2, 2 | 2, 2 | 2, 2 |
| Family members ≥ 18 years   | 0, 0, 0, 0, 0, 0, 0, 0, 0 | 0 | 2 | 2 | 2 |
| Total                       | 0, 0, 0, 0, 0, 0, 0, 0, 0 | 1, 2, 2 | 2, 2 | 2, 2 | 2, 2 |
| Respondent category         | Viscera type consumed in uMzinto (n = 15)     | Viscera type consumed in Shongweni (n = 18) |
| Father                      | 0, 0, 0, 0, 0, 0, 0, 0, 0 | 0, 0, 0, 0, 0, 0, 0, 0, 0, 0 | 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0 | 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0 |
| Mother                      | 0, 0, 0, 0, 0, 0, 0, 0, 0 | 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0 | 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0 | 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0 |
| Family members ≥ 18 years   | 0, 0, 0, 0, 0, 0, 0, 0, 0 | 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0 | 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0 | 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0 |
| Total                       | 0, 0, 0, 0, 0, 0, 0, 0, 0 | 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0 | 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0 | 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0 |

Chicken viscera types are denoted as follows; 1 = gizzard; 2 = heart; 3 = liver; lungs = 4; kidney = 5; intestines = 6
respondents consuming chicken viscera was highest in SH (90%, 18/20), followed by OZ (80%, 16/20), MZ (75%, 15/20), and GI (60%, 12/20). The proportion and category of respondents consuming the combination of all chicken viscera are shown in Table 4 and Fig. 2a–c.

**Molecular results**

All the 30 brain samples were negative for *T. gondii*. *Toxocara canis* larvae were detected and confirmed through sequencing in the right pectoral muscle, liver, left thigh, and lungs of FRC from Gingindlovu (GI) (*n* = 1), uMzinto (MZ) (*n* = 1), and Shongweni (SH) (*n* = 2). There were no *Toxocara* larvae found in FRC from Ozwathini (OZ). The summary of molecular results is shown in Table 5. The 761-bp 18S rRNA sequences of *T. canis* from GI showed 99.74% similarity, with *T. canis* JN256976 from China, with *T. canis* U94382.1 from the USA, and with *T. canis* AF036608.1 from the UK with 100% homology (Table 5). The 773-bp long 18S rRNA sequences of *T. canis* from MZ showed 99.09% similarity, with *T. canis* JN256976 from China, with *T. canis* U94382.1 from the USA, and with *T. canis* AF036608.1 from the UK with 100% homology (Table 5). The 740- and 786-bp 18S rRNA sequences of *T. canis* from SH showed 99.46% and 94.66% similarity respectively with *T. canis* JN256976.1 from China, U94382.1 from the USA, and AF036608.1 from the UK with 100% homology (Table 5).

**Table 5** The sample ID, sequence length, accession number, and homology of sequences with those of GenBank for *Toxocara canis* from dogs all showing 100% query coverage

| Sample ID | Sequence length (bp) | Accession #         | % Similarity | Country |
|-----------|----------------------|---------------------|--------------|---------|
| C(GI)     | 761                  | JN256976.1          | 99.74        | China   |
|           | 761                  | U94382.1            | 99.74        | USA     |
|           | 761                  | AF036608.1          | 99.74        | UK      |
| C(SH)     | 740                  | JN256976.1          | 99.46        | China   |
|           | 740                  | U94382.1            | 99.46        | USA     |
|           | 740                  | AF036608.1          | 99.46        | UK      |
| C(SH)     | 786                  | JN256976.1          | 94.66        | China   |
|           | 786                  | U94382.1            | 94.66        | USA     |
| C(MZ)     | 773                  | JN256976.1          | 99.09        | China   |
|           | 773                  | U94382.1            | 99.09        | USA     |
|           | 773                  | AF036608.1          | 99.09        | UK      |

**Discussion**

Several studies have reported the vulnerability of resource-poor communities who keep FRC to zoonotic diseases such as toxoplasmosis and toxocariasis (Neghina 2010; Santarém et al. 2011; Mirza and Rathore 2019). Socio-demographic factors have been reported to influence the food and meat-gathering practices of people in a way that predisposes them to parasitic infections (Simeone 2008; Drescher et al. 2012; Goyette et al. 2014). Our study showed that a quarter of the respondents in the study areas had only completed primary education. This is consistent with reports from other rural
regions of South Africa where low levels of education have been reported (Mwale and Masika 2009; Spaull 2015).

