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Stopping dengue: recent advances and new challenges

Cover image: Protoscolex of Echinococcus granulosus, showing row of hooklets; obtained from liver aspirate of a case of hydatid cyst. Courtesy of Associate Professor Rob Baird, Royal Darwin Hospital.
Welcome to the first issue of Microbiology Australia for 2016.

As members of The Australian Society for Microbiology know, our electronic issues are freely available. To be notified of a new issue go to our publisher’s website http://microbiology.publish.csiro.au/ and register to get alerts when each issue becomes available.

ASM members receive print copies by mail if they have opted to receive them that way. Contact the ASM Office if you wish to receive print copies. Additional copies are often made for special issues. For example, ~1000 copies were made for delegates of the ISHAM conference in Melbourne last year. The print copies are highly valued by many members and are great to share with others.

The online issues are highly accessed as a reference and learning resource. Individual articles as well as entire issues can be readily downloaded from the above website.

Themes of issues and Guest Editors are chosen by the Editorial Board who meet by teleconference five times each year. The Board always values input from ASM members for future issues. All articles are peer-reviewed and invited by the Guest Editors who have a deep understanding of the themes they present. Themes are chosen to be topical and of interest to ASM members: authors write to reach a broad audience.

On occasions urgent updates are needed and such updates are presented as Hot Topics. However, we are aware that there is a need to have an occasional non-themed issue. Please contact us if you have suggestions for articles that will have broad interest to ASM members.

We are grateful for the support the Editorial Board and for Guest Editors who are experts in their fields. Guest Editors present the latest knowledge in their field through the selection of contributors who write compact articles that are readable and informative to the ASM’s diverse membership. We also thank the reviewers, who provide valuable comments on each contribution. They also have expertise in the articles they review, and they provide valuable comments to contributors to ensure the articles are appropriately presented. We also thank the authors for their contributions. They provide a great amount of current information about their field and seek to educate us all in the exciting developments of microbiology.

We thank The Australian Society for Microbiology and those who lead it. In the challenging world of publishing it has taken boldness to enable Microbiology Australia to be produced as a publication that is freely available to all. We know from the download data provided by CSIRO Publishing, that Microbiology Australia is highly accessed around the world, and that the articles continue as an ongoing resource many years after their first publication. Articles published more than a decade ago still enjoy thousands of downloads every year. There is no doubt that they serve as resource material for teachers, students and those seeking reliable scientific information from experts.

We trust that you enjoy your reading of this year’s issues, which will cover:

- Parasitic Infections
- Education to enhance microbiology graduate employability
- Diseases of Aquaculture
- Microbiology of Travel [a special joint issue with The Microbiology Society in the UK].

Jo, Ian, and Hayley Macreadie

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Parasitic infections: overlooked, under-diagnosed and under-researched

Professor George Nelson (1924–2009) once stated that, ‘Parasitology is the preserve of the diagnostically destitute’. Little has changed to this day, with potentially relevant parasitic causes of illnesses often not being considered early in the differential diagnoses of clinical presentations. Parasitic infections are sometimes overlooked as causes of morbidity and (in some cases) mortality in both the medical and veterinary fields. In Australia there remain significant problems associated with giardiasis, cryptosporidiosis, strongyloidiasis and other parasitic diseases, particularly in remote, underserved and tropical regions of the country and also in the immuno-compromised individuals (HIV, immunosuppressive drugs etc.). The burden of many parasitic diseases is greater in tropical and subtropical areas of non-industrialised countries. With increasingly adventurous travel and dining, increasing numbers of Australians returning from travel overseas with added souvenirs of common or exotic parasitoses every year and refugees and migrants arriving in Australia, these infections are becoming increasingly important.

Recent advances in diagnostic techniques for the detection of parasitic infections have revolutionised how we undertake such diagnostic investigations. These methodologies often provide greater sensitivity as well as in some cases providing additional epidemiological data. As we increasingly move towards the use of molecular methodologies and away from traditional morphological diagnosis, new challenges have emerged for clinicians, veterinarians and laboratory staff. A focus of several articles in this edition is consideration of the advantages and disadvantages of these new methods, in what circumstances they are best applied and how the results of such investigations should be best interpreted. Molecular methods are not without potential sources of error, hence in some situations, morphological and serological techniques still remain relevant.

This edition also considers zoonotic parasitic infections, the treatment of parasitic infections, both from the current WHO recommendations for mass drug administration in highly endemic settings to the rise of resistance to anti-parasitic agents in protozoa such as malarial parasites and Giardia intestinalis. Whilst antimicrobial resistance is greatly investigated in bacterial infections, its emergence and prevalence in parasitic infections of human and veterinary importance will require further investigation and attention in the future.

We hope that this edition of Microbiology Australia will update knowledge and serve to inform all our readers of the importance and relevance of parasitic disease. Whether one is involved in medical, veterinary, food, environmental or other microbiological work, it is likely that aspects of your work will at some stage involve this important and sometimes neglected field of our scientific discipline.

As guest editors, we are grateful and excited to be involved in the planning and execution of this edition. We would like to thank the editorial staff and all of the authors and reviewers who have kindly contributed their time and expertise into the preparation of this edition. We hope that all members of the society will find it helpful, interesting and that it may spark the interest of many into this most fascinating and under-researched area.

Biographies

The biography for Dr Harsha Sheorey is on page 49.
The biography for Dr Richard Bradbury is on page 9.
The laboratory diagnosis of Strongyloides stercoralis

Matthew R Watts A,*, Gemma Robertson B,† and Richard S Bradbury C,D

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It is estimated that over 30 million people worldwide are infected by the nematode, Strongyloides stercoralis1. It is endemic in sub-tropical and tropical parts of Australia, with high rates of infection documented in some indigenous communities2. Due to the potential for chronic autoinfection, that may persist for decades, migration leads to the presence of the infection in non-endemic areas1. Transmission to humans is generally through the penetration of larvae through the skin, following contact with faecally contaminated soil1. Disease severity ranges from asymptomatic chronic carriage to an overwhelming illness, where large numbers spread throughout the body, usually triggered by immunosuppression1.

Clinicians are advised to consider strongyloidiasis in patients prior to immunosuppression, or with indicative symptoms, if there is a history of probable exposure in an endemic area, regardless of the elapsed time since exposure1,4. Eosinophilia is not an accurate marker of strongyloidiasis, with a retrospective study finding that only a quarter of patients with Strongyloides infection had a raised eosinophil count5. The detection of strongyloidiasis is optimised by appropriate test ordering, clinical notes, specimen transportation, and processing by the receiving laboratory.

