Changes in toxoplasma diagnosis

Jean M. W. Chatterton, Susan McDonagh, Neil Spence and Darrel O. Ho-Yen
Scottish Toxoplasma Reference Laboratory, Microbiology Department, Raigmore Hospital, Inverness IV2 3UJ, UK

The serological laboratory workload in detecting toxoplasma infection may be expected to change with changes in the clinical profile of patient populations. We have examined the clinical information and laboratory results for patients referred to the Scottish Toxoplasma Reference Laboratory in April–March 1999–2000 and 2009–2010. Numbers of patient sera submitted for testing were similar (1624 and 1552) but there was a change in the clinical profile, with a significant fall in patients with symptoms of current infection (612 versus 335; \( P<0.0001 \)) and a significant rise in immunocompromised patients (275 versus 531; \( P<0.0001 \)). Although the percentage of patient samples with toxoplasma antibody decreased (53.9 % versus 37.5 %; \( P<0.0001 \)), the number of positives increased with age, demonstrating the continuing risk of toxoplasma infection. More cases of current infection were identified in 2009–2010 than in 1999–2000 (48 versus 35). This increase was significant both for all females with current infection (\( P=0.0253 \)) and also for those in the childbearing 20–39 years age group (\( P=0.0388 \)). Our literature search did not find any published information on toxoplasma workload in the last decade. In summary, we have shown that there have been significant changes in the laboratory diagnosis of toxoplasma infection but it is as important as ever that effective diagnostic strategies are maintained.

INTRODUCTION

Seroprevalence of the parasite Toxoplasma gondii varies throughout the world. The clinical spectrum of toxoplasma infection is wide but there are particular diagnostic problems among the pregnant and the immunocompromised (Montoya & Liesenfeld, 2004). A recent attempt using seroprevalence to gauge evidence of past infection estimated that it varied between <10% and >60% of the global population (Pappas et al., 2009). Hospital laboratories have had to develop protocols to diagnose both current infection and immunity (Evans et al., 2002). We are aware of regional consolidation and integration of toxoplasma services and this could become more widespread (Department of Health, 2006).

It has been predicted that the laboratory’s workload would change and that identifying specific toxoplasma IgG antibody among the pregnant and immunocompromised would be a major part of the diagnostic work (Ho-Yen & Joss, 1992). This change from diagnosing current infection to identifying serological evidence of past infection would change the use of specialist laboratories (Ho-Yen, 2001). This is also complicated as there has been an apparent worldwide decline in toxoplasma infection (Jones et al., 2007; Pappas et al., 2009). We have noticed over the last decade that some commercial laboratory tests could not detect low levels of toxoplasma antibody, and therefore there may be a false perception of less toxoplasma infection. As our laboratory methods and staff have remained stable over the last two decades, we believed that our results could identify any significant changes.

METHODS

Samples. Samples are referred to the Scottish Toxoplasma Reference Laboratory from laboratories throughout Scotland and Northern Ireland. For this study, the clinical information and results were examined for all patients whose samples were received in the laboratory in two periods: 1 April 1999–31 March 2000 and 1 April 2009–31 March 2010. Only one specimen from each patient was included in the analysis. Apart from samples from immunocompromised patients, there may have been screening for toxoplasma antibody before referral.

Testing of sera. All sera were tested using an ‘in-house’ IgG ELISA (Joss et al., 1989a), an ‘in-house’ biotin avidin IgM toxoplasma assay (Joss et al., 1989b) and a modification of the Sabin–Feldman dye test (Ashburn et al., 2000). The results of these tests, together with the clinical details, influenced the choice of further tests. All IgM-positive results were confirmed by more specific tests. In 1999–2000, the confirmatory IgM test was the Toxonostika IgM (Organon Technika) (Ashburn et al., 1992). In 2009–2010, the confirmatory IgM test was the AxSym IgM (Abbott) and this was supplemented by an ‘in-house’ IgG avidity test (Ashburn et al., 1998) when timing of infection was important (e.g. pregnancy). Dye test antibody levels \( \geq 8 \text{ IU ml}^{-1} \) are considered positive, 8–125 IU ml\(^{-1} \) are within normal range and \( \geq 250 \text{ IU ml}^{-1} \) are raised and suggest current
infection. Demonstration of specific IgM antibody is considered evidence of primary infection, low IgG avidity is considered evidence of infection acquired in the last 3 months, borderline avidity infection 3–6 months ago and high avidity infection 6 months ago. A raised dye test result without specific IgM or low IgG avidity is considered evidence of reactivated infection in immunocompromised patients. Where appropriate, statistical analysis was carried out using Fisher’s exact test and the Kolmogorov–Smirnov (KS) test.

