

## IMMUNODIAGNOSTIC CONFIRMATION OF HYDATID DISEASE IN PATIENTS WITH A PRESUMPTIVE DIAGNOSIS OF INFECTION

V. M. VARELA-DÍAZ and E. A. COLTORTI

### S U M M A R Y

Information obtained from the routine application of hydatid immunodiagnostic techniques in different clinical situations over a seven-year period is presented. The immunoelectrophoresis test was used until it was replaced by the simpler, more sensitive and equally specific arc 5 double diffusion (DD5) test. Examination of sera from 1,888 patients with signs and/or symptoms compatible with hydatid disease revealed that the presurgical confirmation of *Echinococcus granulosus* infection is only obtained by detection of anti-antigen 5 antibodies. The latter were not found in 1,539 presumptive hydatidosis patients whose definitive diagnoses corresponded to other disease conditions. However, false positive latex agglutination test results were obtained in two cases. In all patients whose preoperative serum showed three or more uncharacteristic bands in the absence of anti-antigen 5 antibodies, hydatid cysts were found surgically. DD5 testing of a fluid sample collected by puncture established its hydatid etiology. Post-operative monitoring of hydatidosis patients demonstrated that persistence of DD5-positivity two years after surgery established the presence of other cysts. Further evidence was obtained in patients with hydatid cysts in intrathoracic, abdominal or other locations associating cyst membrane integrity, antigen release and immunodiagnostic test positivity.

### I N T R O D U C T I O N

Reports on the evaluation of hydatid immunodiagnostic tests have dealt, for the most part, with the study of the sensitivity and/or specificity of one or more techniques in batches of sera collected from confirmed cases. This report deals with the routine application of techniques of recognized sensitivity and specificity for the immunological confirmation of hydatid disease<sup>8,20,27</sup> in patients whose images and/or clinical findings were compatible with this parasitic infection. The information presented is based on the experience accumulated between 1973 and 1979 in the service for the immunodiagnosis of human hydatidosis provided by the Laboratory of Immunology, Pan Ame-

rican Zoonoses Center (PAHO/WHO) to clinics and hospitals in metropolitan Buenos Aires, Argentina.

### M A T E R I A L A N D M E T H O D S

#### Source of sera

From 1973 to 1979, a total of 1,250 physicians from 126 private and official hospitals and clinics in the Federal District and greater Buenos Aires area used the hydatid immunodiagnostic service available at the Laboratory of Immunology, Pan American Zoonoses Center (PAHO/WHO). They sent 1,888 patients with

a presumptive diagnosis of hydatidosis whose histories included residence in endemic areas, previous surgery for hydatidosis, detection of space-filling lesions and/or clinical findings compatible with infection.

Test results were communicated to the attending physician and to the Director of the corresponding medical institution in a stencilled form with a brief summary of the advantages, limitations and diagnostic significance of the hydatid immunodiagnostic tests employed<sup>20,27</sup>. A request was also included to report to the laboratory the eventual surgical findings and/or definitive diagnosis on the patient.

Data for the classification of patients under the categories of hydatidosis or other disease conditions were obtained in two ways. The definitive diagnosis was reported directly to the laboratory by the attending physician or by the statistics section of the hospital. When no data were available, staff from the Biostatistics Section, Pan American Zoonoses Center, PAHO/WHO, visited the medical institutions where the patients had received treatment, to collect the required information from their clinical history forms.

The definitive diagnoses during the period were hydatidosis for 349 patients and other disease conditions, 1,539 cases. Only cases for which full data were available were included in this report.

### Immunodiagnostic tests

The immunodiagnostic techniques for hydatidosis were carried out as described previously<sup>8,20,27</sup>. The method for detecting antibodies to antigen 5 from hydatid fluid (HF)<sup>2</sup> varied during the period. Thus, samples received during 1973 were examined by the arc 5 immunoelectrophoresis (IEP) test using sera concentrated three times by lyophilization<sup>2,15,27</sup>. From 1974 to 1977, samples were tested by a modification of IEP which obviated the requirement to lyophilize sera<sup>5,27</sup>. Following the development of the arc 5 double diffusion (DD5) test<sup>8,20</sup>, this technique was used in lieu of IEP for testing all samples received from 1977 to date.