The gold standard for the diagnosis of strongyloidiasis is the morphological identification of larvae in stool, tissue biopsies, and other clinical specimens such as bronchoalveolar lavage. However, in chronic infections, detection can be limited by low larval output in stool, leading to false negative results6. Consequently, in validation studies for serological and nucleic acid tests there is a tendency to define heavier infections as ‘true positives’. This affects serological cut-offs, measurements of sensitivity and specificity, and positive and negative predictive values7. Recognition of these limitations is important for the interpretation of negative diagnostic test results, where clinical suspicion remains. Here, we will give an overview of currently available conventional and molecular tests for the diagnosis of strongyloidiasis.

Stool microscopy and culture methods

Specimen transport and storage have a major impact on the efficacy of culture techniques in the laboratory diagnosis of S. stercoralis from faecal samples. Fresh, unrefrigerated samples should be delivered to the laboratory for culture as soon after collection as possible, as the viability of larvae decreases incrementally with storage at 4°C over a 72-h period6. Rhabditiform and filariform larvae will be found along with free-living adults of S. stercoralis in
older cultures (Figure 1). Larval stages must be differentiated from those of hookworms, which may also be recovered.

Microscopic methodologies such as examination of Kato-Katz preparations, FLOTAC, and formalin/ethyl acetate concentrates have a low yield compared to culture. A modified formalin/ethyl acetate method proposed by Anamnart et al. improved rates of detection. Overall, however, microscopic techniques alone are insensitive and not sufficient for the exclusion of strongyloidiasis. In one of these studies, though 30 of 254 participants were diagnosed with strongyloidiasis by either agar plate culture (APC; Figure 2) or Baermann culture techniques, no infections were identified by microscopy using the Kato-Katz technique.

APC is possibly the easiest culture to perform in the context of high volume diagnostic testing. Results are available within two days, although extended incubation up to four days increases yield. Two studies comparing 48 h APC with Baermann culture found an improved recovery of *S. stercoralis* larvae in APC. Recovery rates improve markedly with multiple stool cultures.

Serological diagnosis

Several tests for the serological diagnosis of strongyloidiasis have been described, using both crude and recombinant antigens. Two commercial ELISA kits employing somatic antigens are available from BORDIER (*Strongyloides ratti* antigen) and IVD Research (*S. stercoralis* antigen), respectively. Recently, two recombinant antigens (32 kD recombinant antigen, called NIE and *S. stercoralis* immunoreactive antigen, SsIR) have been employed for serological testing in both ELISA and luciferase immunoprecipitation system assay (LIPS) platforms. The reported sensitivity and specificity of various serological platforms ranges from 56-100% and 29-100%, dependent upon the method, antigens, cut-offs, study populations, and reference methods employed. *Strongyloides* serology using a crude larval extract antigen was shown in one study to be less sensitive for the diagnosis of returned travellers (73%) compared to patients who have lived for an extended period in an endemic area (98%). No definitive study of serological methods has been conducted to date, and much of the available data is subject to flaws in methodology, particularly the use of microscopy only as a reference.

Figure 1. Life stages of *Strongyloides stercoralis* in agar plate culture: (a) rhabditiform larva; (b) a free-living adult male with filariform larva adjacent; and (c) a gravid, free-living adult female with filariform larva adjacent.
standard for positive specimens and varying *S. stercoralis* exposure rates amongst tested serum groups. A 2010 study with a reference standard of a combination of three culture methods and sedimentation concentration found NIE LIPS had a sensitivity of 97.8% (cut-off 37.89 LU) in a study population with high endemicity but from regions without filarial infection. Lower sensitivity resulted when testing the same samples by NIE ELISA (84%), NIE-SsIR LIPS (91.2) and a *S. stercoralis* crude antigen extract ELISA (97%). All assays tested showed 100% specificity in this study. A more recent study compared an in-house crude *S. stercoralis* filariform larvae immunofluorescent antibody test (IFAT) with the ELISAs from IVD Research, Bordier, and a recombinant antigen NIE ELISA and LIPS. This study used reference samples identified as positive by culture as well as microscopy, and also a composite reference standard of concordant results in at least three of five serological tests. The in-house IFAT was found to be the most sensitive (93.9%) when used in a test subject group with no known previous exposure to *S. stercoralis* and using the composite reference standard, whilst NIE LIPS was found to be the most specific test (100%). Furthermore, when tested against subjects with potential previous exposure and using the composite reference standard, NIE LIPS was almost 100% specific and 84.6% sensitive (cut-off value 1388 LU). In testing against the same sample group, the Bordier and IVD ELISAs maintained a high specificity (almost 100%), but a lower sensitivity (70% and 79%, respectively) and the NIE ELISA showed the highest specificity (99%), but a low sensitivity (45%) (cut-off 76.5 U/mL). Seroreversion following treatment of many, but not all, patients was noted in a study using a *Strongyloides ratti* antigen ELISA. However, this effect is not universal and varies between studies. Immunosuppression was demonstrated to cause a reduction in serological sensitivity (62% vs previously determined 92%), when testing haematological patients on antineoplastic therapies. NIE LIPS did not cross-react with antigens from other parasites in the study by Bisoffi et al., whereas IFAT and the two commercial ELISAs did yield false positives, particularly from *Mansonella perstans* infection. Such cross-reaction may be decreased by pre-incubation of serum in an extract of *Onchocerca gutturosa*.

### Nucleic acid tests

Nucleic acid tests complement non-molecular methodologies for the diagnosis of *S. stercoralis*, and allow the use of refrigerated, frozen, or preserved specimens. This simplifies specimen transportation, particularly where collection occurs some distance from the testing laboratory, and there is no risk of laboratory-acquired strongyloidiasis. DNA extraction and amplification can be performed within 1 day, however, laboratories may batch specimens according to demand.

### DNA extraction

It is important that DNA extraction methods for stool specimens are effective at removing the numerous nucleic acid test inhibitors in stool. A comparison of 5 methods of DNA extraction demonstrated that two column-based methods were the most effective for the PCR detection of DNA from *Strongyloides ratti* that had been spiked into human stool. These were the MoBio PowerSoil kit (MoBio Laboratories, Carlsbad, CA, USA) and a method based on modifications of

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*Figure 2. Larval tracks of *Strongyloides stercoralis* on Koga agar plate culture.*
the QiaAmp Tissue kit (Qiagen, Hilden, Germany) by Verweij et al., which has been successfully automated. The comparison found that bead beating prior to the use of the NucliSens EasyMag (BioMerieux, Marcy l’Etoile, France) was less effective, which indicates the method of sample pretreatment prior to automated extraction will impact upon test sensitivity. Other investigators have used a variety of different extraction methods for *Strongyloides* PCR, including in-house methods, the Qiagen stool kit (unmodified and modified), and the Nucleospin Soil kit (Macherey-Nagel, Duren, Germany). One of the inherent limitations of the molecular diagnosis of *S. stercoralis* is the sampling error that can occur when relatively small amounts are extracted in the context of low larval output. For example, 2g of stool can be used for agar plate culture, whereas 250 mg of specimen is recommended for the MoBio PowerSoil kit. Methods that concentrate larger amounts of stool prior to DNA extraction have the potential to increase test sensitivity, if they remove inhibitors and retain larvae.