Also, this study showed that the overall percentage of zoonosis transmission awareness was comparable to 30.1% recorded from cattle farmers in Senegal (Tebug et al. 2015) but higher than the awareness level of 19.1% of the zoonotic risk associated with livestock in Ibadan Nigeria (Awosanya and Akande 2015). However, it was lower than the 69% and 79.74% that were reported in Cambodia and Punjab respectively (Osbjer et al. 2015; Singh et al. 2019). Although no significant associations were found between knowledge of zoonosis transmission and considered variables in all the localities, the high level of knowledge obtained among respondents with high school education disagrees with the report from Western Ethiopia where KAP scores were higher among people with tertiary education (Tamiru et al. 2022). This can be attributed to the fact that the majority (45%) of the respondents in this study have a high school education. Similarly, regarding occupation, the higher level of knowledge observed among unemployed respondents disagrees with the report from Western Ethiopia where good KAP scores were recorded among people with good job types (Tamiru et al. 2022). This can be attributed to the large percentage of unemployed (82.5%) constituting the respondents. A similar explanation is responsible for the high knowledge of zoonosis transmission observed among women in the study locations where most of the respondents (61.3%) were women.

Furthermore, this study revealed that ownership of FRC in the study locations was 65% (52/80), which is higher than the 57.7% (41/71) reported in Ethiopia (Sambo et al. 2015) but lower than the 93.5% and 84% poultry (duck and chicken) ownership observed in the Eastern Cape province of South Africa and Cambodia respectively (Mwale and Masika 2009; Osbjer et al. 2015). Also, the average flock size (17.2 ± 1.4) observed in this study is higher than (16 ± 2.1), reported in the Eastern Cape province of South Africa (Mwale and Masika 2009), but lower than (22.03 ± 2.85) in Limpopo province and (28.40 ± 2.57) earlier reported in the KwaZulu-Natal province respectively (Malatji et al. 2016). Regarding consumption patterns, the majority (76.3%, 61/80) of respondents reported the practice of consumption of FRC viscera in their households. The reason for the high demand for chicken viscera in the study area is however unknown. Studies have identified the role of poor socio-economic factors as well as globalization as important factors in meat consumption patterns (Tambi 2001; Simeone 2008; Goyette et al. 2014; Robertson et al. 2014). Additionally, the viscera of chicken and other avian animals have been reported to be rich in essential nutrients for humans (Schönfeldt and Gibson 2008).

Chickens are used as sentinel agents in monitoring the prevalence of infections in the environment due to their ground-feeding habits (Dubey et al. 2005). Molecular techniques employing non-coding 529 base pairs DNA fragment and the internal transcribed spacer 1 (ITS-1) of the rRNA gene have proved effective in the identification of T. gondii and several organisms to species level due to the variations of the (ITS-1) of the rDNA (Santos et al. 2010), resulting in a higher detection rate (Chehoh et al. 2016). Also, PCR has been used unequivocally for the detection of animal and human toxocariasis (Dewair and Bessat 2020).

