INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
20–1674/2021/25–4–881–887
DOI: 10.17957/IJAB/15.1742
http://www.fspublishers.org

Full Length Article

Copro-Antigenic Sandwich ELISA based Epidemiological Survey on Prevalence of *Echinococcus granulosus* Infection in Dogs: First Insight from Pakistan

Mughees Aizaz Alvi[1], Li Li[1], Muhammad Saqib[2], John Asekhaen Ohiolei[1], Muhammad Haleem Tayyab[2], Muhammad Masood Tahir[3], Warda Qamar[4], Waqas Altaf[3], Muhammad Usman[2], Ali Hassan[2], Muhammad Rashid Khalid Bajwa[2], Bao-Quan Fu[1], Hong-Bin Yan[1*] and Wan-Zhong Jia[1*]

[1]*State Key Laboratory of Veterinary Etiological Biology, National Professional Laboratory of Animal Hydatidosis, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou 730046, People's Republic of China*
[2]*Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad 38000, Punjab, Pakistan*
[3]*Independent Researcher, Rawalpindi, Pakistan*
[4]*Department of Pathobiology, University of Veterinary and Animal Sciences, Lahore (Jhang Campus), Pakistan*
[*]For corresponding authors: jiawanzhong@caas.cn; yanhongbin@caas.cn
Received 25 November 2020; Accepted 26 December 2020; Published 25 March 2021

Abstract

Echinococcus granulosus (Eg) infection is a neglected tropical disease of humans and livestock with serious economic losses. Dogs as the definitive hosts are responsible for contaminating the environment through feces containing eggs. Using coproantigen ELISA, we tested 368 dogs randomly sampled from three Pakistani cities for infection with Eg. The highest prevalence was found in Lahore (9.80%) with an overall prevalence of 6.79%. Prevalence was higher in females (8.72%, odds ratio OR 1.71), young dogs (≤ 3 years) (6.93%, OR 1.06), stray/feral dogs (9.72%, OR 1.60), dogs fed with raw offal (8.28%, OR 2.63) and dogs with no anthelmintics treatment history (8.98%, OR 1.90). Prevalence was also higher in dogs with Body Condition Score (BCS) of 1–3 (10.86%; OR 5.75) and Grey Hound breed (15.62%; OR 6.94). Statistically significant association ($P < 0.05$) was found between copro-positivity and different variables investigated except for sex, dog breed and history for anthelmintic treatment ($P > 0.05$). Significant statistical differences (Binary logistic regression) were observed for age, companionship, feed type, BCS and previous intestinal illness. Since dogs are responsible for contaminating the environment, the Eg prevalence in this study indicates a potential risk for human and livestock populations in the study areas and suggests a proactive approach in CE management.

Keywords: *Echinococcus granulosus*; Prevalence; Copro-ELISA; Dogs; Pakistan

Introduction

Echinococcus granulosus is an important helminth of dogs that causes cystic echinococcosis (CE) in humans and livestock. CE is an emerging and potentially avertable zoonotic disease of veterinary and public health importance spreading into echinococcosis-free regions of the world (Benito and Carmena 2005; Lahmar *et al*. 2007; Rossi *et al*. 2012). It has been reported that CE affects at least one million people across the world putting annual economic loss at about 3 billion US dollars in terms of human treatment and losses in livestock production through organ condemnation, carcass weight loss, decreased milk production, and poor fecundity rate (Rashid *et al*. 2018). This burden is likely to be an underestimation due to poor investigations and surveillance systems in some endemic countries (WHO 2015; Dakkak *et al*. 2017).

E. granulosus is an obligate endoparasite with an indirect type of life cycle involving two mammalian hosts. Both domestic and feral dogs serve as definitive hosts that harbor the adult tapeworm in their small intestine, releasing into the environment (*via* feces) eggs containing infective oncosphere, leading to the contamination of pastures (Acosta-Jamett *et al*. 2010). Viable eggs in the environment can survive for a long period, thus increasing the risk of exposure and chances of infection among intermediate hosts (domestic herbivores and wild ungulates) including humans (Hidalgo *et al*. 2019). After ingestion of eggs, the onchosphere develops into the larva stage metacestode (Thapa *et al*. 2017; Ingole *et al*. 2018; Mulinge *et al*. 2018).

Echinococcus infection in dogs has been reported in many Asian countries including those sharing borders with Pakistan like China, Iran, and India (Zhang *et al*. 2006; Ghabdian *et al*. 2017; Thapa *et al*. 2017). To the best of our

To cite this paper: Alvi MA, L Li, M Saqib, JA Ohiolei, MH Tayyab, MM Tahir, W Qamar, W Altaf, M Usman, A Hassan, MRK Bajwa, BQ Fu, HB Yan and WZ Jia (2021). Copro-antigenic sandwich ELISA based epidemiological survey on prevalence of *Echinococcus granulosus* infection in dogs: first insight from Pakistan. *Intl J Agric Biol* 25:881–887

knowledge, no study on the prevalence of echinococcosis in dogs has been conducted in Pakistan. Thus, this study was designed to assess the prevalence of *E. granulosus* and the risk factors associated with the infection in dogs.