**PCR**

Current PCR methods most commonly target one of four regions: the 18S rRNA small subunit (SSU); the internal transcribed spacer region 1 (ITS-1); the 28S rRNA gene; or the cyclooxygenase gene (*cox1*). Published sensitivities and specificities for *Strongyloides* PCR vary according to the reference methods and are listed in Table 1. The majority of *Strongyloides* PCR publications have used a real-time method with primers and probe published by Verweij *et al.*. This has also allowed for the development of multiplexed PCR. Some studies evaluating the diagnostic accuracy of these PCR methods have used both morphological diagnosis and detection of PCR products as their reference standards, and are not reviewed here. Their methodology precludes the calculation of sensitivity and specificity based on gold-standard, according to an FDA Guidance.

In the absence of a consistent gold standard in chronic infection, positive nucleic acid test results, where conventional tests are negative, may be due to greater sensitivity or false positive results. No PCR studies have reported false positive results when analytical specificity has been tested using DNA extracted from bacteria, viruses, fungi, protozoa, and other helminths. Studies have also assessed the specificity of the PCR products by sequence analysis, with all finding 100% sequence homology with the target sequence of *S. stercoralis*. Sitta *et al.* found a number of false positives, using published genus and species-specific primers, based on non-target sized bands on gel electrophoresis. The genus-specific primers amplified sequences that generated non-target bands on electrophoresis in specimens that contained *Blastocystis* and other helminths on microscopy, and the species-specific primers amplified sequences that generated non-target bands on electrophoresis in specimens positive for hookworm on microscopy. Similar accounts of cross-reactivity have not yet been reported, so further data will be useful to monitor the specificity of PCR in different populations.

### Table 1. Sensitivity and specificity of stool PCR for human strongyloidiasis.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Target</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Reference method</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>18S</td>
<td>61.0%</td>
<td>92.4%</td>
<td>Coproculture; Baermann</td>
</tr>
<tr>
<td>32</td>
<td>18S</td>
<td>58.6%/96.6%</td>
<td>ND</td>
<td>McMaster</td>
</tr>
<tr>
<td>26</td>
<td>18S</td>
<td>61.0%</td>
<td>92.7%</td>
<td>APC; Baermann</td>
</tr>
<tr>
<td>34</td>
<td>18S</td>
<td>100%</td>
<td>100%</td>
<td>Direct microscopy</td>
</tr>
<tr>
<td>23</td>
<td>18S</td>
<td>33.0%</td>
<td>99.0%</td>
<td>Harada-Mori</td>
</tr>
<tr>
<td>25</td>
<td>18S</td>
<td>84.8%/78.8%</td>
<td>ND</td>
<td>APC</td>
</tr>
<tr>
<td>10</td>
<td>18S</td>
<td>11.6%</td>
<td>90.6%</td>
<td>Baermann</td>
</tr>
<tr>
<td>29</td>
<td>18S</td>
<td>93.8%</td>
<td>86.5%</td>
<td>FEAC; APC; Harada-Mori</td>
</tr>
<tr>
<td>36</td>
<td>18S</td>
<td>90.0%</td>
<td>85.7%</td>
<td>APC</td>
</tr>
<tr>
<td>27</td>
<td>18S/cox1</td>
<td>100%</td>
<td>91.6%</td>
<td>FEC; APC</td>
</tr>
<tr>
<td></td>
<td>18S</td>
<td>84.7%</td>
<td>95.8%</td>
<td></td>
</tr>
</tbody>
</table>

*The first value relates to a species-specific primer, the second to a genus-specific primer.*

*Nested PCR. APC, agar plate culture; FEAC, formalin-ethyl acetate concentration; FEC, formalin-ether concentration.*
In Focus

**LAMP**

Loop-mediated isothermal amplification (LAMP) is an additional nucleic acid detection method. LAMP uses a DNA polymerase with strand-displacement activity, so it doesn’t require the temperature cycling of PCR, and can be performed with a simple source of constant temperature such as a heating block. LAMP has been successfully applied in resource limited-settings for the detection of pathogens.

The *Strongyloides* LAMP assay uses primers that are genus specific and bind to the 28S rRNA gene. The reaction runs at 60°C for 1 hour. Pre-heating of the reagents and DNA template to 95°C, prior to the addition of enzyme, increases the limit of detection and eliminates the need to pre-heat the template and keep it at 4°C. A novel use of Syto-82 dye (Life Technologies, Carlsbad, CA, USA) enables the detection of positive results in real-time or visually on completion of the reaction.

Analytical sensitivity and specificity are comparable to PCR, according to the method of Verweij et al.

Further validation of the LAMP assay with clinical specimens is currently in progress.

**Conclusion**

The diagnosis of strongyloidiasis can be made through the morphological identification of larvae, usually in the stool, serological testing, and nucleic acid tests. While each methodology has advantages, there are limitations that need to be taken into account when assessing the significance of negative test results. Often the most important aspect of patient management is to consider the possibility of *S. stercoralis* infection.

**References**


Biographies

Matthew Watts is an Infectious Diseases Physician and Clinical Microbiologist based at the Centre for Infectious Diseases and Microbiology, Pathology West-ICPMR and the Marie Bashir Institute, University of Sydney, Westmead Hospital. His interests include parasitic and zoonotic infections.

Gemma Robertson is a final year microbiology trainee at Melbourne Pathology. She has an interest in tropical medicine and parasitology, and will be undertaking a PhD to continue her research into soil-transmitted helminthiases in Aboriginal communities.

Dr Richard Bradbury is an Australian Parasitologist with an interest in all fields of parasitology. He was recently appointed as the Team Lead in the Parasite Diagnostics and Biology Laboratory of the Centers for Disease Control and Prevention in Atlanta, USA. He is writing this work in both his personal capacity and in his capacity as an adjunct academic at Central Queensland University.
Current WHO protocols for mass drug administration in helminth control

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Soil transmitted helminths (STH), comprising *Ascaris*, *Trichuris*, *Strongyloides* and the hookworms remain a significant cause of morbidity amongst people in many parts of the world, including Australia. Other important helminth infections include lymphatic filariasis (LF), schistosomiasis and onchocerciasis. Preventive chemotherapy (mass drug administration [MDA]) campaigns are frequently conducted for these helminth infections in endemic areas, but the target population groups, duration of campaigns, cointerventions (e.g. vector control) criteria for inclusion, drugs used and doses of drugs differ.

The benefits of deworming individuals, especially children, who are infected with soil-transmitted helminths and schistosomiasis, include reduction in anaemia and improved growth. Rarer, but more severe presentations, such as intestinal obstruction with *A. lumbricoides* and rectal prolapse due to *T. trichiura* infection, will also be reduced. Treatment for filariasis and onchocerciasis in childhood will prevent the development of later severe consequences of these diseases including lymphoedema, hydrocoele, elephantiasis and blindness.