This study is the first to consider the prevalence of T. gondii in free-range chickens from the KZN province, South Africa, using a molecular approach. The absence of T. gondii observed in the brain tissues of chicken in this study might be an indication that the FRC sampled in our study may have not been exposed to T. gondii oocysts. This is consistent with a report from a study conducted on retail turkey meat products where T. gondii DNA was not detectable using magnetic capture PCR (Koethe et al. 2015). Another study reported a low prevalence of T. gondii infection in feral rodents and insectivores (Meerburg et al. 2012). The absence of T. gondii in this study may be attributed to the non-survival of T. gondii oocysts in the environment due to hot and dry temperatures (Lukášová et al. 2017). Toxoplasma gondii infections thrive in mild temperature climates than in a hot and dry environment (Dubey 1998; Gilot-Fromont et al. 2012) or dry and very cold winter (Smallbone et al. 2017). For instance, T. gondii oocysts survived for 32 days at 35 °C, 9 days at 40 °C, and only 1 day at 45 °C (Dubey 1998). The chickens used in this study were obtained between March and July which is usually characterized by the highest temperature and lowest temperature respectively (Masemola et al. 2020). The mean daily minimum temperature in KZN is around 35 °C in March and 16.76 °C in July (Dzikiti et al. 2022).

On the other hand, the presence of T. canis observed in this study agrees with Zibaei et al. (2017) who isolated Toxocara canis larvae from the liver, skeletal muscles, duodenum, and brain of broiler chickens and Okada et al. (2021) who reported the occurrence of T. cati and T. tanuki from the thigh and breast meat from chicken, respectively. Similarly, Davidson et al. (2012) reported T. cati oocysts survived for 32 days at 35 °C, 9 days at 40 °C, and only 1 day at 45 °C (Dubey 1998). The chickens used in this study were obtained between March and July which is usually characterized by the highest temperature and lowest temperature respectively (Masemola et al. 2020). The mean daily minimum temperature in KZN is around 35 °C in March and 16.76 °C in July (Dzikiti et al. 2022).

The prevalence of Toxocara infection observed in this study (9.5%: 4/42) is consistent with reports from other studies where low prevalence has been reported. Zibaei et al. (2017) reported 15.2% (5/33) from broiler chickens while Okada et al. (2021) reported 4% (2/50) from culled chickens from a commercial farm and Davidson et al. (2012) reported a prevalence of 1% (1/100) from a pig in a slaughterhouse from Norway. The occurrence of T. canis in FRC observed in this study indicates that the chickens are being exposed to environments contaminated with T. canis eggs from dogs in the localities studied. Considering the high rate
of consumption of chicken viscera practiced by communities in this study, although most preferred “well-cooked,” it is important to create awareness of the role of FRC as paratenic hosts of important zoonotic parasites of dogs and cats such as *T. gondii* and *Toxocara* spp. Furthermore, the application of the PCR technique used in this study could be employed in routine detection methods for *Toxocara* larvae in organs of suspected infected animals, especially in toxocariasis endemic areas.

We also recommend the participation of all stakeholders through a One Health approach in designing control and prevention strategies for zoonotic pathogens affecting these communities using the findings from this study as a basis.

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**Author contribution** All authors contributed to the study conception and design. Adejumoke O. Omonijo and Samson Mukaratirwa conceived and designed the study. Adejumoke O. Omonijo wrote the article and Samson Mukaratirwa reviewed the article. Both authors read and approved the final manuscript.

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**Data availability** The data used to support the findings of this study are available from the corresponding author upon request.

**Declarations**

**Ethics approval** The study was conducted under the Human and Social Sciences Research Ethics committee of the University of KwaZulu-Natal protocol reference number HSS/1655/018D.

**Consent to participate** We confirm the confidentiality of each participant’s answer, and the data will be treated with complete confidentiality, and only for research and statistical purposes only.

We confirm that their participation in the study was voluntary, with no financial compensation.

We confirm the participants’ right to not answer any question they do not want to, and their right to withdraw from the study at any time they wish without giving reasons without any negative consequences being applied to them.

Written consent was obtained from each participant before their participation in the study.

**Consent for publication** The corresponding author confirms that the manuscript has been read and approved for submission by all the co-authors.

**Conflict of interest** The authors declare no competing interests.

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Further Reading

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