Materials and Methods

Study area

Three cities *viz.*, Faisalabad, Islamabad, and Lahore were selected for this study (Fig. 1). Geographical quadrants (latitudes and longitudes) of the study districts are mentioned in Table 1 (Pakistan Meteorological Department 2019). Outdoor Patient Departments of the Veterinary Teaching Hospitals of the University of Agriculture, Faisalabad, the University of Veterinary and Animal Sciences, Lahore and private pet clinics located in Islamabad were visited for sample collection.

Sample collection

Fecal samples were collected from owned dogs brought to the above-mentioned teaching hospitals and clinics through their owners who were requested to bring fresh feces to the Teaching Hospitals/pet clinics on their next visit, and also from feral dogs captured and brought to the same hospitals for experimental purposes. A total of 368 (owned-dogs n = 296, stray/feral dogs n = 72) fecal samples were collected. Each sample weighed approximately 25 grams. Owners were requested to put fecal samples in phosphate-buffered saline. Samples were then transported to the Laboratory of Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan, where the samples were stored at −80°C for a minimum of 5 days before testing (Liu *et al*. 2015; WHO/OIE 2001).

Risk factors investigation

Dog owners were also requested to complete a questionnaire containing the following information: age of dogs (< 3 years and ≥ 3 years), sex of dog (male and female), breed, feed type (raw meat, leftovers from owner's kitchen, or commercial dog feed), purpose (pet or guard) and deworming status (yes or no). Body condition scoring was determined as described previously (Baldwin *et al*. 2010).

The age of stray/feral dogs was determined by dentition (Anonymous 1996) and the dogs were considered not to have undergone any deworming treatment. Regarding feed type, data from the stray dog population were not included in the analysis as we were unaware of their feeding pattern (whether animal offal or disposed kitchen waste).

Copro-antigenic ELISA

All fecal samples were subjected to copro-antigenic sandwich ELISA kit purchased from Zhuhai Haitai Biological Pharmaceuticals Co., Ltd., Zuhai, China having good sensitivity, specificity, and substantial kappa value (Wang *et al*. 2021).

Briefly, the antigen was separated from each fecal sample by mixing 1g of feces with 1 mL of the sample treatment solution and centrifuged at 4000 rpm for 15 min. The sample supernatant was carefully pipetted and stored in a 1.5 mL tube to avoid contamination from other fecal materials. Two wells in the ELISA plate precoated with *E. granulosus*-specific antibody were designated for positive and negative control samples and 100 μL of controls was dispensed into those wells. In the test wells, 80 μL of sample diluent and 20 μL of each sample were dispensed (except negative and positive control wells). After incubation at 37^{o}C for 30 min, the plate was washed four times with 300 μL of washing solution (0.05% PBS-Tween 20) according to the manufacturer's instruction. Afterward, 100 μL of enzyme working solution (anti-E.g. specific antibodies conjugated with horseradish peroxidase-HRP) was dispensed in each well and incubated again at 37^{o}C for 30 min followed by washing. Thereafter, 100 μL of Chromogen-A and Chromogen-B (provided in the kit with 3,39,5,59- tetramethylbenzidine, TMB) were dispensed in each well. The reaction was allowed to stand for 10 min at 37^{o}C. Finally, 50 μL of stop solution was added and the plate was read within 5 min in a Bio-Rad microplate reader (iMark^{TM}Microplate Absorbance Reader). Optical density (OD) was measured at 450 nm.

Test validation

The test was validated if the mean OD of negative and positive controls was less than 0.5 and greater than 0.8, respectively. The samples with OD ≥ critical value (CV = mean OD negative control × 2.1) were considered positive while those with OD < CV were negative.

Statistical analysis

Prevalence was estimated at 95% confidence interval (CI) (Newcombe 1998). Chi-square test (χ2-test) was used to perform test of significance between variables and results were significant at $P < 0.05$. Univariable analysis and odds ratios (OR) were also carried out. Finally, a binary logistic regression analysis was conducted to assess the association between copro-prevalence of *E. granulosus* and the significant variables at the initial screening. All tests were carried out in IBM S.P.S.S. Statistics 17.0 for Windows® (IBM Corporation, Route 100 Somers, New York, U.S.A.).

Results

The overall copro-prevalence of *E. granulosus* in dogs' feces was 6.79% in the understudied areas. The highest copro-prevalence was recorded in Lahore (9.80%) followed by the twin cities Islamabad/Rawalpindi (7.97%) and Faisalabad (5.02%) (Table 2).

