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RESEARCH ARTICLE

Epidemiology of Bovine Hydatidosis: Urbanization, Dogs, Animal Care and Proximity to Slaughterhouses are Important Risk Factors for Cattle

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A B S T R A C T

Cystic echinococcosis, a neglected tropical disease caused by *Echinococcus granulosus*, is of One-Health importance. The disease has significant impact on the economy of Pakistan, where livestock is an important pillar of farming. Given the large socio-economic and zoonotic importance of cattle, we explored echinococcosis prevalence in livestock, focusing on three previously little-studied districts of Punjab (Narowal, Sheikhupura and Sialkot), Pakistan. We screened in total 1168 slaughtered cattle for presence of hydatid cysts. The collected hydatid cysts were subjected to microscopy, histopathology, and PCR. Overall disease prevalence was 7.7% (n=1168), significantly higher in Narowal (9.6%) than in Sheikhupura (7.6%) and Sialkot (5.7%). The oldest cattle group (>5 years) had significantly higher prevalence (11.8%) than younger animals (6.8% in 3-5-year and 4% in 1-3-year-olds). Females had significantly higher prevalence (9.1%) than male (4.9%) cattle. Significantly more cysts occurred in cattle lungs (71.4%) rather than the liver (28.5%), and the number of fertile cysts was significantly higher in lungs (56.9%) compared to liver (50%). PCR and sequencing of one cyst confirmed the species to be *Echinococcus granulosus*, with phylogenetic analysis clustering our ND1 sequence with the G1-G3 lineages. We found hydatid cysts to cause histopathological changes in the host tissue surrounding the cysts, including atrophy, fibrosis, cell degeneration and leucocytic infiltration. General Linear Models revealed that animals (cattle and dogs) kept near slaughterhouses, particularly in urban and semi-urban areas, significantly increased the risk of cystic echinococcosis in cattle. Based on these findings, we recommend a public health campaign to increase awareness of zoonotic infections.

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INTRODUCTION

Livestock are affected by many infectious diseases that adversely reduce their growth and production. Cystic echinococcosis or hydatidosis is one such zoonotic disease, caused by the cestode parasite *Echinococcus granulosus* that infects both animals and humans (Ali *et al.*, 2015). This is a neglected tropical disease (NTD) of One Health importance. The dog (*Canis familiaris*) is the definitive host for *Echinococcus granulosus*, whereas intermediate hosts are herbivores and omnivores including humans. The disease is transmitted to the intermediate host by ingestion of the cestode eggs expelled by the definitive host (Lawson and Gemmell, 1983). Humans act as accidental hosts by acquiring the infection through close contact with dogs, or by ingesting water or food contaminated with parasitic eggs (Nakao *et al.*, 2010; Torgerson *et al*., 2011). In intermediate hosts, the parasite develops into a fluid filled hydatid cyst, typically in the liver and lungs and rarely in the kidney, brain and bone marrow, resulting in morbidity and mortality (Battelli 2009). In humans, the infection causes bile duct obstruction and pleural fistula disorders (Daali *et al.*, 2001).

Cystic echinococcosis has a cosmopolitan distribution (Haleem *et al.,* 2018), and is highly prevalent in herd keeping areas across the world (Bekele and Butako 2011), ranging from 12% in India (Grakh *et al.,* 2020), 13.9% in Iran (Vaisi-Raygani *et al.,* 2021) and 22% in Ethiopia (Shumuye *et al.,* 2021) to 53.9% in China (Fan *et al.,* 2022; Yang *et al*., 2022). Within Pakistan, prevalence in different host species ranges from 2.4 to 65.4% (Tasawar *et al.,* 2014). The parasite is responsible for huge economic losses due to reduced milk and meat production, and condemned meat (Lemma *et al.,* 2014; Basinger *et al*., 2021). It causes an estimated loss of Rs 26.5 million annually to the livestock sector of Pakistan (Latif *et al.,* 2010), approximately USD 1.65 per organ (Shafiq and Athar 2004). X-ray Computed Topography (CT) scans (e.g. Díaz-Menéndez *et al.,* 2012), ELISA and PCR (Khan *et al.,* 2023) can be exploited for diagnosis of this disease, but to reduce prevalence, further information is needed on the distribution and risk factors associated with *E. granulosus.*

There is limited data on the epidemiology of cystic echinococcosis in remote areas of Northern Punjab, Pakistan, as well as on the histopathological changes associated with this disease. The present study was thus designed to explore the prevalence, histopathology, and associated risk factors of the causative agent of bovine cystic echinococcosis while sampling slaughtered cattle in three districts of the Punjab Province in Pakistan.

