

DISTRIBUTION OF HYDATIDOSIS AND CYSTICERCOSIS IN DIFFERENT PERUVIAN POPULATIONS AS DEMONSTRATED BY AN ENZYME-LINKED IMMUNOELECTROTRANSFER BLOT (EITB) ASSAY

PEDRO L. MORO, ANGELA GUEVARA, MANUELA VERASTEGUI, ROBERT H. GILMAN, HORTENCIA POMA, BEATRIZ TAPIA, VICTOR C. W. TSANG, HECTOR H. GARCIA, ROSA PACHECO, CECILIA LAPEL, ELBA MIRANDA, AND THE CYSTICERCOSIS WORKING GROUP IN PERU (CWG)*

Laboratorio de Parasitología, Universidad Peruana Cayetano Heredia, Lima, Peru; Department of International Health, Johns Hopkins University School of Public Health, Baltimore, Maryland; Departamento de Farmacología, Universidad Nacional Mayor de San Marcos, Lima, Peru; Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia; Departamento de Microbiología, Universidad San Antonio Abad del Cuzco, Cuzco, Peru

Abstract. A serosurvey for human hydatidosis and cysticercosis was performed in different regions of Peru. Those regions included a known endemic area for cystic hydatid disease, a cooperative in the central Peruvian Andes near the city of Tarma, Department of Junin; three areas endemic for cysticercosis in the Departments of Ancash, Cuzco, and San Martín, where the status of hydatid disease is not well defined; and an urban shantytown near Lima, where neither zoonosis is known to be present. A seroprevalence for hydatidosis 1.9% (6 of 309) was found with both the enzyme-linked immunoelectrotransfer blot (EITB) and double diffusion assays in the area endemic for hydatidosis. Seroprevalence in the other zones tested was zero using only the EITB assay. Cysticercosis seroprevalence was high in pig-raising zones but low in the high-altitude, sheep-raising areas and in the seaport of Callao. No cross-reactions between *Echinococcus granulosus* and cysticercosis were noted in any of the regions studied. Hydatid infection remains a major health problem in the central Peruvian Andes where sheep raising is widely practiced; however, in those regions where mainly swine are raised, human hydatid infection is not a problem.

Human hydatid disease caused by *Echinococcus granulosus* has been recognized for decades as a serious medical problem in sheep raising areas of Peru.¹ Official figures probably underestimate the true extent of the problem, since no serologic surveys have been performed for more than 11 years.^{2,3} Previous surveys done in disease-endemic regions of Peru have used the in-

direct hemagglutination assay, immunoelectrophoresis (IEP) assay, and/or the double diffusion test (DD5). The latter two detect the antigen-antibody reaction known as arc S.⁴

In Peru, the DD5 test is the most commonly used method because of its ease of performance, low cost, and high specificity. The major limitation of the DD5 test is its low sensitivity.^{5,6}

Recently, we developed an enzyme-linked immunoelectrotransfer blot (EITB) assay for the diagnosis of *E. granulosus* that is more sensitive and has a lower rate of cross-reactions with antibody to cysticercosis than does the DD5 assay.⁶ This hydatid EITB assay was used to determine the prevalence of hydatid disease in different population centers of Peru, including rural sheep- and pig-raising areas and urban areas.

MATERIALS AND METHODS

Study sites (Figure 1). *Sheep-raising area.* Department of Junin. Tupac Amaru is a farm-

* The CWG members are H. H. Garcia, F. Diaz, J. Naranjo, C. Carcamo, T. Montenegro, P. Torres (Universidad Peruana Cayetano Heredia); J. Pilcher (Centers for Disease Control and Prevention, Atlanta, GA); C. Evans (Cambridge University, UK); A. E. Gonzalez (Universidad Nacional Mayor de San Marcos); M. Martinez, J. Altamirano, M. Alvarado, E. Orrillo, S. Escalante, L. Palomino, G. Alban, N. Rios-Saavedra, J. M. Cuba, H. Estrada (Instituto Nacional de Ciencias Neurológicas); A. Terashima, J. Cabrera, P. Campos (Hospital Cayetano Heredia); U. Rocca (Hospital Guillermo Almenara); M. Lescano, L. E. Vasquez, L. Samaniego, J. Matsuoko (Instituto de Medicina Tropical de San Martín).



FIGURE 1. Map of Peru showing location of the study sites.

ing cooperative (population 40,452) located at an altitude of approximately 4,200 m in the central Andes. Sheep are the major animal raised while pigs are rarely found. Villagers were asked to volunteer for serologic testing at the health center after listening to a talk concerning hydatid disease. They were questioned about sheep-raising practices, dog ownership, infected viscera disposal, and a history of hydatid disease.

