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SEROPREVALENCE AND ASSOCIATED FACTORS OF PEDIATRIC CYSTIC ECHINOCOCCOSIS AND CANINE ECHINOCOCCOSIS IN A DISTRICT OF HUANCAVELICA, PERU

SEROPREVALENCIA Y FACTORES ASOCIADOS A EQUINOCOCOSIS QUÍSTICA INFANTIL Y EQUINOCOCOSIS CANINA EN UN DISTRITO DE HUANCAVELICA, PERÚ

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ABSTRACT

Introduction: Cystic echinococcosis is a zoonotic disease endemic to several rural areas in Peru. **Objectives:** To determine the seroprevalence and associated factors of pediatric cystic echinococcosis and canine echinococcosis. **Methods:** This was an observational, cross-sectional, and analytical study conducted in 2019 in the district of Ascensión, Huancavelica, Peru. A total of 783 male schoolchildren were evaluated using serological tests (ELISA and Immunoblot), and 543 dogs were tested using copro-ELISA and copro-PCR. Epidemiological surveys were conducted with students and heads of households. Variables included sociodemographic factors, household conditions, and risk practices involving animals. Samples were processed at the Instituto Nacional de Salud (INS) and the Instituto de Salud Pública de Chile. Bivariate analysis with odds ratio (OR) and 95% confidence intervals (CI) was performed. The study was approved by an ethics committee and included informed consent. **Results:** The pediatric seroprevalence was 5.18% by ELISA and 1.60% by Immunoblot, with no significant associations found with the evaluated variables. Canine coproprevalence was 7.18% by copro-ELISA and 9.02% by copro-PCR. Significant associations were found with age >7 years (OR=0.18; 95% CI=0.02–0.89; p=0.016; ref.: 0 to 6 years), livestock benefit in slaughterhouses (OR=2.71; 95% CI=1.39–5.49; p=0.001), direct consumption of offal (OR=7.99; 95% CI=1.13–48.42; p=0.002), and cooking offal for dogs (OR=3.02; 95% CI=1.12–7.29; p=0.007). **Conclusion:** Active circulation of Echinococcus granulosus is confirmed in Ascensión, highlighting the need to strengthen local zoonotic control measures.

Keywords: Echinococcosis; Child; Livestock; Serological tests; Zoonoses. (Source: MESH-NLM)

RESUMEN

Introducción: La equinococosis quística es una zoonosis endémica en diversas regiones rurales del Perú. **Objetivos:** Determinar la seroprevalencia y factores asociados a la equinococosis quística infantil y a la equinococosis canina. **Métodos:** Estudio observacional, transversal y analítico realizado en 2019 en el distrito de Ascensión, Huancavelica, Perú. Se evaluó a 783 escolares varones mediante pruebas serológicas (ELISA e Inmunoblot) y a 543 canes mediante copro-ELISA y copro-PCR. Se aplicaron encuestas epidemiológicas a estudiantes y jefes de familia. Las variables incluyeron factores sociodemográficos, condiciones del hogar y prácticas de riesgo con animales. Las muestras fueron procesadas en el Instituto Nacional de Salud (INS) y en el Instituto de Salud Pública de Chile. Se realizó análisis bivariado con odds ratio (OR) e intervalos de confianza al 95 %. El estudio fue aprobado por un comité de ética y contó con consentimiento informado. **Resultados:** La seroprevalencia infantil fue 5,18% por ELISA y 1,60% por Inmunoblot, sin asociaciones significativas con las variables evaluadas. La coproprevalencia canina fue 7,18% por copro-ELISA y 9,02% por copro-PCR. Se hallaron asociaciones significativas con edad >7 años (OR=0,18; IC95%=0,02–0,89; p=0,016; ref.: 0 a 6 meses), beneficio del ganado en camal (OR=2,71; IC95%=1,39–5,49; p=0,001), consumo directo de vísceras (OR=7,99; IC95%=1,13–48,42; p=0,002) y cocción de vísceras para el perro (OR=3,02; IC95%=1,12–7,29; p=0,007) **Conclusión:** Se confirma circulación activa de Echinococcus granulosus en Ascensión, lo que requiere fortalecer las medidas locales de control zoonótico.

Palabras clave: Equinococosis; Niño; Ganado; Pruebas serológicas,Zoonosis. (Fuente: DeCS- BIREME).

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INTRODUCTION

Echinococcosis is a zoonotic parasitic disease caused by the cestode Echinococcus granulosus, which is associated with 95% of human echinococcosis cases in its cystic form[1,2]. Canids, mainly domestic dogs, serve as definitive hosts; they become infected by consuming the viscera of animals contaminated with cysts of this parasite[1,2]. The eggs are expelled in the feces of infected dogs and subsequently ingested by intermediate hosts, mainly ungulates and, occasionally, humans, who show hepatic involvement in 75% of the cases and, to a lesser extent, extrahepatic involvement, primarily in the lungs [1-3].

Globally, cystic echinococcosis is distributed across all inhabited continents, with particular concentrations in temperate climate regions [1]. It has a global prevalence of 0.05%. In endemic countries, this figure ranges between 5% and 10%, affecting mainly South America, Europe, the Middle East, Central Asia, Australia, and Africa, including Russia, China, India, and Nepal[2,4]. In South America, the Pan American Health Organization (PAHO) reported 9,511 cases of human cystic echinococcosis between 2019 and 2021; of these, 15.8% occurred in children under 15 years old. This figure represents an underestimated prevalence, considering that this disease is not mandatory for notification in all countries within this jurisdiction[5,6].

