Cystic hydatid disease (CHD), still a neglected disease despite being highly endemic in many livestock-raising regions around the world, is a zoonotic disease caused by the larval stage of the dog tapeworm *Echinococcus granulosus*. Diagnosis is based on imagenological tools (abdominal ultrasound, chest X-rays, or computed tomography [CT] scan). Serological antibody-detecting assays, using diverse native antigens, have been used as a supportive diagnostic tool, but their sensitivities and specificities differ greatly. The use of synthetic peptides as antigens should provide more reliability and allow better assessment and comparison of test formats and case series. The synthetic peptide p176, corresponding to the N-terminal extreme of the subunit of antigen B (AgB8/1), has shown promising performances for diagnosis of CHD. We evaluated the performance of the synthetic peptide p176 for the diagnosis of pulmonary hydatid disease in an enzyme-linked immunosorbent assay (ELISA) format. Sixty-one serum samples from patients with a diagnosis of pulmonary hydatidosis confirmed by surgery and 128 from healthy volunteers were tested. The overall sensitivity and specificity of the p176 ELISA for lung CHD were 78.69% and 96.88%, respectively. On bivariate analysis, positive serum antibody reactions were associated with the presence of complications and with the number of cysts (single/multiple). Only the presence of persistent complications significantly associated with seropositivity on multivariate logistic regression analysis (odds ratio [OR], 9.58; 95% confidence interval [CI], 2.15 to 42.6; \( P = 0.003 \)). The p176 ELISA performs well for the diagnosis of lung CHD and adds an easily reproducible diagnostic assay to the existing diagnostic tools.

Cystic hydatid disease (CHD), still a neglected disease despite being highly endemic in many livestock-raising regions around the world, is a zoonotic disease caused by the larval stage of the dog tapeworm *Echinococcus granulosus* (8, 14). In the natural life cycle of the tapeworm, humans play the role of an intermediate aberrant host, becoming infected by accidental ingestion of eggs from infected dog feces (25). Human infection frequently occurs during childhood, whereas clinical manifestations, basically determined by growth of or complications caused by the cyst(s), are more frequent in adulthood (4). CHD mainly involves the liver or lungs, with a liver/lung ratio of 6:1 in asymptomatic subjects and 3:1 in symptomatic subjects (1, 11). The increased proportion of lung cases in symptomatic subjects is determined by the higher morbidity of lung cysts, particularly in the presence of complications (rupture or infection) (2, 17).

Diagnosis of lung hydatid disease is based on chest imaging using X-rays or computed tomography (CT). Serological tools are used only to confirm the diagnosis because of low sensitivity and incomplete specificity (17). Assay performance is mainly dependent on the test format and the nature of the antigen used but also varies according to disease characteristics such as the organs involved, the number of cysts, and the presence of any cyst complications (25, 26). Differences in assays and also differences in how the clinical and radiological information are reported preclude sound comparison of serological assays in cases of CHD. Synthetic peptides or recombinant antigens derived from sequences of the two major components of cystic fluid, antigen B (AgB) and Ag5, have been previously proposed for use as reproducible antigens to improve test reliability and allow better standardization (5, 16, 22). The p176 antigen, derived from AgB, is a 38-mer corresponding to the N-terminal extension of the subunit AgB8/1 (12). An enzyme-linked immunosorbent assay (ELISA) using p176 has demonstrated good performance for diagnosing CHD, with sensitivities between 74% and 80% and reported specificity between 79% and 93% (12, 13). However, data on serological diagnosis of lung CHD are scarce, and the published p176 studies do not allow estimations of its sensitivity or provide further details for pulmonary cases. We applied this p176 ELISA in a series of known cases and compared the responses of those patients to the responses of noninfected controls to provide further information on the test performance of the assay for the diagnosis of lung CHD as well as its performance in relation to disease characteristics.

**MATERIALS AND METHODS**

**Study population.** Sera from 61 patients with surgically confirmed lung CHD (disease group) and 128 healthy volunteers (control group) were tested. CHD samples were collected between July 2003 and November 2005 from patients hospitalized in the department of Thoracic and Cardiovascular Surgery of the Hospital Nacional Hipólito Unanue and in the Hospital Nacional Dos de Mayo, both located in Lima, Peru. Negative controls were selected from a group of 170 asymptomatic volunteers after exclusion of three samples with a positive serology result for hydatidosis as determined by a different assay (21). All the serum samples had been collected in previous studies which had been approved by the Ethics Com-

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mittee of the Universidad Peruana Cayetano Heredia (FWA 00000525), Lima, Peru, and we obtained explicit permission from participants for further use of their samples in diagnostic studies in the corresponding signed consent forms.

