



Molecular Characterization of Hydatid Cysts in Slaughtered Sheep from the Rayalaseema Region of Andhra Pradesh

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Abstract

Cystic echinococcosis (hydatidosis) is an important zoonotic parasitic disease of considerable veterinary, public health and economic significance worldwide. The disease is caused by the larval stages of *Echinococcus* spp., which have a complex life cycle involving ungulates and other mammals as intermediate hosts and domestic or wild carnivores as definitive hosts. The present study investigated the prevalence, epidemiological distribution and molecular characteristics of *Echinococcus* spp. in slaughtered sheep from the Rayalaseema region of Andhra Pradesh, India. A total of 3,683 slaughtered sheep from abattoirs located across eight districts were examined for hydatid cysts, of which 87 were positive, resulting in an overall prevalence of 2.36%. Tirupati recorded the highest district-level prevalence at 4.20% (25/595), whereas Sri Satya Sai recorded the lowest at 1.17% (5/424). The prevalence was higher in ewes (3.07% (45/1464)) than in rams (1.90% (42/2219)). Hydatid cysts were detected more frequently in the lungs (1.25% (46/3683)) than in the liver (1.11% (41/3683)). Among the 87 cysts examined, 39 (44.83%) were fertile, 28 (32.18%) were sterile and 20 (22.99%) were calcified. Prevalence increased with age, reaching 4.47% (41/917) in sheep older than 5 years compared with 1.19% (19/1587) in sheep aged 0–3 years. Significant differences were observed according to sex ($P < 0.05$) and age ($P < 0.01$). Molecular characterization was performed by PCR amplification of a partial fragment of the mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) gene, followed by sequence analysis. The isolates were confirmed as *Echinococcus granulosus* and clustered within the G1/G3 genotype group. These findings demonstrate the continued circulation of cystic echinococcosis among sheep in Rayalaseema, while the occurrence of fertile cysts suggests that infected sheep may contribute to the local transmission of *Echinococcus granulosus* fertile cysts suggests that infected sheep may contribute to the local transmission cycle of *E. granulosus*.

Keywords: Hydatidosis; Meat-Borne Zoonotic Disease; *Echinococcus granulosus*; Rayalaseema Region; Mitochondrial Cytochrome Oxidase 1 Gene (COX1)

Introduction

Cystic echinococcosis (CE), also known as hydatidosis, is a widely distributed zoonotic parasitic disease of considerable public health and veterinary importance [1]. The disease is particularly important in regions where livestock production and animal husbandry are major economic activities. According to the World Health Organization, echinococcosis remains a significant health concern in endemic regions, where incidence may exceed 50 cases per 100,000 population. In livestock, cystic echinococcosis is also responsible for substantial economic losses through condemnation of infected organs, reduced productivity, and costs associated with diagnosis and control [2].

Sheep play a particularly important role in maintaining the transmission of *Echinococcus granulosus* because they frequently harbour fertile and viable hydatid cysts. Consequently, the dog–sheep cycle is considered one of the most efficient and epidemiologically important transmission pathways for sustaining the parasite in endemic areas [3]. Domestic dogs become infected by consuming parasite-containing viscera from infected livestock and subsequently contaminate the environment with *Echinococcus* eggs, thereby facilitating continued transmission to susceptible intermediate hosts.

Cystic echinococcosis has been reported from several regions of India and both epidemiological and molecular studies have demonstrated the circulation of *E. granulosus* among livestock populations [3-5]. Molecular characterization is particularly important because identification of circulating genotypes provides insight into parasite transmission patterns and the host species involved in maintaining local transmission cycles. Previous studies from India have identified several genotypes of *E. granulosus*, including the commonly reported G1 and G3 genotypes.

Despite the importance of sheep production in southern India, information on the prevalence and molecular characteristics of cystic echinococcosis in sheep from the Rayalaseema region of Andhra Pradesh remains limited. Therefore, the present study was undertaken to determine the prevalence and epidemiological distribution of hydatid cysts in slaughtered sheep from different districts of Rayalaseema and to characterize *Echinococcus* isolates molecularly using PCR amplification and sequence analysis of the mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) gene.

Materials and Methods

Ethical approval

The study was approved by the Institutional Animal Ethics Committee (IAEC), College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India (Approval No. 281/GO/ReBi/S/2000/CPCSEA/CVSc/VPT/074/Veterinary Parasitology/2025).

