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IMMUNODIAGNOSIS OF PARASITIC DISEASES

WITH EMPHASIS ON HYDATIDOSIS

Immunodiagnosis of parasitic diseases with emphasis on hydatidosis*

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Introduction

The immunodiagnosis of parasitic diseases has been a subject of increasing interest since the turn of the century. It is particularly important to those diseases where the presence of the parasite cannot be readily ascertained through traditional parasitological techniques, like blood or stool examinations. From the clinician's viewpoint an immunological confirmation of the disease is

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often helpful or necessary for the differential diagnosis since the symptoms of the parasitosis often are common to other diseases. Immunodiagnostic techniques may also be of value in epidemiological studies to determine the prevalence of a parasitic infection in a given area or to determine the changes in the incidence of the disease during a control campaign.

At the Pan American Zoonoses Center, PAHO/WHO, emphasis has centered on the immunodiagnosis of hydatid disease, a cestode infection of worldwide significance and of particular importance to human health in the Americas.[1,2] Since the immunodiagnostic techniques employed for the various parasitic diseases are basically similar, as are the problems involved in their interpretation and in the assessment of their sensitivity and specificity, this presentation deals with our experience with hydatid immunodiagnosis.

The main problem encountered when reviewing the literature on the subject, some of it uncritical, some lacking in adequate experimental design, is

the fact that different techniques, antigens and diagnostic criteria for positivity have been employed by the different investigators. During the past year we have endeavoured to evaluate in our laboratory, the different immunodiagnostic tests for hydatidosis to obtain information on their relative value and limitations and our results to date are the subject of this presentation.

The visual identification of the Echinococcus granulosus-specific "arc 5"[3] has been found to provide a reliable criterion of positivity to the immunoelectrophoresis (IEP) test for the diagnosis of human hydatid disease.[3] The antigen fraction 5, responsible for the formation of this characteristic arc, is found in the hydatid cyst fluid (HCF) obtained from several host species.[3-7]

In the experience of some workers, the best source of this fraction was HCF from fertile horse liver cysts[3,4] and others[5] have reported that many lots of ovine HCF are inadequate for use in the IEP test because of their

deficient antigenic composition. Since the antigenicity of HCF has been reported to vary according to its host or organ source in other immunodiagnostic tests for hydatid disease,[9] a study was designed to assess the relative frequency of the fraction 5 antigens in different HCF pools obtained from livers and lungs of naturally infected sheep. This information was considered of interest to determine if HCF collected from either or both of these sources was equally suitable for use as antigen in the IEP test for human hydatidosis in areas, such as ours, where hydatid cyst material of ovine origin is most readily available.

Capron et al[3,4] consider that a HCF lot should be selected for use in the IEP test if it contains a maximum of parasite antigens (including the arc 5) and a minimum of host contaminants. This criterion for antigen selection was also re-examined.

The antigens for the E. granulosus-specific arc 5 were revealed in 95% of the HCF pools obtained from 46 different sheep livers and lungs by IEP against rabbit anti-sheep HCF serum. This high frequency indicates that ovine HCF is indeed a good source

of antigen for the diagnosis of hydatidosis by the IEP test based on the arc 5 criterion of positivity.[3,4] These findings therefore seem to be at variance with the reportedly poor antigenicity of ovine HCF for this purposes.[3-5]

Furthermore, the additional detection of this antigen fraction in a ten-liter pool of sheep HCF indicates that it may be possible to prepare large stocks of antigen for diagnostic and reference purposes in laboratories located in areas where ovine hydatidosis is common. In our laboratory we have been using sheep HCF antigens in the IEP test for these purposes with excellent results.

These observations on the frequent distribution of the E. granulosus fraction 5 antigens in HCF from fertile liver and lung sheep cysts, their presence in fertile equine liver cysts[4] and both fertile and sterile bovine cysts[7,8] suggest that the selection of a pool of HCF for use in the IEP test need not necessarily rest on a priori considerations of cyst fertility or its host or organ source but rather on its appropriate antigenic composition.

Capron et al[3,4] recommend the selection of a pool of HCF for use in the IEP test on the basis of its containing a maximum of parasite antigens (including the arc 5) and a minimum of host contaminants. The question arises however, on the need to determine the number of other parasite or host components in a HCF pool that contains the antigen fraction 5, when it is to be used on a test in which the criterion of positivity consists in the detection of the arc 5 in the patient's serum. HCF containing a maximum of parasite antigens on the other hand, may be indicated when a positive IEP test is based, instead, on a quantitative estimate of the precipitin bands formed. Such an antigen may be of value in the study of post-operatory sera where the number of bands has been reported[3-5] to gradually diminish until eventually disappearing in the absence of other cysts.

The presence of host contaminants in HCF is of interest since they have been associated with false positive reactions in other immunodiagnostic tests for hydatid disease.[10] Attempts to purify hydatid antigens[9,11]

or the selection of HCF antigens containing a minimum of these components[3,4] have been suggested to increase the diagnostic specificity of these tests.

Several authors[3-8] differentiate host from parasite antigens in HCF by IEP analysis using antisera against the host organ from which the cyst fluid is obtained. In our study we have done likewise and also examined the HCF pools by IEP against a rabbit antiserum to normal sheep serum. This was done because host serum components are found in the HCF[12,13] where we have shown they penetrate from the surrounding host tissues.[14]

The results showed that fewer host antigens are detected in HCF by rabbit antisera to normal sheep liver and lung than by antisera to sheep serum components, suggesting that the former estimate of host contamination does not accurately reflect the situation. Since practically all serum components are found in HCF and we had previously shown that the levels of IgG and albumin do not vary markedly from cyst to cyst[13], the discarding of an HCF antigen on the basis of its degree of host contamination does not seem advisable at the moment, especially if the fraction 5 antigens are present.