Pig-raising areas. Department of Ancash. Pomabamba (population 13,000) is an Andean town surrounded by rural communities where pigs are the primary farm animal, but sheep and cattle are also raised. Pomabamba is located in the central Peruvian Sierra at an altitude of 2,948 m. Serum samples were collected from asymptomatic persons who volunteered to be tested after attending a presentation on cysticercosis at the municipal hospital.

Department of Cuzco. Haparquilla (population 635) is a rural village located in the Anta Valley at an altitude of 3,300 m. Pigs are the most common animal raised. Sheep are rare. Se-

rum specimens were collected from consenting persons at home and at a school.

Department of San Martin. Churusapa (population 282) is located in the high jungle of the Andean slopes at an altitude of 333 m. Swine rearing is common but sheep are not raised. Serum specimens were collected from at least one person in 81% (44 of 54) of the households. Hydatidosis is not endemic in this area, and no cases have been reported, based on more than 10,000 abdominal ultrasonographic examinations that were performed on villagers at the local hospital (Samaniego L, unpublished data).

Urban population. Acapulco (pop 15,000) is an urban shantytown located near the Lima airport adjacent to the seaport of Callao. The serum samples were obtained as part of a cholera survey. Farm animals were not present.

Cajamarca slaughterhouse. The city of Cajamarca (Department of Cajamarca, population 97,452) is located in the northern Peruvian Andes at an altitude of 2,720 m. Swine, sheep, and cattle are raised in the rural areas of the city. We

took blood samples from 107 urban slaughterhouse workers.

Sample collection. Blood samples were obtained by venipuncture; they were stored at -20°C .

Serologic tests. Double diffusion test. The DD5 test was performed on samples from Tupac Amaru and Haparquilla as previously described.^{7,8} This test was not performed in other areas because of insufficient volumes of serum.

Hydatid EITB assay. An EITB assay for human hydatid disease diagnosis that identifies three specific bands (8, 16, and 21 kD) was performed as previously described.⁹

Cysticercosis EITB assay. An EITB assay for human cysticercosis was also performed on the serum samples from all study groups to determine if dual infection or cross-reacting antibody was present.^{9,10}

RESULTS

Sheep-raising area. Tupac Amaru. One hundred thirty-one males and 178 females (age range 10–66 years; median age 24 years) responded to the questionnaire; serum sample were obtained from each. More than half (57%) of the families owned dogs and 41% of the individuals were in regular and close contact with dogs belonging to others. Eleven percent fed viscera to dogs; 23% of those did so without cooking it (Table 1). None of the 309 persons questioned reported previous hospitalizations or surgery for cysts or tumors.

Six (1.9%) of 309 persons studied were positive for antibody specific for hydatidosis. All six seropositive persons were asymptomatic and none were from the same family. Three were males, 12, 23, 54 years of age, and three were female, 35, 45, and 60 years of age. Three persons tested positive in both the DD5 and EITB assays. Two participants tested positive only in the EITB assay and one tested positive only in the DD5 test (Table 2). Of the five persons whose serum tested positive by the EITB test, the three diagnostic bands (8, 16, and 21 kD) were present.

Two (0.6%) of 309 participants had a positive EITB test result for cysticercosis. Neither had a positive test result for hydatidosis.

Pig-raising areas. All study samples from the three pig-raising villages were seronegative for hydatidosis. In contrast, 13% (15 of 112), 8% (4

TABLE I
Slaughtering and dog-feeding practices at the farming cooperative of Tupac Amaru, a sheep-raising area where *Echinococcus granulosus* is endemic

	No. (%)
Persons interviewed	309
Families	221
Education level	
Illiterate	25 (8)
Elementary school	199 (64)
Secondary school	67 (22)
Beyond high school	18 (6)
Association with dogs	
No. of dogs	215
No. of families owning dogs	125 (57)
Mean no. of dogs/family	2
No. of persons who play with dogs	126 (41)
Infected viscera disposal*	
No. who have seen water bags† in viscera	
No. who burn or bury infected viscera	210 (68)
No. who throw infected viscera into streams	128/210 (61)
No. who feed viscera to dogs	74/210 (35)
No. who feed infected raw viscera to dogs	35/309 (11)
	8/35 (23)
Farm animal raising	
No. of families that raise farm animals	141/221 (64)
Animal raised by family	
Sheep	63/221 (29)‡
Cattle	104/221 (47)‡
Horse	62/221 (28)‡
Pig	23/221 (10)‡

* Individual responses.

† Water bags is the term used in the questionnaire for hydatid cysts.

‡ Approximate no. of sheep, cattle, horses, and pigs raised by whole village are 45,312, 2,068, 215, and 34, respectively.

of 50), and 7% (7 of 97) of serum samples from Pomabamba, Haparquilla, and Churusapa, respectively, were seropositive for cysticercosis (Table 2).