MATERIALS AND METHODS

Ethical approval and sample size determination: All experimental procedures were approved by the Institutional Guidelines of Ethical Review Committee of UVAS, Lahore, vide letter No. 939-1, Dated 05-09-2019.

Samples size for determination of *Echinococcus* occurrence was calculated by considering previous prevalence of 45.4% (Shahzad *et al.,* 2014) with 95% confidence interval and 5% absolute precision. The formula used to calculate the sample size (Thrusfield 2018) was: $N = (1.96)^2 P (1\text{-}P)/d^2$, where N = required sample size, P is previous prevalence and d is desired absolute precision, such that N = (3.84) 0.45 (1-0.45) / (0.0025)=380. We collected 400 samples from each district to allow for any samples that might subsequently have to be excluded from data analysis.

Sample collection and questionnaire: From three districts in Northern Punjab, Pakistan (Narowal, Sheikhupura and Sialkot, Fig. 1), 1200 cattle (n=400/district) were examined in the main slaughterhouse for each district through random sampling for the presence of hydatid cysts in the liver, lungs, kidneys, and spleen between December 2019 to November 2020. The animals were categorised based on age as <1, 1-3, 3-5 and >5 years old. We subsequently excluded animals younger than 1 year from statistical analyses due to the small sample size (31), as cattle of this age are rarely slaughtered. Cysts were collected in sterilized containers with 70% ethanol (for DNA extraction) or 10% neutral buffered formalin (for histology). Samples for histopathology (individual cysts from eight different animals) were processed according to Belina *et al.* (2015). A questionnaire based on 20 simple close-ended questions about owner and animal details was used for risk factor analysis. All 1168 farmers who completed the survey (one farmer for each surveyed cattle, and each animal/farmer in this study originated from a different farm) were asked about district of their farm, farm habitat (peri-urban, urban and rural), whether they kept dogs, reason for keeping dogs (guard dog, companion and hunting), if they dewormed their dogs, home slaughtering of animals, disposal of offal (buried, left open or undisclosed), feeding dogs with viscera, disposal of dog faeces, distance from abattoir, deworming of cattle, and animal feeding conditions (confined/mixed/grazing) in a face-to-face discussion at the slaughterhouses. We also recorded the species, age and sex of the animal, and season of the slaughter (spring - March, April, and May; summer - June, July, and August; autumn - September, October, and November; and winter - December, January, and February). Post-slaughter, we recorded the presence of cysts (yes or no), cyst location (liver, lungs or other) and fertility (fertile, sterile, or calcified).

Parasite molecular identification: Hydatid cysts were characterized according to Haleem *et al.* (2018). To confirm parasite species, DNA was extracted from one randomly selected cyst sample using a commercially available DNA extraction kit **(**WizPrep™ gDNA Tissue kit Wizbiosolutions, South Korea, W71060-300). A 226 bp fragment of the mtDNA ND1 gene was targeted by using primers Eg1F81 5´-GTT TTT GGC TGC CGC CAGAAC-3´, Eg1R83 5´-AAT TAA TGG AAA TAA TAACAA ACT TAA TCA ACA AT-3´ (Boufana *et al.,* 2013). PCR was performed in a T100 Thermal Cycler (Bio-Rad, Hercules, CA, USA) as described previously (Mahmood *et al.,* 2022). Briefly, a total reaction volume of 50 µL included 25 µL of 2X AmpMaster™ Taq master mix (GeneAll®, Exgene™, catalogue number 541-001), 10 µL Ultrapure™ DEPC water (Invitrogen, 750023), 5 µL each of the forward and reverse primer (50 µM each), and 5 µL DNA extract, with PCR conditions: initial denaturation at 94°C for 3 minutes followed by 28 cycles (denaturation for 30 s at 94°C, annealing at 59.8°C for 30 s, extension at 72°C for 1 minute) and final extension step at 72°C for 5 minutes. The PCR product was then run on a 2% agarose gel (1 h at 120 V), stained with SYBR safe DNA (catalogue no. 2291850; Invitrogen, Waltham, MA, USA) and viewed under a transilluminator (Trans Lum SOLO, Biotop China, serial no. 21102053).

