Evaluation in clinical practice of the fluorescent amoebic antibody test

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SYNOPSIS Serum fluorescent antibody levels against *Entamoeba histolytica* were determined in patients with amoebic infection and in control cases. Titres of 1:64 or above were obtained only in cases of active or recent amoebic infection, and were recorded in 75.0% of intestinal cases and in 95.2% of extraintestinal cases. Titres given by symptomless carriers of *Entamoeba histolytica*, though not exceeding 1:32, were proportionately higher than those obtained in normal subjects or in patients with non-amoebic disorders.

In preliminary reports of the application of the indirect fluorescent antibody technique to the detection of serum antibody to *Entamoeba histolytica* (Jeanes, 1966; Mullan, Ward, Shah, and Jeanes, 1967) it was shown that sera obtained from patients with hepatic amoebiasis were reactive to a titre which clearly distinguished them from the sera of randomly selected patients with diseases or disorders other than amoebiasis. Similar studies have been reported by Goldman (1966) and by Boonpucknavig and Nairn (1967). The purpose of the present paper is to report the titres obtained in a larger series of cases of intestinal and extraintestinal amoebic infection, and to compare them with the titres obtained in normal subjects, patients with diseases other than amoebiasis, and symptomless carriers of *Entamoeba histolytica*.

MATERIALS AND METHODS

THE SERA TESTED Samples of serum were obtained during or soon after the acute stage of the disease, from 40 cases of intestinal and 61 cases of extraintestinal amoebic infection. In all cases, the diagnosis was based on the clinical evidence, the results of other investigations, and the response of the patients to anti-amoebic therapy.

Control sera were obtained from 128 blood donors living in the London area, 116 donors of African race from the Durban area, and 270 patients attending or admitted to Guy's Hospital, London, with diseases or disorders other than amoebiasis. The latter group included 38 cases of non-amoebic liver disease and four cases of non-specific ulcerative colitis. In addition, sera were obtained from 37 patients who had no symptoms attributable to amoebic infection, but whose stools were found on routine examination to contain cysts or trophozoites with the characteristics of *Entamoeba histolytica*.

Finally, further samples of serum were obtained from each of three patients with hepatic amoebiasis, at intervals of 20 months, 16 months, and 11 months respectively, following the commencement of anti-amoebic therapy.

TITRATION OF SERA Serial dilutions of each sample of serum were tested against *Entamoeba histolytica* by the indirect fluorescent antibody technique, and the titre was expressed as the highest dilution of serum giving definite (++) fluorescent staining of the amoebae. Details of the method used have been described previously (Jeanes, 1966).

RESULTS

The Table shows the distribution of serum fluorescent antibody titres against *Entamoeba histolytica* in patients with amoebic infection and in the control cases.

AMOEBIC INFECTION In cases of intestinal amoebic infection, the titres ranged from 1:8 to 1:1,024. Thirty-eight (95.0%) of the 40 cases gave a titre of 1:32 or above and 30 (75.0%) gave a titre of 1:64 or above. Proportionately higher titres, ranging from 1:8 to 1:16,000, were given by cases of extra-intestinal amoebic infection: in 59 (96.8%) of the 61 cases, the titre was 1:32 or above, and in 58 cases (95.2%) it was 1:64 or above.

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TABLE

SERUM FLUORESCENT ANTIBODY TITRES IN PATIENTS WITH AMOEBIC INFECTION AND IN CONTROL CASES

<table>
<thead>
<tr>
<th>Fluorescent Antibody Titre</th>
<th>Amoebic Infection</th>
<th>Normal Subjects</th>
<th>Non-amoebic Disorders</th>
<th>Symptomless Carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intestinal</td>
<td>Extraintestinal</td>
<td>British</td>
<td>African</td>
</tr>
<tr>
<td>1:16,000</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1:8,000</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1:4,000</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1:2,048</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1:1,024</td>
<td>2</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1:512</td>
<td>2</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1:256</td>
<td>4</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1:128</td>
<td>11</td>
<td>15</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1:64</td>
<td>11</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1:32</td>
<td>8</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1:16</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1:8</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>Less than 1:8</td>
<td>0</td>
<td>0</td>
<td>106</td>
<td>100</td>
</tr>
<tr>
<td>Total cases</td>
<td>40</td>
<td>61</td>
<td>128</td>
<td>116</td>
</tr>
</tbody>
</table>

In four cases of amoebic infection, the titre was less than 1 : 32. In one of these, clinically diagnosed as hepatic amoebiasis, serological tests for amoe- 
biasis, including complement-fixation, gel-diffusion, and haemagglutination tests, were reported as negative by all other laboratories participating in an 
international trial (Dr I. G. Kagan, personal com- 
munication, 1966). In the second case, diagnosed as amoebic empyema, the amoebic gel-diffusion precipi-
tin test was reported to be positive (Dr S. J. 
Powell, personal communication, 1966). In the 
remaining two cases, diagnosed as amoebic dysen-
tery, no other serological tests were performed.

NORMAL SUBJECTS: BRITISH One hundred and 
twenty-six (98·5%) of the 128 subjects in this group 
had a titre of 1 : 8 or less, and none had a titre 
higher than 1 : 16.