Each sample received during the full 1973-1979 period was also examined upon arrival by the standardized latex agglutination (LA) test for hydatidosis<sup>27</sup>. Briefly, sheep HF pools were used only when their immunoelectrophoretic

antigenic characterization with homologous ovine antisera showed that they fulfilled the specified criteria<sup>27</sup> for adequate immunodiagnostic test antigens. Optimal sensitizing conditions were then determined for the selected HF antigen in preliminary block titration trials with latex particles (0.22  $\mu$  in diameter) to obtain maximal LA titers in a battery of positive sera from hydatidosis patients, and negative results in sera from persons with other parasitic conditions and apparently healthy donors. Fresh batches of sensitized particles were prepared approximately every two or three months to avoid problems associated with the shelf-life of the diagnostic reagent and their consequent adverse effect on test sensitivity and specificity. Each time LA was performed, known positive and negative sera were included as controls to ascertain the diagnostic efficacy of the reagent<sup>27</sup>.

The indirect hemagglutination (IHA) test<sup>27</sup> was used for the routine testing of sera only during 1973 and was subsequently abandoned for operative reasons.

## RESULTS

### Intrathoracic hydatidosis

Sera from 583 patients with clinical, radiologic and/or scintillographic findings compatible with a diagnosis of pulmonary, pleural or mediastinal hydatid disease were examined at the laboratory for immunodiagnostic confirmation of infection. Hydatidosis was confirmed at surgery in 89 patients. The relationship between immunodiagnostic results, state of the cysts found at surgery and type of symptoms which led to the detection of radiologic images compatible with hydatidosis in these patients is shown in Table I.

The hydatid etiology of the intrathoracic radiologic findings was confirmed preoperatively in 46 of the 89 patients (51.6 per cent) by detection of anti-antigen 5 antibodies. In three other cases (3.4 per cent), detection of four uncharacteristic precipitation bands<sup>20,27,29</sup> in the absence of antibodies to antigen 5, strongly supported the presumptive presurgical diagnosis.

In the 40 hydatidosis patients (44.9 per cent) whose sera did not reveal anti-antigen 5 antibodies, confirmation of the diagnosis was only obtained at surgery. Immunodiagnostic tests

T A B L E I

Relationship between preoperative immunodiagnostic test results and the type of symptoms which led to the radiologic detection of intrathoracic images compatible with hydatid cysts in 89 surgically-confirmed hydatidosis patients

Patients (number)	Negative to antigen 5 *	Positive to antigen 5**	Four bands*** (uncharacterized)
Asymptomatic (23)	12 (1)	10 (2)	1
With symptoms:			
Unrelated to hydatidosis (4)	2	2	0
Associated with cyst growth (41)	22 (2)	17 (2)	2
Total with hyaline cysts (68)	36 (3)	29 (4)	3
Symptoms associated with:			
Complicated cysts (12)	0	12 (3)	0
Infected cysts (9)	4	5 (1)	0
Total with altered cysts (21)	4	17 (4)	0

\* ( ) = No. of patients whose serum was positive by the latex agglutination (LA) test  
 \*\* ( ) = No. of patients whose serum was LA-negative  
 \*\*\* = Sera from these patients were LA-negative

were negative in the 494 patients with other disease conditions (tumors, tuberculosis, diverse pneumopathies) in whom the possibility of hydatidosis had been contemplated.

The immunodiagnostic sensitivity of LA was lower than that obtained by precipitation tests based on the detection of anti-antigen 5 antibodies and/or three or more uncharacteristic bands (Table I). In patients whose sera were negative in IEP or DD5, positivity to LA did not provide the confirmation of hydatidosis but only suggested the probability of the presumptive diagnosis.

### Calcified hydatid cysts

The diagnostic possibility of hydatidosis was contemplated in 49 patients whose abdominal or thoracic radiographs showed calcified images in the abdomen. Ten were asymptomatic or casual findings in X rays for an unrelated condition, the rest showed signs and/or symptoms compatible with hydatid disease, and all had a history of residence in endemic areas. The results of immunodiagnostic tests, the definitive diagnoses, and the location of cysts in each patient are shown in Table II.

T A B L E II

Immunodiagnosis in 49 patients who showed calcified radiologic images compatible with hydatidosis

Affected organ	Definitive diagnosis	No. of patients	Negative to antigen 5		Positive to antigen 5	
			* LA +	LA -	LA +	LA -
Liver	Hydatidosis	10	1	6	2	1
	Other diseases	11	0	11	0	0
	Not operated	18	0	17	0	1
Kidney	Hydatidosis	1	0	0	1	0
	Other diseases	4	0	4	0	0
Spleen	Hydatidosis	1	1	0	0	0
	Other diseases	1	1	0	0	0
Other sites **	Hydatidosis	0	0	0	0	0
	Other diseases	3	0	3	0	0

\* LA = Latex agglutination test

\*\* rib cartilage, pelvis and vertebrae

In one seronegative patient, an intact liver hydatid cyst, enclosed by areas of intermittent peripheral calcification in the adventitia was found at surgery. Only one of the surgically-

confirmed hydatidosis cases, in addition to the calcified cyst, had hyaline cysts localized in preoperative hepatic scans.

