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Investigation of zoonotic pathogen in slaughterhouse environments using molecular and conventional methods

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Abstract
Close interfaces between humans, animals and the environment especially in settings like slaughterhouses play a critical role in zoonotic disease transmission. This study aimed to detect selected parasitic zoonotic pathogens in slaughterhouse environments using conventional and molecular diagnostic techniques. The investigation included 346 environmental samples collected over a period of 7 months from slaughterhouses located in Hyderabad, Telangana, India. Samples collected from key points were assessed for contamination and potential zoonotic risk by conventional microscopic and PCR-based assays targeting specific genes. Microscopy revealed Ascarid (1.35%) eggs in water samples, along with other incidental parasites. Molecular detection identified *T. gondii* in 5.78% (20/346) of samples, *Giardia* spp. and *T. canis* each in 0.57% (2/346) and *Echinococcus* spp. in 0.28% (1/346) samples. The results indicate that slaughterhouse environments can act as important sources of zoonotic parasites, posing potential threats to food safety, worker health and the surrounding environment.

Keywords: Zoonoses, slaughterhouse, polymerase chain reaction (PCR)

Introduction
Zoonotic diseases are those infections which are naturally transmitted between vertebrate animals and humans (WHO, 2020) [15]. The close linkage between human and animal health is central to global disease ecology. In countries like India, socio-economic conditions and agricultural practices create a high-risk environment of zoonotic diseases (Grace *et al.*, 2012) [5]. Among the various human-animal interfaces, slaughterhouses play a critical role in zoonotic disease transmission. These facilities involve direct and repeated handling of live animals, carcasses, blood and waste materials, making them potential hotspots for pathogen exposure (Vial, 2019) [14]. Animal slaughterhouses often harbor a wide range of pathogens capable of contaminating meat, equipment and effluents, thereby endangering workers surrounding communities and the environment (Chhabra & Singla, 2009; Jenkins *et al.*, 2013) [3, 7].

Several parasitic organisms of zoonotic importance have been identified in slaughterhouse environments. Studies have detected *Toxoplasma gondii* DNA in meat and wastewater samples, confirming its environmental persistence (Thakur *et al.*, 2017; Lass *et al.*, 2022) [9, 12]. Similarly, *Giardia duodenalis*, is commonly found in abattoir effluents and wastewater, indicating fecal contamination routes that contribute to zoonotic transmission (Abbas *et al.*, 2018; Ma *et al.*, 2019) [2]. Other parasitic zoonoses of concern including *Echinococcus granulosus* and *Ascarid* spp. with its eggs that can persist in the environment for long periods involving domestic and wild animal in their cycle have been identified (Vaidya *et al.*, 2015; Abadilla & Paller, 2018) [1, 13]. Conventional microscopy techniques which remain valuable for identifying parasitic stages in samples; however, they often lack sensitivity when pathogen loads are low or when environmental matrices are complex (Dubey & Frenkel, 1976) [4]. By focusing on genetic markers that are unique to each species,

molecular methods, particularly polymerase chain reaction (PCR), have significantly improved the accuracy of detection. This has led to enhanced pathogen surveillance in slaughterhouses, resulting in more reliable and sensitive findings (Kalambhe 2017; Garcia - Diez 2023) [6, 8]. While numerous livestock slaughterhouses operate in India, comprehensive monitoring of zoonotic pathogens in the environment has not been conducted in many of these facilities. Therefore, the present study aims to detect parasitic zoonotic pathogens by integrating conventional and molecular techniques which offers a comprehensive understanding of zoonotic contamination in in organized and unorganized slaughterhouses. Thus, establishing sentinel points for disease surveillance will assist slaughterhouse management in making strategic decisions regarding enhancing hygiene practices, improving waste management, and ensuring ongoing monitoring within the slaughterhouse environment.

Materials and Methods

Prior to commencing this research, approval was obtained from Biosafety Committee of the ICAR-National Institute of Veterinary Epidemiology and Disease Informatics. The study was conducted for over 7 months period from December 2024 to July 2025 in 2 organized and 4 unorganized slaughterhouse facilities located in Hyderabad, Telangana, India. These facilities were selected based on slaughter capacity, hygiene status and accessibility. A total of 346 environmental samples, consisting of 272 surface swabs and 74 water samples (Table 1) were collected from slaughter house key points such as unloading pens, lairage, slaughter halls, effluent treatment plants, drainage channels and workers hands to assess contamination and potential zoonotic risk. Sterile cotton swabs pre-moistened with phosphate-buffered saline (PBS, pH 7.4) were used for surface sampling, while water samples (500 mL each) were collected in sterile bottles. All the samples were transported to the parasitic laboratory of ICAR-NIVEDI, Bengaluru under cooled conditions and processed within 24-36 hours of collection.