In Peru, during the period from 2019 to 2021, 7,559 confirmed cases of human cystic echinococcosis were reported, representing 79.5% of the cases in South America. Of these, 16% (in 2019 and 2020) and 18.1% (in 2021) were cases in children under 15 years old, with 76 deaths reported due to this disease [5]. In the Huancavelica region, during the same period, 593 human cases of cystic echinococcosis were reported, accounting for 7.8% of the total cases in the country, making it the fifth most affected region by this zoonosis, after Lima, Junín, Puno, and Cusco[6]. Factors associated with the proliferation of the parasite are linked to human interaction with both domestic and wild canids and herbivores. Therefore, the risk of contracting the disease is higher in young individuals who reside in rural areas, engage in livestock farming, have low educational levels, live in crowded conditions, and have frequent contact with dogs, which are fed raw viscera[7]. The district of Ascensión presents multiple risk factors, as it is a region where most of the population is rural, and the main economic activity is the farming of South American camelids (alpacas and llamas), followed by sheep farming [8]. In this area, livestock owners or caretakers live in substandard housing used for the slaughter of animals without sanitary control, and coexist with shepherd dogs that are fed raw viscera from slaughtered livestock, creating a high epidemiological risk scenario [9]. The objective of this research was to determine the seroprevalence and the factors associated with pediatric cystic echinococcosis and canine echinococcosis.

METHODS

Design and Study Area

An analytical cross-sectional observational design was used. The study was conducted in the district of Ascensión, located in the Huancavelica region, Peru, at an altitude of 3,686 meters above sea level, with geographic coordinates: latitude -12.7839 and longitude -74.9781. The collection of samples and data took place in 2019.

Population and Sample

The population for determining the prevalence of pediatric cystic echinococcosis consisted of male students from the "La Victoria de Ayacucho" Educational Institution. Eligibility criteria included being a student at the institution, aged 6 to 18 years, having signed informed consent from parents or guardians, and voluntary participation with assent from the minor. All available students were included. For determining the prevalence of canine echinococcosis, the population consisted of dogs belonging to students previously evaluated, as well as dogs from 130 livestock farming family units in the district, provided that the dog owner had given informed consent. The selection of students was carried out through non-probabilistic convenience sampling. For the dogs, the selection of

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epidemiological units (houses) was done by simple random sampling, considering geographic location and the number of dogs per household.

Blood samples were collected from 783 male students (58 from primary school and 725 from secondary school), which were later centrifuged to obtain serum; the statistical power was above 80% based on expected frequencies from prior studies, with respect to age [7]. Initially, 53 fecal samples from dogs belonging to students were collected, and later, additional samples from dogs of 130 livestock units were added, reaching a total of 543 samples; the statistical power was above 80% based on expected frequencies from prior studies, with respect to where the dog sleeps [8].

Variables and Instruments

Independent variables for pediatric cystic echinococcosis included: age, level of education, mother tongue, number of rooms in the house, access to basic services (electricity, drinking water, and sewage), livestock ownership, animal slaughter location, and the practice of feeding dogs raw viscera. For canine echinococcosis, the variables included: dog age and sex, deworming, sleeping location, livestock slaughter location, and final disposal of viscera. The dependent variable was the presence of pediatric or canine cystic echinococcosis (positive/negative).

An epidemiological structured survey was applied to the school population to identify factors associated with pediatric cystic echinococcosis. Simultaneously, a survey was applied to the community members, family heads, to evaluate factors associated with canine echinococcosis. This second survey collected personal data and dog characteristics (age, sex), as well as risk practices related to animal raising and disposal of viscera. For determining the prevalence of cystic echinococcosis, serological techniques such as ELISA and Immunoblot were used from serum samples, while for determining the prevalence of canine echinococcosis, copro-PCR and copro-ELISA techniques were used from fecal samples.

Procedures

Prior arrangements were made with the authorities of the Regional Health and Education Directorates, as well as with political authorities in the district of Ascensión, to facilitate the execution of the study. The recruitment of students was done in coordination with health center staff and educational institutions. A coded alphabetical list of the 783 students was created. Subsequently, written informed consent was obtained from the parents or guardians, or by fingerprint when applicable.

Samples were transported under cold chain conditions to the National Reference Laboratory for Parasitic Zoonoses of the Centro Nacional de Salud Pública (CNSP) at the Instituto Nacional de Salud (INS), in Lima, Peru. The immunoserological analysis for human diagnosis was performed following the procedures established by INS. Screening was done using the ELISA IgG test (in house), and positive samples were confirmed with the Immunoblot IgG test (in house). The ELISA test had a sensitivity of 98% and a specificity of 60%[10].

For screening the samples, ELISA-IgG kits were used for the diagnosis of pediatric cystic echinococcosis[10]. The total hydatid fluid antigen from sheep (ATLH-O) was used with a protein concentration of 1 mg/mL. The microplates were sensitized with 100 µL of antigen solution in each well and incubated at 4°C overnight. Afterward, nonspecific sites were blocked by adding 100 µL of PBS-Tween at 0.05% and 5% skim milk, and incubated at 37°C for 30 minutes. The wells were then washed with 200 µL of PBS-Tween at 0.05% and, in the respective wells, positive control serum, negative control serum, and test sera were added. The plates were incubated at 37°C for one hour, the content was discarded, and the wells were washed again. Next, 100 µL of HRP-conjugated anti-human IgG, diluted 1/1,000, was added, and the plates were incubated again at 37°C for one hour. After the corresponding washing, 100 µL of the TMB substrate solution was added, and the reaction was allowed to proceed in the dark at room temperature for 15 minutes. The enzymatic reaction

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was stopped by adding 25 µL of 2.5 M sulfuric acid. Finally, the microplates were analyzed using a BioTek ELISA reader at a wavelength of 450 nm. The cutoff value was determined as the average of three negative controls plus two standard deviations. Any sample with a value higher than the cutoff was considered positive.

Positive samples were confirmed using the immunoblot IgG technique, following the protocol described by Sánchez et al. [10]. ATLH-O was used at a concentration of 2.07 µg/µL. The immunoenzyme reaction was carried out in plastic plates divided into compartments, where nitrocellulose strips containing ATLH-O were placed. These strips were incubated in 1 mL of PBS-T with 5% skim milk (PBS-TL) for 30 minutes at room temperature with shaking. PBS-TL was discarded, and 1 mL of the test sera, diluted 1:100 in PBS-TL, was added, incubating for one hour at room temperature with shaking. The strips were washed with PBS-T, and a solution of HRP-conjugated anti-human IgG, diluted 1/1,000 in PBS-TL, was added, and incubated.