Sequencing and phylogenetics: The PCR product was sequenced by a commercial sequencing facility (1st BASE Pte Ltd., Singapore) using the forward primer. The resulting chromatogram was trimmed to delete low quality bases, and the resulting 162 bp sequence submitted to GenBank (Accession Number: OM935772). For comparison, we downloaded 13 ND1 sequences of *Echinococcus* from GenBank, covering the principal lineages of *Echinococcus granulosus* and closely related lineages (Bowles *et al.,* 1992). The downloaded ND1 sequences were aligned with our new sequence in Genious Prime (version 2022.2.2; www.geneious.com) using the

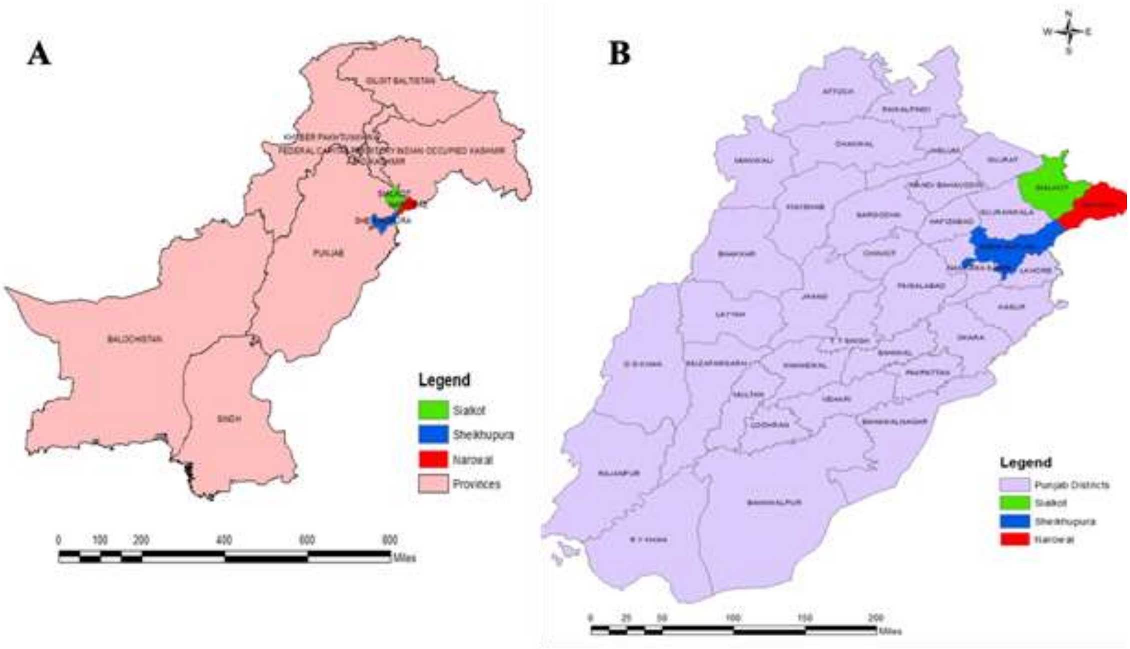

Fig. 1: Sampling districts in the current study. (A) Provinces of Pakistan and (B) districts within the province of Punjab. Maps constructed in QGIS software (3.28.1).

MUSCLE plugin. A maximum likelihood phylogenetic tree was reconstructed using IQ-tree webserver (version 2.2.0) (Trifinopoulos *et al.,* 2016). The most suitable substitution model for our alignment was determined by the built-in model finder function (yielding the HKY+I model with empirically determined base frequencies, based on the Bayesian Information Criterion) (Kalyaanamoorthy *et al.,* 2017). Statistical support for branches was determined from 1000 ultrafast bootstrap replicates (Hoang *et al.,* 2018).

Statistical analyses: All statistical analyses were performed using RStudio version 4.2.2. To understand the relationship between the chance of cysts being present within cattle and key environmental variables, we developed binomial generalized linear models (GLMs) with a logit link function, with the dependant variable being the presence or absence of cysts in slaughtered animals. We ran three models to analyse the data. In Model 1, the independent variables were district, age of animal and sex. Model 2 included habitat, deworming practice in cattle, farming type, animal feeding, home slaughtering, disposal of offal, seasons, and distance from abattoir as independent variables. Model 3 included keeping of dogs, reasons for keeping dogs, deworming of dogs, feeding dogs with viscera, and disposal of dog faeces. Removal of non-significant terms was performed to ensure model refinement (Thomas *et al.,* 2017). The odds ratios were extracted from GLMs. Finally, Pearson Chi square tests assessed the association between cyst location and fertility. We excluded calcified cysts from the analysis, as only one calcified cyst was recovered.