Antisera to HCF prepared in a different host species from that from which the HCF was obtained is known to form antibodies to the contaminating host antigens.[3-13] Several approaches have been used to differentiate host from parasite antigens in HCF: an identity may be established between the precipitin bands obtained in IEP with anti-HCF sera and antisera to host components[3-9]; an anti-HCF serum may be absorbed with normal host components[12] or host contaminants may be recognized using antisera to host antigens.[3-9,11]

We then prepared an antiserum to ovine HCF in a sheep, since it was reasoned that the host antigens in the HCF inoculum would not readily elicit an antibody response in the homologous host species. IEP tests revealed no antibody activity to normal sheep serum, liver and lung in this antiserum while in HCF, the arc 5 and 12 other precipitation bands were observed. These bands may thus be considered as reactions to antigens of parasite origin. Further work is in progress to ascertain the value of such an antiserum as a reference serum in the selection of antigens for the IEP test for human hydatidosis in terms of their parasite antigen composition.

Knowledge of the antigenic structure of a parasite and on its antigenic communities with other species is of interest from the immunologic and phylogenetic viewpoints and some of this information is presently available on E. granulosus.[9,10,15,16] Since parasites live in immunologically competent hosts and depend on the latter for their nutrition, the presence of host antigens (like those in HCF and hydatid cyst membranes,[10-18] for example) is the source of difficulty in establishing the parasitic origin of an antigen by immunoprecipitation tests. In the absence of successful methods for the host-antigen-free, in vitro cultivation of parasites to obtain strictly parasitic antigens, the use of antisera to parasites which are prepared in the host-species from which they are collected may be a useful tool in their accurate antigenic characterization.

The similar physicochemical characteristics of the host and parasite components of HCF[9,11,12] may account in part, for the difficulties encountered in obtaining host-free parasite antigens[9,11]. The observed

apparent specificity of the sheep antiserum to ovine HCF for parasite antigens in the IEP test in our study suggests the possibility of obtaining E. granulosus antigens in purified form using immunoabsorbents prepared with such antisera. Work along these lines is currently the subject of interest in our laboratory.

The immunoelectrophoresis test

The immunoelectrophoresis test was first applied to the diagnosis of several mycotic and parasitic infections by French investigators at Lille[19,20] with excellent results. The test, in various forms, has been applied to the diagnosis of hydatid disease by many workers in different areas of the world and the differences in methodolody and results are shown in Table I.

As may be seen in Table I, positive reactors have nt been observed in non-hydatid sera, when the test result is based on the detection of the Echinococcus granulosus-specific "arc 5" with whole (WHF) hydatid cyst fluid antigens,[3] or the presence of bands A and/or B with the purified (PHF) hydatid

fluid antigens. A higher sensitivity, however has been reported[3,7,21] with the antigens for the arc 5 and this has been attributed[21] to the use of agar and a higher proportion of pulmonary hydatid cases in the evaluation of the PHF antigen.

The IEP test, as used in these studies[3-7], however, is not uniform in terms of several parameters: agarose[3-8] or agar[11,21] are used as supporting media; the antigen wells may be rectangular[3-8] or circular in shape[11,21], the antigens employed have been WHF obtained from equine or bovine liver cysts or sheep WHF at a concentration of 200 mg dry weight per ml[3-8], sheep WHF at 50 mg protein per ml[21] or PHF at 10 mg protein per ml.[11,21]

Thus the different technical conditions, together with the use of sera with varying degrees of serological reactivity in the above studies[3-7] render a judgement on the comparative sensitivity and specificity of the WHF and PHF antigens in the IEP test difficult to assess. Therefore several technical variants of the IEP test were evaluated in our laboratory to determine the optimal

standard conditions for performing the test simultaneously with both antigens. This information was considered necessary for a subsequent evaluation of the relative sensitivity and specificity of these two antigens in the diagnosis of hydatidosis by the IEP test.

The results of this evaluation, which will be illustrated with slides, allowed the standardization of the IEP test for the simultaneous use of the whole and purified antigens. The sensitivity and specificity of these antigens in the IEP test was then determined using sera from patients with hydatidosis, other parasitic and non-parasitic diseases and from healthy donors.

The results demonstrated that the purified antigen is more sensitive than the WHF in the diagnosis of hydatid disease. Thus, the E. granulosus-specific arc 5 was only revealed in 81.5% of those hydatid sera giving a positive reaction to the PHF antigen. These results are in agreement with our previous observations that the PHF is more sensitive than the antigens for the arc 5 in the IEP test of immunoglobulin fractions of sera from hydatid disease patients.[22]

Ten of the hydatid sera (13.3%) examined in this evaluation did not reveal the arc 5 nor bands A and B to the WHF and PHF, respectively (Table I). The absence of detectable antibody activity in persons with hydatid disease by the various immunodiagnostic tests is well known and has been associated with the localization[10,21] and physical condition[4,7,10] of the cysts or the time elapsed after surgery[3-5]. The sensitivity of hydatid serology is therefore dependent in part, on the degree of antibody activity to the antigens in hydatid fluid in the groups of sera tested. If the negative reactors in our study would have been included in a different proportion, it is evident that the sensitivities obtained with either antigen would have more closely paralleled those in the literature[3-8,21]. This may account for the lower sensitivity of the antigens for the arc 5 in the preoperative sera in this study (66%) when compared with other reports[3-8] in which it has varied from 75.7% to 91.7%.

These considerations may similarly apply to the higher sensitivity of the PHF (86.6%) in the present study than that (49.5%) reported previously.[21]

This difference may also be accounted for, in part, by our use of a higher antigen concentration and agarose as a supporting medium.

None of the 102 non-hydatid sera in our study revealed the arc 5 by IEP against the WHF, a finding in agreement with the observed specificity of this reaction to E. granulosus infection.[3-8] False positive reactions to the PHF, however, were obtained in 39.0% of the sera from persons with other parasitic disease and in 17.0% of patients with non-parasitic diseases. This 22.4% non-specificity of the PHF in the non-hydatid sera tested is in contrast to the absence of such reactions in the sera studied by Williams et al[21] and may also be explained, in part, by our use of optimal test conditions and more reactive non-hydatid sera.