**Literature search.** Literature searches were carried out for the period 1990–2010 using Ovid, Cambridge Scientific Abstracts, Geobase, BioSS and Web of Science. The searches were limited to human studies published in English. Toxoplasm* was used as a keyword with ‘prevalence’, ‘reactivat*’, ‘antibod*’, ‘IgG’, ‘IgM’, ‘laboratory’ or ‘dye test’.

**RESULTS**

The number of samples referred to the laboratory for testing was not significantly different between the years examined: 1624 in 1999–2000 and 1552 in 2009–2010. The age and sex of the patients were recorded and the data were divided into eight clinical groups: possible current infection (including lymphadenopathy, flu-like illness, respiratory infection, malaise, pyrexia, fatigue, atypical blood picture, hepatitis); infection in the immunocompromised (transplant and HIV); infection in pregnant women; congenital infection; ocular infection; central nervous system infection; other presentations (including rash, joint pains, diarrhoea and vomiting); and no clinical details stated. Results in the different clinical groups are as in Table 1.

Current infections are shown in Table 2. There were more females with current infection ($P=0.0138$) and the increase was in women with primary infection ($P=0.0253$). This increase was found to be greatest in the 20–39 year age group ($P=0.0388$; Table 3).

**Dye test distribution**

Dye test results of 8–125 IU ml$^{-1}$ are considered to be indicative of past infection and were found to be significantly reduced in 2009–2010 compared with 1999–2000 ($P<0.0001$; Table 4). Those with dye test $<8$ IU ml$^{-1}$ are considered susceptible, and increased in 2009–2010, compared with 1999–2000 ($P<0.0001$). These differences were also significant in males only and females only. The

### Table 1. Clinical presentation and dye test results in patients referred for toxoplasma testing in 1999–2000 and 2009–2010

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Current*</td>
<td></td>
<td>612 (37.7)</td>
<td>335 (21.6)</td>
<td>&lt;0.0001</td>
<td>359 (58.7)</td>
<td>112 (33.4)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Immunocompromised</td>
<td></td>
<td>275 (16.9)</td>
<td>531 (34.2)</td>
<td>&lt;0.0001</td>
<td>113 (41.1)</td>
<td>132 (24.9)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Pregnant</td>
<td></td>
<td>272 (16.8)</td>
<td>213 (13.7)</td>
<td>0.018</td>
<td>115 (42.3)</td>
<td>84 (39.4)</td>
<td>0.577</td>
<td></td>
</tr>
<tr>
<td>Congenital</td>
<td></td>
<td>32 (2.0)</td>
<td>65 (4.2)</td>
<td>0.0003</td>
<td>18 (56.2)</td>
<td>13 (20)</td>
<td>0.0005</td>
<td></td>
</tr>
<tr>
<td>Ocular</td>
<td></td>
<td>98 (6.0)</td>
<td>110 (7.1)</td>
<td>0.251</td>
<td>55 (56.1)</td>
<td>49 (44.5)</td>
<td>0.1263</td>
<td></td>
</tr>
<tr>
<td>Central nervous system</td>
<td></td>
<td>21 (1.3)</td>
<td>26 (1.7)</td>
<td>0.303</td>
<td>12 (57.1)</td>
<td>12 (46.2)</td>
<td>0.5612</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td>128 (7.9)</td>
<td>70 (4.5)</td>
<td>&lt;0.0001</td>
<td>75 (58.6)</td>
<td>39 (55.7)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>No clinical details</td>
<td></td>
<td>186 (11.4)</td>
<td>202 (13.0)</td>
<td>0.193</td>
<td>129 (69.4)</td>
<td>141 (69.8)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>1624 (100)</td>
<td>1552 (100)</td>
<td></td>
<td>876 (53.9)</td>
<td>582 (37.5)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

*Patients tested for current infection.
‡% of total number.
§% of group number.

### Table 2. Current infections (primary and reactivated) in females and males during 1999–2000 and 2009–2010

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primary</td>
<td>Reactivated</td>
<td>Cases/total (%)</td>
<td>Primary</td>
</tr>
<tr>
<td>Female</td>
<td>13*</td>
<td>3</td>
<td>16/681 (2.3)</td>
<td>27*</td>
</tr>
<tr>
<td>Male</td>
<td>17</td>
<td>2</td>
<td>19/889 (2.1)</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>5</td>
<td>35/1570 (2.2)</td>
<td>36</td>
</tr>
</tbody>
</table>

$*P=0.0253$.
$†P=0.0138$. 