Lung CHD cases were grouped according to the locations of cysts (lungs only/other organs also involved), number of cysts (one/multiple; patients classified as having multiple cysts could have two or more lung cysts or at least one lung cyst plus a cyst(s) elsewhere), presence or absence of cyst complications (no cyst complication/ at least one cyst complication, regardless of whether it was in the lung or elsewhere), and total volume of cysts. Volume was calculated using the approximate mean diameter, divided by 2, and cases were classified into groups of those individuals with no abdominal ultrasound evaluation, those 46, 44 had at least one lung cyst complication and 2 had no lung cyst complications but had at least one liver cyst complication. Of the 41 patients who also had liver involvement, classification of liver cysts according to the WHO guidelines showed cystic echinococcosis type 1 (CE1) in three cases, CE2 in four cases, CE3 in five cases, CE4 in one case, and CE5 in one case.

In the p176 ELISA, 48 patients and 4 controls were seropositive (sensitivity for lung CHD, 78.69%; specificity, 96.88%); exclusion of patients with liver involvement resulted in a decrease in sensitivity to 74.19%, whereas specificity remained without variation. Bivariate analysis (Table 1 and Fig. 1) showed that individuals who had at least one cyst with complications were more frequently seropositive (41/46 [89%]) than those without evidence of complications (7/15 [47%]) (odds ratio [OR], 9.4; 95% confidence interval [CI], 2.4 to 37.1; P = 0.001, chi-square test). Since individuals with no abdominal ultrasound evaluation could have had hidden liver cyst complications, we repeated the analysis, excluding those individuals with no abdominal ultrasound evaluation,

### RESULTS

There were effectively no differences in age or sex between the cases (35/61 male [57%]; mean age, 27.5 ± 15.5 years) and controls (36/68 male [53%]; mean age, 31.5 ± 8.1 years). Of the 61 cases, a total of 49 (80.32%) were evaluated by abdominal ultrasound, and 14 of them demonstrated additional cysts in the liver. One case had spleen involvement, and another had cutaneous involvement.

Most (38/61 [62.30%]) patients had a single lung cyst, and 23 patients had multiple lung lesions (mean, 1.61 ± 1.28 cysts; range, 1 to 10 cysts). Among 38 patients with a single lung cyst, 13 patients also presented liver involvement and 7 were not evaluated by abdominal ultrasound. Forty-six (75.41%) patients had at least one cyst complication (infected, n = 21; ruptured, n = 25); of those 46, 44 had at least one lung cyst complication and 2 had no lung cyst complications but had at least one liver cyst complication. Of the 14 patients who also had liver involvement, classification of liver cysts according to the WHO guidelines showed cystic echinococcosis type 1 (CE1) in three cases, CE2 in four cases, CE3 in five cases, CE4 in one case, and CE5 in one case.

| Variable | Group including individuals without abdominal ultrasound evaluation | Group excluding individuals without abdominal ultrasound evaluation |
|----------|---------------------------------------------------------------|---------------------------------------------------------------|
|          | OR    | 95% CI | P value | OR    | 95% CI | P value |
| Organ involved | Lung only | 1 | Reference | Reference | 1 | Reference | Reference |
|                | Lung and others | 6.6 | 0.79–55.05 | 0.082 | 5.9 | 0.67–51.86 | 0.109 |
| Presence of complications | No | 1 | Reference | Reference | 1 | Reference | Reference |
|                       | Yes | 9.4 | 2.36–37.07 | 0.001 | 9.84 | 2.18–44.40 | 0.003 |
| No. of cysts | One | 1 | Reference | Reference | 1 | Reference | Reference |
|                      | Two or more | 4.5 | 1.20–16.88 | 0.026 | 4 | 0.96–16.69 | 0.048 |
| Total cyst vol | <1,000 ml | 1 | Reference | Reference | 1 | Reference | Reference |
|                  | ≥1,000 ml | 3.5 | 0.42–166.14 | 0.221 | 2.6 | 0.28–127.05 | 0.377 |

*OR, odds ratio; CI, confidence interval.*

**ELISA.** We performed an indirect antibody-detecting ELISA according to the method described by González et al. (12) with minor modifications as follows: ELISA plates (Nunc Maxisorp) were sensitized with p176 antigen (100 µl/well [20 µg/ml]) in carbonate bicarbonate buffer at 4°C overnight, blocked with phosphate-buffered saline (PBS) (200 µl/well; pH 7.2) and milk (1%) for 1 h at 37°C, and then washed four times with PBS–0.05% Tween 20 (PBS-T). Serum samples diluted 1:200 in PBS-T–1% milk (100 µl/well) were incubated at 37°C for 1 h. Following a second washing step, conjugated anti-human IgG (γ-specific chain) (100 µl/well) linked to peroxidase and diluted 1:1,000 in PBS-T–5% milk was added, incubated at room temperature for 75 min, and washed again. TMB (3,3',5,5' tetramethylbenzidine) was used as the substrate for incubation in a dark environment at room temperature for 15 min. A reading was then performed at 650 nm in an ELISA reader (Vmax; Molecular Devices Inc., Sunnyvale, CA).