In situations of moderate to high endemicity, it has been considered more efficient and cost-effective to treat the entire eligible population in particular age groups or communities for these diseases, rather than first testing individuals to determine who is infected. The goals of such MDA are morbidity control in some cases and interruption of transmission through vectors in others. For these reasons, MDA for STH is usually undertaken for school age children, while for schistosomiasis, MDA is performed either in children or in eligible people of all ages, depending on the endemicity level.

For the insect borne helminths, onchocerciasis and LF, the goal is transmission interruption and the entire community is eligible for MDA.

The frequency of MDA for STH in school-age children is dependent on the prevalence of infections in a given population (Table 1). Current WHO protocols for STH control recommend MDA with a single oral dose of albendazole (400 mg), mebendazole (500 mg) or levamisole (80 mg). Mebendazole is more effective than albendazole for *T. trichiura*, whilst albendazole is slightly more effective against hookworm than mebendazole. The two drugs have equally

<table>
<thead>
<tr>
<th>Prevalence</th>
<th>Regularity of MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥50%</td>
<td>Twice per year, or every 4 months if at the high end of prevalence</td>
</tr>
<tr>
<td>20 and &lt;50%</td>
<td>Once yearly</td>
</tr>
<tr>
<td>&lt;20%</td>
<td>Treat on case-by-case basis</td>
</tr>
</tbody>
</table>

Table 1. Current recommended regularity of mass drug administration (MDA) for helminth (STH) infections in school-age children (adapted from WHO 2011).

For control of schistosomiasis

<table>
<thead>
<tr>
<th>Prevalence</th>
<th>Regularity of MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥50%</td>
<td>Once per year, or every 4 months if at the high end of prevalence</td>
</tr>
<tr>
<td>≥10 and &lt;50%</td>
<td>Once every 2 years</td>
</tr>
<tr>
<td>&lt;10%</td>
<td>Treat on case-by-case basis</td>
</tr>
</tbody>
</table>

*Usually defined as children between 5 and 14 years of age.
*As determined by parasitological methods; cut-off is ≥30% if based only on questionnaires for visible haematuria.
high efficacy when used against *Ascaris lumbricoides*. Despite these differences, in practice when administered biennially over a number of years, either drug used on its own is effective for overall STH control.

Onchocerciasis control and/or elimination requires annual or biannual treatment with ivermectin for many years (up to 20 in some cases), while lymphatic filariasis uses annual administration with albendazole and either ivermectin or diethylcarbamazine (DEC) with at least 65% population coverage for at least five years. Thus ivermectin and/or albendazole administration may occur in some communities annually or biannually as part of onchocerciasis or LF elimination programs. Where this occurs, STH control programs should be harmonised to ensure that there is a 6 month delay between albendazole administrations. In communities with endemic schistosomiasis, the addition of praziquantel (40 mg/kg) is recommended (Table 1). Reductions in the frequency of MDA may be considered after 5–6 years of consistent >75% population coverage, after testing of the residual prevalence of helminths in that population. Such a decision is based on several factors, specific details of which may be found in the World Health Organization guide for managers of control programmes.

Reliance on albendazole and mebendazole in WHO recommendations for MDA will result in a lower impact on the clearance of *Strongyloides stercoralis*, for which ivermectin and thiabendazole are more effective drugs. Thiabendazole was discontinued in Australia in 2003 and due to the lower rate of side effects, ivermectin has been recommended by some as the treatment of choice. Due to the auto-infective cycle of this helminth, some authors have recommended re-treatment at one and two months to ensure elimination. Only one randomised trial has been performed thus far, in which treatment twice at 2 weeks apart was found to have no greater benefit than a single dose. Both the authors of this paper and others recommend further studies into the optimal dose schedules for ivermectin in the control of Strongyloidiasis within a larger cohort of participants.

Recently, there has been discussion about the outcomes and optimal age range for MDA programs to control STH and more evidence on the impacts is required; this controversy is outside the scope of this review. A novel concept of elimination of STH by MDA ‘one village at a time’ rather than by wide-scale MDA has been proposed for remote areas with low populations, such as many remote Australian Aboriginal communities. This approach is currently being trialled in a remote area of the Solomon Islands. It advocates allowing individual communities to act as autonomous units and to employ control options specifically tailored to the geographic, cultural, economic, aetiological and environmental factors influencing STH transmission in their own community (Figure 1).

*Figure 1. A Solomon Islander researcher undertaking a community wide STH prevalence survey as part of an integrated STH control program (photograph by Richard Bradbury).*

Deworming of school-age children has been a mainstay of helmint control for many years. Discussion continues on the optimal method of MDA for this purpose. In some cases, such as where high rates of strongyloidiasis or onchocerciasis are present, the addition of other drugs may be warranted. Further consideration of new therapies, combination therapies, the reconsideration of use of the use of ‘old’ anti-helmintic therapies have all been postulated as mechanisms by which to improve absolute cure rates in MDA programs and to reduce the possible development of antihelmintic resistance. Ways to improve MDA participation, as well as paediatric formulations of praziquantel for schistosomiasis prevention in preschool children may also be added to this list. The current WHO protocols for MDA provide an important baseline guide to those undertaking MDA for helmint control in endemic areas.

**References**

Zoonotic tissue parasites of Australian wildlife

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Increasing use of bushlands for recreational, commercial and scientific activities fosters movement across the urban-bushland interface. This may facilitate the transmission of parasitic diseases from wildlife to humans (zoonoses). The fashionable trend to consumption of game meats such as feral pig and crocodile, and raw fish such as sushi, sashimi and pickled herring has exacerbated the zoonotic potential of parasites of wildlife.

**Transmission from wildlife to humans**

**Angiostrongyliasis**

Angiostrongyulus cantonensis is a nematode parasite of the pulmonary arteries and right ventricle of Rattus rattus and R. norvegicus in Australia. It is the causative agent of eosinophilic meningoencephalitis, a zoonotic infection of humans. The life cycle includes an obligatory period of larval development in terrestrial or aquatic snails and slugs, and also may involve a range of paratenic or transport hosts (freshwater prawns, land crabs, planarians, frogs, lizards), which feed on gastropods. Rats become infected by ingesting intermediate or paratenic hosts. In the rat, the nematode undergoes an obligatory migration through the spinal column and brain en route to the final site in the pulmonary arteries of the lungs. Humans become infected by accidentally or deliberately eating infected gastropods or paratenic hosts, or unwashed salad greens containing these. The parasite has been reported from domestic and zoo animals, mammalian and avian wildlife and humans in Brisbane and Sydney. The clinical signs of headache, vomiting, paralysis and sometimes death are induced as a consequence of the obligatory period of development of the parasite in the central nervous system. This occurs in young children who deliberately or accidentally ingest snails or slugs containing infective larvae, or foolish young adults who do so for a bet.