In our study, not all hydatid sera reacted to the WHF or PHF and different numbers of bands were revealed to these antigens by different sera. (Tables II and III). Williams et al[21] have also reported variations in the number and type of bands formed to the PHF by different hydatidosis sera and others[3-5,7]

have observed a relationship between the localization or physical condition of the cyst or the interval after surgery and the number of bands to the WHF in the IEP test. These observations suggest that the degree of antigenic stimulation by the hydatid antigens in the different patients (and their degree of immunological responsiveness) determines the degree of the ensuing antibody response which may then be detectable by a serological test. The same considerations seem to apply to the degree of antibody activity to cross-reacting hydatid cyst fluid antigens observed in the non-hydatid patients in this study since for example, not all the sera from persons infected with the same non-hydatid parasite elicited a positive reaction to the PHF.

This suggests that, in the selection of sera from non-hydatid cases to determine the absolute specificity of an antigen (or technique) for the diagnosis of hydatid disease, these should preferably contain cross-reacting antibodies to hydatid fluid antigens (which may be detectable by another antigen or test). If the non-hydatid sera selected in the present study would not have been reactive to hydatid antigens, an accurate estimate

of the diagnostic specificity of the PHF may not have been attained. The absence of false positives to the arc 5 in non-hydatid sera containing antibodies to the PHF supports previous results[3-7] on its specificity for E. granulosus.

Other workers[23] have observed the formation of electropositive precipitation bands in the IEP test only with sera from hydatidosis patients but others[24] have detected such bands with non-hydatid sera as well. These results are difficult to assess since the sera, antigen concentrations and supporting media differed in both cases. Under the conditions of our study however, all bands revealed by both hydatid and non-hydatid sera were in the anodic portion of the antigen well, an observation apparently confirming that of the latter authors.[24]

Another criterion of positivity to the IEP test is based on the mere formation of bands by the serum against hydatid cyst fluid.[21,23] By this criterion however non-specific reactions were observed by Williams et al[21] and De Rosa et al[25] in 1 of 80 and 13 of 73 non-hydatid sera using sheep hydatid

fluid at concentrations of 50 and 320 mg protein per ml, respectively.

In our study, eleven of 82 sera from persons with other parasitic and non-parasitic diseases revealed one band other than the arc 5, while another serum from the latter group formed two bands to WHF by the IEP test. In contrast 26 of the 75 hydatid sera revealed more than two bands with the same antigen. This suggested that the number of bands formed to the WHF may be a useful criterion in the differentiation of hydatid from non-hydatid sera by the IEP test.

Examination of the data presented by Sorice and Castagnari[23] reveals that in only nine of the 40 non-hydatid sera were precipitation bands formed and in no case was their number larger than two, while 18 of the 28 hydatid sera revealed more than two bands under their test conditions. The data reported by De Rosa et al[24] similarly shows several hydatid sera with more bands than non-hydatid sera. Although several reports[3-8,21,24] do not provide details on the numbers of bands observed in non-hydatid sera,

mean numbers of four to six bands (including the arc 5) have been observed with hydatid sera by different investigators.[3-5,7]

Further work to determine the threshold in the number of bands that would differentiate hydatid from non-hydatid cases with a high degree of accuracy, seems indicated, since three preoperative hydatid sera in this study revealed more bands than the non-hydatid sera in the absence of the diagnostic arc 5 (Table IV). Such a criterion would be equivalent to a serological titer of diagnostic significance in agglutination reactions and as such it is anticipated that hydatid sera of low antibody reactivity will escape detection by this criterion. It also seems reasonable to suggest that WHF used for these purposes should be standardized as described by Capron et al[4] to ascertain the presence of a large, relatively constant number of parasite antigens in the different WHF lots used.

Whether the uncharacterized bands in the IEP test for hydatid and non-hydatid sera seen in this and other studies[3-7,23,24] are reactions to the host

and/or the parasite antigens in hydatid fluid, remains to be determined. This would be of interest since the presence of host components in hydatid antigens has been associated with the occurrence of false positive reactions.[10] Should host factors be indeed involved, prior absorption of sera with these components or the simultaneous characterization of these bands with antisera to the host antigens, may identify bands formed to non-parasite antigens, thus increasing the test specificity.

In general, however, our study illustrates the superiority of the antigens responsible for the formation of the arc 5 as a positivity criteria in the IEP test for human hydatid disease (Tables II and IV). Furthermore, it suggests that quantitation of a reaction for achieving specificity in hydatid immunodiagnosis, although useful in cases of relatively high antibody activity, may not be as satisfactory a diagnostic criterion as basing it on a qualitatively characteristic reaction (Table IV). This point is further illustrated by two sera in this study in which the arc 5 was detected in the absence of other precipitation bands.

Since previous efforts to purify Echinococcus specific antigens have not been successful,[10,11] the immunoelectrophoretic individualization of the E. granulosus arc 5 of Capron et al[3] seems to be the only available method at the moment for the specific diagnosis of hydatid disease. No false positive reactions have been recorded to date with this criterion of positivity in this and other studies.[3-7]

The indirect haemagglutination test

The indirect haemagglutination test (IHA) has been used by many workers in different areas of the world for the immunodiagnosis of human hydatid disease.[10] Antigens and red cells from various host sources, different procedures for coupling the antigen to the red cells and different sera have been employed with corresponding variations in sensitivity and specificity.

At the present time, tannic acid,[25-33] glutaraldehyde,[5,34] benzidine,[3,4] and formol-[21,35] treated red cells have been used. It is not known, however, if these IHA test variants are equally effective in the diagnosis of hydatid infection in man.

Therefore, we designed another study to compare the relative sensitivity and specificity of the IHA test using tannic acid-,[25] glutaraldehyde-,[34] benzidine-[4] and formol-[21] treated cells in the immunodiagnosis of this zoonotic disease. The same lot of sheep hydatid cyst fluid (HCF) antigen and sera from hydatid and non-hydatid persons were employed in the evaluation of each test.