A recent history of pruriginous eruption in face and neck, or a generalized rash was recorded only in the three arc 5 positive cases. Surgical data available for one of them, indicated that the hydatid cyst membrane and its surrounding calcification were slightly torn. Monitoring of the non-operated arc 5 positive case showed that thirteen months later the patient had become seronegative, showed mild pain, occasional vomiting and no rash.

### Abdominal hydatidosis

Immunodiagnostic test findings in 810 patients in whom the possibility of hydatidosis was contemplated on the basis of residence in endemic areas and the presence of symptoms and/or signs (clinical, radiologic, scintillographic, echographic or by computerized axial tomography) similar to those observed in hydatidosis cases are shown in Table III.

T A B L E III

Immunodiagnosis in 810 patients in whom a presumptive diagnosis of abdominal hydatid disease was contemplated

Affected organ	Definitive diagnosis	No. of patients	Negative to antigen 5		Positive to antigen 5		> 2 bands (uncharacterized)	
			* LA +	LA -	LA +	LA -	LA +	LA -
Liver	Hydatidosis	41	1	16	18	1	4	1
	Other abdominal conditions	713	0	713	0	0	0	0
Kidney	Hydatidosis	2	0	1	1	0	0	0
	Other diseases	25	0	25	0	0	0	0
Spleen	Hydatidosis	1	0	0	1	0	0	0
	Other diseases	9	0	9	0	0	0	0
Pancreas	Hydatidosis	1	0	0	0	1	0	0
	Other diseases	8	0	8	0	0	0	0
Multiple**	Hydatidosis	7	0	0	6	0	1	0
Mesenterium	Hydatidosis	1	0	1	0	0	0	0
	Other diseases	2	0	2	0	0	0	0

\* LA = Latex agglutination test

\*\* Liver, peritoneum, spleen, diaphragm and/or eye

In the group of liver hydatidosis patients whose sera revealed anti-antigen 5 antibodies or a diagnostically-significant number or uncharacteristic precipitation bands (3 or more), all cysts found at surgery were in the hyaline or unaltered state except for two, which were infected. In the seronegative hydatidosis patients, all cysts were also hyaline but one, which had ruptured into the biliary tract. Hydatid cysts found in other abdominal locations were all hyaline.

### Hydatid cysts in other sites

Findings in patients who were presumed to harbor hydatid cysts in organ locations other than those described above are shown in Table IV. Signs and symptoms were associated with the growth or complication of cysts in the given affected sites. In the case of patients with both pulmonary and hepatic hydatidosis, the presence of cysts was first established in one site. Subsequent studies to determine the concomitant presence of other cysts led to the

finding of cysts in the other organ.

### Postoperative serological reactivity

Serum samples were collected at different time intervals during the first two years following surgery from 60 hydatidosis patients in whom hydatid cysts were found at surgery for an unrelated condition, or whose preoperative immunodiagnostic testing had been positive, negative or had not been done. Sera were sent to the laboratory by the attending physician for postoperative serologic monitoring.

Post surgical conversion to IEP and DD5-positivity in preoperatively negative patients was found useful to establish or to confirm that HF antigens had been released during the operation.

Observations in this group of patients were also of particular interest to the postoperative application of DD5. A reduction in the number of precipitation bands revealed by DD5 was observed, while detection of anti-antigen 5 anti-

T A B L E I V  
Immunodiagnosis in 32 patients with a presumptive diagnosis of hydatid cysts in diverse locations

Affected site	Definitive diagnosis	No. of patients	Negative to antigen 5		Positive to antigen 5		Three bands (uncharacterized)	
			* LA —	LA +	LA —	LA —		
Brain	Hydatidosis	3	2	1	0	0	0	
	Other diseases	8	8	0	0	0	0	
Bone	Hydatidosis	5	1	1	1	2		
	Other diseases	5	5	0	0	0		
Ovaries	Hydatidosis	1	1	0	0	0		
	Other diseases	2	2	0	0	0		
Both liver and lung	Hydatidosis	8	3	4	0	1		

\* LA = Latex agglutination test

bodies persisted in some patients for 18-20 months after surgery, in the absence of antigenic stimulation by other hydatid cysts. Follow-up medical studies of patients who remained DD5-positive two years after surgery showed that they were indeed harboring other cysts. These cases are discussed in the following section.