Microscopic Examination

Preliminary screening for parasitic pathogens was performed using standard sedimentation and flotation techniques as described by Soulsby (1982) [11]. Concentrated samples were examined under light microscopy at 10X and 40X magnifications to detect helminth eggs and protozoan cysts. Identification was based on morphological characteristics and size measurements (Soulsby *et al.*, 1982) [11]. The detection rates were expressed as percentages of total samples examined.

Molecular Detection

Genomic DNA was extracted from 200 µL of each processed concentrated sample using the QIAamp DNA Mini Kit (Qiagen, Germany) following the manufacturer's instructions. DNA concentration and purity were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA) and extracts were stored at -20°C until PCR analysis. Pathogen-specific PCR assays were performed targeting the specific genes for selected parasitic zoonotic pathogens (Table 2). PCR constituents and cyclical conditions were optimized using control and negative samples with varied concentration of PCR master mixture and cyclical conditions. Finally, PCR reaction mixture was prepared for 25 µL containing 2X master mix (12.5 µL), forward and reverse primers (1 µL each), DNA template (1 µL) and nuclease-free water (9.5 µL). Amplification of species-specific gene was carried out in a thermal cycler (Bio-Rad, USA) under routine cycling conditions consisting of initial denaturation at 94°C for 7 min followed by 30 cycles each of denaturation at 94°C for 45 sec, annealing temperature (refer Table 1 for pathogen-specific gene) and extension at 72°C for 45 sec and the final extension at 72°C for 7 min. PCR products were visualized by electrophoresis on 1.5% agarose gels stained with ethidium bromide and documented using a gel documentation system (Bio-Rad, USA).

Positive control DNA for each target zoonotic pathogen was obtained from accredited institutions to validate the screening assays. DNA for *Giardia* spp. and *Toxoplasma* spp. was obtained from the Centre for One Health, GADVASU, Ludhiana. *Echinococcus* spp. DNA was retrieved from a goat hydatid cyst sample maintained at ICAR-NIVEDI. Positive control DNA for *Toxocara canis* was procured from the Department of Veterinary Parasitology, PVNRTVU, Hyderabad and nuclease-free water served as a negative control in every PCR run. All steps were performed under aseptic conditions using a biosafety cabinet.

Results

All the 346 environmental samples (272 swab and 74 water) when subjected to standardized conventional microscopy and molecular techniques (PCR) revealed following results.

Microscopic Examination

Microscopic examination of 74 water samples revealed 1.35% (1/74) occurrence of *Ascarid* eggs (Fig 1) from drainage water sample collected from slaughter hall by standardized sedimentation and flotation techniques. Besides, helminth eggs comprising strongylid eggs (Fig. 2a & 2b) were identified in 2 out of 74 water samples collected from drainage areas showing 2.7% occurrence and *Trichuris* spp. (Fig 3) showed an occurrence of 1.35% (1/74) from water samples collected from ETP inlet. No protozoan cysts were observed in any of the water sample. All the other samples were found to be negative for any of the parasitic egg or larvae or adult worm. The presence of *Ascarid*, strongylid eggs and *Trichuris* spp. indicated the fecal contamination and persistence of helminthic stages within abattoir wastewater.

Molecular detection of zoonotic pathogens

Among 346 samples subjected to PCR assays using species-specific primers (Table 2), the study revealed the occurrence of *T. gondii* in 5.78% samples in which 20 samples (including both swabs and water) collected across unloading sections, lairage and slaughter hall area showed positive amplicons (Fig 4). *Giardia* spp. showed an occurrence of 0.57% in 2 (Fig 5) swab samples collected across unloading section and slaughter hall floor. *Echinococcus* spp. showed an occurrence of 0.28% (Fig 6) in a swab sample collected across slaughter hall area, and *Toxocara canis* showed an occurrence of 0.57% in 2 (Fig 7) swab samples collected across slaughter hall floor giving an overall PCR positivity of 7.22% (25/346) for zoonotic parasitic pathogen occurrence. Slaughter hall drainage and floor samples

contributed the majority of pathogens. None of the hand swab samples collected from slaughterhouse workers found to carry any parasitic pathogen selected for study.