RESULTS

Epidemiological study: From a total of 1168 slaughtered cattle from three districts in Pakistan, gross examination revealed 90 (7.7%) were infected with hydatid cysts (Fig. 2A and 2B). Infection rate was significantly higher in the Narowal district (9.6%) followed by Sheikhupura (7.6%) and Sialkot (5.7%) (Table 1). The oldest animals (>5 years) were significantly more prone to infection (11.8%) than those aged 3-5 years (6.8%) and 1-3-years (4%) (Table 1). Males were significantly less infected (4.9%) than females (9.1%) ($P<0.001$, Table 1). Cysts were more common in lungs (71.4%) compared to liver (28.5%), and cyst fertility was also significantly higher in lungs (56.9%) than liver (50%) (chi-square test, χ^2=1203.7, $P<0.05$). Prevalence was significantly higher in winter (11.2%) compared to autumn (8.0%), spring (6.1%) and summer (5.4%) (Table 1).

The species of *Echinococcus* was confirmed through PCR and Sanger sequencing of one sample. Phylogenetic analysis (Fig. 3) revealed that the obtained ND1 sequence clustered with high statistical support (ultrafast bootstrap support: UF=100) within the wider G1/G3 lineage, identifying it as *E. granulosus sensu stricto* (Vuitton *et al*., 2020). Our short alignment provided only limited resolution about clustering within this G1/G3 lineage, suggesting clustering of our sequence with moderate support (UF=74) with the G2/G3 types. The alignment however contained one diagnostic site that allowed us to distinguish between G1 and G3, and our sequence grouped with G3 (at site 82 of our submitted GenBank sequence, G1 had a C, and all other sequences included in the alignment, including G3 and our sequence, had a T).

Sociodemographic survey regarding disease risk factors: Infection was significantly higher in urban (29.4%), and peri-urban (25.4%) areas compared to rural locations (5.4%; Table 1). Infection in cattle was significantly higher where farmers kept dogs at home or with other animals; ($P<0.001$) and higher when these were hunting (30.7%; $P<0.27$) or companion (69.3%; $P<0.001$) dogs compared to guard dogs (7.9%), and if the owners dewormed their dogs this decreased prevalence ($P<0.001$). The practice of home slaughtering did not increase the risk

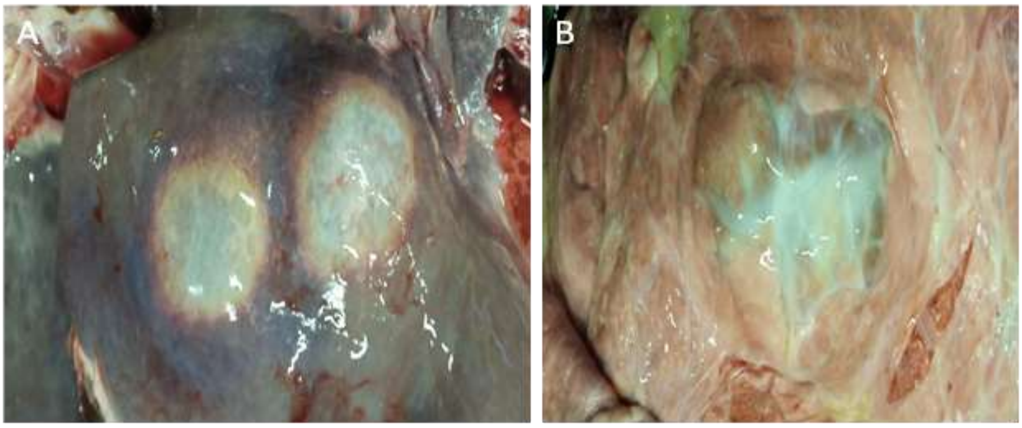

Fig. 2: Photographs showing multiple hydatid cysts present in the (A) liver and (B) lungs of cattle, encountered during gross examination at slaughterhouses.