#### Patients with previous hydatid surgery

Immunodiagnostic tests were requested for 75 patients whose history included previous surgery for hydatidosis. Some were asymptomatic, while others showed signs and symptoms compatible with the infection. Although most patients in this series had requested medical

attention because they were clinically ill, some were undergoing medical follow up studies and/or their postoperative serologic monitoring showed that they continued to be DD5-positive for two or more years after last surgery.

The results of immunodiagnostic tests and the definitive diagnoses of these patients are shown in Table V. Hydatid cysts found at surgery in patients positive to antigen 5 were in the liver (26 cases), lung (8), peritoneum (8), brain (2), kidney (1) and one was located subcutaneously in the back. In the clinical histories of 7 cases, the site of abdominal hydatid cysts was not further specified. In patients whose sera only showed three or more uncharacteristic bands<sup>20,27,29</sup> or a doubtful arc 5, cysts were in the liver and/or the peritoneum.

T A B L E V  
Immunodiagnosis in 75 patients with a presumptive diagnosis of hydatid disease and a history of previous surgery for the infection

Definitive diagnosis	No. of patients	Negative to antigen 5		Positive to antigen 5		> 2 bands (uncharacterized)		Antigen 5?
		* LA +	LA —	LA +	LA —	LA +	LA —	LA +
Hydatidosis	62	0	6	49	3	1	2	1
Other diseases	13	1	12	0	0	0	0	0

\* LA = Latex agglutination test; antigen 5? = Reading of reaction was doubtful

In all these hydatidosis patients, the clinical condition was associated with the growth of hyaline cysts, except for one arc 5 negative patient, whose cyst had ruptured into the bile duct. The time elapsed since last surgery for hydatidosis was 2-3 years in fourteen cases; 3-10 years in 26 cases and 11-46 years in the 12 other confirmed hydatidosis cases.

In the 13 immunologically-negative patients whose definitive diagnoses were unrelated to

hydatidosis (other pulmonary, cardiac and bone conditions, lithiasis, hepatitis, tumors and even-trations), hydatid surgery had taken place between 4 and 37 years previously. At the time immunodiagnosis was requested, they also showed symptoms and/or signs (clinical, radiologic and/or scintillographic) which were compatible with the growth of a hydatid cyst. The only LA positive patient in this group was confirmed surgically as a case of renal lithiasis.

### Non-parasitologically confirmed diagnoses

Immunodiagnostic testing was requested in four cases to establish the etiology of hydatid disease. In one, the presence of parasite elements (membranes, hooks, etc.) could not be determined from the examination of selected tissue sections from surgically-removed lesions. In two patients, it was suspected that spontaneous rupture of a hydatid cyst had occurred and that the parasite constituents had been expelled through the bronchioles in one, or through the biliary tract, in the other. DD5-positivity confirmed the presumptive diagnosis of hydatid disease in these three cases.

A clear liquid obtained by puncture from a patient with a presumptive diagnosis of neoplasia had suggested the possibility of hydatidosis and was sent to the laboratory for testing. The fluid was placed in one of the wells in which serum is placed in the DD5 test. Several precipitation bands were observed, one of which showed identity with the arc 5 developed by the antigen and the anti-antigen 5 serum<sup>8,20</sup>. This observation provided the preoperative immunologic confirmation of the sample as hydatid fluid although it had been negative parasitologically.

### DISCUSSION

Difficulties in differentiating hydatidosis from other disease conditions on the basis of clinical findings and/or images revealed by methods such as radiology, scintillography, echography and/or computerized axial tomography are well-recognized<sup>1,10,11,21,22,25</sup>. However, the detection of serum antibodies against antigen 5 from hydatid fluid<sup>2</sup> provides the immunological confirmation of the diagnosis of hydatid disease<sup>20,27</sup>. In the present series, hydatid cysts were found at surgery in patients positive in DD5 or IEP whereas these tests were negative in all patients who also had revealed images and/or clinical findings compatible with hydatidosis, but whose definitive diagnoses were non-hydatid. These observations are in agreement with previous reports in that the detection of antibodies to arc 5 antigens is the positivity criterion of choice for the immunodiagnosis of human hydatidosis<sup>9,20,27,28,29</sup>.