Urban area. In Acapulco, the majority of the population is composed of young people born in the city of Callao or in nearby Lima (median age = 18 years). All 250 persons tested were seronegative for both hydatidosis and cysticercosis (Table 2).

Cajamarca slaughterhouse workers. Of the Cajamarca slaughterhouse employees, 7.4% (8 of 107) of the workers had a positive EITB test result for cysticercosis; none tested seropositive for hydatidosis.

TABLE 2
Seropositive rates of human hydatidosis and cysticercosis

Sites (Department)	No. of serum samples	Main domestic animal	Hydatidosis		Cysticercosis	
			No. (%) positive	CI*	No. (%) positive	CI*
Tupac Amaru† (Junin)	309	Sheep‡	6 (1.9)	0.4-3.6	2 (0.6)	0.3-1.4
Pomabamba§ (Ancash)	112	Swine‡¶	0	0	15 (13.4)	7.1-19.7
Haparquilla‡§	50	Swine	0	0	4 (8.0)	2.2-19.2
Churusapa§ (San Martin)	97	Swine	0	0	7 (7.2)	2.9-14.3
Urban	250	None	0	0	0	0
Cajamarca slaughterhouse (Cajamarca)	107	NA*	0	0	8 (7.4)	2.4-12.4

* CI = 95% confidence intervals.

† Sites studied with both the hydatid enzyme-linked immunoelectrotransfer blot assay and the double diffusion assay.

‡ Cattle are also raised.

§ Pomabamba, Haparquilla, and Churusapa have significantly more cysticercosis seropositive persons than Tupac Amaru and Callao.

¶ Sheep are also raised.

* NA = not applicable. In Cajamarca town, pigs are the domestic animal.

DISCUSSION

In this study, diagnostic serology (EITB and DD5 tests) showed that hydatid infection remains endemic in the central Peruvian Andes but does not appear to be a significant problem in the city of Lima or in areas where pigs are the primary farm animal. Although exact figures are not available, urban-acquired hydatid disease occurs in Lima to a small extent.¹¹

Prevalence of sheep hydatidosis in the study area varied from 4% to 66% (unpublished data). In a recent study, approximately 30% of the sheep slaughtered at the Tupac Amaru abattoir had hydatid cysts of the lung and liver (unpublished data).

In hydatid-endemic areas of Peru, feeding raw, infected viscera to dogs and the indiscriminate disposal of viscera within reach of dogs is still being practiced, thus allowing ongoing transmission of *E. granulosus*. These same slaughter practices were documented more than 25 years ago.¹ Better public health intervention is required if the transmission of hydatid disease is to be stopped.

Our data show that transmission of hydatid disease remains high. A chest radiographic study done in 1976, adjacent to Tupac Amaru, found that 3% of the radiographs had images compatible with hydatid cysts.⁴ Previous studies performed in 1974 and 1976 in Tupac Amaru using the IEP test demonstrated seroprevalences of 0.1% and 0.98%, respectively.⁴ Using more sen-

sitive techniques, we found a prevalence of 1.9% by the EITB and DD5 tests, which suggested that hydatid infection is at least as common now as it was nearly two decades ago.

At a high altitude, hydatid cysts appear to be found more often in the lung than in the liver.¹² Lung cysts provoke a much weaker immunologic response than do those found in the liver.^{3,6} In hospital-based cases, only about half of the persons with lung cysts have detectable antibodies to *E. granulosus* compared with 80-90% of those with liver cysts. In an African community, only 50% of the persons with liver cysts detected by ultrasonography tested seropositive.¹³ Given the high rate of pulmonary cysts and the low sensitivity of immunologic tests for hydatidosis, the true prevalence of hydatid disease is probably at least 2-3 times higher than the seroprevalence of 1.9% found in our study.

The hydatid EITB assay has been found to cross-react in patients with cysticercosis.^{6,8} In this study, no one reacted to both antigens.

Our study also demonstrates that pig-raising areas appear to be free of hydatidosis, even though pigs can be infected with *E. granulosus*.¹⁴ In Peru, we have found that most ovine hydatid cysts are fertile, whereas pig and bovine cysts usually are not (unpublished data). The difference in the fertility of sheep and pig hydatid cysts may in part explain the absence of human hydatid infection in areas where only pigs and cattle are raised. In contrast, at least 7% of all

persons living in pig-raising areas were infected with cysticercosis. In fact, the only two areas where cysticercosis infection was not highly endemic were in the seaport of Callao and in the high-altitude, sheep-raising area of Tupac Amaru. The high percentage of cysticercosis infection in three widely separated and ecologically different regions of Peru supports previous studies demonstrating that *Taenia solium* infection is highly endemic in Peru.^{10, 15, 16}

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Authors' addresses: Pedro L. Moro, Angela Guevara, Manuela Verastegui, Hector H. Garcia, Cecilia Lapel, and Elba Miranda, Laboratorio de Parasitologia, Universidad Peruana Cayetano Heredia, Lima, Peru. Robert H. Gilman, Department of International Health, Johns Hopkins University School of Public Health, Baltimore, MD 21205. Hortencia Poma and Beatriz Tapia, Departamento de Farmacologia, Universidad Nacional Mayor de San Marcos, Lima, Peru. Victor C. W. Tsang, Immunology Branch, Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA 30341-3724. Rosa Pacheco, Departamento de Microbiologia, Universidad San Antonio Abad del Cuzco, Cuzco, Peru.