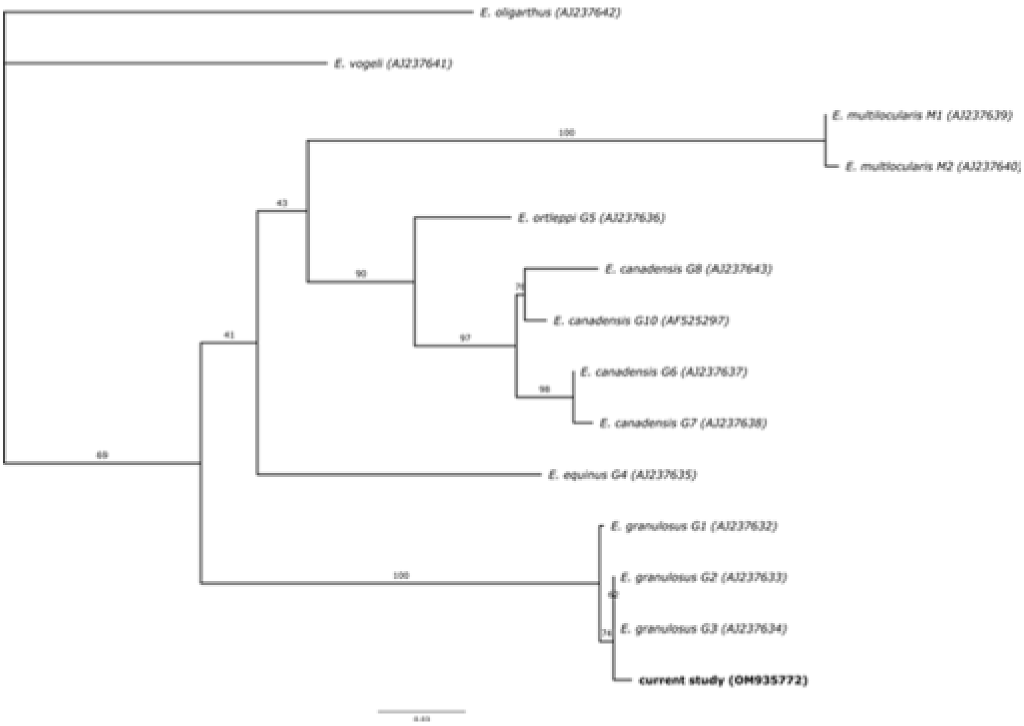

Fig. 3: Maximum likelihood phylogeny of ND1 sequences of main lineages of *Echinococcus granulosus (s.l.)* plus outgroups with GenBank accession numbers in brackets, along with the sequence obtained in the current study. Numbers on branches denote ultrafast bootstrap support values for the inferred groupings.