After verifying that the coefficient of variation between plates was lower than 5%, a cutoff value of 0.1215 was calculated using a receiver operating characteristic (ROC) curve on the basis of the optical density (OD) of the samples. This cutoff was applied to evaluate the sensitivity and specificity of the test as well as the associations between positive serology results and disease characteristics.

**Statistical analysis.** Fisher’s exact test and the chi-square test were used to evaluate differences between the proportions of seropositive cases in each group. Bivariate and multivariate logistic analyses were performed to evaluate associations between CHD characteristics and serological results. In multiple logistic regression analysis, the best model for multivariate analysis was chosen based on the goodness-of-fit test. We considered statistically significant differences with a confidence interval of 95% or higher (P ≤ 0.05). Analyses of associations between specific disease characteristics and serological responses were performed before the exclusion of subjects with lung CHD but without abdominal ultrasound and were repeated with those cases included to exclude any effect of this subgroup with undefined liver compromise.
with similar results (OR, 9.8; 95% CI, 2.2 to 44.4; P = 0.003, chi-square test). Also, individuals with more than one cyst were more frequently seropositive (32/36 [89%]) than those with a single cystic lesion (16/25 [64%]) (OR, 4.5; 95% CI, 1.2 to 16.9; P = 0.026, chi-square test). Significance persisted after exclusion of individuals with no abdominal ultrasound evaluation (OR, 4; 95% CI, 0.96 to 16.7; P = 0.048, chi-square test).

There was a trend toward a greater frequency of negative serological results in individuals with only pulmonary hydatid disease (31/43 [72%]) compared to those with cysts in lung plus another organ (17/18 [94%]) (OR, 6.5; 95% CI, 0.81 to 297.5; P = 0.085, Fisher’s exact test). This trend persisted after the exclusion of individuals without abdominal ultrasound evaluations (OR, 5.9; 95% CI, 0.7 to 277.5; P = 0.127, Fisher’s exact test). Seropositivity was also assessed in relation to total cyst volume, and there was a trend for individuals with higher cyst volume to be more frequently seropositive (14/15 [93%]) than those with a lower volume cyst (34/46 [74%]) (OR, 4.9; 95% CI, 0.6 to 226; P = 0.155, Fisher’s exact test). Analysis after exclusion of individuals without abdominal ultrasound evaluation confirmed this trend (OR, 3.9; 95% CI, 0.43 to 182.9; P = 0.258, Fisher’s exact test). On multivariate analysis (Table 2), only the effect of having at least one cyst with complications was significantly associated with seropositivity with respect to p176 (OR, 9.58; 95% CI, 2.15 to 42.6; P = 0.003).

**DISCUSSION**

Among several parasitic infections that may affect the human lungs, CHD is likely the most common cause of symptomatic disease (17). Community X-ray surveys in Peru have demonstrated a 1.1% to 2% prevalence of pulmonary cases in the general population (11, 15), with the majority of them being asymptomatic. The proportions of symptomatic cases vary greatly, from 27% to 59% (7, 17). Morbidity of lung CHD is higher than that of liver cases, probably due to rapid growth of cysts in the loose lung tissue and the development of a thinner cystic membrane that makes these cysts more likely to rupture or to host an additional bacterial or fungal infection (2, 20). Diagnosis of lung CHD is based on imaging examinations, including chest X-rays, computed tomography (CT), and magnetic resonance imaging (MRI) (17). Because of low sensitivity and incomplete specificity, the use of serology is restricted to case confirmation, especially among symptomatic patients with an atypical lung lesion. Serology may also be of use for patient follow-up as an indicator of relapse or recurrence (10, 16, 24).

