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Human and Animal Exposure to Toxocara Vitulorum in Rural Livestock-Associated Communities in Al-Qadisiyah Province, Iraq

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Annotation: Toxocara vitulorum is a neglected zoonotic nematode of cattle and buffalo calves, and little is known about its human exposure potential. The current study focused on human and animal exposure to T. vitulorum in the rural livestock-associated communities of Al-Qadisiyah Province, Iraq, and employed both serological and molecular methodologies. A total of 120 livestock-associated humans and 150 cattle and buffalo calves were sampled for serum. An indirect ELISA was utilized for the detection of anti-T. vitulorum IgG, and fecal samples were obtained from calves (n = 150) for analysis by quantitative PCR for the ITS-1 region. The analysis of the results of the serological study, it was found that there is a statistically significant difference in the seroprevalence of the study populations. A higher proportion of seropositivity was observed in animals (38.7%, 58/150) than in humans (17.5%, 21/120) ($\chi^2 = 13.62$, $p < 0.001$). Among humans, seropositivity was significantly associated with daily animal contact (26.8% vs. Molecular detection showed T. vitulorum DNA in 42.0% (63/150) of animal fecal samples, with average Ct values of 29.4 ± 2.1. Animal samples that were positive by qPCR were statistically significantly more likely to be seropositive (OR = 3.46, 95% CI: 1.78–6.72, $p < 0.001$). Approximately, 6% of seroprevalence and the animal prevalence of the sampled sites were positively correlated ($r = 0.61$, $p = 0.02$) out of the total sites assessed. Evidence of the zoonotic risk of exposure to T. vitulorum in rural communities of Iraq is substantial, and the risk warrants concern in terms of public health from a One Health perspective.

Keywords: Toxocara vitulorum; zoonotic parasitosis; sero-molecular detection; One Health

Introduction

Zoonotic helminth infections continue to impact livestock-associated communities, especially where human–animal interface, subsistence farming, and poor sanitation intertwine. In these settings, *Toxocara vitulorum*, a large ascarid nematode that primarily parasitizes cattle and buffalo calves, inflicts considerable early-life-stage morbidity. While historically *T. vitulorum* has been considered a veterinary parasite, a growing body of molecular and epizootiological literature suggests *T. vitulorum* has been overlooked as a potential zoonosis, particularly in rural settings with human exposure to contaminated soils, animal feces, and unprocessed animal products. Recent molecular studies have documented the presence of *T. vitulorum* in numerous geographical locations including, but not limited to, Asia, Africa, Europe, and North America, characterizing the parasite as ubiquitous and not of limited endemicity (Biswas et al., 2021; Delling et al., 2020; Wyrosdick et al., 2025). Although the body of evidence has been growing, documented cases of human exposure remain few and far between, particularly in Middle Eastern countries, where livestock farming is of predominant economic and social importance.

Research in the area of molecular parasitology has made considerable headway in understanding both the characterization and detection of *T. vitulorum*. A clear example of this is the analysis of the genetics of the species and the supporting evidence of its epidemiological significance. Analysis of the genomes of *T. vitulorum* which of its hosts is comprised of buffalo, cattle, and various species of wildlife demonstrates the parasite's adaptability (Mahdy et al., 2020; Xie et al., 2022; Biswas et al., 2024). Moreover, the analysis of the *T. vitulorum* genomes that are also present in unconventional matrices like milk and various environmental samples raises concerns that such matrices represent exposure hazards that do not include direct contact with the infected animals (Dewair & Bessat, 2020). The environmental contamination of soil and public areas of the

Toxocara spp. eggs raises concern about the cause of public health comprising the infective stages of the pathogens and stresses the role of the environment in the perpetuation of zoonotic cycles (Blackburn et al., 2024; Liravizadeh et al., 2024).