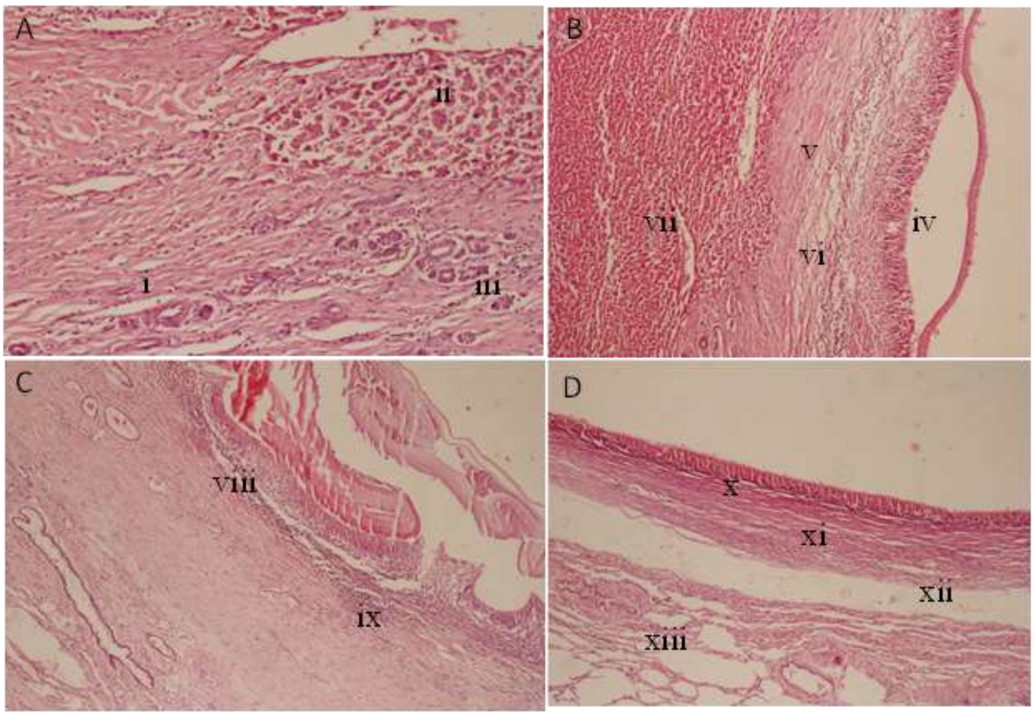

Fig. 4: Light microscope images of cattle tissue infected with *Echinococcus granulosus* stained with haematoxylin and eosin (40X magnification). (A) Liver showing (i) fibrosis, (ii) atrophy of hepatocytes and (iii) bile duct hyperplasia. (B) Liver with (iv) cyst lining, (v) infiltration of mononuclear inflammatory cells, (vi) proliferation of fibrous connective tissue and (vii) atrophy. (C) Lung showing (viii) degeneration of cells and (ix) leucocytic infiltration. (D) Lung with (x) proliferation of fibrous connective tissue, (xi) infiltration of mononuclear inflammatory cells, (xii) cystic wall and (xiii) atelectasis.

Table 1: GLM analyses of prevalence of hydatidosis of slaughtered cattle and risk factors based on data from sociodemographic survey from farmers completed in parallel to sample collection.

Risk Factors	Response	No. of Positive/ Total (%)	SE	Z. value	Odds ratios	P Value
Models 1 and 2: Risk factors related to location and cattle. Reference categories Narowal and >5 years old, peri-urban, confined, 10-20 km, buried and autumn.						
	Narowal	38/395 (9.6)				
Districts	Sheikhupura	30/393 (7.6)	0.34	1.01	1.41	0.31
	Sialkot	22/380 (5.7)	0.40	0.55	1.24	0.58
	1-3	11/272 (4.04)	0.43	-3.70	0.20	0.001
Age (years)	3-5	38/541 (6.7)	0.35	-2.38	0.43	0.01
	>5	42/356 (11.79)				
Sex	Male	20/401 (4.97)	0.26	-3.23	0.42	0.001
	Female	70/767 (9.12)				
Habitat	Peri-urban	29/114 (25.43)				
	Rural	56/1037 (5.40)	0.40	-6.79	0.06	0.001
	Urban	5/17 (29.41)	0.67	-1.10	0.47	0.27
Feeding	Confined	23/192 (11.97)				
	Mixed	65/917 (7.08)	0.39	-3.15	0.28	0.001
	Grazing	2/59 (3.38)	0.98	-0.26	0.77	0.79
Deworming cattle	Yes	61/779 (7.83)	0.31	-1.55	0.61	0.12
	No	29/389 (7.45)				
Home slaughtering	Yes	49/409(11.98)	0.31	1.32	1.51	0.18
	No	41/759 (5.40)				
Distance from abattoir	3-5 km	25/183 (13.66)	0.31	3.55	3.01	0.001
	5-10km	12/177 (6.77)	0.50	-2.43	0.29	0.01
	10-20km	53/808 (6.55)				
Disposal of offal	Buried	15/280 (5.35)				
	Left open	34/129 (26.35)	0.33	5.66	6.50	0.001
	Unknown	41/759 (5.40)	0.31	0.02	1.0	0.97
Season of slaughter	Autumn	23/286 (8.04)				
	Spring	18/295 (6.10)	0.36	0.38	1.15	0.70
	Summer	16/293 (5.46)	0.38	-0.14	0.94	0.88
	Winter	33/294 (11.22)	0.33	2.07	2.01	0.03
Model 3: Risk factors related to dogs. Reference categories for reason of keeping dog was guard dog, no dog for deworming and feeding viscera to dogs and unknown for faeces disposal.						
Keeping of dogs	Yes	67/427(15.69)	0.24	7.10	5.89	0.001
	No	23/741 (3.10)				
Reasons for keeping dogs	Guard dog	29/365 (7.94)				
	Companion	34/49 (69.38)	1.29	4.99	6.34	0.001
	Hunting	4/13 (30.76)	2.36	1.09	1.88	0.27
	No dog	23/741 (3.10)	900.88	0.02	2.88	0.97
Deworming of dogs	Yes	41/363 (11.29)	1.33	3.85	5.79	0.001
	No	26/64 (40.62)				
	No dog	23/741 (3.10)				
Feeding dogs with viscera	Yes	52/80 (65)	1.15	4.10	1.12	0.001
	No	15/347 (4.32)				
	No dog	23/741 (3.10)				
Disposal of dog faeces	Yes	16/348 (4.59)	900.8	0.01	4.19	0.98
	No	51/79 (64.55)	900.8	0.02	1.84	0.98
	Unknown	23/741 (3.10)				

Table 2: Prevalence of *Echinococcus granulosus* in cattle from two provinces (Punjab and KPK=Khyber Pakhtunkhwa) and different districts of Punjab, Pakistan.