**Muspiceoidosis**

Haycocknema perplexum is a minute muspiceoid nematode living as adults inside individual skeletal muscle cells of humans in Australia. Eight cases have been documented, four in Tasmania and four in north Queensland. Gasser (personal communication). Eight to twelve eggs hatch inside the uterus of the female, develop to third-stage infective larvae and burst from the head region killing the adult, an efficient mechanism for auto-re-infection. Escaped larvae invade uninfected muscle cells. The occurrence of H. perplexum in intramyofibres results in eosinophilic polymyositis but no reaction within the invaded cell itself. Progressive myopathy occurs and infection becomes life threatening. Early human diagnosis by muscle biopsy is imperative in cases of progressive myopathy associated with blood eosinophilia and elevated creatine kinase levels. Steroid
treatment of patients exacerbates their infections to a life-threatening illness and may delay diagnosis by masking key diagnostic features. Treatment with albendazole, 400 mg twice daily for 8–9 weeks, is recommended. *Haycocknema perplexum* is considered a zoonosis although the source of infection of humans – water, soil, plants or animals – remains unknown.

**Halicephalobus gingivalis**

*Halicephalobus gingivalis* formerly known as *Micronema deletrix*, is a free-living nematode of soil, manure and decaying humus known to cause opportunistic infections, primarily in horses but also in humans. The majority of cases in horses have been fatal and usually not diagnosed before necropsy. All human cases have involved fatal meningoencephalitis including the first human case in Australia, a 74-year-old woman from Eyre Peninsula, South Australia. In tissues, only ova, larvae and adult females are seen, reproduction in the parasitic phase presumed to be by parthenogenesis. It is not known how *H. gingivalis* infects humans or horses although exposure through an oromaxillary route may explain common neurological involvement.

**Transmission from domestic animals to humans and wildlife**

**Toxoplasmosis**

Felids, domestic cats in particular, are the only definitive host of the obligate intracellular protozoan parasite, *Toxoplasma gondii*. Most species of mammals and birds are susceptible to infection and may act as intermediate hosts. Infection is usually systemic resulting in a short period of rapid multiplication in various tissues followed by the establishment of tissue cysts in the muscles and brain. These are transmitted only if ingested by predation or scavenging or if passed vertically across the placenta from mother to foetus.

The localisation of *T. gondii* cysts in the forebrain of rats and mice together with the immune reaction to the cysts is related to altered behaviour, in particular the attenuation of predator odour aversion and anxiety. This facilitates ingestion of the intermediate host by the cat definitive host and perpetuation of the life cycle.

Humans become infected with *T. gondii* mainly by ingesting uncooked meat containing viable tissue cysts or by ingesting food or water contaminated with oocysts from the faeces of infected cats. Transplacental transmission may occur in women during their first trimester of pregnancy and this infection is then passed to the developing embryo via the placenta. This frequently results in severe brain and eye lesions in the newborn or death of the developing embryo. Research results from the past 15 years have shown that *T. gondii* infection is associated with several neuropsychiatric diseases and behavioural changes in humans as well as animals. Although the mechanisms are unknown a growing body of data indicates that they are complex, comprising humoral, immune, neurotransmitter, epigenetic, genetic, and structural effects.

**Echinococcosis (hydatid disease)**

Australia has, on average, >80 new cases of human hydatidosis per annum caused by the larval stage of the cestode, *Echinococcus granulosus*. The parasite was introduced into Australia with domestic livestock and dogs. However, a cycle in wildlife is maintained through a predator/prey interaction between dingoes, wild dogs and less importantly foxes and wallabies, less importantly feral pigs (*Sus scrofa*). The establishment of a dingo/wild dog-macropod cycle, which effectively maintains parasite transmission, acts as a spill-back reservoir of infection for sheep and cattle. This is a major problem for control strategies focussed on human education and husbandry practices to break the domestic ‘dog-sheep’ cycle. In contrast to the situation in livestock, hydatid cysts occur primarily in the lungs rather than the liver of marsupials. Infection occurs predominantly in the eastern States but the parasite has established recently in wildlife in water catchment and forestry areas outside Perth. Western grey kangaroos and feral pigs act as intermediate hosts. This focus of transmission may have been initiated through *E. granulosus*-infected domestic pig hunting dogs.
from the eastern states. Transmission appears to be perpetuated by
dogs of local pig hunters infected through being fed the offal of
locally shot kangaroos.21 Hydatid disease is also an important
conservation issue, especially for small endangered species and
populations of Macropodidae by severely reducing effective lung
volume.24,25 Such reductions impact the fitness of the animals
enhancing susceptibility to predation, thus ensuring perpetuation
of the cycle.

Several potential zoonotic tissue parasite infections in Australia are
listed in Table 1.

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Infect. Dis. 57, 1158–1161. doi:10.1093/cid/cit444

Biography

Dave Spratt is an Honorary Fellow at the Australian National
Wildlife Collection, National Research Collections Australia, CSIRO,
in Canberra. The major themes of Dr Spratt’s research are the study
and understanding of the diseases of wildlife including disease
ecology, parasitic taxonomy, helminth biodiversity and zoonoses.
Research topics of interest include metastastrongyloid, filarioid, trichi-
nelloid and muspiceoid nematodes, pentastomes, and small mam-
mal succession and recolonisation of their helminth communities
following wildfire.
Assessing enteric helminths in refugees, asylum seekers and new migrants

Currently there are 59.5 million people forcibly displaced worldwide as a result of conflict, human rights violations, generalised violence or persecution. Of these, 19.5 million are refugees and 1.8 million are asylum seekers. Each year Australia accepts 13,750 refugees through the offshore Humanitarian program, and in 2016 that number will almost double with the addition of 12,000 refugees from Syria and Iraq. Many refugees have complex medical needs and have reached Australia after a difficult journey, often involving time in refugee camps and exposure to traumatic events including physical hardship and illness. Refugees often come from parts of the world where parasitic and tropical infectious diseases are prevalent and untreated. This article provides a review of enteric helminth infections in refugees, including asylum seekers and those from a refugee-like background.

Parasitic infections in refugees and new migrants reflect the underlying epidemiology of parasites in areas where refugees may have been exposed, including the country of origin, migration journey to Australia, and place of detention. Factors such as poverty, disruption of basic services, poor sanitation/hygiene (e.g. quality of drinking water, access to running water, access to footwear) and insufficient access to adequate health care and treatment may significantly increase the risk of exposure to intestinal parasitic disease in the refugee population. Other practices such as the use of night-soil as fertiliser, dietary habits, and past occupational exposures are likely to play an important role in increasing the burden of disease. Soil transmitted helminth (STH) infections are very common in those living in resource constrained settings. Treatment of refugees and asylum seekers is often empirical, in refugee camps, before departure as part of the pre-departure health check, or after arrival in Australia. More serious infections, such as strongyloidiasis, schistosomiasis, opisthorchus and Taenia solium, require diagnosis and specific treatment. Table 1 summarises the findings of recent prevalence studies of strongyloidiasis and schistosomiasis in refugee groups from Australia and overseas.