According to dog sex, females were more copro-

Table 1: Geographical quadrants of study areas

City	Quadrants	
	Latitudes	Longitudes
Faisalabad	31º26'	73º08'
Rawalpindi / Islamabad	33º68'	73º04'
Lahore	31º35'	74º24'

Table 2: Prevalence of *E. granulosus* in dogs sampled from three districts of Punjab province, Pakistan

District	Positive/ Tested	Prevalence (95% CI)	OR (95% CI)	Statistics
Lahore	5/51	9.80 (4.26-20.97)	1.95 (0.63-6.03)	
Islamabad	11/138	7.97 (4.51-13.71)	1.23 (0.41-3.68)	$\chi^2 = 1.66$
Faisalabad	9/179	5.02 (2.67-9.28)	-	*P*-value= 0.435
Total	25/368	6.79 (4.64-9.83)		

CI confidence interval; OR odds ratio

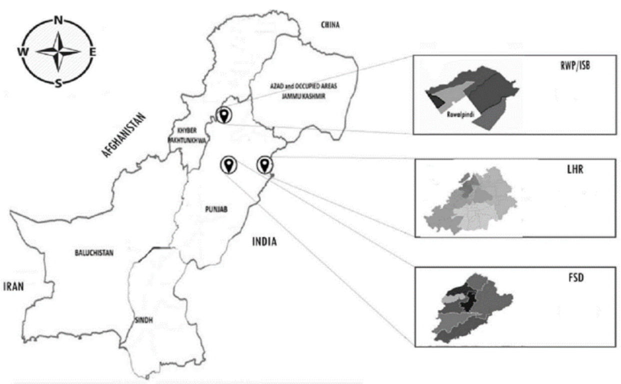

Fig. 1: Map of Pakistan. Sampling areas chosen for collection of dogs' fecal samples in this study are zoomed-in

positive (8.72%; 95% CI 5.36–13.89) than males (5.10%; 95% CI 2.79–9.13). Dogs ≤ 3 years of age were more copro-positive (6.93%; 95% CI 4.48–10.57) than those > 3 years (6.38%; 95% CI 2.96–13.23). Dogs without de-worming history were found to be more copro-positive (8.98%; 95% CI 5.61–14.1) compared to those with a history of anthelmintic treatment (4.73%; 95% CI 2.51–8.76).

Regarding dog captivity status, higher prevalence was found in feral/stray dogs (9.72%; 95% CI 4.79–18.73) compared to domestic/captive dogs (6.08%; 95% CI 3.88–9.41). Furthermore, companion dogs fed with raw meat demonstrated higher prevalence (8.28%) than those fed with commercially available/non-fleshy items (rice or bread). Unfortunately, the feeding status of stray/feral dogs was undefined and thus not included in the current findings. Dogs falling in Body Condition Score (BCS) in class 1–3 showed higher prevalence (10.86%; 95% CI 7.06–16.34) than those in class 4–6 (3.57%; 95% CI 1.53–8.09) and 7–9 (1.89%; 95% CI 0.33–9.95). Also, dogs with apparent lower intestinal clinical diseases were more copro-positive (10.22%; 95% CI 6.64–15.41) for *E. granulosus* compared to healthy dogs which showed only 3.33% copro-positivity.

In this study, copro-prevalence variation in relation to dog breeds (13 different breeds) was observed as follows: Grey Hound (15.62%; 95% CI 6.87–31.76), Bulldog (10.00%; 95% CI 3.96–23.05), stray/feral (9.72%; 95% CI 4.79–18.73), Alsatian (8.33%; 95% CI 1.49–35.38), Bull Terrier (8.00%; 95% CI 2.22–24.97), German Shepherd (5.66%; 95% CI 1.94–15.37), Labrador (5.00%; 95% CI 1.38–16.50) and owned non-descript (4.00%; 95% CI 1.1–13.46) dogs. None of the dogs from Siberian Husky, Doberman, Cocker Spaniel, Rottweiler, and Shih Tzu breed was found positive.

Univariate analysis (Table 3) of the study variables revealed that dogs age ≤ 3 (OR 1.06), female (OR 1.71), stray/feral dogs (OR 1.60), dogs fed with raw meat (OR 2.63), BCS 1–3 (OR 5.75), dogs with intestinal illness (OR 3.06), Grey Hound breed (OR 6.94) and dogs without deworming history (OR 1.90) showed a higher likelihood of being copro-positive.

A statistically significant association ($P < 0.05$) was observed between copro-prevalence and the variables investigated except for breed, anthelmintic history and sex of the dogs.

All variables found significant ($P < 0.05$) were included in the final binary logistic regression analysis; however, sex, breed and deworming history were excluded from the model at subsequent steps ($P > 0.05$). The following variables or factors were significantly associated with copro-prevalence of *E. granulosus* in the understudied dog population: Age, stray dog status, feeding habit (raw meat), BCS 1–3, and previous intestinal disease status (Table 4).

Owned/domesticated dogs' results

The highest prevalence was found in dogs > 3 years of age (9.52%; OR 3.55). Female dogs (6.45%; OR 1.11), dogs with no anthelmintic treatment history (12.00%; OR 6.84), those in BCS 1–3 (11.11%; OR 6.22) and previous lower intestinal condition (11.40%; OR 4.15) were mostly positive. The sex of dogs was the only variable that was found to be statistically insignificant ($P > 0.05$) while the others were associated significantly ($P < 0.05$) with prevalence (Table 5).