Discussion

The present study highlights the occurrence and environmental distribution of major parasitic zoonotic pathogens within slaughterhouse settings in Hyderabad, Telangana. Using a combination of conventional microscopy and PCR-based assays, the study identified environmentally resilient parasites, confirming that slaughterhouses serve as significant contamination points at the human-animal interface (Vial, 2019; Jenkins *et al.*, 2013) [7, 14]. Pathogens including *T. gondii*, *T. canis*, *Echinococcus* spp. and *Giardia* spp. were detected across key operational areas. Although the overall PCR positivity was moderate (7.22%), the presence of these robust parasites underscores their persistence in slaughterhouse environments and the potential for transmission to workers, animals and surrounding communities (Grace *et al.*, 2012; Garcia-Diez, 2023) [5, 6].

Among the detected pathogens, *T. gondii* showed the highest occurrence (5.78%), consistent with earlier findings that demonstrate its environmental stability in moist and organically contaminated settings (Lass *et al.*, 2022; Thakur *et al.*, 2017) [9, 12]. Detection of *T. gondii* in both swabs and water samples indicates possible contamination from infected tissues, wastewater splash and organic material entering slaughter areas. Although *Giardia* spp. (0.57%), *Echinococcus* spp. (0.28%) and *T. canis* (0.57%) were detected at lower levels than previously reported in other regions (Abbas *et al.*, 2016; Abadilla & Paller, 2018) [1, 2], these pathogens are epidemiologically significant due to their high resilience and low infectious dose. The identification of *Toxocara* and *Echinococcus* DNA further raises concerns regarding inadequate waste disposal and the access of stray dogs and cats to raw offal - a well-established factor in sustaining transmission cycles (Vaidya *et al.*, 2015) [13]. In addition to stray dogs and cats, the presence and habitat of rats, rodents, reptiles, and birds in the slaughterhouse environment play a crucial role in the dissemination of pathogens over a wider geographical area.

Microscopy revealed Ascarid, strongylid and *Trichuris* spp. eggs in wastewater and drainage samples, indicating fecal contamination along effluent pathways. The occurrence of Ascarid eggs (1.35%) reflects the known durability of geohelminths under harsh environmental conditions (Soulsby, 1982) [11]. While the prevalence observed was lower than levels reported in earlier studies (Habluetzel *et al.*, 2003; Abadilla & Paller, 2022) [1], the findings align with evidence that abattoir drainage systems often accumulate helminth stages due to intermittent cleaning and incomplete removal of organic material (Chhabra & Singla, 2009) [3]. The absence of protozoan cysts on microscopy, despite their detection by PCR, further reinforces the superior sensitivity of molecular diagnostics in detecting low-level contamination within complex environmental matrices (Kalambhe, 2017) [8].

Overall, the findings demonstrate that slaughterhouses play a critical role in the maintenance and dissemination of zoonotic parasites through contaminated surfaces, wastewater and worker contact points. Contamination across key points highlights multiple risk areas that require targeted intervention. This study addresses a major surveillance gap in India, where environmental monitoring in slaughterhouses is rarely performed and most systems rely solely on clinical or post-mortem reporting (WHO, 2020) [15]. By generating baseline environmental data from organized urban abattoirs, the study underscores the need for strengthened sanitation, improved waste management, routine molecular surveillance and integrated One Health-based interventions to enhance food safety and reduce community and occupational health risks.

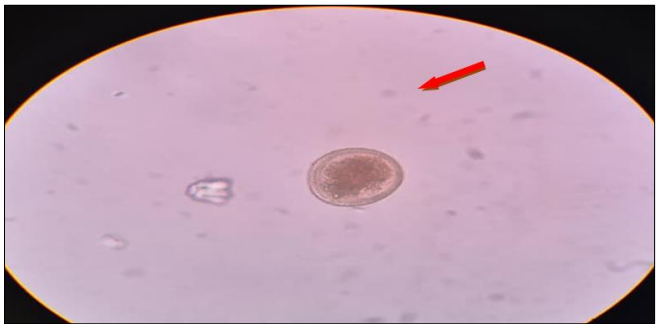

Fig 1: Microscopic image showing egg of Ascarid spp.