Different degrees of sensitivity and cross-reactivity were observed with each test. The serum titers for each patient varied with the IHA test variant employed (Table V) and the various techniques were more or less sensitive, depending on whether the sera were obtained from preoperative or post-operative hydatid cases and on the positivity criterion used (Table VI).

Table V shows that the degree of cross reactivity also varied with the serum dilution selected as positive, the type of IHA test and the non-hydatid sera studied. The reasons for these variations are not known but they could be related, to qualitative and/or quantitative differences of the antigens in HCF which are bound to the cells by each technique, and/or to the specificity of the

antibodies to these antigens which were produced by the different patients included in the study. These observations also indicate, that previous studies[3-5,10,21,25-33,35-36] on the comparative sensitivity and specificity of the IHA test and other immunodiagnostic tests for hydatid disease are difficult to assess when besides different antigens and sera, different technical variants of these tests have been employed.

The situation is more complex since different criteria for test positivity have been used in the interpretation of the IHA tests for hydatid disease. Thus a serum may be considered positive to the IHA test if it shows a diagnostic titer ("criterion D"), which is higher than the last dilution where no false positive reactions are observed in non-hydatid sera.[3,4,27,31,32,35,36] Alternatively, a positive IHA test may be based on the highest serum titer where a minimum inespecificity is obtained ("criterion M"),[10,28-30,36] or may also be based on the lowest serum titer where positive haemagglutination is observed ("criterion R").[25,26,36] It seems evident that there is less probability that a positive IHA reactor may indeed be affected by hydatid disease by the last two positivity criteria than by criterion D (Table VI).

These findings also indicate that the selection of a positive titer depends on the IHA test and non-hydatid sera employed in its determination (Table V). A higher proportion of sera from persons with other parasitic diseases showed cross reactions, of varying magnitude, with the tannic acid and glutaraldehyde tests, than did sera from patients with non-parasitic diseases (Table VI). The opposite was the case, however, with the benzidine and formol tests, which also detected more cross-reactions in the group of sera from healthy donors. These considerations seem to indicate that a diagnostic titer needs to be determined in each laboratory for a given technique, using sera from persons affected with the non-hydatid diseases prevalent in the area. If the glutaraldehyde IHA test, for example would have been evaluated in an area free from other parasitic diseases, its diagnostic titer would have been lower, and its sensitivity for hydatid cases consequently higher, than obtained in our study where sera from such persons were included.

Not all sera from persons with the same non-hydatid disease showed the same degree of cross-reactivity to the

IHA tests studied (Table V). This suggests, as was the case with the IEP test that in the determination of the cross-reactivity of hydatid serology in a given area, the inclusion of sera from non-hydatid diseases is of limited value, if these do not contain antibody activity to HCF antigens. The use of non-reactive sera from other diseases[27,37] may not contribute the required information on the background cross-reactivity of the IHA test and would further increase the chances of subsequent false positive results.

Theoretically however, the possibility always remains that a highly cross-reacting serum from a non-hydatid person will show a titer above the established positivity criterion. Vernes and Capron[19] working with the immunofluorescence test for hydatidosis, increased their established diagnostic titer for this test to obtain a maximum of specificity when a false positive result was obtained with a non-hydatid serum. A similar situation has been observed with IHA titers in non-hydatid sera by other workers.[10,25]

The results with the benzidine test (Table VI) most clearly illustrate the reduction in the sensitivity of the test by selecting a positive diagnostic titer which completely

eliminates cross-reactions. A superior specificity at the expense of sensitivity is thus obtained with criterion D which may account for its preferential use by those investigators interested in confirming a presumptive diagnosis of hydatid disease in a patient.[3-8] The other criteria, which favours test sensitivity, although less specific, seem to be more adequate for seroepidemiological or screening purposes as will be shown later.

These considerations suggest that if the IHA test is to be used in a clinical situation, sera from patients with diseases which clinically resemble hydatidosis should be selected to evaluate the test because of their importance in the differential diagnosis. In areas where other parasitic diseases are common, a stool examination of the patient may contribute to the interpretation of serological results. If the test is to be used for screening purposes, however, its evaluation using a wider representative selection of sera from non-hydatid diseases common in the region seems indicated. This baseline information seems particularly important prior to the application of the test in seroepidemiological studies in a given area.

Although some authors[9,32] have reported variations in antigenicity among different batches of HCF, Hariri et al,[39] by block titrations of HCF antigens from several hosts did not find marked differences in the IHA test among HF from most host sources studied. In order to minimize the risk of encountering poor batches of antigen, the collection and evaluation of a large pool of HF seems desirable. Furthermore, in evaluating an antigen for use in the IHA test for the pre-operative diagnosis of hydatid disease, presurgical sera from cases subsequently confirmed at surgery seems indicated since the use of unconfirmed sera render the results of such studies difficult to assess. Similar complications are found when comparisons are made using different techniques with different sera[4,5,26,28-30] since technical differences and sera of varying reactivities are involved.

Although data obtained with pre-surgical sera seems more pertinent in the evaluation of IHA tests for hydatidosis a comparable range of titers was obtained in our study with pre and post-operative sera (Table V). This may be related to the degree of antigenic stimulation on the patient, which has been associated with the localization[10,21]

and physical condition[4,7,10] of the cyst or the time elapsed since the cyst(s) was surgically removed.[3-5] Post-operative sera should be included however, in studies on the sensitivity and specificity of hydatid serology to ascertain their value in the post-surgical evolution of antibody activity. As mentioned earlier, hydatid serology has been found to be of value in this period since sera from patients with residual cysts tend to remain positive longer than those from persons with a single cyst removed at surgery.[4,5,32]

It is generally recognized that a laboratory diagnosis of hydatid disease should be based in more than one test to increase the specificity of the results.[4,10,32,38] In our study more hydatid cases were serologically positive (to one or more types of IHA tests) when all sera were examined by the four types of IHA tests than by either test alone. This observation may account for the absence of detectable antibody activity in some hydatid sera in this and previous studies employing only one type of IHA test and supports the notion that several tests varying in sensitivity and measuring different antigen-antibody systems increase the sensitivity of hydatid immunodiagnosis.