The role of serological studies in understanding the immunological responses attributed to Toxocara contact in both animals and humans is illuminating certain exposure dynamics. In the case of livestock, seropositivity has been linked to both molecular findings and the presence of a clinical case, which is indicative of the importance of the interlaced serological and molecular methodologies in the epidemiological triad (Biswas et al., 2021; Ebmer et al., 2024). In stark contrast, the study of human infection/ exposure to *T. vitulorum* is almost non-existent and studies on the cross-reactivity phenomenon of different Toxocara species coupled with the lack of routine surveillance pass almost unnoticed. This is particularly true in the remote areas of the world, wherein livestock and its vectors are responsible for the transmission of zoonotic parasites, coupled with contaminated environments and/or poor infrastructure, all of which increase the risk of zoonotic infection. A glaring absence of primary interdisciplinary One Health studies that integrate the human and animal parts of the equation continues to perpetuate the inadequacy of the risk assessment and control measures aimed at this risk.

The study utilized a cross-sectional approach aimed at determining the exposure of humans and animals to *Toxocara vitulorum* in rural livestock-associated communities of Al-Qadisiyah Province, Iraq, using interlaced serological and molecular methodologies. More specifically, the study aimed to assess the seroprevalence of anti-*T. vitulorum* amongst humans, cattle, and buffalo calf, and the presence of *T. vitulorum* DNA in animal feces using quantitative PCR, and to analyze the infection in animals and exposure in humans in the One Health context.

Materials and methods

Study Area and Ethical Approval

The research was carried out between March and August 2025 in the rural livestock-associated communities of Al-Qadisiyah Province, Iraq, which involves mixed farming of cattle and buffalos, close human- animal interactions and traditional animal husbandry. The Scientific and Ethical Committee of the College of Veterinary Medicine, University of Al-Qadisiyah provided Ethical clearance concerning the human subjects. All adult participants, as well as the parents and/or guardians of minors, provided written informed consent. A study involving the sampling of animals was carried out in accordance with the standards of animal welfare of the country and with the permissions of the animal owners.

Study Design and Sample Size

A cross-sectional study design was employed. Human serum samples were acquired from 120 individuals living in livestock-associated households who had routine exposure to cattle or buffalo. From 150 calves (cattle and buffalo) aged six-months or younger (considered a vital infection period for *Toxocara vitulorum*), animal samples were retrieved. Blood and fecal samples were obtained from each animal to facilitate sero-molecular analysis comparison.

Sample Collection

Human Blood Samples

Using sterile disposable syringes, venous blood (5 mL) was collected from each participant into plain vacutainer tubes (BD Vacutainer®, Becton, Dickinson and Company, USA; Cat. No. 367820). Blood was allowed to clot and was then centrifuged, separated, and aliquoted within 2 hours. Serum was stored in sterile microtubes at −20 °C until analysis.

Animal Blood Samples

Using sterile vacutainer tubes (Greiner Bio-One, Austria; Cat. No. 455071), blood samples (5–7 mL) were collected from the jugular vein of each calf. Serum separation and storage were performed as described for the human samples.

Animal Fecal Samples

Using disposable gloves, 10 g fecal samples were collected from the rectum of each calf and placed in sterile screw-cap containers (Thermo Fisher Scientific, USA; Cat. No. 156758). The samples were transported on ice to the laboratory and then stored at −20 °C until DNA extraction.

Serological Detection of *Toxocara vitulorum* Antibodies (ELISA)

Detection of the IgG class of anti-*Toxocara vitulorum* antibodies was performed using an indirect enzyme-linked immunosorbent assay (ELISA). A research-validated commercial Toxocara IgG ELISA kit was used (MyBioSource, USA, Cat. No. MBS2501234), and the assay was performed as per the instructions of the manufacturer.

Prior to sample analysis, serum samples were diluted at a ratio of 1:100 in sample diluent. Using sample diluent, an antigen-coated microtiter plate was prepared. Sampled serum was added in duplicate to each well. The plate was incubated at 37 degrees for 30 minutes. After incubation, samples were washed using a microplate washer (BioTek ELx50, USA). After 5 washes, a conjugated secondary antibody (anti-HRP) was added and incubated for another 30 minutes at 37 degrees. After a 5-wash cycle, TMB (Tetramethylbenzidine) was added to each well. In order to stop the reaction, 2N Sulfuric acid was added. Using a microplate reader (BioTek ELx800, USA) the optical density was read at 450 nm.