Downloaded from www.microbiologyresearch.org by IP: 54.70.40.11
On: Thu, 08 Nov 2018 05:23:21
dye test results for the patient populations are shown in Fig. 1. This shows a normal distribution in both time periods, but the distribution shifted significantly, with a larger number of samples in the dye test 8–15 IU ml\(^{-1}\) range and fewer in the 30–125 IU ml\(^{-1}\) range in 2009–2010 compared to 1999–2000 (KS=0.203, \(P<0.05\)).

**Age distribution**

The age in both populations ranged from under 10 years to greater than 80 years. Although positive results increased with age in both time periods, there was no significant difference in the rate of antibody acquisition (KS=0.026) (Fig. 2). There was a significant decrease in positive results between 1999–2000 and 2009–2010 in all age bands up to the age of 70 years. When the patients were examined in age groups <20, 20–40 and >40 years, there was a significant fall in seropositivity in all groups (\(P<0.0001\)).

**Literature review**

A comprehensive literature search identified 6415 articles on toxoplasma in humans published in English between 1990 and 2010. Of the 573 papers identified using 'IgM' as a keyword, current infection was considered in 35 of these publications: 21 were methods-based; 8 were seroprevalence studies; and 6 were case studies. Using 'dye test' as a search term identified 61 papers but all had been identified in other searches. 'Reactivation' identified 166 papers; however, 128 were research- or methodology-based and 38 were case studies or reviews. No studies were identified which looked at the incidence of reactivation in different patient populations. Searches using ‘laboratory’ as a keyword did not add any studies and there was only one on laboratory workload over the last two decades (Mavin et al., 2000).

**DISCUSSION**

*T. gondii* infects up to a third of the world’s population (Montoya & Liesenfeld, 2004). For the laboratory, the identification of toxoplasma-specific antibody is important in several clinical groups (Ho-Yen & Joss, 1992). We found that when 2009–2010 was compared to 1999–2000, there was a significant fall in samples referred for testing current infection (\(P<0.0001\)) and a rise in samples for testing immunocompromised patients (\(P<0.0001\)). Our results show a fall of specific antibody from 41.1 % to 24.9 % in the immunocompromised group (Table 1). Among immunocompromised patients who are HIV-positive or bone marrow transplant patients, this is good news as toxoplasma infection is usually reactivated (Montoya & Liesenfeld, 2004). However, among organ transplantation patients, a toxoplasma-positive donor and a negative recipient requires prophylactic treatment so pre-transplant testing remains important (Ho-Yen & Joss, 1992).

Our review of the literature has shown little information on laboratory workload. We found a large decrease in samples from the possible current infection group (37.7 % versus 21.6 %; \(P<0.0001\)) (Table 1), yet the number of cases of current infection identified increased from 35 to 48. Current infections may be primary, or reactivated, and Table 2 shows that among women, primary infections have significantly increased. This is important as primary infections can cause congenital toxoplasmosis, whereas reactivated infections only do so if there is significant immunocompromise (Ho-Yen & Joss, 1992). Moreover, if the sex and age of the patients are considered, it was found that the increase was among females in the 20–39 year age group (Table 3), a major period for child-bearing.

The more women (20–39 years) with infection has meant that there are more cases referred for testing for congenital toxoplasmosis (\(P=0.0003\); Table 1). Yet, there have been fewer with infection (13 instead of 18; Table 1), and this

**Table 3. Sex and age of patients with current infections (primary and reactivated) in 1999–2000 and 2009–2010**

<table>
<thead>
<tr>
<th>Gender</th>
<th>1999–2000</th>
<th>2009–2010</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;20</td>
<td>20–39</td>
</tr>
<tr>
<td>Female</td>
<td>3</td>
<td>8*</td>
</tr>
<tr>
<td>Male</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>17</td>
</tr>
</tbody>
</table>

\(P=0.0388\).