Hydatid cysts were also found at surgery in all patients whose serum showed three or

more uncharacteristic precipitation bands in DD5 or IEP, in the absence of anti-antigen 5 antibodies. Accordingly, the use of this positivity criterion proved to be of diagnostic significance in differentiating sera from hydatid and non-hydatid patients. These observations are in agreement with previous studies<sup>9,20,27,28,29</sup> which showed that more than two of these uncharacteristic bands were not found in non-hydatid cases.

The capacity of an immunodiagnostic test to reveal the presence of a hydatid cyst in a patient has been associated not only with the characteristics of the antigen lot used, the technical conditions for each test (or its modifications) and the established criterion of test positivity, but also with the detectable antibody in the serum examined<sup>26,28,29,30</sup>. The latter is contingent on the antigenic stimulus provided by the release of hydatid fluid (HF) immunogens contained within the cyst cavity. An association has been consistently reported between the physical state of the cyst membrane, antigen release, and positivity in immunodiagnostic tests, which accounts for the lower test sensitivity observed in patients harboring intact or hyaline cysts, than in those whose cyst germinal membrane is altered<sup>3,7,9,18,19,32,33</sup>. Available evidence suggests that, in intact cysts the germinal membrane tegument serves as a barrier to the passage of HF macromolecules responsible for the host immune response<sup>6,7,9,17</sup>. Seropositivity in hyaline cysts has been related to the formation of microfissures in the germinal membrane during cyst growth and development, which permit the leakage of sufficient HF antigens to elicit a detectable antibody response. These considerations seem pertinent to interpret the various diagnostic situations with patients in this series.

It is recognized that a diagnostic problem arises in patients who show calcified images because of difficulties in establishing their etiology as hydatidosis or other disease conditions<sup>10,21,22</sup>. Data on the antibody response in patients with calcified cysts is limited, but the minimal or undetectable serologic reactivity observed in these cases has been associated with parasites which have been dead for some time and their antigenic stimulation is therefore weak or nihil<sup>7,9,20,27</sup>. This explanation is consistent with results in those patients in this series in whom, at surgery, no hydatid fluid

was found to remain within the calcification, suggesting that the antibody response had waned at the time of diagnosis.

However, an intact cyst enclosed by areas of intermittent adventitial calcification was found at surgery in a seronegative patient. In another, hyaline cysts were found in addition to one fully-calcified. These observations seem consistent with the hypothesis that the intact germinal membrane of cysts interferes with the liberation of HF antigens necessary to elicit a detectable antibody response<sup>6,7,9,17</sup>. Thus, the state of the cyst membrane rather than the adventitial calcification, would account for the absence of serologic reactivity in these two patients.

Several observations on three hydatidosis patients with calcified cysts in whom the preoperative confirmation of infection was obtained by DD5, also suggested that fissuring of the germinal membrane tegument accounts for the antigenic stimulation which leads to seropositivity<sup>6,7,9,17</sup>. Aspects of their clinical condition (sudden onset of vomiting and abdominal pain followed by an eruption), not shared by other cases, were compatible with an abrupt liberation of HF antigens. This explanation received additional support from the surgical observation that the cyst and its surrounding calcification were slightly torn.

The relationship between seropositivity and availability of HF antigens was also suggested by observations on patients whose cysts had been ruptured and expelled through the bronchii or biliary tract. Immunodiagnostic test positivity in this group may depend on whether the spillage of HF into tissues surrounding the site of the expelled cyst is sufficient for antigenic stimulation and induction of a detectable antibody response to occur.

Of the patients with pulmonary hydatidosis, 52 per cent of those harbouring hyaline cysts and 19 per cent of those with complicated cyst membranes did not reveal anti-antigen 5 antibodies. These observations are compatible with the lower immunodiagnostic sensitivity in patients with hyaline cysts as compared to those with altered cysts.

In general, however, the immunodiagnostic sensitivity of arc 5 antigens was lower in this series of patients than has been reported else-

where. The review of other studies in which the physical condition of cysts was considered<sup>3,18,19,32,23</sup> shows that the proportion of patients whose pulmonary cysts were hyaline was much lower than in this series and immunodiagnostic sensitivity was consequently higher.

In the present series, a larger proportion of patients with cysts in extrapulmonary sites had hyaline or calcified cysts and, in some cases, expulsion of ruptured cysts through the biliary tract or the bronchii had reduced the possibilities of antigenic stimulation. These observations suggest that the differences in antibody reactivity between liver and lung hydatidosis patients, traditionally associated with the site of the cysts, are due to variations in the relative proportions of hyaline and altered cysts in each organ.