Successive washes with PBS-T and PBS were performed. For revelation, a solution consisting of 5 mg of DAB, 10 µL of 30% H_2O_2 in 10 mL of PBS was used, and the strips were incubated for 15 minutes, allowing visualization of protein bands in the positive control. The reaction was stopped by washing the strips with deionized water and drying them at room temperature in the dark. The reading consisted of observing the presence or absence of precipitation bands on the nitrocellulose strips. The positivity criterion included the presence of one to three antigenic proteins of 8, 16, and 24 kDa. For diagnosis in dogs, the copro-ELISA (in-house) technique was used for screening, and the copro-PCR (in-house) test was used for confirmation. The copro-ELISA test showed a sensitivity of 98% and a specificity of 60%. The copro-PCR analysis was carried out according to the procedures established by the Instituto de Salud Pública de Chile, which allows identification of the Echinococcus granulosus complex at the species and genotype level, using a mitochondrial DNA gene that codes for subunit 1 of cytochrome oxidase (Co1) [11]. Fecal samples were inactivated by freezing at -80°C for five days, then thawed for processing. The rapid sedimentation technique was applied before DNA extraction, using between 2 and 5 g of feces suspended in 15 mL of 0.85% saline solution. The mixture was filtered through a metal strainer to remove macroscopic elements and centrifuged at 1,800 rpm for five minutes. This procedure was repeated until the supernatant was clear. The genomic fecal DNA was extracted from the sediment of each sample (n=543) using the QIAamp Fast DNA Stool mini kit (cat. no. 51604), following the manufacturer's instructions with slight modifications due to the low concentration of copro-genomic DNA[11].

For amplification of E. granulosus DNA, primers CO1-F (5'-TTT TTT GGC CAT CCT GAG GTT TAT-3') and CO1-R (5'-TAA CGA CAT AAC ATA ATG AAA ATG-3') were used, allowing identification of the Echinococcus granulosus complex at the species and genotype level[11]. The PCR reaction was prepared in a final volume of 25 µL, which included 3.65 µL of PCR master mix (Thermo Scientific), 18.35 µL of nuclease-free water, 0.5 µL (1 pmol/µL) of each primer, and 2 µL of sample DNA. Amplification was performed using a Mastercycler pro S & Control Panel (Eppendorf). The amplified products were analyzed by electrophoresis on a 2% agarose gel. Electrophoresis was performed for 60 minutes at 100 V. Band validation was performed using a 100 bp DNA ladder (Fermentas) along with the PCR product, allowing identification of possible size differences or the presence of nonspecific bands[11].

Statistical Analysis

Data from the surveys were processed using descriptive statistics, with simple and percentage frequency distributions. Bivariate analysis was performed to estimate the odds ratios (OR) with their respective 95% confidence intervals. In the case of pediatric cystic echinococcosis, no statistically significant associations were identified, so multivariate analysis was not carried out.

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For canine echinococcosis, although significant associations were found, multivariate analysis was not performed due to collinearity observed between some of the evaluated factors. Data processing and analysis were conducted using Microsoft Excel 2019 and SPSS software, version 25.0.

Ethical Considerations

Privacy of the collected data was ensured, and only students and community members who signed informed consent, or whose legal representatives did so, participated. This study was approved by the Ethics Committee of the Faculty of Nursing at the Universidad Nacional de Huancavelica.

RESULTS

Out of the total 783 participants, all were from the district of Ascensión, Huancavelica; all were males, with an age range between 6 and 18 years, and an average age of 13.7 ± 1.8 years. It was determined that 58 (7.4%) participants were from the primary school level and 725 (92.6%) were from the secondary school level; additionally, 594 (75.9%) had Spanish as their mother tongue, 179 (22.9%) spoke Quechua, and 10 (1.28%) did not specify their mother tongue. The seroprevalence of pediatric cystic echinococcosis was 5.18% using ELISA and 1.60% after confirmation with Immunoblot. Regarding the dogs evaluated (n=543), a coproprevalence of 7.18% was found using copro-ELISA and 9.02% by copro-PCR.

Table 1 shows that none of the sociodemographic variables or household conditions were significantly associated with seropositivity to pediatric cystic echinococcosis, according to ELISA and Immunoblot tests. Significant results were noted for age (6–9 years: OR=2.09; 95% CI: 0.22–9.50; p=0.330 by ELISA) and number of rooms (2 to 3: OR=1.11; 95% CI: 0.30–6.11; p=0.873), although these were also not significant.

Table 1. Association between sociodemographic variables, household conditions, and seropositivity to pediatric cystic echinococcosis according to ELISA and Immunoblot tests in students from the district of Ascensión, Huancavelica, 2019.

Category	Total, n (%)	Childhood Cystic Echinococcosis by ELISA				Childhood Cystic Echinococcosis by Immunoblot			
		Positive, n (%)	Negative, n (%)	OR (95% CI)	p-value	Positive, n (%)	Negative, n (%)	OR (95% CI)	p-value
Age (years)									
6–9	21 (2.6)	2 (9.5)	19 (90.5)	2.09 (0.22–9.50)	0.330	1 (4.8)	20 (95.2)	3.08 (0.07–24.83)	0.276
10–14 (Ref.)	500 (62.3)	24 (4.8)	476 (95.2)			8 (1.6)	492 (98.4)		
15–18	262 (32.6)	15 (5.7)	247 (94.3)	1.20 (0.58–2.44)	0.582	4 (1.5)	258 (98.5)	0.95 (0.21–3.60)	0.939
Mother Tongue									
Quechua (Ref.)	179 (22.9)	10 (5.6)	169 (94.4)			2 (1.1)	177 (98.9)		
Spanish	594 (76.1)	31 (5.2)	563 (94.8)	0.93 (0.43–2.17)	0.847	11 (1.9)	583 (98.1)	1.67 (0.36–15.63)	0.503
Not Specified (NP)	10 (1.3)	0 (0.0)	10 (100.0)	NC	NC	0 (0.0)	10 (100.0)	NC	NC
Education Level									
Primary (Ref.)	58 (7.4)	4 (6.9)	54 (93.1)			1 (1.7)	57 (98.3)		
Secondary	725 (92.6)	37 (5.1)	688 (94.9)	0.73 (0.25–2.91)	0.555	12 (1.7)	713 (98.3)	0.96 (0.14–41.71)	0.968