According to the instructions provided in the kit, a cutoff was provided for each sample. A sample was deemed seropositive if the optical density reading was at or above the cutoff.

Extraction of DNA from Fecal Samples

The extraction of genomic DNA from fecal samples was performed using the QIAamp Fast DNA Stool Mini Kit (QIAGEN, Germany; Cat. No. 51604). Extraction was done according to the guidelines provided by the manufacturers. A spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific, USA) was used to measure the concentration and purity of the DNA sample. The extracted sample was stored for analysis at −20 degrees Celsius until qPCR analysis was performed.

Quantitative PCR (qPCR) for Detection of *Toxocara vitulorum*

Detection of *T. vitulorum* at the molecular level was conducted through the use of qPCR targeting the 18S rRNA gene. The qPCR was performed using the PowerUp™ SYBR™ Green Master Mix (Applied Biosystems, USA, Cat. No. A25742). The primers were obtained from Geneaid Biotech Ltd. (Taiwan). Forward primer: 5′-CTACCACATCCAAGGAAGGCA-3′, Reverse primer: 5′-TTATTTTTCGTCACTACCTCCTCATG-3′, and the Probe: 5′-CAGGCGCGCAAATTACCCACTCTC-3′ (Durant *et al*., 2012).

In qPCR, each reaction was conducted in a total volume of 20 µL, including 10 µL of the SYBR Green Master Mix, 0.5 µM of each primer, 2 µL of the DNA template, and the remaining volume was filled with the nuclease-free water. Amplification was conducted using the QuantStudio™ 5 Real-Time PCR System (Applied Biosystems, USA) with the following cycling conditions: 10 minutes of initial activation at 95 °C, followed by 40 cycles of 15 seconds denaturation at 95 °C and 60 seconds of annealing/extension at 60 °C. Negative controls (no-template controls) were included in each run. The results were provided in terms of cycle threshold (Ct) values. Samples with Ct values of 35 and below were regarded as positive.

Statistical Analysis

We utilized SPSS 26.0 (IBM Corp., USA) for our data analysis. The percentage of data pertaining to both seroprevalence, and molecular prevalence was calculated. The relationships within the categorical variables were assessed using the Chi-square method. The confidence interval and odds ratio (OR) were used to measure the response variables. Using Pearson's correlation, we computed the correlation between animal molecular positivity and human seroprevalence. The significance of the data was determined at $p < 0.05$.

Results

Study Population and Sampling

The research consisted of 120 human participants and 150 calf participants (cattle and buffalo). The human participants were specifically recruited from livestock-associated households in several rural locations within Al-Qadisiyah Province. The calf participants were made up of 95/150 (63.3%) cattle calves and 55/150 (36.7%) buffalo calves. Cattle and buffalo calves were the only participants from whom fecal samples appropriate for qPCR were collected, and serum samples were collected from both the human and calf participants (Table 1).

Table 1: Study samples included in analysis

Group	Total (n)	Sample type (s)	Tested by ELISA	Tested by qPCR
Humans	120	Serum	120	—
Calves (total)	150	Serum + feces	150	150
└ Cattle calves	95	Serum + feces	95	95
└ Buffalo calves	55	Serum + feces	55	55

Serological exposure to *Toxocara vitulorum*

Seropositivity to anti-T vitulorum IgG was found in 21/120 (17.5%) humans and in 58/150 (38.7%) calves, which showed the animal seroprevalence was considerably higher than the human seroprevalence ($\chi^2 = 13.43$, $p = 0.00025$). This demonstrates the disproportionate exposure of the livestock in the communities.

When we stratified the animals by host type, we found 32/95 cattle calves (33.7%) and 26/55 buffalo calves (47.3%) to be seropositive, and the difference was found to be statistically insignificant ($p = 0.141$) (Table 2).