Drawbacks of serology in hydatid disease in general include poor reproducibility, incomplete specificity, and low sensitivity, which is particularly low in cases of pulmonary CHD. Most assays have sensitivities for the diagnosis of lung CHD of around 70%, with a high range of 44% to 100% (3, 10, 18, 21, 23, 24). The high variability is mostly due to the use of cyst fluid as the antigen. The performance of cyst fluid analysis differs from batch to batch due to heterogeneity of cyst fertility and antigenicity between host species and individual infected animals or even between cysts from the same host. ELISA performance and the reproducibility of several tests for CHD diagnosis were previously examined by González et al. in surgically confirmed cases. In that case series, p176 ELISA had the best performance, with a sensitivity of 80% and a specificity of 93% (12). Later, Lorenzo et al. compared the performance results determined in a double-blind multicenter study using six major *E. granulosus* antigens in ELISA, including p176 ELISA, which demonstrated 74% sensitivity and 79% specificity. Surprisingly, the diagnostic efficiency of p176 ELISA showed the largest interlaboratory variation (±6.3%). Both of the case series described above mostly examined cases of patients with liver CHD, which are assumed to be more frequently seropositive than lung CHD cases (13).

Given the reported performance of p176, and since the inter-laboratory variation observed is attributable to the results determined in only three of the laboratories where the peptide was used, we choose to apply the synthetic peptide-based ELISA to this series of samples, looking for a sensitive and reproducible test which could be of help in the diagnosis of lung CHD. The test performed quite well, with a sensitivity of 79% and specificity of 97%. Previous studies using p176 ELISA did not report its specific performance according to disease and cyst characteristics (e.g., the organ involved, the presence or absence of complications, and number and volume of the cyst[s]). In other assays using a variety of antigens and techniques, serology has been associated with the location, number, and complications of the cysts (10, 18, 21, 23, 24). The most important factor associated with seropositivity was the presence of complications. This association was evident upon bivariate analysis (89% versus 53% seropositivity) and persisted after adjustment by number of lesions and organ involvement upon multivariate logistic regression analysis. The effect of cyst complications on increasing antibody seropositivity had previ-

![Image: FIG 1 Reactivity of the serum sample panel to peptide p176 as assessed by ELISA. Control group, sera from healthy donors; SNC, single cyst with no complications; SC, single cyst with complications; MNC, multiple cyst with no complications; MC, multiple cyst with complications. Cutoff, 0.1215. The data represent results for only those patients with an ultrasound evaluation.](image-url)
ously been described in reports of other studies using either ELISA or Western blotting (9, 18, 19, 21). The hydatid cysts isolate their contents from the immune system by developing a very thick collagen layer, leading to minimal or nil antigen release and subsequent minimal or nil antibody responses. Rupture of the membrane or aggregated infection exposes parasite antigens and unmask the host’s humoral response (5, 22, 26).

Exclusion of patients who also had liver involvement resulted in a marginal decrease in sensitivity (from 79% to 74%). A decrease in test sensitivity among those with only lung involvement compared with subjects with lung and liver involvement had also been shown in studies using cystic fluid with ELISA (69% to 60%) (19) and Western blotting (94% to 88%) (18). In the bivariate analysis, seropositivity for p176 was associated with having more than one cyst. The association between numbers of cysts and seroprevalence was previously described by Dueger et al., who used Western blotting in a study performed with naturally infected sheep (9), and by Cohen et al., who used ELISA in a study performed with humans (6). In this case series, the significance of the bivariate associations of serology with numbers of cysts and organ involvement did not persist upon multivariate analysis. This could have been due to sample size limitations or perhaps can be explained for the most part by the presence of complications. Previous studies had not reported adjusted analyses. The specificity of p176 ELISA was found to be almost 97%, and only 4 controls were seropositive, although no further imaging evaluations were performed for these subjects, we assume that their results represented false positives, since they had no related symptoms, were seronegative in analyses performed using an enzyme-linked immunoelectrotransfer blot (ELISA), and lived in an area where the disease is not endemic. A previous study using p176 ELISA detection found 93% specificity (12) when evaluating false-positive results, including cross-reactions with alveolar hydatidosis (3/27), schistosomiasis (1/6), toxocariasis (2/10), Chagas’ disease (1/4), and syphilis (1/2). We assessed specificity only in samples from healthy donors, so no further information on cross-reactions was obtained.

The sensitivity of the p176 ELISA for the diagnosis of lung CHD cases was almost 80%. Despite the fact that the restricted numbers of samples with isolated pulmonary CHD prevented a more precise assessment of sensitivity, the overall sensitivity was comparable to those of all other tests used for diagnosing lung CHD, although it was slightly lower than that previously obtained using EITB in serum samples from the same panel of surgical lung CHD cases (18). The simpler, cheaper, semiquantitative ELISA format and the potential for better reproducibility make this ELISA a good alternative for the diagnosis and posttreatment follow-up of lung CHD.

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