Dogs kept as pets showed higher prevalence (7.38%; OR 1.55) than the working/shepherd dogs (4.76%) but this difference was statistically insignificant ($P > 0.05$). Further, the prevalence in dogs fed with raw meat was higher (11.85%) than those fed with non-meat items and was statistically significant ($P < 0.05$). About breed susceptibility, Grey Hound was the most copro-positivity breed (15.62%) but association of prevalence and breed difference was statistically non-significant ($P > 0.05$).

Stray/feral dogs' results

Prevalence was higher in young dogs with ≤ 3 years (15.56%; OR 9.07). Female dogs (11.76%; OR 1.29), those in BCS 1–3 (23.08%; OR 8.58), dogs with previous

Table 3: Risk factors and univariable analysis for the copro-prevalence of *Echinococcus granulosus* antigen in owned and stray/feral dogs

Variables	Category	Positive/ Tested	Prevalence (95% CI)	OR (95% CI)	Chi-square	*P*-value
Age	Up to 3	19/274	6.93 (4.48-10.57)	1.06 (0.42-2.79)	27.43	0.037*
	More than 3	6/94	6.38 (2.96-13.23)	-		
Sex	Female	15/172	8.72 (5.36-13.89)	1.71 (0.75-3.90)	21.77	0.334
	Male	10/196	5.10 (2.79-9.13)	-		
Anthelmintic medication	No	16/178	8.98 (5.61-14.1)	1.90 (0.82-4.39)	14.66	0.360
	Yes	9/190	4.73 (2.51-8.76)	-		
Companionship	Stray/feral	7/72	9.72 (4.79-18.73)	1.60 (0.65-3.95)	23.65	0.029*
	Pet/domesticated	18/296	6.08 (3.88-9.41)	-		
Raw meat	Yes	14/169	8.28 (5.00-13.42)	2.63 (0.85-8.15)	26.95	0.018*
	No	4/127	3.14 (1.23-7.82)	-		
BCS	1-3	19/175	10.86 (7.06-16.34)	5.75 (0.77-43.21)	7.75	0.0208*
	4-6	5/140	3.57 (1.53-8.09)	3.04 (1.11-8.32)		
	7-9	1/53	1.89 (0.33-9.95)	-		
Apparent intestinal status	Diseased	19/186	10.22 (6.64-15.41)	3.06 (1.20-7.83)	5.95	0.0147*
	Healthy	6/180	3.33 (1.53-7.08)	-		
Breed	Grey Hound	5/32	15.62 (6.87-31.76)	6.94 (0.39-123.52)	8.14	0.7740
	Bulldog	4/40	10 (3.96-23.05)	1.56 (0.39-6.19)		
	Stray	7/72	9.72 (4.79-18.73)	1.61 (0.48-5.38)		
	Alsatian	1/12	8.33 (1.49-35.38)	1.88 (0.21-16.41)		
	Bull Terrier	2/25	8 (2.22-24.97)	1.95 (0.36-10.60)		
	German Shepherd	3/53	5.66 (1.94-15.37)	2.76 (0.63-12.14)		
	Labrador	2/40	5 (1.38-16.50)	3.13 (0.58-16.82)		
	Owned ND	2/50	4 (1.1-13.46)	3.91 (0.73-20.97)		
	Siberian Husky	0/3	0 (0-56.15)	-		
	Doberman	0/5	0 (0-43.45)	-		
	Cocker Spaniel	0/6	0 (0-39.03)	-		
	Rottweiler	0/10	0 (0-27.75)	-		
	Shih Tzu	0/20	0 (0-16.11)	-		

*****statistically significant ($P < 0.05$); CI confidence interval; OR odds ratio

Table 4: Final binary logistic regression analyses for the prediction of *Echinococcus granulosus* in dogs from three districts (Lahore, Islamabad and Faisalabad) of Punjab Province, Pakistan

Variable	Comparison	*P*-value
Age ≤ 3 years (*n* = 274)	> 3 years (*n* = 94)	0.038*
Feral dog (*n* = 72)	Companion dog (*n* = 296)	0.022*
Raw meat feeding (*n* = 169)	Other feed stuff (*n* = 127)	0.042*
BCS 1-3 (*n* = 175)	BCS 4-9 (*n* = 193)	0.031*
Previous intestinal disease (*n* = 186)	No previous intestinal disease (*n* = 180)	0.029*

*****statistically significant ($P < 0.05$)

intestinal disease condition (18.18%; OR 7.09). Sex was the only variable that was found to be associated with prevalence but was non-significantly ($P > 0.05$) (Table 6).

Discussion

Dogs have proven to be the most successful among other canids' species because of their domestication and proximity to man as companion animals (Knobel *et al*. 2008; Paul *et al*. 2010). On the contrary, their close association with humans and behaviors remain a leading risk to public health. Several parasites are harbored by dogs, thus posing a potential risk of disease transmission to humans and livestock (Moro and Abah 2018). In Pakistan, information on echinococcosis in dogs is scarce. However, a few studies conducted in limited geographical areas on hydatidosis have confirmed the presence of CE in ruminants (Mirani *et al*. 2002; Iqbal *et al*. 2012).