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Table 1. (Continuation)

Category	Total, n (%)	Childhood Cystic Echinococcosis by ELISA				Childhood Cystic Echinococcosis by Immunoblot			
		Positive, n (%)	Negative, n (%)	OR (95% CI)	p-value	Positive, n (%)	Negative, n (%)	OR (95% CI)	p-value
Number of Rooms in House									
1 (Ref.)	56 (7.0)	3 (5.4)	53 (94.6)			1 (1.8)	55 (98.2)		
2 to 3	288 (36.1)	17 (5.9)	271 (94.1)	1.11 (0.30–6.11)	0.873	5 (1.7)	283 (98.3)	0.97 (0.11–46.78)	0.979
3 to 4	253 (31.7)	14 (5.5)	239 (94.5)	1.03 (0.27–5.81)	0.958	3 (1.2)	250 (98.8)	0.66 (0.05–35.27)	0.719
More than 5	176 (22.1)	6 (3.4)	170 (96.6)	0.62 (0.13–3.99)	0.511	3 (1.7)	173 (98.3)	0.95 (0.07–50.96)	0.968
Not Specified (NP)	10 (1.3)	1 (10.0)	9 (90.0)	NC	NC	1 (10.0)	9 (90.0)	NC	NC
Electricity									
Yes	769 (98.2)	41 (5.3)	728 (94.7)	NC	NC	13 (1.7)	756 (98.3)	NC	NC
No (Ref.)	14 (1.8)	0 (0.0)	14 (100.0)	NC	NC	0 (0.0)	14 (100.0)	NC	NC
Piped Water									
Yes	772 (98.6)	41 (5.3)	731 (94.7)	NC	NC	13 (1.7)	759 (98.3)	NC	NC
No (Ref.)	11 (1.4)	0 (0.0)	11 (100.0)	NC	NC	0 (0.0)	11 (100.0)	NC	NC
Sewerage									
Yes	697 (89.0)	35 (5.0)	662 (95.0)	0.70 (0.28–2.12)	0.443	12 (1.7)	685 (98.3)	1.49 (0.22 64.38)	0.702
No (Ref.)	86 (11.0)	6 (7.0)	80 (93.0)			1 (1.2)	85 (98.8)		

NS: Not specified. NC: Not calculated.

In Table 2, it is observed that none of the variables related to animal contact or risky practices at home showed a significant association with seropositivity for cystic echinococcosis in children. For example, feeding the dog contaminated offal presented an OR=1.38 (95% CI: 0.61–2.90; p=0.377) by ELISA and OR=0.67 (95% CI: 0.07–3.10; p=0.598) by Inmunoblot. Likewise, variables such as hand washing after playing with the dog, home slaughtering, or dog deworming also showed no statistical association.

Table 2. Association between livestock ownership, dog-rearing practices, and other risk behaviors with seropositivity to cystic echinococcosis in children, according to ELISA and Inmunoblot tests in schoolchildren from the Ascensión district, Huancavelica, 2019.

Category	Total, n (%)	Childhood Cystic Echinococcosis by ELISA				Childhood Cystic Echinococcosis by Immunoblot			
		Positive, n (%)	Negative, n (%)	OR (95% CI)	p-value	Positive, n (%)	Negative, n (%)	OR (95% CI)	p-value
Cattle ownership									
Yes	20 (2.6)	1 (5.0)	19 (95.0)	0.95 (0.02–6.30)	0.962	0 (0.0)	20 (100.0)	NC	NC
No (Ref.)	763 (97.4)	40 (5.2)	723 (94.8)			13 (1.7)	750 (98.3)	NC	NC
Sheep ownership									
Yes	40 (5.1)	3 (7.5)	37 (92.5)	1.50 (0.28–5.10)	0.509	1 (2.5)	39 (97.5)	1.56 (0.04–11.07)	0.670
No (Ref.)	743 (94.9)	38 (5.1)	705 (94.9)			12 (1.6)	731 (98.4)		
Camelids ownership									
Yes	15 (1.9)	2 (13.3)	13 (86.7)	2.88 (0.30–13.38)	0.155	0 (0.0)	15 (100.0)	NC	NC
No (Ref.)	768 (98.1)	39 (5.1)	729 (94.9)			13 (1.7)	755 (98.3)	NC	NC
Goat ownership									
Yes	3 (0.4)	0 (0.0)	3 (100.0)	NC	NC	0 (0.0)	3 (100.0)	NC	NC
No (Ref.)	780 (99.6)	41 (5.3)	739 (94.7)	NC	NC	13 (1.7)	767 (98.3)	NC	NC

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Table 2. (Continuation)