The large number of cross-reactions revealed by the benzidine test and the lower detection of hydatid cases with the glutaraldehyde test in this study suggest that these are less useful than the more sensitive and specific formol and tannic acid techniques in the diagnosis of hydatid disease by the IHA test. Benzidine has the added disadvantage of being potentially cancerinogenic but, the greater simplicity and reproducibility of the tannic acid over the formol test tends to favour it, in our opinion, as the technique of choice for the IHA test in hydatidosis. Our results apply however, only to the four techniques employed since nothing is known on the comparable sensitivity and specificity of other variants of these tests not included in the preset evaluation.

Table VI shows the results obtained in our laboratory with the tannic acid IHA test (by two positivity criteria) when compared with the IEP test in the same hydatid sera. The IHA test was found to be more sensitive than the IEP test. IEP-positive hydatid sera were encountered which were negative to the IHA test, suggesting that these cases would escape immunological confirmation by the IEP test if a preliminary screening would rely only on the IHA test. Several

low-reacting sera were IEP-positive and these would also be missed if the positivity criterion for the IHA test were based on titers above which no cross-reactions are observed in non-hydatid sera.

Although several types of latex agglutination (LA) tests have been used for hydatid disease, we routinely use the technique developed at the Pan American Zoonoses Center by Williams and Prezioso.[40] More hydatid sera reacted in the IHA test than in the LA test but no false positives were obtained with the latter test (Table VIII). When a positive IHA test was based on the titer which was specific for hydatidosis, however, the LA test was more sensitive. The promising results on the apparent specificity of this latex test for hydatid sera are the subject of continuing study in our laboratory.

The LA test was also found to be more sensitive than the IEP test (Table IX). Several IEP-positive sera were negative to the LA test, a situation similar to that observed with the IHA test. This latter observation should also be taken into consideration when planning a seroepidemiological survey on the basis of this test, since sera which could be immunologically confirmed by the IEP test would escape detection.

Table X shows the results obtained with both the LA and IHA tests on the hydatid sera studied. Again, sera negative to both these tests were immunologically confirmed as hydatid disease with the IEP test.

In general, we have seen that some hydatidosis patients are negative to one or more tests, yet positive to other(s) and we have attributed this to the involvement of different antigen-antibody systems in the various tests or perhaps to the differantial sensitivity of each test in the detection of antibody activity. In any case, if a screening of several patients (or of a population) were to rest in the LA and/or the IHA tests, hydatid infections which could be immunologically confirmed by the IEP test would be missed. This presents a problem since the complexity of the latter test renders it unsuitable for use in large populations.

In order to avoid the loss of these individuals we are presently evaluating the following approach: Sera are first screened by the LA and/or the IHA tests and the reactors are then examined by the IEP test. Those sera which are negative to the LA and IHA tests are not discarded as negative but are next screened for the presence of precipitating antibodies by the simpler double

diffusion (DD) or crossed-over electrophoresis (COE) tests. Those reactors to either of the latter two tests are then examined by the IEP test. This procedure, however is quite cumbersome in areas with a high prevalence of other parasitic diseases where many sera, subsequently negative to the IEP test, are positive to the DD or COE tests. The rate of false positive results is usually high with the DD and COE tests, which are highly sensitive and in which it is not possible to differentiate hydatid from non-hydatid sera. Therefore, in our experience to date these tests are not suitable for screening purposes in general, although they may turn out to be useful for a secondary screening of LA or IHA-negative sera, prior to their examination by the IEP test. In the case of patients in which a differential diagnosis for hydatid disease in required, however, we routinely examine the serum with the IEP test obviating the DD or COE tests.

The possibility of detecting IHA (and/or LA) test-negative, IEP test-positive sera by the incorporation of the immunofluorescence test as an additional screening technique is the subject of present study in our laboratory.

Intradermal tests

The intradermal test for hydatidosis has been extensively studied since its introduction by Casoni in 1911 and it has been generally reported to show a high sensitivity for the detection of hydatid disease infection in man.[10,21,32] The test, as performed by the different investigators differs by several parameters, among them: the source, type, preparation and concentration of antigen employed; the use or not of an appropriate control inoculation; the amount and physical location of the inoculum; the time elapsed before reading the reaction; the criteria for test positivity. Another difficulty often encountered is the absence of confirmation of the disease in skin-tested individuals which seems necessary for the interpretation of the results if these are to provide evidence of the effectivity of the skin test.

False positive reactions of varying degree have also been reported by most investigators in patients with other parasitic and non-parasitic diseases.[10,32,41] This has been attributed to the known antigenic communities which exist among different parasite species, some of which, particularly other taenids, have also shown a good sensitivity when used as antigens in the detection of hydatid infections by the intradermal test.[10,32]

Kagan et al[28] reported that the rate of non-specific Casoni reactions was reduced by lowering the antigen concentration. Further work seems necessary to confirm this association, however, since the various antigen concentrations in their study were evaluated in different groups of patients who may have differed in their reactivity to hydatid fluid antigens. Cherubim,[41] however, skin-tested non-hydatid persons (schistosomiasis, normal individuals, etc.) with several HF antigens of different nitrogen content and found a proportion of reactors comparable to that found in hydatid patients by other workers. He also found that the antigens with a low nitrogen content had no advantage over the others. It should also be considered that techniques based on antigen dilutions and/or degree of reactivity to reduce inespecificity ultimately involve a loss in diagnostic sensitivity since weak reactors may be lost. This may be seen in the data shown by Kagan et al[28] and Williams et al.[21]

Aside from the difficulties in ascertaining whether a positive skin test individual indeed has hydatid disease, the problem remains that not all hydatidosis patients give a Casoni positive reaction. This may be accounted for since

the intradermal test is generally used as a measure of immediate hypersensitivity, which is IgE-mediated, while the serological tests are associated with the circulating serum antibodies of the IgM, IgG and IgA immunoglobulin classes. Melli et al[42] found an association between skin test activity and the IgA fraction of serum but it could not be absorbed with anti-IgA serum. This may suggest that this activity lies in the IgE immunoglobulin class but recent work by Huldt et al[43] did not find a consistent association between large skin reactions and the in vitro detection of IgE antibodies by the radioallergoabsorbent test. Further characterization of the Casoni reaction therefore seems desirable.