Copro-ELISA is a widely used technique for field surveys and field diagnosis of CE in dogs and has been applied effectively in previous studies (El-Shazly *et al*. 2007; Acosta-Jamett *et al*. 2010; Carmena and Cardona 2014). The main advantage of copro-ELISA over antibody detection in serum is its correlation with current infection (Adediran *et al*. 2014).

To the best of our knowledge, there is no report on canine echinococcosis in Pakistan, although some studies are available on hydatidosis in livestock. In this study, the overall prevalence was found to be 6.79% which is quite high considering the zoonotic potential of *Echinococcus*. Meanwhile, the overall prevalence observed in this study is comparable to other studies conducted in different geographical regions of the world. For instance, Prathiush *et al*. (2008) found an overall *Echinococcus* copro-prevalence of 4.35% in dogs from India. Svobodová and Lenska (2002) also reported an 8.1% copro-prevalence of *Echinococcus* in dogs in the Czech Republic while another study in Argentina demonstrated 7.3% prevalence (Cavagión *et al*.

Table 5: Risk factors and univariable analysis for the copro-prevalence of *Echinococcus granulosus* antigen in owned dogs

Variables	Category	Positive/ Tested	Prevalence (95% CI)	OR (95% CI)	Chi-square	*P*-value
Age	More than 3	14/147	9.52 (5.76-15.35)	3.55 (1.15-10.99)	5.37	0.0205*
	Up to 3	4/149	2.68 (1.05-6.69)	-		
Sex	Male	8/124	6.45 (3.3-12.21)	1.11 (0.43-2.88)	0.05	0.8313
	Female	10/172	5.81 (3.19-10.37)	-		
Anthelmintic medication	No	15/125	12 (7.41-18.86)	6.84 (1.95-24.04)	11.60	0.0007*
	Yes	3/171	1.75 (0.6-5.02)	-		
Purpose	Pet	11/149	7.38 (4.17-12.73)	1.55 (0.59-4.10)	0.79	0.3747
	Working	7/147	4.76 (2.32-9.5)	-		
Raw meat	Yes	16/135	11.85 (7.43-18.38)	9.54 (2.17-42.04)	12.73	0.0004*
	No	2/161	1.24 (0.34-4.41)	-		
BCS	1-3	15/135	11.11 (6.85-17.52)	6.22 (1.40-27.61)	9.68	0.0079*
	4-6	1/49	2.04 (0.36-10.69)	5.44 (0.71-41.49)		
	7-9	2/112	1.79 (0.49-6.28)	-		
Apparent intestinal status	Disease	13/114	11.40 (6.78-18.53)	4.15 (1.45-11.91)	8.00	0.0047*
	Sick	5/182	2.75 (1.18-6.27)	-		
Breed	Grey Hound	5/32	15.62 (6.87-31.76)	6.94 (0.39-123.52)	7.96	0.7168
	Bulldog	4/40	10 (3.96-23.05)	1.56 (0.39-6.19)		
	Alsatian	1/12	8.33 (1.49-35.38)	1.88 (0.21-16.41)		
	Bull Terrier	2/25	8 (2.22-24.97)	1.95 (0.36-10.60)		
	German Shepherd	3/53	5.66 (1.94-15.37)	2.76 (0.63-12.14)		
	Labrador	2/40	5 (1.38-16.50)	3.13 (0.58-16.82)		
	Owned ND	2/50	4 (1.1-13.46)	3.91 (0.73-20.97)		
	Siberian Husky	0/3	0 (0-56.15)	-		
	Doberman	0/5	0 (0-43.45)	-		
	Cocker Spaniel	0/6	0 (0-39.03)	-		
	Rottweiler	0/10	0 (0-27.75)	-		
	Shih Tzu	0/20	0 (0-16.11)	-		

*****statistically significant ($P < 0.05$); CI confidence interval; OR odds ratio

Table 6: Risk factors and univariable analysis for the copro-prevalence of *Echinococcus granulosus* antigen in stray/feral dogs

Variables	Category	Positive/ Tested	Prevalence (95% CI)	OR (95% CI)	Chi-square	*P*-value
Age	Up to 3	7/45	15.56 (7.75-28.79)	9.07 (0.52-156.90)	3.99	0.0458*
	More than 3	0/27	0 (0-12.46)	-		
Sex	Female	2/17	11.76 (3.29-34.33)	1.29 (0.24-7.02)	0.09	0.7694
	Male	5/55	9.09 (3.95-19.58)	-		
BCS	1-3	6/26	23.08 (11.04-42.05)	8.58 (0.49-149.84)	6.66	0.0357*
	4-6	1/29	3.45 (0.61-17.18)	6.69 (0.78- 57.22)		
	7-9	0/17	0 (0-18.43)	-		
Apparent intestinal status	Diseased	6/33	18.18 (8.61-34.39)	7.09 (0.83-60.26)	4.06	0.0439*
	Healthy	1/39	2.56 (0.450-13.17)	-		