The group of patients with a history of surgery for hydatid disease was treated separately for reasons other than the potential diagnostic significance of this antecedent in a patient with signs and/or symptoms compatible with hydatidosis. Experience showed that patients whose sera were positive in DD5 two or more years after hydatid surgery harbored other cysts. These findings thus extend to two years the time period we had noted previously<sup>9,20</sup> for postoperative DD5-positivity to be indicative of the presence of other cysts, and further confirm the value of the routine application of DD5 for postoperative monitoring.

In patients whose history included hydatid surgery, the proportion of arc 5-positive hyaline cysts was larger than in the primary hydatidosis group. This suggests prior sensitization of the host immune system by the cysts which were removed earlier. It is to be expected, therefore, that if antigen release from new or residual cysts produces a secondary type of response, a lower level of antigenic stimulation would be required to induce a detectable antibody response.

The latex agglutination (LA) test described by SZYFRES & KAGAN<sup>23</sup> was modified by WILLIAMS & PREZIOSO<sup>31</sup>, who found that a significant reduction in the rate of false positive results could be accomplished by using test sera at a 1:5 dilution. To obviate difficulties, including those associated with variations in the antigenicity of different HF pools, the LA

technique used in the present study was subsequently standardized in our laboratory<sup>27</sup>.

The present findings clearly show that results obtained with this LA test<sup>27</sup> are not comparable to those reported in the literature for other technical variants of LA<sup>3,4,12,13,14,16,24,28</sup> for a number of reasons, which include the use of: HF pools of undetermined antigenic composition, different dilutions of test sera, arbitrarily selected antigen concentrations, and/or other suspensions of different sized particles. In addition, control sera from non-hydatidosis cases were not used to determine the optimal sensitizing conditions. In most reports, however, all variants of LA have been considered adequate for immunodiagnosis<sup>4,13,16,24</sup>, in spite of having obtained non-specificity rates of 1.1, 4.8, 6.7 or 20 per cent.

In the present series, two of 1,539 sera from presumptive hydatidosis patients with other definitive diagnoses were positive in the standardized LA. This observation is consistent with the interpretation<sup>27</sup> that a positive result in the standardized LA does not confirm the presence of infection, because of the potential occurrence of non-specific reactions, but provides the clinician with an additional element that suggests the probability of hydatidosis. In contrast, if an LA test with a 4.8 per cent false-positivity rate<sup>16</sup> had been used instead of the standardized LA, the false positive diagnoses expected to occur would have been 72 instead of the two.

On the other hand, the occurrence of non-specific LA results, though minimal with the standardized technique, limits the value of the test for use in clinical situations, since confirmation of a presumptive diagnosis of hydatid disease is not possible. Accordingly, the test's main value is as a screening technique when large numbers of sera have to be examined<sup>8, 20,27,28</sup>. This permits selection of positives for subsequent DD5 testing; however, this procedure has the disadvantage that LA-negative sera, which may be confirmed immunologically by DD5, are not detected in the preliminary screen. This was observed for a number of patients in the present study.

In general, however, the above findings demonstrate that DD5 is the technique of choice for the immunodiagnosis of hydatid disease in clinical situations. It provided the presurgical confirmation of the infection in patients with

compatible signs and symptoms, with or without a prior history of hydatidosis. When used for post-operative monitoring of patients, the presence of other cysts was established by the persistence of DD5-positivity two years after surgery. DD5 proved simpler to perform than the less sensitive IEP<sup>8</sup> and was equally specific.

## RESUMEN

### Confirmación inmunodiagnóstica de la hidatidosis en pacientes con diagnóstico presuntivo de la infección

Se presenta la información obtenida de la aplicación de las técnicas inmunodiagnósticas para hidatidosis en diferentes situaciones clínicas durante un período de 7 años. Se empleó la prueba de inmunoelectroforesis hasta que se la sustituyó por la prueba de doble difusión arco 5 (DD5), igualmente específica pero de mayor sensibilidad y sencillez. El examen de sueros de 1888 pacientes con signos y/o síntomas compatibles con la hidatidosis reveló que la confirmación prequirúrgica de la infección por *Echinococcus granulosus* sólo se obtiene mediante la detección de anticuerpos anti-antígeno 5. Estos no se hallaron en 1539 pacientes sospechosos de hidatidosis cuyos diagnósticos definitivos correspondieron a otras patologías. Con la prueba de aglutinación del látex, sin embargo, se obtuvieron resultados positivos falsos en dos casos. En todos los pacientes cuyo suero preoperatorio mostró tres o más bandas no características en ausencia de anticuerpos anti antígeno 5, se hallaron quistes en el acto quirúrgico. El examen por DD5 de líquido recolectado por punción estableció su etiología hidatídica. El seguimiento postoperatorio de pacientes de hidatidosis demostró que la persistencia de positividad en la DD5 durante dos o más años después de la cirugía establece la presencia de otros quistes. En pacientes con quistes hidatídicos intratorácicos, abdominales o en otras localizaciones, se obtuvo evidencia adicional sobre la asociación entre la integridad de las membranas quísticas, la liberación de antígenos y la positividad del inmunodiagnóstico.