Whether the immunoglobulins associated with the intradermal or serologic tests for hydatidosis are antibodies to Echinococcus-specific antigens, host antigens or antigens common to other helminths, cannot be ascertained unless their activity is removed by absorptions with parasite-specific antigens. This has not been achieved to date partly because host and parasites components in HF have similar physicochemical

characteristics, as discussed previously. The only serological test, however, which is based on the detection of an E. granulosus-specific antigen is the immunoelectrophoresis test of Capron et al[3]. Using this test we have demonstrated[22] an association between the parasite-specific antigens responsible for the formation of the diagnostic "arc 5" and the IgG fractions of sera from hydatidosis patients.

It is considered that the use of more than one test increases the sensitivity of hydatid immunodiagnosis[10] and this may be expected, theoretically, when the tests differ in sensitivity and/or measure different antigen-antibody systems. The combined use of the Casoni reaction and a serological test has been reported to increase the sensitivity of the diagnosis in known hydatid cases.[31] This may be explained if both hypersensitivity (IgE) and humoral (IgM, IgG and IgA) responses are being measured. In practice, however, the confirmation of a diagnosis of hydatid disease in a Casoni-positive, serologically-negative person is not a sound basis especially when the known inespecificity of the Casoni reaction is considered.

It has also been suggested that the Casoni skin test may be useful as a screening technique in epidemiological

studies.[10,29] The above considerations on the inherent inespecificity of the test and the fact that non-specific reactions may vary from one area to another, depending on the relative prevalence of diseases which may give rise to cross-reactions, seem to limit the indiscriminate use of the Casoni test for these purposes.

The LA and the IHA tests have been found to be sensitive in the detection of hydatidosis cases. High titers in the IHA test may strongly suggest hydatid disease but the only available immunological confirmation of this disease to date, relies on the IEP test which is based on the presence of the E. granulosus-specific arc 5 in the person's serum. If persons are screened with the Casoni skin test and the reactors selected for subsequent serological testing Casoni-positive, serologically-negative cases are at a dead-end in terms of the immunological confirmation of the disease. At the same time, Casoni-negative serologically positive cases are missed and the only hydatid cases which could be serologically diagnosed would be those positive to both types of tests. Non reactors would escape detection by these techniques. The essence of this situation may be summarized as an attempt to detect IgM, IgG and IgA antibodies to HF antigens on the basis of a previous selection for IgE.

Data obtained from the work reported by several investigators, which shows the differential reactiv ty of the same hydatidosis patients to the IHA (and/or the IEP) and the intradermal tests are presented in Table XI. This information seems useful to illustrate the above considerations.

The comparative value of immnnodiagnostic and non-immunological methods in the determination of the prevalence of human hydatid infection in an area is not known. Necropsy and surgical findings have been used as indicators of the importance of the disease to human health in an area. Radiological surveys favour pulmonary infections while hydatid serology, least sensitive in pulmonary cases, has shown a high sensitivity for liver cases, suggesting that simultaneous application of serological and radiological screenings may increase the accuracy of estimates on the prevalence of this zoonosis in human populations. Further work along these lines seems necessary.

A laboratory guide[44] on the techniques for the immunodiagnosis of human hydatid disease was prepared at the Pan American Zoonoses Center for use in the

Course on Epidemiology and Immunodiagnosis of Hydatidosis, sponsored by the Pan American Health Organization at the Universidad Nacional de San Agustín, Arequipa, Peru, 16-20 October 1972 and is available, on request to the Director, at the Pan American Zoonoses Center.

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Table I. The immunoelectrophoresis test in the diagnosis of human hydatidosis. Antigen = hydatid cyst fluid (HF)

Reference	Antigen source	Antigen concentration	Hydatid sera (P_os/studied)	% pos.	Positivity criterion	non-hyd. sera (Pos/studied)	Supporting medium	Antigen well
3	Horse liver HF	200 mg dry wt/ml	19/21	94.7	arc 5	0/80	agarose	rectangular
23	Sheep HF	200 mg prot. (Lowry)/ml	21/28	75	electropositive bands	0/90	agarose	circular
45	Horse liver HF	200 mg dry wt/ml	88/100	88	arc 5	not reported	agarose	rectangular
24	Sheep HF	320 mg prot. (Lowry)/ml	24/31	77.4	presence of bands	13/73	agar	circular
4	Horse liver HF	200 mg dry wt/ml	159/188	84.6	arc 5	not reported	agarose	rectangular
21	Sheep HF	50 mg prot. (Lowry)/ml	52/91	57	presence of bands	1/80	agar	circular
21	Purified HF	10 mg prot. (Lowry)/ml	45/91	49.5	arcs A and/ or B	0/80	agar	circular
5	Sheep HF	200 mg dry wt/ml	62/68	91.2	arc 5	0/180	agarose	rectangular
8	Bovine HF	200 mg dry wt/ml	50/66	75.7	arc 5	not reported	agarose	rectangular
7	Bovine HF	200 mg dry wt/ml	75/111	67.5	arc 5	not reported	agarose	rectangular
12*	Sheep HF	0.333mg N/ml	13/16	81.2	number of bands glyco-prot.No.4	not reported	agar	circular

* Not used for diagnostic purposes.