*****statistically significant ($P < 0.05$); CI confidence interval; OR odds ratio

2005). In contrast, higher prevalence has been reported in Uruguay 22.7% (Craig *et al*. 1995), Libya 21.6% (Buishi *et al*. 2005), and Peru where copro-prevalence ranged between 46 and 82% (Moro *et al*. 1999; Lopera *et al*. 2003; Moro *et al*. 2005). Additionally, prevalence of *E. granulosus* infection up to 35.3% has been reported in dogs in Sidi Kacem Province of Morocco (Dakkak *et al*. 2017). In some cases, the prevalence differs between local areas within a region or country but the values are often non-significant. For example, the report from different Libyan districts; Alkhums, Tripoli and Azahwia (38.7, 17.5 and 38.7%, respectively) (Buishi *et al*. 2005). This observation is in agreement with our findings which showed a prevalence of 9.80, 7.97 and 5.02% in Lahore, Islamabad and, Faisalabad districts, respectively with no significant differences ($P > 0.05$).

In this study, a statistically significant negative correlation ($P < 0.05$) was observed between copro-positivity and age which is in disparity with the results of Adediran *et al*. (2014). Dogs up to 3 years in age were found to be more likely to be copro-positive than older dogs. This is in agreement with reports of high worm burden in young dogs compared to adults due to the development of acquired immunity over time (Lahmar *et al*. 2007). Moreover, age-related variations in dog behavior and management also advocate for differences in prevalence between young and adult dogs (Torgerson *et al*. 2003). Also, immunocompromised status increases susceptibility to infection and the captive dogs from rural areas fall under this category as they lack proper nutrition and medical attention.

Higher prevalence in female than in male dogs was observed in the current study and is in line with the findings of Adediran *et al*. (2014) but contrast the report of Budke (2004). However further analysis showed that there was no association ($P > 0.05$) between sex and prevalence of canine echinococcosis which has also been demonstrated in previous studies (Siavashi and Motamedi 2006; Öge *et al*. 2017).

In the current study, we investigated dog breeds as a potential risk factor for *Echinococcus* infection and samples collected from 13 different breeds demonstrated the highest prevalence in Grey Hound (15.62%) breed and lowest in client-owned non-descript breeds (4.00%). However, the difference in prevalence was statistically non-significant ($P < 0.05$). To best of our knowledge, this result presents variation in prevalence according to dog breeds for the first time and as risk factor for canine echinococcosis. We also found that previous de-worming status is not the significant predictor ($P > 0.05$) of copro-positivity which is in contrast to the findings of Acosta-Jamett *et al*. (2014) who found significantly higher ($P < 0.05$) copro-prevalence in dogs which were not dewormed previously. The results of this study showed that prevalence in stray dogs was higher and statistically different ($P < 0.05$) when compared to restrained/companion dogs which is expected as free-roaming dogs are apparently more exposed due to easy access to hydatid offal (Buishi *et al*. 2005).

Conclusion

The results of this study demonstrate that echinococcosis is prevalent among dogs hosted in different prefectures of Pakistan and suggest that more epidemiological and molecular studies focusing on intermediate hosts including humans are warranted to further ascertain the risk posed by canine population in order to design effective control strategies.

Acknowledgments

This study was part of a PhD research supported by Lanzhou Veterinary Research Institute, Lanzhou, People's Republic of China. We would like to thank the National Key Research and Development Program of China (2017YFD0501301), National Key Basic Research Program (973 Program) of China (2015CB150300) and the Central Public-interest Scientific Institution Basal Research Fund (1610312017001; 1610312016012) for their funding support. We are also thankful to the Veterinary Assistants at the Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad for their assistance during sample collection.

Author Contributions

Conceptualization, Mughees Aizaz Alvi, Muhammad Saqib, Li Li, Wan-Zhong Jia; methodology, Mughees Aizaz Alvi, Muhammad Saqib, Hong-Bin Yan, Warda Qamar; formal analysis, Mughees Aizaz Alvi, Muhammad Haleem Tayyab, Muhammad Masood Tahir; investigation, Mughees Aizaz Alvi, Warda Qamar, Waqas Altaf, Muhammad Usman, Ali Hassan, Muhammad Rashid Khalid Bajwa; resources, Wan-Zhong Jia; data curation, Mughees Aizaz Alvi, John Asekhaen Ohiolei; writing—original draft preparation, Mughees Aizaz Alvi; writing—review and editing, John Asekhaen Ohiolei, Hong-Bin Yan, Wan-Zhong Jia; project administration, Bao-Quan Fu, Wan-Zhong Jia ; funding acquisition, Wan-Zhong Jia.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article