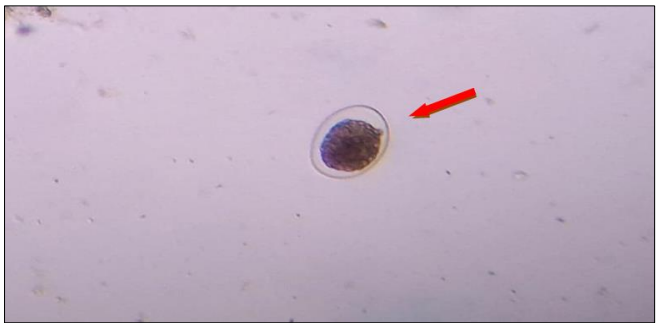

Fig 2a: Microscopic image showing *Strongylid* egg

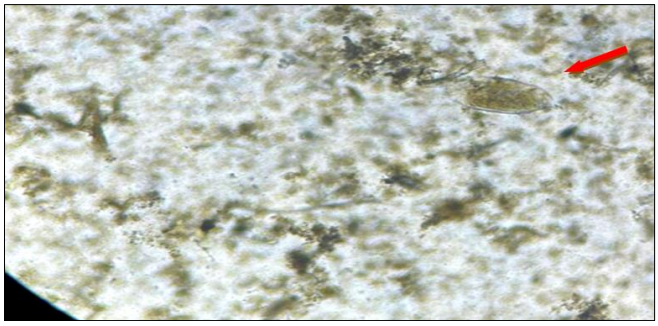

Fig 2b: Microscopic image showing *Strongylid* egg

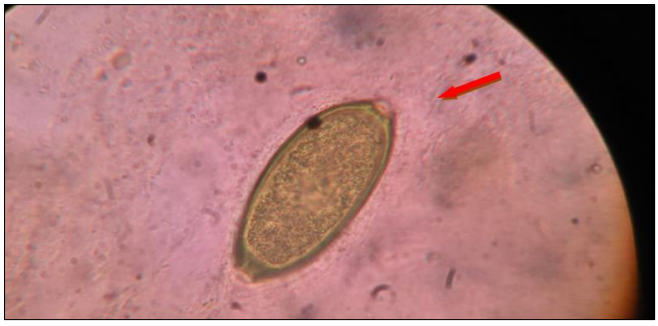

Fig 3: Microscopic image showing egg of *Trichuris* spp. (40X)

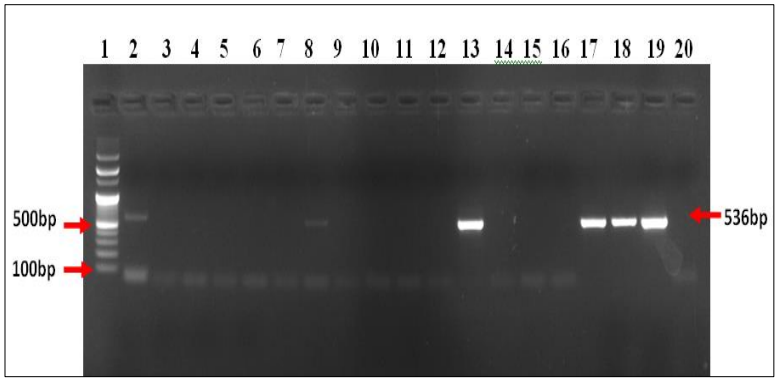

Fig 4: PCR product of *Toxoplasma gondii* targeting *B1* gene on agarose gel

Lane 1: 100 bp DNA ladder, Lane 2: Positive control, Lane 3: Negative control, Lanes 13, 17, 18 and 19: *B1* gene amplicons of test samples

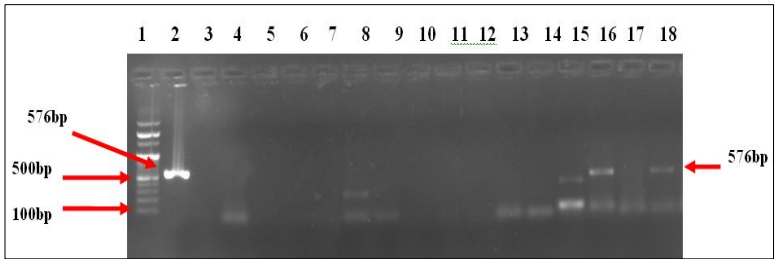

Fig 5: PCR product of *Giardia* spp. targeting *tpiA* gene on agarose gel

Lane 1: 100 bp DNA ladder, Lane 2: Positive control, Lane 3: Negative control, Lanes 16 and 18: *tpiA* gene amplicon from test samples