Strongyloidiasis

The highest prevalence of Strongyloides stercoralis infection occurs in refugees from Africa and South-East Asia. Of those arriving in the last decade, the Burmese groups (e.g. Karen, Chin) have the highest prevalence (26.0%). Earlier data show an even
higher prevalence in Lao and Cambodian refugees. Infections may persist for decades after leaving an endemic area due to a continual cycle of auto-infection. Although many patients are asymptomatic or have minimal clinical symptoms, they remain at risk for subsequent hyperinfection if immunosuppressed. Strongyloides antibodies decline after effective treatment.

### Schistosomiasis

The highest prevalence of schistosomiasis is found in Africa, accounting for an estimated 95% of global cases. Species involved are *S. mansoni*, *S. haematobium* and *S. intercalatum*. *S. japonicum* is present along the Yangtze River in China and the Philippines. *S. mekongi* is found in the Mekong river valley. Schistosoma infection may also be encountered in other areas such as parts of Indonesia, the Caribbean, the Arabian Peninsula, Madagascar, the Middle east and Turkey. Prevalence of schistosomiasis infection in refugees in Australia, as determined by serology, has been shown to range from 5.4% in Burmese to 37% in Africans.

Schistosoma antibodies are thought to persist in those from endemic areas despite prior treatment. A long-term study of schistosomiasis serology post-treatment showed an immediate increase in titre and then a fourfold decline in most travellers after 6–12 months.

### Table 1. Prevalence of strongyloidiasis and schistosomiasis in refugee and asylum seeker groups.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Sample size</th>
<th>Country/region of origin</th>
<th>Country of settlement</th>
<th>Prevalence of Schistosomiasis</th>
<th>Prevalence of Strongyloides</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>International studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>700</td>
<td>Latin America, Sub-Saharan Africa</td>
<td>Spain</td>
<td>5.9%</td>
<td>56.1%</td>
</tr>
<tr>
<td>22</td>
<td>1063</td>
<td>Europe, Eastern Mediterranean, Africa</td>
<td>Canada</td>
<td>15%</td>
<td>3%</td>
</tr>
<tr>
<td>23</td>
<td>208</td>
<td>Brazil</td>
<td>USA</td>
<td>27.7%</td>
<td>5.8%</td>
</tr>
<tr>
<td>24</td>
<td>350</td>
<td>Africa (46%), Asia (28.6%)</td>
<td>Canada</td>
<td>N/A</td>
<td>4.6%</td>
</tr>
<tr>
<td>16</td>
<td>1376</td>
<td>Middle East, Africa, Asia</td>
<td>USA</td>
<td>0.8% (Africa)</td>
<td>2.0% (SE Asia) 2.5% (Africa)</td>
</tr>
<tr>
<td>20</td>
<td>176</td>
<td>Southeast Asia (59%), Africa (27%), Middle East (14%)</td>
<td>USA</td>
<td>8%</td>
<td>24%</td>
</tr>
<tr>
<td><strong>Australian studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>1136</td>
<td>Burma (Karen refugees)</td>
<td>Australia</td>
<td>7%</td>
<td>20.8%</td>
</tr>
<tr>
<td>21</td>
<td>187</td>
<td>Asia</td>
<td>Australia</td>
<td>17%</td>
<td>5.7%</td>
</tr>
<tr>
<td>27</td>
<td>182</td>
<td>Africa</td>
<td>Australia</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>7</td>
<td>234</td>
<td>Cambodia</td>
<td>Australia</td>
<td>N/A</td>
<td>35%</td>
</tr>
<tr>
<td>9</td>
<td>156</td>
<td>Burma</td>
<td>Australia</td>
<td>5.4%</td>
<td>26%</td>
</tr>
<tr>
<td>10</td>
<td>239</td>
<td>Africa</td>
<td>Australia</td>
<td>37%</td>
<td>–</td>
</tr>
<tr>
<td>28</td>
<td>258</td>
<td>Sudan, Liberia</td>
<td>Australia</td>
<td>12%</td>
<td>9%</td>
</tr>
<tr>
<td>3</td>
<td>361</td>
<td>Cambodia, East Africa</td>
<td>Australia</td>
<td>11% East African</td>
<td>2% East African 42% Cambodian</td>
</tr>
<tr>
<td>29</td>
<td>135</td>
<td>East Africa</td>
<td>Australia</td>
<td>2%</td>
<td>11%</td>
</tr>
<tr>
<td>5</td>
<td>95</td>
<td>Laos</td>
<td>Australia</td>
<td>N/A</td>
<td>23%</td>
</tr>
</tbody>
</table>

N/A, not available.
However, for immigrants, serology remained elevated even three years after effective treatment in a proportion of patients.11

Soil-transmitted helminths (STH)
Many refugees will have received empirical albendazole as part of the predeparture medical assessment conducted by the International Organisation of Migration on behalf of the Australian Government. This has significantly altered the prevalence and patterns of intestinal helminths in refugees.12 However, albendazole has limited effectiveness against Trichuris trichiura, and is not an effective treatment for some less common helminths found in refugees and asylum seekers (see below).

Less common helminth infections
The majority of Asian refugees currently entering Australia are from Myanmar, from camps on the Thai border. Within Thailand, especially the north east, Opisthorchis viverrini is highly prevalent. Infection results from consumption of raw, uncooked or fermented fish containing metacerciae. Long-term infection may cause cholangitis, obstructive jaundice, cholecystitis, periductal fibrosis and bile duct cancer, contributing to a liver cancer rate in excess of 70 per 100,000 in NE Thailand. There is a paucity of data on faecal microscopy findings for refugees from Myanmar. However, the presence of vector cyprinoid fish and substantial wetlands suggests that infection with Opisthorchis viverrini is also likely.

Other serious infections in refugees from Asia include Taenia solium (pork tapeworm) with the potential risk of cysticercosis for both patient and household members. In Thailand faecal tests for helminth eggs have revealed a prevalence of Taenia spp of 2.3–3.7% in communities with high migrant populations along the Thai border. In the remote western border area of Kanchanaburi three species of tapeworm T. saginata, T. solium and T. asiatica co-exist in the human population.13

Considerations in the Syrian refugee population
In September 2015, it was announced that 12,000 Syrian and Iraqi refugees would be accepted to Australia as part of the Humanitarian Program during 2016–17. The recent prevalence of enteric parasite infections is not well documented in Syria. However, schistosomiasis is considered to have low endemicity in Iraq (0.1%) and Syria (<10% prevalence in 2010).14 There is no information available on the prevalence of S. stercoralis in Syria; however, a hospital-based survey in Iraq reported a prevalence of 24.2%15. Chang et al. reported a low prevalence of other parasitic infections in refugees from Iran and Iraq.16

Diagnosis
In Australia, the number of faecal microscopy tests performed has fallen in some States (e.g. NSW), with more emphasis being placed on empirical treatment of STH and the use of serology for the diagnosis of S. stercoralis or Schistosoma spp. Current recommended diagnostic tests for enteric parasites are shown in Table 2.