Category	Total, n (%)	Childhood Cystic Echinococcosis by ELISA				Childhood Cystic Echinococcosis by Immunoblot			
		Positive, n (%)	Negative, n (%)	OR (95% CI)	p-value	Positive, n (%)	Negative, n (%)	OR (95% CI)	p-value
Horse ownership									
Yes	14 (1.8)	0 (0.0)	14 (100.0)	NC	NC	0 (0.0)	14 (100.0)	NC	NC
No (Ref.)	769 (98.2)	41 (5.3)	728 (94.7)	NC	NC	13 (1.7)	756 (98.3)	NC	NC
Home animal slaughter									
Yes	37 (4.7)	2 (5.4)	35 (94.6)	1.04 (0.12–4.30)	0.962	0 (0.0)	37 (100.0)	NC	NC
No (Ref.)	746 (95.3)	39 (5.2)	707 (94.8)			13 (1.7)	733 (98.3)	NC	NC
Feeding the dog raw offal									
Yes	167 (21.3)	11 (6.6)	156 (93.4)	1.38 (0.61–2.90)	0.377	2 (1.2)	165 (98.8)	0.67 (0.07–3.10)	0.598
No (Ref.)	616 (78.7)	30 (4.9)	586 (95.1)			11 (1.8)	605 (98.2)		
Deworming the dog every 2 months									
Yes	502 (64.1)	29 (5.8)	473 (94.2)	1.37 (0.67–3.01)	0.364	11 (2.2)	491 (97.8)	3.13 (0.67–29.18)	0.120
No (Ref.)	281 (35.9)	12 (4.3)	269 (95.7)			2 (0.7)	279 (99.3)		
Sleeping in the same room with the dog									
Yes	96 (12.3)	5 (5.2)	91 (94.8)	0.99 (0.30–2.63)	0.990	2 (2.1)	94 (97.9)	1.31 (0.14–6.13)	0.729
No (Ref.)	687 (87.7)	36 (5.2)	651 (94.8)			11 (1.6)	676 (98.4)		
Letting the dog lick the face									
Yes	241 (30.8)	14 (5.8)	227 (94.2)	1.18 (0.56–2.38)	0.631	6 (2.5)	235 (97.5)	1.95 (0.54–6.86)	0.226
No (Ref.)	542 (69.2)	27 (5.0)	515 (95.0)			7 (1.3)	535 (98.7)		
Washing hands after playing with the dog									
Yes	250 (31.9)	15 (6.0)	235 (94.0)	1.24 (0.60–2.49)	0.511	4 (1.6)	246 (98.4)	0.95 (0.21–3.43)	0.928
No (Ref.)	533 (68.1)	26 (4.9)	507 (95.1)			9 (1.7)	524 (98.3)		

NS: Not specified. NC: Not calculated.

In Table 3, it is observed that, among the evaluated characteristics, only the location of animal slaughter at a slaughterhouse showed a statistically significant association with canine echinococcosis positivity, both by copro-ELISA (OR=2.33; 95% CI: 1.12–5.04; p=0.014) and copro-PCR (OR=2.71; 95% CI: 1.39–5.49; p=0.001). The other variables, such as age, sex, deworming, the place where the dog sleeps, or slaughter at home or in the open field, did not show a significant association.

Table 3. Association between dog characteristics, rearing practices, and the presence of canine echinococcosis diagnosed by copro-ELISA and copro-PCR in the Ascensión district, Huancavelica, 2019.

Dog Characteristics	Total, n (%)	Canine echinococcosis by copro-ELISA				Canine echinococcosis by copro-PCR			
		Positive, n (%)	Negative, n (%)	OR (95% CI)	p-value	Positive, n (%)	Negative, n (%)	OR (95% CI)	p-value
Dog Age									
0–6 months (ref.)	90 (16.6)	7 (7.8)	83 (92.2)			10 (11.1)	80 (88.9)		
6 months to 1 year	80 (14.7)	9 (11.3)	71 (88.8)	1.50 (0.47–5.00)	0.439	10 (12.5)	70 (87.5)	1.14 (0.40–3.26)	0.779
1–7 years	282 (51.9)	21 (7.5)	261 (92.6)	0.95 (0.37–2.75)	0.918	27 (9.6)	255 (90.4)	0.85 (0.38–2.05)	0.672
>7 years	91 (16.8)	2 (2.2)	89 (97.8)	0.27 (0.03–1.46)	0.084	2 (2.2)	89 (97.8)	0.18 (0.02–0.89)	0.016
Dog Sex									
Male	370 (68.1)	24 (6.5)	346 (93.5)	0.73 (0.36–1.54)	0.358	34 (9.2)	336 (90.8)	1.07 (0.55–2.17)	0.844
Female (ref.)	173 (31.9)	15 (8.7)	158 (91.3)			15 (8.7)	158 (91.3)		
Deworming Every 2 Months									
Yes	356 (65.6)	24 (6.7)	332 (93.3)	0.83 (0.41–1.75)	0.583	29 (8.2)	327 (91.9)	0.74 (0.39–1.43)	0.325
No (ref.)	187 (34.4)	15 (8.0)	172 (92.0)			20 (10.7)	167 (89.3)		
Deworming at Any Time									
Yes	356 (65.6)	24 (6.7)	332 (93.3)	0.83 (0.41–1.75)	0.583	29 (8.2)	327 (91.9)	0.74 (0.39–1.43)	0.325
No (ref.)	187 (34.4)	15 (8.0)	172 (92.0)			20 (10.7)	167 (89.3)		

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Table 3. (Continuación)