Study area

The study was conducted in the Rayalaseema region of Andhra Pradesh, India, comprising the districts of Anantapur, Sri Satya Sai, Kurnool, Nandyal, YSR Kadapa, Annamayya, Chittoor, and Tirupati. The region is characterized predominantly by semi-arid climatic conditions and extensive livestock production, particularly small-ruminant farming. The close interaction between sheep and dogs in livestock-rearing areas may facilitate maintenance of the *Echinococcus* transmission cycle (Figure 1).

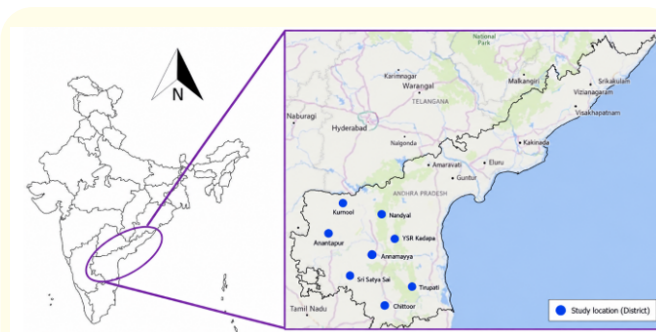


Figure 1: Map showing the study area and sampling locations in the Rayalaseema region of Andhra Pradesh, India.

Sample collection

Hydatid cyst samples were collected over a period of nine months, from July 2025 to March 2026, from sheep slaughtered at government and private slaughterhouses located across the Rayalaseema region. Visceral organs, particularly the liver and lungs, were examined for the presence of hydatid cysts. Sample collection was carried out in coordination with veterinary officers of the Animal Husbandry Department and personnel from the respective abattoirs.

Slaughtered sheep were carefully examined for cystic lesions, particularly in the visceral organs. Organs containing suspected hydatid cysts were excised using sterile surgical blades and scissors while taking care to avoid rupture or contamination of the cysts. The collected cysts were placed in sterile containers containing 0.9% NaCl solution and transported to the laboratory in sealed ice boxes for further examination.

Laboratory analysis

Collection of hydatid cyst fluid

The external surface of each hydatid cyst was cleaned aseptically. The cyst wall was punctured using a sterile 18-gauge needle and the cyst fluid was carefully aspirated for microscopic examination.

Microscopic examination

The collected cyst fluid was centrifuged at 1,000 rpm for 2 min. A drop of the resulting sediment was transferred onto a clean microscopic glass slide, covered with a coverslip and examined microscopically for the presence of hydatid elements, including protoscolices and hooklets.

The fertility of the cysts was determined based on the presence of protoscolices. Cysts containing protoscolices were classified as fertile, whereas cysts without protoscolices were considered sterile. Cysts showing hard and mineralized consistency were categorized as calcified.

Viability of protoscolices was evaluated using 0.1% eosin staining, with viable protoscolices remaining unstained and nonviable protoscolices taking up the stain.

DNA extraction and molecular analysis

Fertile hydatid cysts selected for molecular analysis were subjected to DNA extraction. The concentration and purity of the extracted DNA were assessed using a nano-spectrophotometer. DNA concentrations ranged from 89.58 to 97.73 ng/ μ L, and samples with A260/A280 ratios between 1.7 and 1.9 were selected for further molecular analysis.

Conventional PCR was performed for molecular confirmation of *Echinococcus* spp. by amplification of a partial fragment of the mitochondrial cytochrome c oxidase subunit 1 (*cox1*) gene, using primers described by Pednekar, *et al.* [6]. The *cox1* gene was amplified

using the forward primer 5'-GCCATCCTGAGGTTTATGTGTT-3' and the reverse primer 5'-CGACATAACATAATGAAAATGAGC-3'.

PCR amplification conditions

PCR amplification was performed in a total reaction volume of 20 μ L, containing 10 μ L of 2 $\times$ master mix, 1 μ L each of forward and reverse primers, 1 μ L of genomic DNA, and 7 μ L of nuclease-free water. The PCR cycling conditions consisted of an initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 2 min. A final extension was performed at 72°C for 10 min. The amplified PCR products were examined for the presence of a band corresponding to the expected *cox1* amplicon size.