Challenges and limitations of testing for schistosoma and strongyloides infections in a refugee population
Serology may overestimate the prevalence of disease due to cross-reactivity with other nematode infections and there is difficulty distinguishing recent from past (and cured) infections. Serological titres (OD values) in the equivocal and low positive ranges are difficult to interpret. Follow-up serology should preferably be done in the same laboratory and in parallel with previous specimens where available. The interpretation of Schistosoma serology in

<table>
<thead>
<tr>
<th>Enteric parasite</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strongyloides stercoralis</td>
<td>Positive serology</td>
</tr>
<tr>
<td></td>
<td>Stool microscopy should be performed to rule out other enteric infections</td>
</tr>
<tr>
<td>Schistosoma spp.</td>
<td>Positive serology</td>
</tr>
<tr>
<td></td>
<td>Stool/urine microscopy for ova should be performed in those with positive serology</td>
</tr>
<tr>
<td>Hookworm (Necator americanus and Ancylostoma duodenale)</td>
<td>Microscopic finding of ova in faecal specimens</td>
</tr>
<tr>
<td></td>
<td>Concentration methods are necessary to detect light infections</td>
</tr>
<tr>
<td>Ascaris lumbricoides</td>
<td>Microscopic identification of ova in faecal specimens</td>
</tr>
<tr>
<td>Trichuris trichiura</td>
<td>Microscopic identification of ova in faecal specimens</td>
</tr>
<tr>
<td>Taenia spp.</td>
<td>Microscopic identification of ova and proglottids in faecal specimens</td>
</tr>
</tbody>
</table>
Australia presents several challenges as test methodology varies between laboratories. VIDRL in Melbourne, use the Fumouze indirect haemagglutination test (IHA). This is based on an antigen derived from adult worms of *S. mansoni*. Estimates in one comparison study show that the sensitivity of this test is 76.2% for *Schistosoma mansoni*, and slightly lower for *S. haematobium*, with a specificity of 99%17. However, in NSW at ICPMR, an ‘in house’ ELISA assay is the preferred assay used, based on *S. mansoni* egg antigen. Two commercial assays based on the use of a similar antigen showed 71.4% sensitivity but reduced specificity of 76.9–88.4%. Both ELISA assays showed cross-reactivity with cestode, nematode and trematode infections17. The sensitivity of these assays for other species of Schistosoma, such as *S. baematobium*, *S. intercalatum*, *S. mekongi* and *S. japonicum*, is not specified; however, it is likely to be reduced.

The sensitivity and specificity of Strongyloides stercoralis serology is reported to be up to 94.6% and 99.6% respectively, depending on the assay used16. However, as there is no gold standard test for comparison, these are estimations only. Serological titres decline with effective treatment over a 12 month period7,18.

**Persistence of parasitic infections in refugee populations**

Several studies have demonstrated that serious intestinal parasitic infections may persist for many years after arrival in Australia. A 2002 study of Laotian refugees who had arrived in Australia during 1974–91 showed that strongyloides serology was positive in 24% and 3 carried Opisthorchis, compared to respective prevalences of 19.2% and 41%, on initial screening by faecal microscopy3. As liver flukes survive for approximately 7–10 years, the three cases of Opisthorchis identified may well represent reinfection on subsequent visits to Laos.

In a second study of East African refugees, who arrived in Australia in the late 90s, screening for strongyloides and schistosoma serology was positive in 11% and 15%, respectively, of patients some 16 years later in 20063. In a further aspect of the same study, 42% of 234 Cambodians who had arrived in the late 80s still tested positive for strongyloides serology3. This compared with 7.8% of Cambodians who were found to be positive for strongyloides by microscopy at initial health screening16.

**Post arrival health assessment for refugees and asylum seekers**

Community Health Centres and GP services in suburbs with high rates of migrant and refugee settlement are now responsible for much of the post-arrival refugee health screening, with support from specialist Refugee Health Services. Guidelines for post-arrival assessment for people of refugee-like background were published by the Australian Society for Infectious Diseases in 2009 and are currently being revised19. If possible, a full health assessment of new arrivals is ideally conducted within one month of arrival in Australia, including serology for Strongyloides for all, and Schistosoma in those who have lived or travelled through an endemic area. For many of the clinics in suburbs with high migrant populations, the special pathology requirements are met by private pathology providers.

**Summary**

Intestinal helminth infections in refugees are common and should remain a high priority for health workers. These populations often have specific needs that should be considered in diagnosis and management of these infections. Burden of disease is likely to reflect the country of origin, journey of migration to Australia, pre-departure treatment and place of detention. Other socio-economic and cultural factors are also likely to play a significant role in exposure risk. Helminth infections may be chronic and persist in humans for more than four decades resulting in serious morbidity and mortality and highlighting the need for early diagnosis.

**Acknowledgements**

We thank Ms Christalla Hajisava for technical assistance.

**References**


Biographies

Dr Sarah Hanieh is a paediatric infectious diseases physician and NHMRC Early Career Research Fellow in the Immigrant and International Health Group, at the Peter Doherty Institute for Infection and Immunity, The University of Melbourne. She has interests in child nutrition, infectious diseases and refugee health. Her current research aims to understand the nutritional and infectious causes of impaired child growth and development in resource poor settings.

Norbert Ryan is a Senior Scientist in the Bacteriology Laboratory at Victorian Infectious Diseases Reference Laboratory, Doherty Institute, Melbourne. Areas of interest include parasitology, diagnosis of Legionnaires’ disease and sexually transmitted infections. The use of staining methods for diagnosis of protozoal infections including microsporidia has been a specialty field. He was involved in processing of specimens during the systematic health screening of refugee and family reunion migrant groups conducted by the Victorian Department of health during the late 80s-early 90s.

Professor Beverley-Ann Biggs heads the International and Immigrant Health Group in the Department of Medicine, The University of Melbourne and is an Infectious Diseases Physician in the Victorian Infectious Diseases Service at the Royal Melbourne Hospital. She has a special interest in parasitic and other infectious diseases in refugees and immigrants living in Australia, and has published extensively in this area.

We invite you to attend the 9th International Wolbachia Conference to be held at the O’Reilly’s Rainforest Retreat, Lamington Plateau, Queensland.