**Table 4. Toxoplasma dye test results in 1999–2000 and 2009–2010 among females and males**

<table>
<thead>
<tr>
<th>Dye test (IU ml(^{-1}))</th>
<th>Females (%)</th>
<th>Males (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;8</td>
<td>405 (46.6)</td>
<td>480 (59.8)</td>
<td>(&lt;0.0001)</td>
</tr>
<tr>
<td>8–125</td>
<td>432 (49.7)</td>
<td>287 (35.8)</td>
<td>(&lt;0.0001)</td>
</tr>
<tr>
<td>(\geq 250)</td>
<td>32 (3.7)</td>
<td>35 (4.4)</td>
<td>0.5332</td>
</tr>
<tr>
<td>Total</td>
<td>869</td>
<td>802</td>
<td></td>
</tr>
</tbody>
</table>
highlights the need for definitive, specialist interpretation of results. As with the increase in testing among the immunocompromised (Table 1), the requirement of specialist laboratories is appropriate testing and interpretation (McDonald, 1997) and this is most appreciated by the users of the service (Ho-Yen, 2001).

In the samples referred to us, there has been an increase in patients without specific antibody (dye test $<$8 IU ml$^{-1}$) in both males and females (Table 4). There has also been a fall in both males and females with specific antibody (up to 125 IU ml$^{-1}$; $P<0.001$). This reduction in antibody parallels that in healthy individuals in Britain (Walker et al., 1992; Gilbert et al., 1993; Zadik et al., 1995; Taylor et al., 1997; Allain et al., 1998) and in many other European countries (Pappas et al., 2009).

Toxoplasma antibodies can persist lifelong after infection but levels fall with time. Although there was a normal dye test distribution in both populations, there was a fall in antibody level with peaks at 65 IU ml$^{-1}$ in 1999–2000 and 30 IU ml$^{-1}$ in 2009–2010 (Fig. 1). The shift to lower dye test results in 2009–2010 could suggest that infection in these patients was acquired further in the past than infection in patients in 1999–2000. Thus, the risk of toxoplasma infection was greater in 1999–2000 than in 2009–2010 and supports the fall in specific antibody. The pattern of low antibody levels which increase with age was consistent in both time periods and has been the expected pattern (Williams et al., 1981; Joss et al., 1988; Gilbert et al., 1993; Allain et al., 1998; Nash et al., 2005). Although antibody levels were lower in each age band, the rate of increase was similar (Fig. 2). The increasing antibody levels with age confirms the continuing risk of toxoplasma infection and this is supported by our finding that the number of current infections diagnosed increased between 1999–2000 and 2009–2010. As in 1996–1999 (Mavin et al., 2000), most cases of current infection were in people aged $<$40 years.

We have previously identified contact with raw, undercooked meat, cats and foreign holidays as risk factors in cases of current infection in Scottish patients (Mavin et al., 2000). A European multicentre study identified undercooked and cured meats as the main risk factor for toxoplasma infection in pregnant women (Cook et al., 2000). Changes in diet with wide access to fast-foods and frozen meals suggested as a cause of reduction in seroprevalence from 45.4 % in 1998 to 39.4 % in 2003 in young women in Poland (Nowakowska et al., 2006) could equally apply to the UK. There have also been considerable changes in farming and meat production, which may have affected the level of contamination at source (Tenter et al., 2000, Kijlstra & Jongert, 2009). Indeed, the risk of toxoplasma infection from meat is now taken so seriously a toxoplasma-free meat label has been suggested (Havelaar et al., 2007; Ho-Yen, 2009). Our results show that there is still a cause for concern as we identified more cases of current infection in 2009–2010 than in 1999–2000, and consequently it is as important as ever to provide advice on how to avoid infection, particularly for pregnant women and the immunocompromised.

We have demonstrated significant changes in the laboratory’s workload from 1999–2000 to 2009–2010. This information will be of use to large laboratories, especially if a result of amalgamation of several smaller laboratories, in developing appropriate diagnostic strategies for toxoplasma. These changes will also impact on how specialist laboratories are used and identify a role in dealing with difficult cases of toxoplasma infection among the pregnant and immunocompromised.

![Fig. 1. Distribution of toxoplasma dye test titres (IU ml$^{-1}$) in patients in 1999–2000 (open bars) and 2009–2010 (filled bars).](http://jmm.sgmjournals.org)

![Fig. 2. Variation in toxoplasma seropositivity with age in 1999–2000 (open bars) and 2009–2010 (filled bars) showing the percentage of the age group with dye test $>$8 IU ml$^{-1}$.](http://jmm.sgmjournals.org)
ACKNOWLEDGEMENTS

We are grateful to all our users for their continued help and cooperation. We are grateful to Mr Rob Polson, Highland Health Sciences Library, for assistance with the literature review and to Mrs Barbara Reed for secretarial assistance. We would like to thank Health Protection Scotland and the National Services Division of NHS National Service Scotland for their support.

REFERENCES