	Positive/total animals	Prevalence (%)	Reference
Province			
Punjab	90/1168	7.7	Current study
KPK	85/538	15.8	Haleem *et al.* 2018
KPK	41/189	21.7	Khan *et al.* 2021
District			
Narowal	38/395	9.6	Current study
Sheikhupura	30/393	7.6	Current study
Sialkot	22/380	5.7	Current study
Multan	105/1179	8.9	Mehmood *et al.* 2020
Sargodha	48/857	5.6	Mehmood *et al.* 2020
Islamabad & Rawalpindi	132/3845	3.4	Khan *et al.* 2020

of disease (P<0.18), whereas farmers who improperly disposed of offal (P<0.001) and/or fed dogs with viscera increased the risk of disease (P<0.001). The improper disposal of dog faeces did not increase the infection (P<0.98). Proximity of the home or dairy farm to an abattoir enhanced the risk of disease (P<0.001). There was a higher infection among confined cattle (23/192; 11.9%) compared to those which were kept in mixed conditions (65/917; 7.0%; P<0.001) or grazing (2/59; 3.3%; P<0.79). Surprisingly, deworming of cattle did not significantly (P<0.12) impact prevalence of cystic echinococcosis.

Histopathology of liver and lungs: In the liver, fibrosis, atrophy of hepatocytes and bile duct hyperplasia were common, and at the cyst lining, infiltration of mononuclear inflammatory cells, proliferation of fibrous connective tissue and atrophy were seen (Fig. 4A and B). In the lungs, cellular degeneration, leucocytic infiltration, proliferation of fibrous connective tissue, infiltration of mononuclear inflammatory cells, cystic wall and atelectasis were observed (Fig. 4C and D).

DISCUSSION

The livestock industry is threatened by many infectious diseases, including cystic echinococcosis that causes significant animal losses (Khan *et al.,* 2023). In Pakistan, favourable socio-economic conditions for hydatidosis and high-level of infection in cattle mean that this is one of the most important diseases for cattle in the area (Fikire *et al.,* 2012). We confirmed with NDI sequencing that *Echinococcus granulosus* is present in cattle in Northern Punjab, Pakistan. Phylogenetic analysis revealed clustering within the G1/G3 lineage (which includes the microvariant G2; Vuitton *et al.,* 2020), with one substitution favouring a clustering with G3 rather than G1. Our findings indicate that the utilized primer pair, which was designed to be specific to the G1 lineage (Boufana *et al.,* 2013), may also amplify the G2/G3 lineages, consistent with lineages G1, G2 and G3 being considered a single clade by Latif *et al.* (2010). We note, however, the limited phylogenetic resolution provided by our short fragment, so this finding should be reassessed with longer sequences. A recent phylogenetic study showed that although G1 and G3 are closely related, the two lineages are clearly diagnoseable with the resolution provided by whole mitogenome DNA sequences (Zhao *et al.*, 2022). The G3 lineage has previously been reported in buffalo from India and China (Bowles *et al.* 1992; Guo *et al.* 2023), and the G1/G3 lineages in cattle from Pakistan (Mehmood *et al*., 2020), camels in Nigeria (Samari *et al.,* 2022), and dogs, sheep, and humans in Uzbekistan (Kim *et al.,* 2020). This highlights that the G1 and G3 lineages (at least those identified so far) are present in a variety of intermediate hosts and across a wide geographical distribution.

Within Pakistan, 0.71 million cattle suffer from echinococcosis in three North-East districts of the Punjab Province (Narowal, Sheikhupura and Sialkot), but at a lower prevalence (8%) than in North-West (Khyber Pakhtunkhwa; KPK) areas (Haleem *et al.,* 2018; Khan *et al.,* 2021; see Table 2). The Punjab is warmer than the KPK, and echinococcosis infection is negatively correlated with temperature; hence there is a lower risk of disease in warmer areas (Piarroux *et al.* 2015). The Punjab is also situated at a low altitude, and altitude is positively correlated with disease occurrence (Giraudoux *et al.,* 2013). We speculate that the increased grassland area in the Punjab with lower cattle densities may result in the lower infection levels compared with the KPK. Other climatic variables and variable landscape features might also contribute to the difference in infection levels, but at a local level contact between animals and animal products is the most important risk factor (Hegglin and Deplazes, 2013).