Data Availability Declaration

All the data pertaining to this work is mentioned in the manuscript

Conformation to Ethical Guidelines for Research on Animals

Not applicable

References

Acosta-Jamett G, T Weitzel, B Boufana, C Adones, A Bahamonde, K Abarca, PS Craig, I Reiter-Owona (2014). Prevalence and risk factors for echinococcal infection in a rural area of Northern Chile: A household-based cross-sectional study. *PLoS Negl Trop Dis* 8; Article e3090

Acosta-Jamett G, S Cleaveland, M Barend, AA Cunningham, H Bradshaw, PS Craig (2010). *Echinococcus granulosus* infection in domestic dogs in urban and rural areas of the Coquimbo region, north-central Chile. *Vet Parasitol* 169:117–122

Adediran OA, TU Kolapo, EC Uwalaka (2014). *Echinococcus granulosus* prevalence in dogs in Southwest Nigeria. *J Parasitol Res* 2014:1-6

Anonymous (1996). How to determine a cat's or dog's age [online. Available from: http://www.ruralareavet.org/PDF/Physical_Exam-How_to_Determine_Age.pdf

Baldwin K, J Bartges, T Buffington, LM Freeman, M Grabow, J Legred, D Ostwald (2010). AAHA nutritional assesment guidelines for dogs and cats. *J Amer Anim Hosp Assoc* 46:285–296

Benito A, D Carmena (2005). Double-antibody sandwich ELISA using biotinylated antibodies for the detection of *Echinococcus granulosus* coproantigens in dogs. *Acta Trop* 95:9–15

Budke CM (2004). Echinococcosis on the Tibetan Plateau. *Ph.D. Dissertation*. University of Basel, Switzerland

Buishi I, E Njoroge, O Bouamra, PS Craig (2005). Canine echinococcosis in northwest Libya: assessment of coproantigen ELISA, and a survey of infection with analysis of risk-factors. *Vet Parasitol* 130:223–232

Carmena D, GA Cardona (2014). Echinococcosis in wild carnivorous species: Epidemiology, genotypic diversity, and implications for veterinary public health. *Vet Parasitol* 202:69–94

Cavagión L, A Perez, G Santillan, F Zanini, O Jensen, L Saldía, M Diaz, Guatavo Cantoni, E Herrero, MT Costa, M Volpe, D Araya, NA Rubianes, C Aguado, G Meglia, E Guarnera, E Larrieu (2005). Diagnosis of cystic echinococcosis on sheep farms in the south of Argentina: areas with a control program. *Vet Parasitol* 128:73–81

Craig P, R Gasser, L Parada, P Cabrera, S Parietti, C Borgues, A Acuttist, J Agulla, K Snowden, E Paolillo (1995). Diagnosis of canine echinococcosis: comparison of coproantigen and serum antibody tests with arecoline purgation in Uruguay. *Vet Parasitol* 56:293–301

Dakkak A, I El-Berbri, A Petavy, F Boué, M Bouslikhane, OF Fihri, S Welburn, MJ Ducrotoy (2017). *Echinococcus granulosus* infection in dogs in Sidi Kacem Province (north-West Morocco). *Acta Trop* 165:26–32

El-Shazly AS, S Awad, I Nagaty, T Morsy (2007). Echinococcosis in dogs in urban and rural areas in Dakahlia Governorate, Egypt. *J Egypt Soc Parasitol* 37:483–492

Ghabdian S, H Borji, A Naghibi (2017). Molecular identification of *Echinococcus granulosus* strain in stray dogs from Northeastern Iran. *Vet Parasitol Reg Stud Rep* 9:6–8

Hidalgo A, A Melo, F Romero, J Villanueva, C Carrasco, P Jara, J Venegas, FF Salamanca (2019). A PCR-RFLP assay for discrimination of *Echinococcus granulosus* sensu stricto and *Taenia* spp in dogs stool. *Exp Parasitol* 200:42–47

Ingole R, H Khakse, M Jadhao, SR Ingole (2018). Prevalence of *Echinococcus* infection in dogs in Akola district of Maharashtra (India) by Copro-PCR. *Vet Parasitol Reg Stud Rep* 13:60–63

Iqbal HJ, A Maqbool, M Lateef, M Khan, M Riaz, A Mahmood, FA Atif, Z Ali, MS Ahmad (2012). Studies on hydatidosis in sheep and goats at Lahore, Pakistan. *J Anim Plant Sci* 22:894–897

Knobel DL, MK Laurenson, RR Kazwala, LA Boden, S Cleaveland (2008). A cross-sectional study of factors associated with dog ownership in Tanzania. *BMC Vet Res* 4; Article 5

Lahmar S, B Boufana, H Bradshaw, P Craig (2007). Screening for *Echinococcus granulosus* in dogs: Comparison between arecoline purgation, coproELISA and coproPCR with necropsy in pre-patent infections. *Vet Parasitol* 144:287–292

Liu CN, ZZ Lou, L Li, HB Yan, D Blair, MT Lei, JZ Cai, YL Fan, JQ Li, BB Fu, YR Yang, DP McManus, WZ Jia (2015). Discrimination between *E. granulosus sensu stricto*, *E. multilocularis* and *E. shiquicus* using a multiplex PCR assay. *PLoS Negl Trop Dis* 9; Article e0004084