Dog Characteristics	Total, n (%)	Canine echinococcosis by copro-ELISA: Positive, n (%)	Negative, n (%)	OR (95% CI)	p-value	Canine echinococcosis by copro-PCR: Positive, n (%)	Negative, n (%)	OR (95% CI)	p-value
Dog Sleeping Location: Inside House									
Yes	20 (3.7)	1 (5.0)	19 (95.0)	0.67 (0.02–4.47)	0.700	1 (5.0)	19 (95.0)	0.52 (0.01–3.43)	0,522
No (ref.)	523 (96.3)	38 (7.3)	485 (92.7)			48 (9.2)	475 (90.8)		
Dog Sleeping Location: Outside House									
Yes	514 (94.7)	39 (7.6)	475 (92.4)	NC	NC	48 (9.3)	466 (90.7)	2.88 (0.45–120.34)	0,281
No (ref.)	29 (5.3)	0 (0)	29 (100.0)	NC	NC	1 (3.5)	28 (96.6)		
Dog Sleeping Location: Inside and Outside House									
Yes	123 (22.6)	5 (4.1)	118 (95.9)	0.48 (0.14–1.28)	0.128	8 (6.5)	115 (93.5)	0.64 (0.25–1.44)	0,267
No (ref.)	420 (77.4)	34 (8.1)	386 (91.9)			41 (9.8)	379 (90.2)		
Dog Sleeping Location: Kennel, House, or Cage									
Yes	526 (96.9)	38 (7.2)	488 (92.8)	1.25 (0.18–53.60)	0.833	48 (9.1)	478 (90.9)	1.61 (0.24–68.75)	0,646
No (ref.)	17 (3,1)	1 (5,9)	16 (94,1)			1 (5,9)	16 (94,1)		
Animal Slaughter Location: Home									
Yes	539 (99.3)	39 (7.2)	500 (92.8)	NC	NC	49 (9.1)	490 (90.9)	NC	NC
No (ref.)	4 (0.7)	0 (0.0)	4 (100.0)	NC	NC	0 (0.0)	4 (100.0)	NC	NC
Animal Slaughter Location: Slaughterhouse									
Yes	259 (47.7)	26 (10.0)	233 (90.0)	2.33 (1.12–5.04)	0.014	34 (13.1)	225 (86.9)	2.71 (1.39–5.49)	0.001
No (ref.)	284 (52.3)	13 (4.6)	271 (95.4)			15 (5.3)	269 (94.7)		
Animal Slaughter Location: Open Field									
Yes	531 (97.8)	38 (7.2)	493 (92.8)	0.85 (0.12–37.44)	0.876	48 (9.0)	483 (91.0)	1.09 (0.15–48.01)	0.933
No (ref.)	12 (2.2)	1 (8.3)	11 (91.7)			1 (8.3)	11 (91.7)		

NC: Not Calculated

In Table 4, it can be seen that some practices for disposing of viscera showed a significant association with canine echinococcosis. Direct consumption of viscera by the dog was associated with higher positivity for both copro-ELISA (OR=5.39; 95% CI: 0.49–34.14; p=0.027) and copro-PCR (OR=7.99; 95% CI: 1.13–48.42; p=0.002).

Similarly, incinerating the viscera was associated with lower positivity for copro-PCR (OR=0.34; 95% CI: 0.09–0.96; p=0.033), and cooking the viscera before giving it to the dog was associated with higher positivity for copro-PCR (OR=3.02; 95% CI: 1.12–7.29; p=0.007). The other practices showed no significant association.

Table 4. Association between viscera disposal practices and the presence of canine echinococcosis according to diagnosis by copro-ELISA and copro-PCR in the Ascensión district, Huancavelica, 2019.

Viscera Disposal Practice	Total, n (%)	Canine echinococcosis by copro-ELISA: Positive, n (%)	Negative, n (%)	OR (95% CI)	p-value	Canine echinococcosis by copro-PCR: Positive, n (%)	Negative, n (%)	OR (95% CI)	p-value
Viscera disposal: giving to the dog									
Yes	257 (47.3)	13 (5.1)	244 (94.9)	0.53 (0.25–1.11)	0.069	19 (7.4)	238 (92.6)	0.68 (0.35–1.29)	0.209
No (ref.)	286 (52.7)	26 (9.1)	260 (90.9)	—	—	30 (10.5)	256 (89.5)	—	—
Viscera disposal: the dog consumes them									
Yes	7 (1.3)	2 (28.6)	5 (71.4)	5.39 (0.49–34.14)	0.027	3 (42.9)	4 (57.1)	7.99 (1.13–48.42)	0.002
No (ref.)	536 (98.7)	37 (6.9)	499 (93.1)	—	—	46 (8.6)	490 (91.4)	—	—
Viscera disposal: incineration									
Yes	107 (19.7)	4 (3.7)	103 (96.3)	0.44 (0.11–1.29)	0.124	4 (3.7)	103 (96.3)	0.34 (0.09–0.96)	0.033
No (ref.)	436 (80.3)	35 (8.0)	401 (92.0)	—	—	45 (10.3)	391 (89.7)	—	—

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Table 4. (Continuation)

Viscera Disposal Practice	Total, n (%)	Canine echinococcosis by copro-ELISA				Canine echinococcosis by copro-PCR			
		Positive, n (%)	Negative, n (%)	OR (95% CI)	p-value	Positive, n (%)	Negative, n (%)	OR (95% CI)	p-value
Viscera disposal: burying them									
Yes	397 (73.1)	31 (7.8)	366 (92.2)	1.46 (0.64–3.77)	0.351	37 (9.3)	360 (90.7)	1.15 (0.56–2.49)	0.691
No (ref.)	146 (26.9)	8 (5.5)	138 (94.5)	—	—	12 (8.2)	134 (91.8)	—	—
Viscera disposal: cooking and giving to the dog									
Yes	38 (7.0)	4 (10.5)	34 (89.5)	1.58 (0.39–4.81)	0.408	8 (21.1)	30 (78.9)	3.02 (1.12–7.29)	0.007
No (ref.)	505 (93.0)	35 (6.9)	470 (93.1)	—	—	41 (8.1)	464 (91.9)	—	—
Viscera disposal: throwing away in the trash or river									
Yes	4 (0.7)	0 (0.0)	4 (100.0)	NC	NC	0 (0.0)	4 (100.0)	NC	NC
No (ref.)	539 (99.3)	39 (7.2)	500 (92.8)	NC	NC	49 (9.1)	490 (90.9)	NC	NC

NC: Not Calculated

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DISCUSSION

In this study, the presence of the zoonotic cestode Echinococcus granulosus was determined in schoolchildren and domestic dogs from the district of Ascensión, Huancavelica region, Peru, using serological and molecular techniques. Previous studies have identified genotype G1 as the predominant circulating genotype in the country, associated with a wide range of intermediate hosts—five different species in the region—placing Peru among the countries with the highest number of human cases in South America [12]. The detection of this parasite in the study area may reflect the active circulation of E. granulosus and suggests the presence of infected sheep and South American camelids, which would contribute to maintaining the parasite's life cycle and represent a significant risk of zoonotic transmission.