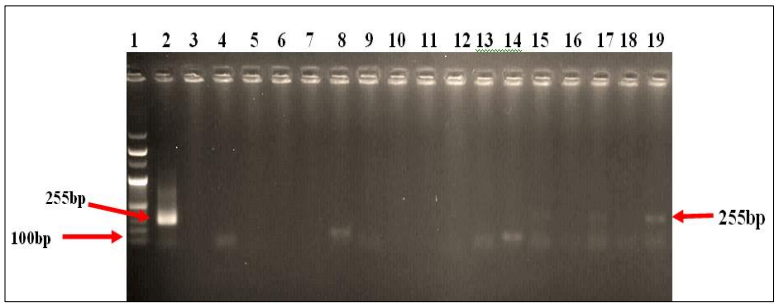

Fig 6: PCR product of *Echinococcus* spp. targeting *rRNA* gene on agarose gel

Lane 1: 100 bp DNA ladder, Lane 2: Positive control, Lane 3: Negative control, Lane 19: *rRNA* gene amplicons of test samples

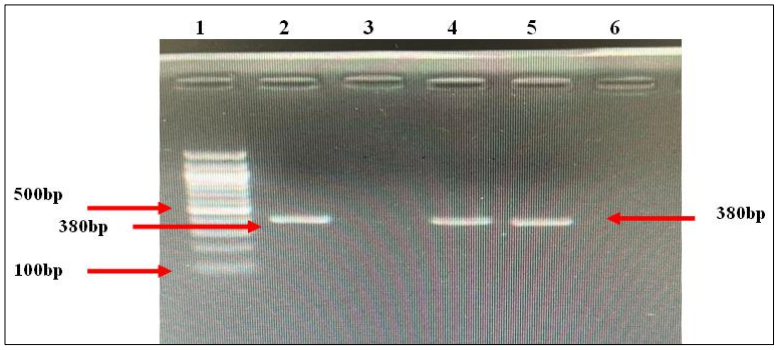

Fig 7: PCR product of *Toxocara canis* targeting *ITS2* gene on agarose gel

Lane 1: 100 bp DNA ladder, Lane 2: Positive control, Lane 3: Negative control, Lane 4 and 5: *ITS2* gene amplicon from test samples

Table 1: Particulars of number of samples collected from different sites within slaughter houses during the study

Sampling area	Sampling sites	No. of swabs sample taken	No. of Water samples
Unloading pen	Floor	30	15
	Wall	30	
	Vehicle surface	16	
Lairage	Floor	30	17
	Wall	30	
Slaughter Hall	Floor	42	30
	Wall	42	
	Table surface	36	
	Hand swabs	16	
Sludge / Effluent plant	Inlet	-	6
	Outlet	-	6
Total number of samples		272	74
Total		346	

Table 2: Details of primers targeting specific genes of respective pathogens

Pathogen	Target gene	Primer sequence (5'-3')	Annealing Temp. (℃)	Product size (bp)	References
Toxoplasma spp.	*B1*	Tg1-F-5'- TGT TCT GTC CTA TCG CAA CG -3'	48℃	536	Kalambhae *et al.* (2017)
		Tg2-R-5'- ACG GAT GCA GTT CCT TTC TG-3'			
		Tg3-F-5'- TCT TCC CAG ACG TGG ATT TC -3'			
		Tg4-R-5'- CTC GAC AAT ACG CTG CTT GA -3'			
Echinococcus spp.	*rRNA*	F-5'-CAT TAA TGT ATT TTG TAA AGT TG-3'	58℃	255	Stefanic *et al.* (2004)
		R-5'-CAC ATC ATC TTA CAA TAA CAC C-3'			
Giardia spp.	*tpiA*	F-5'-CGA GAC AAG TGT TGA GAT G-3'	55℃	576	Abbas *et al.* (2016) [2]
		R-5'- GGT CAA GAG CTT ACA ACA CG-3'			
Toxocara canis	*ITS-2*	F-5'- AGT ATG ATG GGC GCG CCA AT - 3'	55℃	380	Jacobs *et al.* (1997)
		R-5' - TTA GTT TCT TTT CCT CCG CT - 3'			
		R-5'-CAT AAA TAC ACT TTT ATA GTC CTC G-3'			

Conclusion

The study confirms the presence of key zoonotic parasitic pathogens in slaughterhouse environments, demonstrating that both organized and unorganized facilities contribute to the persistence of parasitic stages in surface and wastewater matrices with molecular methods outperforming microscopy in detection. Contamination identified across high-contact zones highlights gaps in hygiene and effluent management practices, underscoring the need for strengthened sanitation and routine molecular surveillance to reduce zoonotic risk. Overall, the study reinforces the need for enhanced One Health-oriented surveillance frameworks to safeguard food safety and public health in slaughterhouse settings.

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