Lopera L, PL Moro, A Chavez, G Montes, A Gonzales, RH Gilman (2003). Field evaluation of a coproantigen enzyme-linked immunosorbent assay for diagnosis of canine echinococcosis in a rural Andean village in Peru. *Vet Parasitol* 117:37–42

Mirani A, S Buoghio, N Akhter (2002). Age and size-wise distribution of echinococcosis in buffaloes slaughtered at the Larkana Abattoir. *Pak J Appl Sci* 2:837–838

Moro KK, AE Abah (2018). Epizootiology of zoonotic parasites of dogs in Abua Area of Rivers State, Nigeria. *Vet Anim Sci* 7; Article 100045

Moro PL, L Lopera, N Bonifacio, A Gonzales, RH Gilman, MH Moro (2005). Risk factors for canine echinococcosis in an endemic area of Peru. *Vet Parasitol* 130:99–104

Moro PL, N Bonifacio, RH Gilman, L Lopera, B Silva, R Takumoto, M Verastegui, L Cabrera (1999). Field diagnosis of *Echinococcus granulosus* infection among intermediate and definitive hosts in an endemic focus of human cystic echinococcosis. *Trans Roy Soc Trop Med Hyg* 93:611–615

Mulinge E, J Magambo, D Odongo, S Njenga, E Zeyhle, C Mbae, D Kagendo, F Addy, D Ebi, M Wassermann, P Kern, T Romig (2018). Molecular characterization of *Echinococcus* species in dogs from four regions of Kenya. *Vet Parasitol* 255:49–57

Newcombe RG (1998). Two-sided confidence intervals for the single proportion: comparison of seven methods. *Stat Med* 17:857–872

Öge H, S Öge, B Gönenç, O Sarımehmetoğlu, G Özbakış (2017). Coprodiagnosis of *Echinococcus granulosus* infection in dogs from Ankara, Turkey. *Vet Parasitol* 242:44–46

Pakistan Meteorological Department [online]. [cited 2019 Oct 30]. Available from: http://www.pmd.gov.pk/Observatories/index.html

Paul M, L King, EP Carlin (2010). Zoonoses of people and their pets: a US perspective on significant pet-associated parasitic diseases. *Trends Parasitol* 26:153–154

Prathiush PR, ED Placid, KJG Ananda (2008). Diagnosis of *Echinococcus granulosus* infection in dogs by a coproantigen sandwich ELISA. *Vet Arhiv* 78:297–305

Rashid M, MI Rashid, H Akbar, L Ahmad, MA Hassan, K Ashraf, K Saeed, M Gharbi (2018). A systematic review on modelling approaches for economic losses studies caused by parasites and their associated diseases in cattle. *Parasitology* 146:129–141

Rossi A, JM Marqués, CM Gavidia, AE Gonzalez, C Carmona, HH García, JA Chabalgoity (2012). *Echinococcus granulosus*: different cytokine profiles are induced by single versus multiple experimental infections in dogs. *Exp Parasitol* 130:110–115

Siavashi M, G Motamedi (2006). Evaluation of a coproantigen enzyme-linked immunosorbent assay for the diagnosis of canine echinococcosis in Iran. *Helminthologia* 43:17–19

Svobodová V, B Lenska (2002). Echinococcosis in dogs in the Czech Republic. *Acta Vet Brno* 71:347–350

Thapa NK, MT Armua-Fernandez, D Kinzang, RB Gurung, P Wangdi, D Deplazes (2017). Detection of *Echinococcus granulosus* and *Echinococcus ortleppi* in Bhutan. *Parasitol Intl* 66:139–141

Torgerson PR, BS Shaikenov, AT Rysmukhambetova, AE Ussenbayev, AM Abdybekova, KK Burtisurnov (2003). Modelling the transmission dynamics of *Echinococcus granulosus* in dogs in rural Kazakhstan. *Parasitology* 126:417–424

Wang L, Q Wang, H Cai, H Wang, Y Huang, Y Feng, X Bai, M Qin, S Manguin, L Gavotte, W Wu, R Frutos (2021). Evaluation of fecal immunoassays for canine *Echinococcus* infection in China. *PLoS Negl Trop Dis* 15; Article e0008690

WHO - World Health Organization (2015). Available at: https://www.who.int/echinococcosis/epidemiology/en/ (Accessed [October 30, 2019]

WHO/OIE (World Health Organization/World Organization for Animal Health) (2001). Echinococcosis in animals: clinical aspects, diagnosis and treatment. *WHO/OIE Manual on Echinococcosis in Humans and Animals: A Public Health Problem of Global Concern*. Chapter 3. , WHO/OIE, Paris, France

Zhang Y, JM Bart, P Giraudoux, P Craig, D Vuitton, H Wen (2006). Morphological and molecular characteristics of *Echinococcus multilocularis* and *Echinococcus granulosus* mixed infection in a dog from Xinjiang, China. *Vet Parasitol* 139:244–248