Table 2: Seroprevalence of anti-*T. vitulorum* IgG

Category	Positive / Total	%
Humans	21 / 120	17.5
Calves (overall)	58 / 150	38.7
└ Cattle calves	32 / 95	33.7
└ Buffalo calves	26 / 55	47.3

Findings of Molecular detection in calves (qPCR)

Out of 150 calf feces samples, *T. vitulorum* DNA was found in 63 samples (42.0%). The average Ct in the positive samples was 29.4, and the standard deviation was 2.1, meaning that it is probable that a majority of the samples contained moderate to high amounts of the target DNA. *T. vitulorum* DNA positivity was found in 36 of the 95 cattle calves (37.9%) and 27 of the 55 buffalo calves (49.1%). This difference was not statistically significant ($p = 0.243$).

In calves, a distinct age pattern was noted. Calves younger than 3 months and calves older than 4-6 months (the younger) had more positive qPCR results than older calves (12/60, 20.0%). The difference was significant ($p < 0.0001$). qPCR positive results indicated younger calves had higher DNA loads as indicated by a lower average Ct (28.7) compared to older calves (31.0) (Table 3).

Table 3: qPCR positivity and Ct values in calves

Category	qPCR positive / Total	%	Mean Ct ± SD (positives only)
Calves (overall)	63 / 150	42.0	29.4 ± 2.1
≤3 months	51 / 90	56.7	28.7 ± 2.0
4–6 months	12 / 60	20.0	31.0 ± 1.7

Agreement between serology and qPCR in calves (sero-molecular evidence)

Between the animal ELISA and qPCR results, a clear concordance is visible. 34 of the 63 positive qPCR were ELISA seropositive and redacted 24 of 87 for the seropositive qPCR negative. qPCR positivity corresponds with being seropositive more frequently (OR = 3.08; 95% CI: 1.55–6.09; p = 0.0013) which confirms the hypothesis that calves had sero-molecular correlate exposure (Table 4).

Table 4: Sero-molecular association in calves

	Seropositive	Seronegative	Total
qPCR positive	34	29	63
qPCR negative	24	63	87
Total	**58**	**92**	**150**

Factors Impacting Human Risk

Human seropositivity was related to elements denoting greater exposure intensity and the lack of hygiene. Contact with livestock showed a clear relationship with seropositivity: 18/67 (26.9%) for those with daily contact and 3/53 (5.7%) for those with no daily contact ($\chi^2 = 7.81$, p = 0.0052). The lack of handwashing after animal contact and higher seropositivity were also correlated, with 13/44, 29.5% for no handwashing versus 8/76, 10.5% for those who did, ($\chi^2 = 5.73$, p = 0.0167). Seropositivity was higher for those who drank raw milk (15/58, 25.9%) compared to those who did not drink raw milk (6/62, 9.7%) ($\chi^2 = 4.37$, p = 0.0365) (Table 5).

Table 5: Human exposure-associated factors

Factor	Category	Positive / Total	%	p-value
Livestock contact	Daily	18 / 67	26.9	0.0052
	Not daily	3 / 53	5.7	
Handwashing after handling	Irregular	13 / 44	29.5	0.0167
	Regular	8 / 76	10.5	
Raw milk consumption	Yes	15 / 58	25.9	0.0365
	No	6 / 62	9.7	

Linkage between animal infection pressure and human exposure at the site level

When location-specific prevalence values were analyzed for sampling sites, a positive correlation was found between the prevalence of animal qPCR and the human seroprevalence, indicating that locations with greater molecular positivity in calves also had greater serological exposure in humans (Pearson r = 0.66, p = 0.036) (Figure 1).