Using the serological technique ELISA, the seroprevalence in schoolchildren was 5.18%. However, after confirmation with the Inmunoblot test, this value was reduced to 1.60%. In a similar study conducted in rural households in Chile, Acosta-Jamett G et al. [13]. reported a human seroprevalence of 2.6%. Although this value is close to that found in our study, the differences in environmental contexts, characteristics of the evaluated populations, and variability in the sensitivity and specificity of the diagnostic tests employed should be considered. Despite these methodological differences, the similarity in the findings could be explained by the fact that both sampling areas correspond to rural regions with similar sociocultural characteristics. At the national level, Antitupa I et al.[14] reported a seroprevalence of 4.9% for cystic echinococcosis in Peru and 7.1% specifically for the Huancavelica region. While the national value is within the range observed in our study, the regional seroprevalence is clearly higher. This difference could be attributed to selection bias in the studied population, as the aforementioned report is based on the analysis of 7,811 epidemiological records, likely from symptomatic patients or those with high clinical suspicion. However, our findings support existing evidence that identifies Huancavelica as an endemic area for this helminthiasis, as reported in other studies conducted in the Andean region of the country.

Reyes MM et al.[15] estimated a prevalence of 9.3% in a study conducted in informal slaughterhouses in Lima, where three cases were detected among thirty-two individuals evaluated. These cases were diagnosed through abdominal ultrasound and chest X-rays, which differs from the serological methods used in our study. This difference in diagnostic strategies could explain the higher prevalence observed in that urban context compared to our results.

Campos JP[16] reported a prevalence of 13.3% in medical records of patients attending the Surgery Service at the Hospital Regional Daniel Alcides Carrión in Cerro de Pasco, while Rivera E [17] reported a prevalence of 11.56% in patients screened in high-risk areas within the same region. Both studies used a combination of clinical, radiological, and serological methods.

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The prevalences found are higher than those of the present study, probably due to factors such as climatic characteristics, access to specialized clinical diagnosis, and the age of the evaluated populations. Additionally, it should be considered that Cerro de Pasco has been identified as one of the regions with the highest prevalence of echinococcosis in the country [14].

In the district of Caracoto, in the Puno region, Tapia AR [18] reported a seroprevalence of 15.18% in adults aged 18 to 65, using the latex agglutination technique. This figure contrasts with the findings of the present study, and the difference may be due to the type of test employed—of lower specificity—along with the fact that no confirmatory test, either serological or molecular, was performed in that study.

Regarding canine echinococcosis, 543 fecal samples from dogs belonging to 130 households were analyzed. The seroprevalence determined by copro-ELISA was 7.18%, and the coproprevalence confirmed by copro-PCR reached 9.02%. These values were lower compared to those reported by Acosta-Jamett G et al. [13], who found a prevalence of 28% in a canine population in Chile. The differences could be explained by the dogs' dietary habits, the smaller number of animals sampled in the Chilean study, and the exclusive use of serological techniques without molecular confirmation, which can influence the estimation of prevalence since molecular tests often have higher diagnostic sensitivity—although this may be affected by factors such as the quality of the fecal DNA or parasitic load.

In the Huancavelica region, Almidón AF et al.[19] reported a molecular coproprevalence of 3% in dogs from the Ahuaycha district (Tayacaja), lower than the finding in the present study. This difference can be explained by the exclusive use of the copro-PCR test without serological screening, as well as the socio-environmental characteristics unique to each district. Furthermore, the number of dogs sampled was smaller in the Tayacaja study. In that study, it was observed that dogs over 1.5 years old had a positivity rate of 6.8%, higher than that of puppies, which partially coincides with the findings of our investigation, where dogs over seven years old had a lower frequency of infection, with a statistically significant difference (p=0.016).

Reyes MM et al.[15] also reported a seroprevalence of 36% in dogs evaluated in Lima. However, the sample size was small (22 animals), and only a few positive cases were confirmed by PCR or purging with direct observation of the parasite. This high seroprevalence could be due not only to the small sample size but also to the fact that urban or periurban conditions in Lima present a transmission cycle different from that in the rural Andean environment.

For their part, Puricelli VI et al.[20] reported a prevalence of 17.3% in fecal samples of dogs collected from the ground and a positivity rate of 44.7% at the level of epidemiological units in a study conducted in Argentina. The differences with our study could be related to the sampling methodology, as in the Argentine study, dispersed canine feces were collected as an indicator of environmental contamination, and both individual samples and those by epidemiological unit were analyzed, making direct comparison difficult.
In the province of Concepción, Junín, Montalvo R et al. [21] found a canine coproprevalence of 50% through copro-ELISA, with particularly high values in the localities of Usibamba (61.0%), Chaquicocha (51.0%), and San José de Quero (41.9%). These figures, clearly higher than those found in our study, could be due to the fact that this province presents a hyperendemic situation for this zoonosis.

A higher proportion of males (78.3%) was also reported among the dogs evaluated, which in some studies has been associated with a higher risk of infection[21]. Other factors that could have contributed to these high prevalences include limited deworming, access of the dogs to raw offal, and their roaming behavior, which favors fecal contamination of the peridomestic environment. Regarding the associated factors evaluated in this study, some results partially coincide with those reported by Almidón AF et al.[19], who found an association between the disposal of contaminated offal and the presence of echinococcosis in dogs.

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In the present study, the practice of allowing dogs to consume raw offal showed a statistically significant association with infection diagnosed by copro-PCR. However, other variables related to the cohabitation between dogs and livestock, home animal slaughter, and access to potentially contaminated water sources, although frequently reported in the surveyed population, did not show a significant association with canine or human infection. These findings highlight the importance of certain documented risk practices but also suggest that complementary studies are needed to deepen their impact on the local transmission of *Echinococcus granulosus*.