Table II. Comparison of the results of the immunoelectrophoresis test simultaneously using whole (WHF) and purified (PHF) hydatid fluid antigens in the same hydatid and non-hydatid sera. Positivity criteria are the presence of the arc 5 with WHF and bands A and B with PHF*

Group	Source of sera	Total of sera examined	PHF(+)WHF(+)	PHF(+)WHF(-)	PHF(-)WHF(+)	PHF(-) WHF(-)
I	Pre-operative from surgically-confirmed hydatid disease	36	20	7	0	9
II	Hydatid disease by clinical-radiological diagnosis	17	15	2	0	0
III	Post-operative from surgically-confirmed hydatid patients	17	15	1	0	1
IV	Post-operative from patients with residual cysts	5	3	2	0	0
	Hydatidosis(total)	75	53	12	0	10
V	Patients with other parasitic diseases	41	0	16	0	25
VI	Patients with non-parasitic diseases	41	0	7	0	34
VII	Healthy donors	0	0	0	0	0
	Non-hydatidosis(Total)	102	0	23	0	59

* (+) indicates a positive and (-) a negative IEP test result to the respective antigen.

Table III. Number of hydatid and non-hydatid sera revealing bands in the immunoelectrophoresis test with the purified (PHF) antigens.

Group	Source of sera	Sera*	Both A and B bands	1 band	3 bands
I	Preoperative from surgically confirmed hydatid disease	27	8	16	3
II	Clinical-radiological hydatidosis	17	7	8	2
III	Postoperative cases	16	7	8	1
IV	Postoperative from patients with residual cysts	5	3	2	0
	Hydatidosis (total)	65	25	34	6
V	Patients with other parasitic diseases	16	2	14	0
VI	Patients with non-parasitic diseases	7	3	4	0
	Non-hydatidosis (total)	23	5	18	0

* Numbers of positive reactors to the PHF among the total number of sera examined in each group.

Table IV. Number of preoperative hydatid sera showing bands other than the arc 5 in the immunoelectrophoresis test with the whole hydatid fluid antigen.

	Number of sera	Number of bands	
		3 and more	two or less
Arc 5 positive	35	14	21
Arc 5 negative	18	3	15
Total	53	17	36

Table V. Number of hydatid and non-hydatid sera showing serological activity in four variants of the indirect hemagglutination test.

Group	Source of sera	Total examined	IHA test	Titers (expressed as reciprocals) 0	32	64	128	256	512	1024	2048	4096	8192	16384	32768	65536
I	Preoperative from surgically confirmed cases	35	TA	13	4	1	6	2	4	1	3	-	1	-	-	-
			G	6	1	3	2	2	1	-	1	-	1	-	-	-
			B	4	7	4	4	1	4	2	3	5	-	-	-	1
			F	10	3	-	1	2	7	3	2	4	-	-	-	3
II	Hydatid disease clinical-radiological diagnosis	18	TA	4	-	2	3	4	3	-	-	1	1	-	-	-
			G	2	3	2	4	1	2	-	2	1	1	-	-	-
			B	2	1	-	5	2	2	1	-	3	2	-	-	-
			F	1	-	-	1	4	-	2	4	2	2	-	1	1
III	Post-operative from surgically	17	TA	2	3	2	3	3	2	1	1	-	-	-	-	-
			G	6	1	3	2	2	1	-	1	-	1	-	-	-
			B	3	-	2	5	2	1	2	1	-	-	1	-	-
			F	3	2	1	1	1	4	1	2	2	-	-	-	-
IV	Post-operative from patients with residual cysts	4	TA	-	2	-	-	1	-	-	-	1	-	-	-	-
			G	-	-	-	2	-	-	1	-	-	1	-	-	-
			B	-	-	-	1	1	-	1	-	-	-	-	-	-
			F	-	-	-	2	-	-	-	1	-	1	-	-	-
	Hydatidosis (total)	74	T	19	9	5	12	10	9	2	4	2	2	-	-	-
			G	14	5	8	10	5	4	1	4	1	4	-	-	-
			B	9	8	6	15	6	7	6	4	8	2	1	-	1
			F	14	5	1	5	7	11	6	9	8	3	-	1	4
V	Patients with other parasitic diseases	38	TA	34	4	-	-	-	-	-	-	-	-	-	-	-
			G	34	1	1	1	1	-	-	-	-	-	-	-	-
			B	26	9	1	2	-	-	-	-	-	-	-	-	-
			F	32	6	-	-	-	-	-	-	-	-	-	-	-
VI	Patients with non-parasitic diseases	40	TA	39	1	-	-	-	-	-	-	-	-	-	-	-
			G	39	1	-	-	-	-	-	-	-	-	-	-	-
			B	21	11	3	5	-	-	-	-	-	-	-	-	-
			F	28	8	4	-	-	-	-	-	-	-	-	-	-
VII	Healthy donors	20	TA	19	1	-	-	-	-	-	-	-	-	-	-	-
			G	19	-	1	-	-	-	-	-	-	-	-	-	-
			B	15	3	2	-	-	-	-	-	-	-	-	-	-
			F	17	1	1	1	-	-	-	-	-	-	-	-	-
	Non-hydatidosis (total)	98	TA	92	6	-	-	-	-	-	-	-	-	-	-	-
			G	92	2	2	1	1	-	-	-	-	-	-	-	-
			B	62	23	6	7	-	-	-	-	-	-	-	-	-
			F	77	15	5	1	-	-	-	-	-	-	-	-	-

Table VI. Comparison of the results obtained with four variants of the indirect hemagglutination (IHA) test for human hydatid disease in the same hydatid and non-hydatid sera by two positivity criteria.*

Group	Source of sera	Total No. of sera examined	Positivity criteria*	Number of sera positive: Tannic acid IHA test	Glutaraldehyde IHA test	Benzidine IHA test	Formol IHA test
I	Preoperative from surgically confirmed cases	35	R	22	22	30	25
			D	18	10	16	21
II	Hydatid disease by clinical radiological diagnosis	18	R	14	16	16	17
			D	14	6	10	16
III	Post-operative surgically confirmed hydatid patients	17	R	15	11	14	14
			D	12	3	7	10
IV	Post-operative from patients with residual cysts	4	R	4	4	4	4
			D	2	2	2	2
	Hydatidosis (total)	74	R	55	53	64	60
			D	46	21	35	49
V	Patients with other parasitic diseases	38	R	4	4	12	6
			D	0	0	0	0
VI	Patients with non-parasitic diseases	40	R	1	1	19	12
			D	0	0	0	0
VII	Healthy donors	20	R	1	1	5	3
			D	0	0	0	0
	Non-hydatidosis (total)	98	R	6	6	36	21
			D	0	0	0	0

* R = serological reactivity detectable at the lowest serum dilution examined (1/32).