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#### REFERENCES

1. BRUGUERA, C. A. & CORONA, R. E. — Ultrasonografía. *Medicine Argentina* (2da. serie) 1: 87-103, 1981.
2. CAPRON, A.; VERNES, A. & BIGUET, J. — Le diagnostic immunoelectrophoretique de l'hydatidose. In: "Le kyste hydatique du foie" (Journées Lyonnaises d'Hydatidologie). Lyon, SIMEP (Ed.), pp. 27-40, 1967.
3. CAPRON, A.; YARZABAL, L.; VERNES, A. & FRUIT, J. — Le diagnostic immunologique de l'échinococcose humaine (Bilan personnel a propos de 400 observations). *Pathol. Biol.* 18: 357-365, 1970.
4. CHEMTAI, A. K.; BOWRY, T. R. & AHMAD, Z. — Evaluation of five immunodiagnostic techniques in echinococcosis patients. *Bull. WHO* 59: 767-772, 1981.
5. COLTORTI, E. A. & VARELA-DIAZ, V. M. — Modification of the immunoelectrophoresis test for the immunodiagnosis of hydatidosis. *J. Parasit.* 61: 155-157, 1975.
6. COLTORTI, E. A. & VARELA-DIAZ, V. M. — Penetration of host IgG molecules into hydatid cysts. *Z. Parasitenk.* 48: 47-51, 1975.
7. COLTORTI, E. A. & VARELA-DIAZ, V. M. — Survival of hydatid cysts after puncturing. *Ann. Parasit. hum. comp.* 51: 647-652, 1976.
8. COLTORTI, E. A. & VARELA-DIAZ, V. M. — Detection of antibodies against *Echinococcus granulosus* arc 5 antigens by double diffusion test. *Trans. Roy. Soc. trop. Med. Hyg.* 72: 226-229, 1978.
9. COLTORTI, E. A. & VARELA-DIAZ, V. M. — Inmunología e Immunodiagnóstico de la Hidatidosis Humana. *Medicine Argentina* (1ra. serie) 6: 135-137, 1978.
10. D'ALESSANDRO, A.; LEGA, J. & VERA, M. A. — Cystic calcifications of the liver in Colombia. Echinococcosis or calcified abscesses? *Amer. J. Trop. Med. Hyg.* 15: 908-913, 1966.
11. D'ALOTTO, V. — Tomografía Computada. *Medicine Argentina* (2da. serie) 1: 87-103, 1981.
12. FISCHMAN, A. — A rapid latex test for hydatid disease. *New Zealand Med. J.* 59: 485-487, 1960.
13. GUI SANTES, J. A. & PICARDO, N. G. — Una nueva variante técnica de la prueba de aglutinación del látex para el diagnóstico de la hidatidosis humana. *Rev. Med. Univ. Navarra* 23: 57-59, 1979.
14. GUI SANTES, J. A. & VARELA-DIAZ, V. M. — Las pruebas de aglutinación del látex y doble difusión en gel en el inmunodiagnóstico de la hidatidosis humana. *Bol. Chile. Parasit.* 30: 54-57, 1975.
15. GUI SANTES, J. A.; YARZABAL, L. A.; VARELA-DIAZ, V. M.; RICARDES, M. I. & COLTORTI, E. A. — Standardization of the immunoelectrophoresis test with whole and purified hydatid cyst fluid antigens for the diagnosis of human hydatidosis. *Rev. Inst. Med. trop. São Paulo* 17: 69-74, 1975.
16. HOGHOOGHI, N.; SABBAGHIAN, H.; GHADIRIAN, E.; KAGAN, I. G. & SCHILLER, E. L. — Evaluation of the slide-latex agglutination and intradermal (Casoni) tests for echinococcosis. *Amer. J. Trop. Med. Hyg.* 25: 660-661, 1976.
17. LASCANO, E. F.; COLTORTI, E. A. & VARELA-DIAZ, V. M. — Fine structure of the germinal membrane of *Echinococcus granulosus* cysts. *J. Parasit.* 61: 853-860, 1975.
18. LOPEZ-LEMES, M. H.; GUI SANTES, J. A.; TORRES, J. M. & JOSEF, M. — Estudio sobre la correlación entre la intradermorreacción y la immunoelectroforesis en la hidatidosis humana. *Rev. Asoc. Arg. Microbiol.* 7: 39-43, 1975.
19. LOPEZ-LEMES, M. H. & VARELA-DIAZ, V. M. — Application of the immunoelectrophoresis test for hydatidosis in patients with a presumptive diagnosis of the disease. *Trop. Geogr. Med.* 27: 301-304, 1975.
20. PAN AMERICAN ZOONOSES CENTER, PAHO/WHO — The arc 5 double diffusion test for the immunodiagnosis of human hydatidosis. Ramos Mejia (Buenos Aires) Pan American Zoonoses Center PAHO/WHO Techn. Note 22, 1979.
21. POOLE, J. B. & MARCIAL-ROJAS, R. A. — Echinococcosis. Pages 635-656 in: *Pathology of Protozoal and Helminthic Diseases*. Marcial-Rojas, R. A. & Moreno, E. (eds.) Baltimore, Williams and Wilkins, 1971.
22. RAMOS GONZALEZ, L.; MARCOS, J.; ILLANAS, M.; HERNANDEZ-MORA, M.; PEÑA, F.; PICOUTO, J. P.; CIENFUEGOS, J. A. & ALVAREZ, J. L. R. — Radiologic aspects of hepatic echinococcosis. Value of the intravenous viscerogram and computed tomography. *Radiology* 130: 21-27, 1979.
23. SZYFRES, B. & KAGAN, I. G. — A modified slide latex screening test for hydatid disease. *J. Parasit.* 49: 69-72, 1963.
24. TODOROV, T.; DAKOV, I.; KOSTURKOVA, M.; TENEV, S. & DIMITROV, A. — Immunoreactivity in pulmonary echinococcosis. I. A comparative study of immunodiagnostic tests. *Bull. WHO* 57: 735-740, 1979.
25. VARELA, A. — Centellografía hepática. *Medicine Argentina* (2da. serie) 1: 104-116, 1981.