Sequence and phylogenetic analysis

PCR products showing a clear band of the expected size were purified using a PCR Product Purification Kit (HiMedia Laboratories) according to the manufacturer's instructions. Purified amplicons were subjected to bidirectional sequencing at Barcode Biosciences India Pvt. Ltd., Bangalore, India. The resulting nucleotide sequences were compared with reference sequences available in the National Center for Biotechnology Information (NCBI) database using the BLASTn algorithm. Sequence similarity and phylogenetic relationships were subsequently evaluated to determine the genotype affiliation of the *Echinococcus* isolates.

Statistical analysis

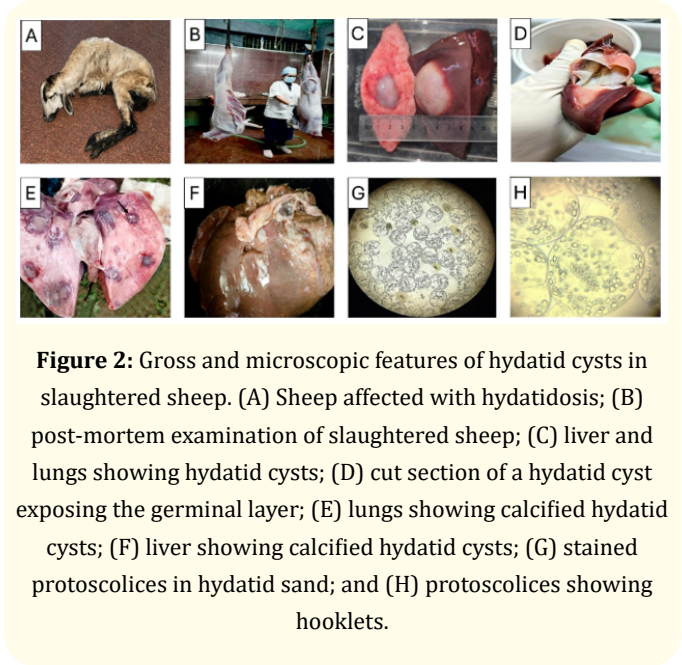
The overall prevalence of cystic echinococcosis was calculated as the proportion of infected sheep among the total number of animals examined. Prevalence was further evaluated according to district, sex and age group. Differences in infection frequencies among the respective categories were assessed using the chi-square test. Statistical analyses were performed using IBM SPSS Statistics and differences were considered statistically significant at the specified probability levels.

Results and Discussion

Prevalence and characteristics of hydatid cysts

Of the 3,683 slaughtered sheep examined, 87 were positive for hydatid cysts, corresponding to an overall prevalence of 2.36%. Gross and microscopic examination demonstrated typical hydatid cyst structures in affected organs (Figure 2). Among the 87 cysts examined, 39 (44.83%) were fertile, 28 (32.18%) were

sterile and 20 (22.99%) were calcified. Fertile cysts contained protoscolices and hooklets and viability assessment using 0.1% eosin differentiated viable, unstained protoscolices from nonviable stained forms.



The presence of fertile cysts is epidemiologically important because viable protoscolices can maintain the parasite life cycle when infected viscera become accessible to definitive hosts, particularly dogs. Similar microscopic criteria based on protoscolices and hydatid sand have been widely used to determine cyst fertility and viability [7-9]. The relatively high proportion of fertile cysts observed in the present study therefore indicates the potential for continued transmission of cystic echinococcosis within the sheep–dog cycle in the study region.

District-wise distribution

The prevalence of cystic echinococcosis varied numerically among the eight districts examined (Table 1). The highest prevalence was recorded in Tirupati [4.20% (25/595)], followed by Chittoor [2.80% (11/392)], YSR Kadapa [2.38% (13/546)] and Annamayya [2.18% (9/412)]. Lower prevalence was observed in Anantapur [1.95% (11/563)], Kurnool [1.87% (8/427)], Nandyal [1.54% (5/324)] and Sri Satya Sai [1.17% (5/424)].

Districts	No. of sheep examined	No. of sheep infected	Prevalence (%)
Anantapur	563	11	1.95
Sri Satya Sai	424	05	1.17
Chittoor	392	11	2.80
Tirupati	595	25	4.20
YSR Kadapa	546	13	2.38
Annamayya	412	09	2.18
Kurnool	427	08	1.87
Nandyal	324	05	1.54
Total	3683	87	2.36
Chi-square value	13.48		
df	7		
p value	0.06		

Table 1: District wise distribution of Hydatidosis in sheep of Rayalaseema region of Andhra Pradesh.