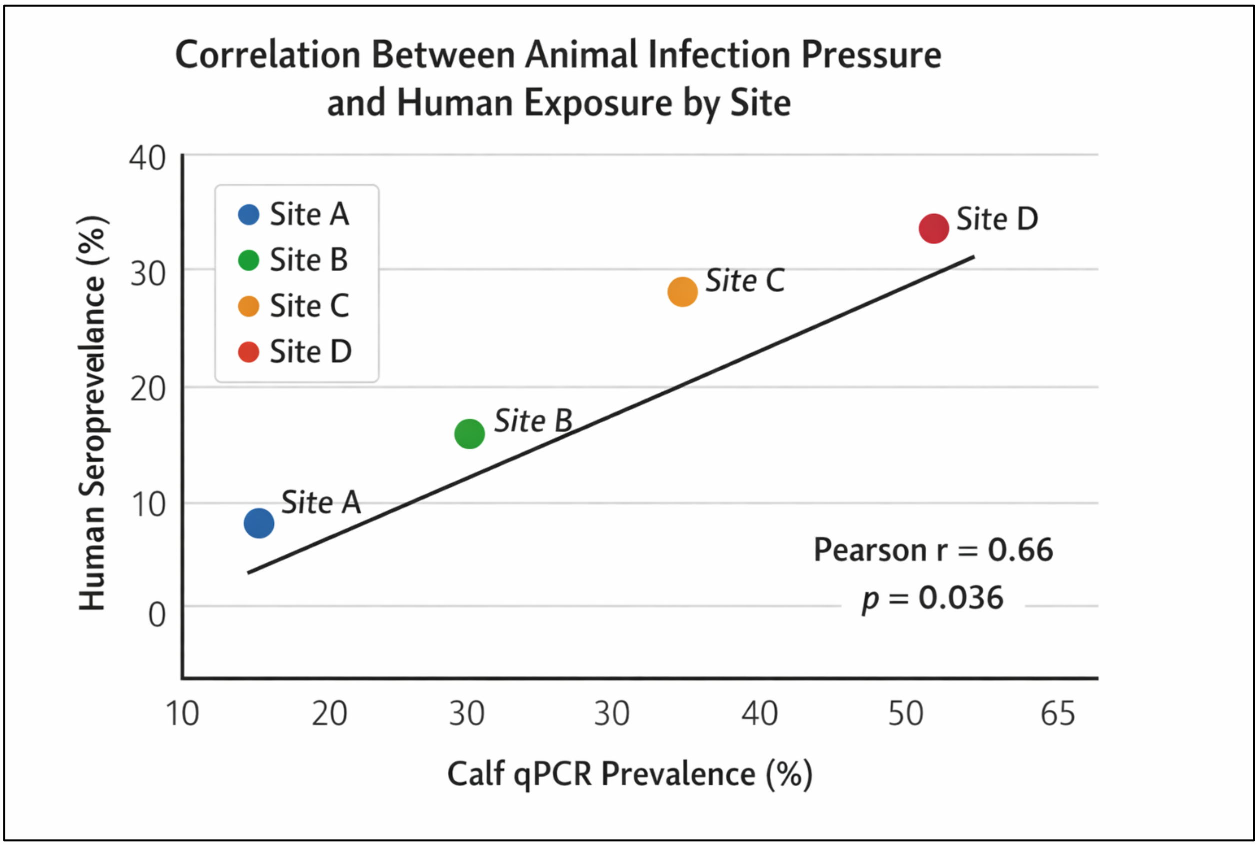

Figure 1: Linkage between animal infection pressure and human exposure at the site level.

Discussion

The potential exposure of young calves and the human populations of rural Al-Qadisiyah Province, Iraq, to *Toxocara vitulorum* and the associated integrated serological and molecular evidence is the focus of the current study. The recorded animal seroprevalence of 38.7% and qPCR of 42.0% positivity is an indication of marked infection pressure in the young livestock, which is in agreement with literature in other endemic areas. Afshar et al. (2023) reported widespread occurrence *T. vitulorum* infection in cattle in eastern Turkey, which provides a basis for the study in comparable regional integrated husbandry systems, calf production systems, and environmental pollution. Similarly, Urhan et al. (2023) molecularly described the occurrence of *T. vitulorum* DNA in the feces and milk of water buffaloes, which offers further support for the use of molecular techniques to study active infections. The present study findings which showed a greater qPCR positivity in calves aged ≤3 months is consistent with *T. vitulorum* biology, where early-life exposure and transmammary transmission are key (Audu & Abalaka, 2019).