REFERENCES

- Otarola SG, 1966. Epidemiologia de la hidatidosis en el Peru. *Bol Oficina Sanit Panam* 60: 144-153.
- Serra I, Reyes H, 1989. Hidatidosis humana en cuatro paises de sudamerica. *Bol Oficina Sanit Panam* 106: 527-530.
- Diaz VM, Guarnera EA, Coltorti EA, 1986. Ventajas y limitaciones de los metodos inmunologicos y de deteccion de imagenes para el diagnostico de la hidatidosis. *Bol Oficina Sanit Panam* 100: 369-383.
- Diaz VM, Naquira F, Coltorti EA, Ocharan AM, Bullon F, Elliot A, Contreras O, Tantalean M, Huiza A, Naquira C, 1984. La hidatidosis en el Peru: importancia del inmunodiagnostico y de los estudios seroepidemiologicos. *Acta Med Peruana* 11: 21-28.
- Mercado R, Atias A, Astorga B, Lorca M, 1988. Reaccion de doble difusion en agar con deteccion del arco 5o en el diagnostico de la hidatidosis. *Bol Oficina Sanit Panam* 105: 159-163.
- Verastegui M, Moro P, Guevara A, Rodriguez T, Miranda E, Gilman RH, 1992. Enzyme-linked immunoelectrotransfer blot test for the diagnosis of human hydatid disease. *J Clin Microbiol* 30: 1557-1561.
- Coltorti EA, Diaz VM, 1978. Detection of antibodies against *Echinococcus granulosus* arc 5 antigens by double diffusion test. *Trans R Soc Trop Med Hyg* 72: 226-229.
- Moro PL, Gilman RH, Wilson M, Schantz PM, Verastegui M, Garcia HH, Miranda E, 1992. Immunoblot (Western blot) and double diffusion (DD5) tests for hydatid disease cross-react with sera from patients with cysticercosis. *Trans R Soc Trop Med Hyg* 86: 422-423.
- Tsang VCW, Brand JA, Boyer AE, 1989. An enzyme-linked immunoelectrotransfer blot assay and glycoprotein antigens for diagnosing human cysticercosis (*Taenia solium*). *J Infect Dis* 159: 50-59.
- Diaz JF, Verastegui M, Gilman RH, Tsang VCW, Pilcher JB, Gallo C, Garcia HH, Torres P, Montenegro T, Miranda E, and The Cysticercosis Working Group in Peru, 1992. Immunodiagnosis of human cysticercosis (*Taenia solium*): a field comparison of an antibody-enzyme-linked immunosorbent assay (ELISA), an antigen-ELISA, and an enzyme-linked immunoelectrotransfer blot (EITB) assay in Peru. *Am J Trop Med Hyg* 46: 610-615.
- Alarcon J, Somocurcio J, Reyes N, Arevalo N, Bustamante E, 1992. Hidatidosis pulmonar: estudio epidemiologico de casos urbanos en el Hospital Hipolito Unanue de Lima. *Rev Peruana Epidemiol* 5: 15-19.
- Uceda del Campo J, 1967. Echinococosis hidatidica en la sierra central. *Anal Fac Med* 50: 500-524.
- MacPherson CNL, Zeyhle E, Romig T, Rees PH, Were JBO, 1987. Portable ultrasound scanner versus serology in screening for hydatid cysts in a nomadic population. *Lancet* ii: 259-291.
- Schwabe CW, 1986. Current status of hydatid disease: a zoonosis of increasing importance. Thompson RCA, ed. *The Biology of Echinococcus and Hydatid Disease*. London: George Allen and Unwin, 81-104.
- Garcia HH, Gilman RH, Martinez M, Tsang VCW, Pilcher JB, Herrera G, Diaz F, Alvarado M, Miranda E, and The Cysticercosis Group in Peru, 1993. Cysticercosis as a major cause of epilepsy in Peru. *Lancet* 341: 197-200.
- Diaz F, Garcia HH, Gilman RH, Gonzales AE, Castro M, Tsang VCW, Pilcher JB, Vasquez LE, Lescano M, Carcamo C, Madico G, Miranda E, and The Cysticercosis Working Group In Peru, 1992. Epidemiology of taeniasis and cysticercosis in a Peruvian village. *Am J Epidemiol* 135: 875-882.