D = serological reactivity at titers above the last serum dilution where antibody activity was detected in the non-hydatid sera studied with each technique.

Table VII. Comparison of the results obtained with the tannic acid indirect haemagglutination (IHA) test* and the immunoelectrophoresis (IEP) test in the same human hydatidosis sera. A positive IEP test is based on the detection of the arc 5.

Group	Source of sera	Total No. of sera examined	IHA Positivity criteria*	Number of sera IHA+ IEP+	IHA+ IEP-	IHA- IEP+	IHA- IEP-
I	Preoperative from surgically confirmed cases	35	R	17	5	1	12
			D	15	3	3	14
II	Hydatid disease by clinical radiological diagnosis	18	R	13	1	2	2
			D	13	1	2	2
III	Post-operative, surgically-confirmed hydatid patients	17	R	13	2	1	1
			D	11	1	3	2
IV	Post-operative from patients with residual cysts	4	R	3	1	0	0
			D	2	0	1	1
	Hydatidosis (total	74	R	46	9	4	15
			D	41	5	9	19

* R = serological activity detectable with the IHA test at the lowest serum dilution examined (1/32) and above.

D = serological activity with the IHA test at titers above the last serum dilution where antibody activity was detected in the non-hydatid sera studied.

Table VIII. Comparison of the results obtained with the latex agglutination (LA) test and with the tannic acid indirect haemagglutination (IHA) test* for human hydatidosis in the same hydatid and non-hydatid sera.

Group	Source of sera	Total sera examined	IHA positivity criterion*	IHA+ LA+	IHA+ LA-	IHA- LA+	IHA- LA-
I	Preoperative from surgically confirmed hydatidosis cases	30	R	16	1	0	13
			D	13	0	3	14
II	Hydatid disease by clinical radiological diagnosis	15	R	11	0	1	3
			D	11	0	1	3
III	Post-operative surgically confirmed hydatid patients	17	R	12	3	0	2
			D	12	0	0	5
IV	Post-operative from patients with residual cysts	3	R	2	1	0	0
			D	1	0	1	1
	Hydatidosis (total)	65	R	41	5	1	18
			D	37	0	5	23
V	Patients with other parasitic diseases	34	R	0	4	0	30
			D	0	0	0	34
VI	Patients with non-parasitic diseases	40	R	0	1	0	39
			D	0	0	0	40
VII	Healthy donors	20	R	0	1	0	19
			D	0	0	0	20
	Non-hydatidosis (total)	94	R	0	6	0	88
			D	0	0	0	94

* R: serological activity detectable with theIHA test at the lowest serum dilution examined (1/32) and above.

D: serological activity detectable with the IHA test at titers above the last serum distribution when antibody activity was detected in the non-hydatid sera studied.

Table IX. Comparison of the results obtained with the latex agglutination (LA) and the immunoelectrophoresis (IEP) tests in the same human hydatidosis sera. A positive IEP test is based on the detection of the arc 5.

Group	Source of sera	Total No. of sera examined	LA+ IEP+	LA+ IEP-	LA- IEP+	LA- IEP-
I	Preoperative from surgically confirmed cases	30	14	2	1	13
II	Hydatid disease by clinical-radiological diagnosis	15	11	1	1	2
III	Post-operative, surgically confirmed hydatid patients	17	12	1	2	2
IV	Post-operative from patients with residual cysts	3	2	0	0	1
	Hydatidosis (total)	65	39	4	4	18

Table X. Comparison of the results obtained with the latex agglutination (LA) and the tannic acid indirect haemagglutination (IHA) test* in hydatidosis patients positive to the immunoelectrophoresis test.

Group	Source of sera	Total sera examined	IHA positivity criteria*	Number of sera IHA+ LA+	IHA+ LA-	IHA- LA+	IHA- LA-
I	Preoperative from surgically confirmed cases	15	R	14	0	0	1
			D	12	0	2	1
II	Hydatid disease by clinical radiological diagnosis	12	R	10	0	1	1
			D	10	0	1	1
III	Postoperative from surgically confirmed hydatid patients	14	R	12	1	0	1
			D	11	0	1	2
IV	Postoperative from patients with residual cysts	2	R	2	0	0	0
			D	1	0	1	0
	Hydatidosis (total)	43	R	38	1	1	3
			D	34	0	5	4

* R = serological activity detectable with the IHA test at the lowest serum dilution examined (1/32) and above.

D = serological activity detectable with the IHA test at titers above the last serum dilution where antibody activity was detected in the non-hydatid sera studied.

Table XI. Correlation between the results of the indirect haemagglutination (IHA) and the Casoni intradermal (ID) tests on the same hydatidosis patients.

Source of data	Total patients	IHA+ ID+	IHA+ ID-	IHA- ID+	IHA- ID-	Total IHA+	Total ID+
Garabedian,G.A., Matossian, R.M., Djanian,A.D. (1959) Am. J.Trop.Med.Hyg. 8:67-71	79	63	6	7	3	69	70
Arabatzis,G. and Papapanagiotou, J. (1963) Bull.Wld.Hlth.Org. 28: 266-268	120	88	20	3	9	108	91
Abou-Daoud, K.T. (1965) Am. J.Trop.Med.Hyg. 14: 760-764	78	38	14	17	9	52	55

Correlation between the results of the immunoelectrophoresis (IEP) and the Casoni intradermal (ID) tests on the same hydatid patients.

Source of data	Total patients	IEP+ ID+	IEP+ ID-	IEP- ID+	IEP- ID-	Total IEP+	Total ID+
Capron, A.,Yarzábal,L., Vernes,A.,Fruit,J. (1970) Path.Biol. 18: 357-365	79	20	7	59	27	27	79