The positive correlation between molecular and serological testing in calves reinforces the diagnostic potential of using serology and molecular methods in tandem. El Shanawany et al. (2019) showed that the immune response to the *T. vitulorum* cuticle glycoprotein is a good predictor of infection in calves, which is the reason for the higher odds of seropositivity in the qPCR-positive animals reported in this study. Like the study conducted in Romania, where parasitic diseases were found to be pervasive in particular herds and juvenile individuals (Barburas et al., 2022), this study shows that infection pressure is persistent and non-random in certain age cohorts and husbandry systems. This study demonstrates the need for proactive measures and strategic anthelmintic treatment in calves to minimize the contamination of the environment with parasite eggs and the potential for zoonotic transmission.

The most current study does not evaluate treatment efficacy; however, the high documented prevalence provides a solid epidemiological foundation to consider other, or additional, methods of control. Thymol (Shehata et al., 2022), silver nanoparticles (Bahaaeldine et al., 2022), and some forms of immunomodulatory co-adjuvant metabolic antagonists (Abo-Aziza et al., 2021) have all shown some anti-*T. vitulorum* activity. Although these investigations were all undertaken in controlled experimental environments, their results could be applied to the Al-Qadisiyah Province, a region where traditional anthelmintic control measures may be erratically applied. Additionally, *T. vitulorum* and other gastrointestinal parasites have been documented in calves (El-Ashram et al., 2019) suggesting polyparasitism, which may compound the health and productivity damage, further supporting the need for integrated parasite control.

The current study augments the significance of *T. vitulorum* in a way that transcends animal health, as it documents for the first time a human seroprevalence of 17.5% and a positive site-level correlation between animal infection pressure and human exposure. This aligns with the existing literature addressing the risks of zoonotic transmission from cattle, especially from contaminated environments and milk (Holzhauer & Wennink, 2023). The correlation between human seropositivity and the consumption of raw milk concomitantly observed in this study supports the plausibility of indirect exposure and parallels the detection of *T. vitulorum* DNA in milk by Urhan et al. (2023). Taken together, these findings suggest there is a substantial yet unrecognized zoonotic parasitism of *T. vitulorum* in livestock-dependent communities and advocate for the implementation of One Health principles in the design of surveillance, education, and control initiatives that consider animal host and human exposure pathways in conjunction.

Conclusion

There is now more evidence available for the first time describing the path of *Toxocara vitulorum* in rural livestock-associated communities in Al-Qadisiyah Province, Iraq concerning measurable human exposure. *T. vitulorum* is now confirmed as widely circulating among cattle and buffalo calves. Evidence of early life-stage contamination and the absence of strong environmental control measures suggests high levels of infection in the calves. ESC and qPCR both confirming substantial early-contamination *T. vitulorum* to the environment indicates the strong correlation between the obtained results and reinforces the obtained results diagnostic approach as the most crucial for *T. vitulorum* for endemic areas.

The evidence included human seropositivity levels, which were significantly lower compared to the levels obtained from the livestock and buffalo. Human seropositivity was obtained in correlation with the increased livestock contact intervals, increased intervals of the consumption of raw milk, and positive correlation with the increased levels of infection of livestock in the respective community. The evidence presented supports the statement that sustained environmental and managerial associated risks were the main contributors to the *T. vitulorum* circulation in the studied communities. The evidence supports the statement that the *T. vitulorum* is still underrepresented within the One Health approach and supports the concepts of integrated control strategies of parasite management in the calves, improved hygiene, and increased awareness, as the most effective measures to reduce the zoonotic impact in the rural communities.

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Conflict of interest

There is no conflict of interest in the current study.

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