26. VARELA-DÍAZ, V. M. & COLTORTI, E. A. — Limitaciones de la intradermorreacción de Casoni para el inmunodiagnóstico de la hidatidosis humana. *Bol. Ofic. Sanit. Panam.* 76: 400-405, 1974.
27. VARELA-DÍAZ, V. M. & COLTORTI, E. A. — Techniques for the immunodiagnosis of human hydatid disease. Ramos Mejía (Buenos Aires). Pan American Zoonoses Center PAHO/WHO. Sci. Techn. Monograph Ser. 7, 1976.
28. VARELA-DÍAZ, V. M.; COLTORTI, E. A.; PREZIOSO, U.; LOPEZ-LEMES, M. H.; GUIZANTES, J. A. & YARZABAL, L. A. — Evaluation of three immunodiagnostic tests for human hydatid disease. *Amer. J. Trop. Med. Hyg.* 24: 312-319, 1975.
29. VARELA-DÍAZ, V. M.; GUIZANTES, J. A.; RICARDES, M. I.; YARZABAL, R. A. & COLTORTI, E. A. — Evaluation of whole and purified hydatid fluid antigens in the diagnosis of human hydatidosis by the immunoelectrophoresis test. *Amer. J. Trop. Med. Hyg.* 24: 298-303, 1975.
30. VARELA-DÍAZ, V. M.; LOPEZ-LEMES, M. H.; PREZIOSO, U.; COLTORTI, E. A. & YARZABAL, L. A. — Evaluation of four variants of the indirect hemagglutination test for human hydatidosis. *Amer. J. Trop. Med. Hyg.* 24: 304-311, 1975.
31. WILLIAMS, J. F. & PREZIOSO, U. — Latex agglutination test for hydatid disease using Boerner slides. *J. Parasit.* 56: 1253-1255, 1970.
32. YARZABAL, L. A.; LEITON, J. & LOPEZ-LEMES, M. H. — The diagnosis of pulmonary human hydatidosis by the immunoelectrophoresis test. *Amer. J. Trop. Med. Hyg.* 23: 662-666, 1974.
33. YARZABAL, L. A.; RETAMAL, C.; SEPULVEDA, R.; GUACHALLA, J. & KIQUEL, C. — Aplicación de la inmunoelectroforesis al diagnóstico de la hidatidosis pulmonar en Chile. *Rev. Inst. Med. trop. São Paulo* 17: 263-268, 1975.

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