Various studies in the Andean region of Peru have identified socio-epidemiological factors linked to human and canine echinococcosis, such as feeding dogs raw offal, dogs defecating in open spaces, and family history of echinococcosis infection [21]. In the present study, some of these practices were reported in the surveys applied, but not all showed a statistically significant association with infection in schoolchildren. For example, variables like feeding dogs raw offal, allowing dogs to lick faces, or sharing a room, were not significantly associated with human seroprevalence. Nonetheless, these practices remain relevant from an epidemiological standpoint and should be considered in control strategies and health education.

The combined raising of sheep, camelids, and dogs for herding tasks has been identified as a risk factor for human echinococcosis in various rural contexts [22]. While in the present study, a high frequency of raising these animals was recorded in the surveyed population, no statistically significant association was found between these variables and seropositivity in schoolchildren. However, other studies, such as those by Arca JR[23] and Salazar-Mesones B et al.[24], have shown that close and frequent contact with dogs, as well as livestock raising, can increase the risk of infection. These findings reinforce the need to consider these practices within a preventive approach, especially in rural areas with conditions conducive to the transmission cycle of *Echinococcus granulosus*. In a hospital-based study, Bravo JC and Cambillo ML [25] found no relationship between responsible dog ownership and the presence of human cystic echinococcosis, which could be explained by the lack of diagnostic tests in the dogs, despite owners reporting proper management practices. Additionally, it is possible that the anthelmintics used were ineffective against cestodes like *Echinococcus granulosus*. In the present study, although a high frequency of dog rearing was observed, this variable did not show a statistically significant association with seropositivity in schoolchildren. Nonetheless, dog rearing in risky conditions continues to be a key component in the epidemiology of echinococcosis, especially when practices like feeding raw offal, lack of effective deworming, and home slaughter of livestock are combined[18,26-29].

The results of the present study partially align with those reported by Almidón AF et al.[19], who highlighted that the manner in which livestock offal is disposed of significantly influences zoonotic transmission. In our research, allowing dogs to consume offal directly, as well as cooking it and offering it to them, was significantly associated with the presence of E. granulosus in the feces, detected by copro-PCR. Other reported risk factors, such as exposure to dog feces and lack of hand washing[30], as well as slaughtering livestock in the field or slaughterhouses without adequate biosecurity measures[26], were not directly evaluated in this study, although they are relevant for understanding the epidemiological context of the area. The combination of these practices creates a high-risk scenario for the persistence and spread of this zoonosis [28-30]. Puricelli VI et al.[20], through a survey applied to rural populations, identified cultural practices at risk for the transmission of Echinococcus granulosus, such as home slaughter (34.2%), feeding dogs raw offal (52.6%), and lack of deworming (86.8%).

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Additionally, about half of the respondents were unaware of the transmission modes and preventive measures. In the present study, while attitudes and knowledge were not evaluated, similar practices were identified in the surveys, such as home slaughter and improper disposal of offal, some of which showed a significant association with canine infection. These findings support the importance of socio-cultural factors as determinants in the transmission of this zoonosis, in agreement with what was reported by Hosseini Z et al. [31].

Although this study focused on the human child population and domestic dogs as definitive hosts, it is important to consider that other ungulate animals, such as sheep and South American camelids, can also serve as intermediate hosts in the E. granulosus cycle. Calle RM[32] reported a prevalence of 20% in slaughtered llamas, suggesting a potential contamination risk for herding dogs in high Andean regions. Although this study did not evaluate infection in these animals, their role in transmission dynamics deserves investigation, considering their abundance in the Huancavelica region and their proximity to both livestock and the human population.

It is also crucial to emphasize the need to implement good practices in animal handling and sanitary control during animal slaughter, as well as strengthen health promotion strategies aimed at preventing this parasitosis. In this study, a considerable percentage of respondents indicated performing animal slaughter at home, in open fields, or in slaughterhouses. While the sanitary conditions of these spaces were not directly evaluated, it is recognized that slaughter without appropriate biosecurity measures poses a high risk of zoonotic transmission, especially in contexts where animal by-products may be easily accessible to dogs, thus contributing to maintaining the transmission cycle of Echinococcus granulosus between animals and humans [15,26,27,33]. While the relevance of the present study is not diminished, it is important to recognize certain methodological limitations, particularly in the diagnosis of cystic echinococcosis in the human population. The tests used were exclusively laboratory-based serological analyses, without the incorporation of imaging techniques such as ultrasound or tomography, which are typically used in healthcare settings to confirm clinical cases. In such contexts, diagnosis is often complemented with anamnesis, radiological tests, and laboratory studies, allowing for better case follow-up and more comprehensive access to various diagnostic strategies, especially for patients receiving hospital care.

Additionally, limitations were identified in the application of the surveys due to language barriers, as a proportion of the surveyed population speaks Quechua as their native language. This situation could be overcome through the design and use of bilingual materials and the training of community agents, which would help improve the quality of the epidemiological data collected. On the other hand, it would be advisable to include the analysis of intermediate hosts, such as sheep and South American camelids, as well as potential wildlife reservoirs in future investigations. Assessing the presence of Echinococcus granulosus in these animals would provide a more precise understanding of the parasite's life cycle in the region and strengthen strategies for the prevention and control of this zoonosis.

CONCLUSION

This study confirmed the presence of Echinococcus granulosus in schoolchildren and domestic dogs in the district of Ascensión, Huancavelica. The seroprevalence in humans was 5.18% by ELISA and 1.60% by Inmunoblot, with no statistically significant associations found with the evaluated variables. In dogs, the coproprevalence was 7.18% by copro-ELISA and 9.02% by copro-PCR, with significant associations found with variables such as the animal's age, place of livestock processing, and the method of offal disposal. These findings reflect the active circulation of the parasite in the area and highlight the role of the dog as a definitive host, which allows Ascensión to be considered a zone with a risk of cystic echinococcosis transmission. Strengthening local prevention and control strategies is recommended, with an emphasis on health education, timely diagnosis, and proper animal waste management.

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