NORMAL SUBJECTS: AFRICAN One hundred and 
fourteen (98·3%) of the normal African subjects 
had a titre of 1 : 8 or less, and in no case was the 
titre higher than 1 : 16. The range and distribution 
of titres was therefore similar to that observed in 
the normal British subjects.

NON-AMOEBOC DISORDERS Of the 270 patients in this 
group, 258 (95·6%) had a titre of 1 : 8 or less, 10 
(3·7%) had a titre of 1 : 16, and two (0·7%) had a 
titre of 1 : 32. There was thus a slight 'upward 
shift' of titres compared with those obtained in 
normal subjects.

Of the two sera with a titre of 1 : 32, one was from 
a patient with carcinoma of the caecum and the 
other from a patient with carcinoma of the breast 
metastasizing to the liver. In 18 other cases of 
malignant disease, including seven with metastases 
in the liver, the titre did not exceed 1 : 16.

In 33 of the total of 38 cases of nonamoebic liver 
disease, the titre was 1 : 8 or less. In the four cases 
of nonspecific ulcerative colitis, the titre was less 
than 1 : 8.

SYMPTOMLESS CARRIERS In 21 (56·8%) of the 37 
symptomless carriers of Entamoebia histolytica the 
titre was 1 : 8 or less, but in 11 (29·7%) it was 1 : 16 
and in five (13·5%) it was 1 : 32. Thus, although the 
titre did not exceed 1 : 32 in any of the carriers, the 
titres were proportionately higher than those ob-
served either in normal subjects or in patients with 
non-amoebic disorders.

FOLLOWED-UP CASES Of the three patients with 
hepatic amoebiasis in whom follow-up studies were 
possible, initial titres during the acute stage of the 
ilness were respectively 1 : 1,024, 1 : 2,048, and 
1 : 1,024. At intervals of 20 months, 16 months, 
and 11 months respectively following the commence-
ment of antiamoebic therapy, the three patients were 
clinically well, and their titres had fallen to 1 : 32, 
1 : 32, and 1 : 8.

DISCUSSION

Following the early observations of Izar (1914) and 
of Craig (1927) on the application of complement-
fixation tests to the diagnosis of amoebiasis, many 
of the further studies in the serology of the disease 
have been handicapped by the difficulty of preparing 
amoebic antigen of standard potency, free from non-
amoebic protein. For this reason, the results of 
serological tests for amoebiasis have often lacked 
uniformity and reproducibility, and the diagnostic 
value of such tests has, in the past, been open to 
question. In recent years, however, the development 
of antigen preparations of a higher degree of purity,
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and the introduction of new immunological techniques, have stimulated further interest in the application of serological methods to the diagnosis of this important disease.

While it is not intended to discuss here technical details at length, it may be said that the outstanding advantage of the fluorescent antibody method is that the tests are read by direct visualization of intact amoebic cells, thus eliminating the risk of false positive results arising from chance reactions of the test serum with impurities in the antigen preparation. The tests are simple to perform, only a single 'drop' of serum is required for a full titration, and the result of a titration may be given less than two hours after receiving the sample in the laboratory.

A disadvantage of the method, as illustrated by the results presented here, is that low-titre reactions are given by a small proportion of normal subjects, and by a rather higher proportion of patients with non-amoebic disorders. It is possible that in some cases, particularly in those from endemic areas, such reactions are due to previous amoebic infection or to the undetected carriage of amoebae in the intestine. The relative distribution of titres in normal British subjects was almost identical, however, with that observed in normal African subjects residing in the Durban area, where amoebiasis is prevalent. It therefore seems likely that the majority of low-titre reactions given by 'non-amoebic' sera do not result from previous or subclinical amoebic infection.

From the results obtained in this study it is seen that a titre of 1 : 32 is suggestive of active amoebic infection, but may also occur in symptomless carriers of *Entamoeba histolytica*, patients with a history of previous amoebic infection, and, very occasionally, in patients with non-amoebic disease. Titres of 1 : 64 or above, however, were obtained only in cases of active or recent amoebic infection and could therefore be regarded as 'diagnostic'. Such titres were recorded in 75-0% of cases of intestinal amoebic infection and in 95-2% of cases of extraintestinal infection.

The laboratory diagnosis of intestinal amoebiasis depends primarily on the identification of the causative organism in the stools, and to a much lesser extent on serological studies. Since 'diagnostic' titres were obtained in a substantial proportion of cases, however, the fluorescent antibody test might well be of confirmatory value in doubtful cases, and a changing titre could serve as a guide to the effectiveness of therapy.

The incidence of generally higher titres in extra-intestinal cases has been noted in many previous studies in the serology of amoebiasis, and suggests that the antibody response varies with the extent of tissue invasion by *Entamoeba histolytica*. The observation is of more than academic interest, since it is in the diagnosis of extraintestinal amoebiasis, so often characterized by an absence of amoebae from the stools, that serological tests are likely to be of most value.

Although patients rarely become infected in Britain, the incidence of amoebiasis among immigrants and returning overseas travellers is sufficiently high to emphasize the need even in Britain for reliable serological tests, if only to exclude the diagnosis. The evidence so far accumulated suggests that the 'fluorescent amoebic antibody test' is suitable for routine use by those with experience of immunofluorescence techniques. For the routine screening of large numbers of sera, each serum may be tested at a single dilution of 1 : 32, and titrated only if reactive at that dilution.

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