(P > 0.05) No significant difference. Chi-square test. Figures in parenthesis indicate percent.

Despite these numerical differences, the overall variation among districts did not reach statistical significance ($\chi^2 = 13.48$, df = 7, P = 0.061). Therefore, the higher prevalence observed in Tirupati should be interpreted cautiously rather than as evidence of a statistically greater infection burden in that district. Geographic variation in cystic echinococcosis may reflect differences in livestock management, access of dogs to infected offal, slaughter practices and environmental contamination with *Echinococcus* eggs. Nevertheless, the detection of infected sheep in all eight districts suggests that transmission is not restricted to a single locality within Rayalaseema.

Association with sex and age

The prevalence of hydatidosis was significantly higher in ewes [3.07% (45/1464)] than in rams [1.90% (42/2219)] ($\chi^2 = 4.83$, P = 0.0279; Table 2). A similar tendency toward greater infection in females has been reported in other livestock studies [10-12]. One likely explanation is the longer retention of breeding females within flocks, resulting in prolonged cumulative exposure to infective *Echinococcus* eggs compared with males that are frequently slaughtered at younger ages. Thus, the observed difference may reflect differences in duration of exposure rather than an inherent sex-related susceptibility.

Name of District	Rams		Ewes	
	Number exam-ined	Number infected (%)	Number exam-ined	Number infected (%)
Anantapur	347	5 (1.44)	216	06 (2.78)
Sri Satya Sai	259	3 (1.15)	165	02 (1.21)
Chittoor	247	6 (2.43)	145	5 (3.45)
Tirupati	351	13 (3.70)	244	12 (4.92) *
YSR Kadapa	329	05 (1.52)	217	08 (3.68)
Annamayya	227	04 (1.76)	185	05 (2.70)
Kurnool	274	04 (1.46)	153	04 (2.61)
Nandyal	185	02 (1.08)	139	03 (2.15)
Total	2219	42 (1.90)	1464	45 (3.07)
Chi-square value	4.83			
df	1			
P value	0.0279			

Table 2: Gender wise distribution of Hydatidosis in sheep of Rayalaseema region of Andhra Pradesh.
(P < 0.05) * Significance difference. Chi-square test. Figures in parenthesis indicate percent.

Age showed an even stronger association with infection (Table 3). Prevalence increased from 1.19% (19/1587) in sheep aged 0–3 years to 2.29% (27/1179) in those aged 3–5 years and 4.47% (41/917) in animals older than 5 years. This age-related increase was highly significant ($\chi^2 = 27.05$, $df = 2$, $P < 0.0001$). The progressive increase in prevalence with age is consistent with the chronic nature of cystic echinococcosis and the cumulative probability of exposure to parasite eggs over successive grazing seasons. Older animals also have more time for hydatid cysts to develop to a detectable size. Similar age-associated patterns have been reported in sheep and other livestock populations [5,11-13].

Molecular identification and phylogenetic analysis

DNA extracted from 25 fertile hydatid cysts was subjected to conventional PCR targeting a partial region of the mitochondrial *cox1* gene. Amplification produced the expected 434-bp product, confirming the presence of *Echinococcus* DNA in the analyzed

Age of the animal	No. of animals examined	No of animals infected with Hydatid cysts	Percentage
0 – 3 yr	1587	19	1.19%
3 – 5 yrs	1179	27	2.29%
>5yrs	917	41	4.47%**
Total	3683	87	2.36%
Chi-square value	27.05		
df	2		
p value	0.00013		

Table 3: Age wise distribution of Hydatidosis in sheep of Rayalaseema region of Andhra Pradesh.
(P < 0.01) ** Highly Significance difference. Chi-square test. Figures in parenthesis indicate percent.

samples (Figure 3). Sequence comparison using BLAST further identified the isolates as *E. granulosus*.