The risk factors identified in this study indicate that keeping cattle close to a slaughterhouse enhances the risk of *E. granulosus* infection. Waste in the form of infected offal from the slaughterhouses can contaminate the surrounding environment for both final and intermediate hosts (Otero-Abad and Torgerson, 2013). Cattle from urban and peri-urban areas are also more likely to be infected with *E. granulosus* than those from rural habitats (Acosta-Jamett *et al.,* 2010). This may be a result of farmers in urban and peri-urban areas living in close contact with canines, the definite host for this parasite. Dogs in urban/peri-urban habitats have greater opportunity to ingest infected organs. Many butchers discard infected tissues (liver and lungs) inappropriately, increasing the risk for canids consuming this meat (Buishi *et al.,* 2006). The rate of infection is higher in dogs whose owners feed them with viscera (Otero-Abad and Torgerson, 2013). Also, just keeping dogs with livestock increases the chance of echinococcosis (current study; Khan *et al.* 2020). Ingestion of eggs from contaminated soil is the primary route of echinococcosis infection for intermediate hosts (Shaikenov *et al.,* 2003). In the current study, cattle maintained under mixed feeding or confined feeding conditions had a higher prevalence of disease, probably due to the higher risk of environmental contamination by dogs, compared to grazing cattle.

The liver and especially lungs were the most infected organs of cattle (current study; Khan *et al.,* 2023). These highly vascularized organs are ideal for parasite growth, and the rich spongy nature of the lungs is probably more permissive for establishment and maintaining fertility of the oncosphere (Abunna *et al*., 2012). Not surprisingly, hydatid cysts cause histopathological changes to surrounding host tissues inducing atrophy, degeneration, and inflammatory cell infiltrations (current study; Beigh *et al.,* 2017). Older animals (>5 years) had more cysts and higher prevalence (Khan *et al.* 2023), likely reflecting increased exposure to the parasite over time. In agreement to Mousa *et al*. (2015), the seasonal prevalence was higher in winter. The higher prevalence in female compared to male animals might be linked to pregnancy, parturition, and lactation, sometimes leading to malnutrition and transient immunosuppression thus enhancing their susceptibility to infection (Haleem *et al.,* 2018).

Deworming of cattle did not appear to reduce the infection rate in the current study. This may be due to use of inappropriate drugs, incorrect administration, drug resistance (Gemmell *et al.,* 2001), or cost. To reduce levels of echinococcosis, it is important to educate farmers about the timing and dosage of the correct cattle dewormers, and to ensure that they have access to effective, affordable dewormers. In agreement with (Mahmood *et al.,* 2022), performing slaughtering at home did not increase the risk of infection in dogs, and reduced offal feeding to dogs reduced infection in cattle (current study; Wilson *et al.,* 2019). Deworming of dogs can help control the disease in dogs (current study), which reduces environmental spread of *Echinococcus* eggs (Hegglin and Deplazes, 2013). Seminars/workshops should be arranged for dog owners and other members of the public to increase awareness of echinococcosis and other zoonotic infections.

Conclusions: Cystic echinococcosis, caused by *E. granulosus,* including lineage G1/G3, is prevalent in Northern Punjab, Pakistan (5.7-9.6%) and causes histopathological changes in vital organs. The disease shows significant association with host age and sex, district, and homes and dairy farms close to slaughterhouses. This study also highlights the disease risk of *E. granulosus* transmission between animals and humans if dogs are kept at home or with other animals. The zoonotic impact of echinococcosis needs urgent attention by governments and stakeholders to reduce livestock loss and safeguard public health. A policy on dog keeping and handling, including registration advice on treatment, is needed, as well as control of stray dogs. Slaughterhouses with appropriate disposal pits and obligatory meat inspections are also highly recommended to reduce the prevalence of this common, but preventable, zoonotic disease.

Authors contribution: HA, MIR and MY designed the experiment. SK performed the field and laboratory experiments, analysed the data, and drafted the manuscript, with input from HA and JC. AI helped with sample collection. FH conducted phylogenetic analyses. HA, JC, and FH reviewed the manuscript.

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