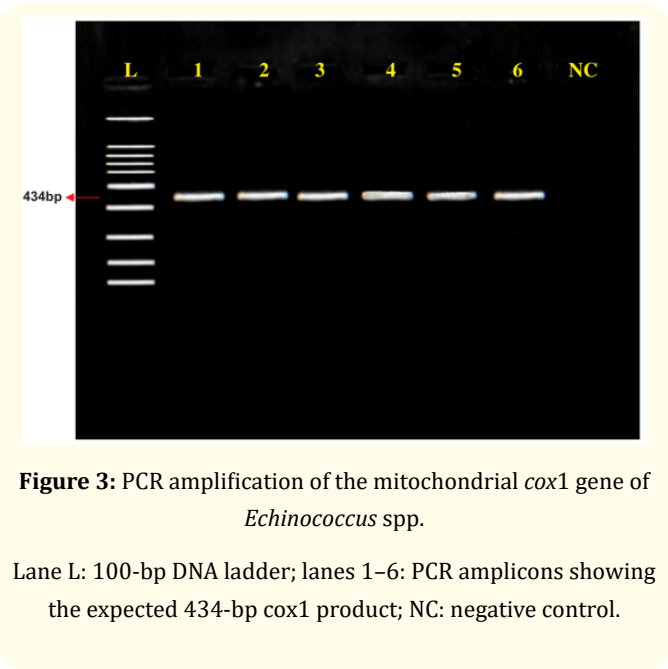


Figure 3: PCR amplification of the mitochondrial *cox1* gene of *Echinococcus* spp.
Lane L: 100-bp DNA ladder; lanes 1–6: PCR amplicons showing the expected 434-bp *cox1* product; NC: negative control.

Phylogenetic analysis was performed using four representative sequences deposited in GenBank under accession numbers PZ025233, PZ027964, PZ028098 and PZ028099 (Figure 4). The sequences showed 99.87–100% identity, with low genetic divergence among the study isolates. The Andhra Pradesh isolates clustered with reference sequences belonging to the G1/G3 genotype group of *E. granulosus sensu stricto*.

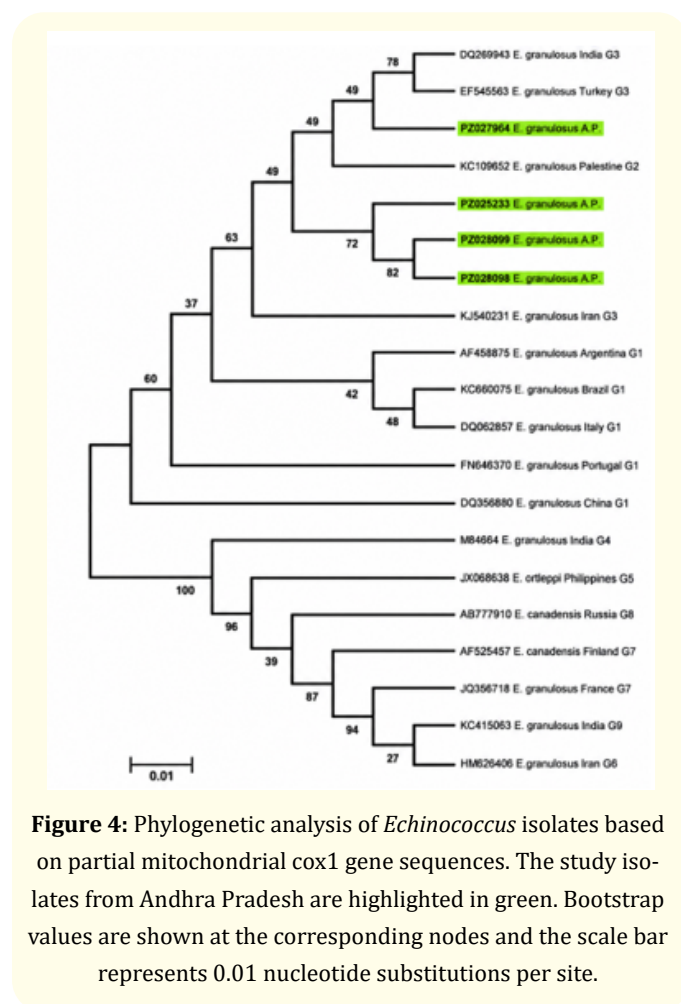


Figure 4: Phylogenetic analysis of *Echinococcus* isolates based on partial mitochondrial cox1 gene sequences. The study isolates from Andhra Pradesh are highlighted in green. Bootstrap values are shown at the corresponding nodes and the scale bar represents 0.01 nucleotide substitutions per site.

The occurrence of the G1/G3 group is consistent with previous molecular studies from India, where these genotypes have been frequently detected in livestock [4,6,14,15]. Similar clustering of sheep-derived isolates within the G1–G3 complex has also been reported from other endemic regions [16]. Because sheep are important intermediate hosts for *E. granulosus sensu stricto*, the molecular findings, together with the occurrence of fertile cysts, support the continued involvement of the sheep–dog transmission cycle in the Rayalaseema region.

The identification of both G1 and G3 genotypes in the present study is epidemiologically important because these genotypes are commonly designated as the common sheep strain (G1) and Indian buffalo strain (G3), respectively. G1 is widely associated with the sheep–dog transmission cycle, whereas G3 has been predominantly associated with buffaloes in India. However, these designations represent genotype–host associations rather than strict host specificity, as both G1 and G3 have been reported from different livestock species. Molecular studies in India have demonstrated the circulation of G1 and G3 among sheep, buffaloes, cattle, goats and other intermediate hosts, indicating that these genotypes may participate in overlapping transmission cycles [6,17]. Therefore, the detection of G1/G3 genotypes in sheep in the present study suggests that both sheep-associated and buffalo-associated *Echinococcus granulosus* lineages may be circulating in the Rayalaseema region. Nevertheless, as the present study was based on isolates obtained from sheep, the direct contribution of buffaloes to transmission cannot be established from the present findings alone. Further molecular characterization of hydatid cysts from buffaloes, other livestock and dogs in the region would help clarify the host distribution and transmission. This finding also indicates the possibility of interconnected transmission cycles involving sheep, buffaloes and dogs, although the present study, being based on sheep isolates, cannot establish the contribution of buffaloes or other intermediate hosts directly. Further molecular characterization of hydatid cysts from buffaloes, other livestock and dogs in the region would be useful to clarify the host distribution and transmission dynamics of G1 and G3 genotypes.

Overall, the combined epidemiological and molecular findings demonstrate ongoing circulation of cystic echinococcosis among slaughtered sheep in Rayalaseema. Although the overall prevalence was relatively low, the presence of fertile cysts and G1/G3-associated *E. granulosus sensu stricto* isolates indicates that conditions necessary for parasite transmission remain established in the region. The findings of the present study provide important epidemiological and molecular insights into the maintenance and transmission of cystic echinococcosis in the Rayalaseema region. The detection of fertile hydatid cysts in 44.83% of infected sheep is epidemiologically important because sheep harbouring fertile cysts may serve as a source of infection for dogs when infected viscera are improperly disposed of or become accessible to them. Infected dogs can subsequently disseminate

Echinococcus granulosus eggs into the environment, facilitating infection of grazing sheep and thereby maintaining the sheep-dog transmission cycle [18-20]. The molecular characterization of the isolates identified *E. granulosus sensu stricto* within the G1/G3 genotype group, which has been reported from livestock hosts including sheep and buffalo [6,17]. Although the present study confirms the occurrence of this genotype group in sheep, the direct involvement of buffalo in transmission cannot be established from the present sheep-based dataset and warrants further investigation through molecular surveillance of other potential intermediate hosts. From a zoonotic perspective, the occurrence of fertile *Echinococcus* cysts in slaughtered sheep indicates a potential role of infected livestock in maintaining the parasite cycle and indirectly, in sustaining the risk of human exposure through environmental contamination by infected dogs [19]. The integration of abattoir surveillance, assessment of cyst fertility and molecular characterization in the present study therefore provides useful baseline information for understanding local transmission patterns and developing regionally appropriate prevention and control strategies. Strengthening meat inspection, ensuring safe disposal of infected offal, preventing dogs from accessing raw livestock viscera, appropriate dog management and deworming, and increasing awareness among livestock owners and abattoir personnel could help reduce opportunities for continuation of the parasite life cycle [19,20]. Continued surveillance involving sheep, other livestock hosts, and definitive hosts would further strengthen the epidemiological evidence required for effective control of cystic echinococcosis in the region [20].

Conclusion

The present study demonstrates the continued occurrence of cystic echinococcosis in slaughtered sheep from the Rayalaseema region of Andhra Pradesh, with an overall prevalence of 2.36%. Infection was more frequent in ewes and increased significantly with age, while district-level variation was not statistically significant. The detection of fertile hydatid cysts indicates the potential for continued parasite transmission in the region. Molecular analysis of the mitochondrial *cox1* gene confirmed the isolates as *Echinococcus granulosus sensu stricto*, clustering within the G1/G3 genotype group. These findings support the continued involvement of the sheep-dog transmission cycle and emphasize the value of combining abattoir surveillance with molecular characterization for understanding local transmission patterns and guiding control measures.

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Conflict of Interest

The authors do not have any conflicts of interest for this manuscript.

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