We are pleased to advise that the Call for Symposia is now open on the Wolbachia website. If you would like to suggest a symposium please complete the form at wolbachia2016.org. The deadline for submissions has been extended to Monday 15 February 2016. The Call for Abstracts is also available on the website. The deadline for receipt of abstracts is 15 April.

If you have not already completed an Expression of Interest, we encourage you to complete the form on the website.

Best wishes, we look forward to meeting you all at O’Reilly’s in June 2016!

wolbachia2016.org
Free living amoebae and human disease

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Pathogenic FLA are ubiquitous protozoans and despite frequent human contact remain a rare cause of often devastating infection with poor prognosis. Given changes in climate, human encroachment into the environment, increasing immunosuppression, and improving diagnostic capacity, it is likely we will see increased cases in the future. Early diagnosis is challenging but crucial to achieving a favourable outcome. It is best facilitated by improved awareness of FLA disease, appropriate clinical suspicion and early diagnostic testing.

Free living amoebae (FLA) are a cosmopolitan group of protozoan organisms that do not require a host to survive. Despite their ubiquitous nature, these organisms are uncommon human pathogens. However, four genera contain species known to cause invasive disease in humans: *Acanthamoeba*, *Naegleria*, *Balamuthia* and *Sappinia*. *Acanthamoeba* infections may present as granulomatous amoebic encephalitis (GAE), disseminated disease (e.g. cutaneous, sinus or pulmonary infection) or keratitis, with *Balamuthia mandrillaris* causing similar cutaneous infections and GAE. *Naegleria fowleri* is responsible for the rapidly progressive primary amoebic meningoencephalitis (PAM). In addition, a single case of human central nervous system (CNS) infection with *Sappinia pedata* has been reported¹, along with isolated cases of corneal infection with *Vahlkampfia* spp., *Hartmannella* spp. and *Paravahlkampfia* spp.². This review aims to provide an overview of human disease caused by the three most common genera involved, *Acanthamoeba*, *Naegleria* and *Balamuthia*, including their laboratory diagnosis.

**Epidemiology**

Pathogenic FLA are found worldwide and serological studies suggest human exposure is common³–⁵. Three cases of *Acanthamoeba* GAE have been described in Australia⁶–⁸. *Acanthamoeba* sp tolerate a wide range of temperature, pH and osmolarity⁹ and may be found in air, soil and water samples. They are one of the most commonly isolated FLA in the environment¹⁰, and the most common in human infection⁹. The strongest risk factor for GAE or disseminated infection is immunodeficiency, while keratitis most commonly affects immunocompetent contact lens wearers who often have a history of poor lens hygiene. Exposure is thought to occur by inhalation, mucosal contact or direct inoculation¹⁰. *Acanthamoeba* species were previously classified based on morphology, but are now grouped into 17 genotypes based on 18S rRNA sequencing, with the majority of pathogenic species belonging to the T4 genotype⁹,¹¹.

*Naegleria fowleri* may be found in rivers, lakes and soil but does not survive in sea water. As thermophiles, their presence in fresh water is related to temperature and they may even be recovered from thermally polluted waters at high latitudes¹². Human exposure occurs through contact with intact or disrupted nasal mucosa, commonly through recreational or nasal ablution practices¹³ and contaminated drinking water has been implicated as a source of infection in some cases¹⁴. Australia has featured prominently in the history of *Naegleria fowleri* infection, with cases described in Queensland, New South Wales and Western Australia following the first description of the disease in 1965 by two South Australian pathologists, Fowler and Carter¹⁵,¹⁶. This discovery was related to an outbreak of 20 cases, attributed to a contaminated overland water pipeline which reached optimal temperatures for *Naegleria* proliferation during the summer months. Cases continued from 1947 to 1972 when public health measures including adequate chlorination were applied¹⁷. Several cases in Western Australia have also been associated with overland water pipelines, and Australian drinking water guidelines suggest a *Naegleria* monitoring and response protocol for water supplies that seasonally exceed 30°C, or 25°C continually¹⁸. Today, despite significant advancement in
knowledge regarding risk reduction measures, the incidence of infection with *Naegleria fowleri* appears to be increasing worldwide, with factors such as global warming, substandard water management and sanitation, and changing recreational practices likely involved.

*Balamuthia mandrillaris* is most commonly found in soil, though it may also be recovered from water samples. Infection tends to occur in immunocompetent individuals, most commonly children, probably through inhalation or nasal or cutaneous inoculation. Transmission via organ transplantation has also been described. Six human cases have been described in Australia, Tasmania, Victoria and Queensland, with another being described in a Victorian dog.

**Clinical manifestations**

*Acanthamoeba* sp.

The predominant clinical manifestations of *Acanthamoeba* infection are disseminated disease (e.g. cutaneous, nasopharyngeal or pulmonary infection), GAE and keratitis. With the exception of rare case reports, GAE and disseminated disease occur in the immunocompromised or debilitated. The incubation period is unknown, but thought to be weeks to months in duration. Cutaneous infection usually begins as fibronodular lesions, which progress to non-healing ulcerated lesions over time. While GAE may be fatal within days of symptom onset, it generally assumes a more chronic course, with slow progression over weeks to months. Clinical features of GAE are myriad and include fever, symptoms of meningism, personality and mental status change and, later, focal neurological deficits, coma and death. Imaging of the brain may show single or multiple space occupying lesions which can be ring enhancing.

*Acanthamoeba* keratitis is usually a disease of immunocompetent patients, with the strongest risk factor being contact lens use and poor lens hygiene. The disease is usually unilateral, with symptoms including lacrimation, pain, photophobia and foreign body sensation. Signs include a typical corneal ring infiltrate, stromal infiltrates, epitheliopathy and hypopyon. In the absence of effective therapy, it may progress to corneal perforation and loss of vision. The clinical diagnosis of AK can be difficult, as lesions may resemble bacterial or fungal disease, or the dendritic ulcer of HSV infection. Further- more, the clinical course may be characterised by periods of temporary remission, leading to false impressions of response to antibacterial or viral agents.

*Naegleria fowleri*

*Naegleria fowleri* causes primary amoebic meningoencephalitis. Symptoms generally occur 2–5 days after exposure and may begin with changes in taste or smell, followed by fever, nausea, vomiting, photophobia and headache. The disease is fulminant, with rapid progression to coma and death.

**Balamuthia mandrillaris**

*Balamuthia mandrillaris* causes GAE in immunocompromised and immunocompetent individuals. The onset of meningoencephalitis is often subacute or chronic, with symptoms developing over a period of 2 weeks to 2 years. It also has the propensity to cause cutaneous lesions that may precede CNS involvement and are similar in appearance to those of *Acanthamoeba* sp. These lesions appear as poorly defined plaques and may be single or with bordering satellite lesions. They often involve the central face and appear to be more common in South America. Cutaneous disease generally progresses to CNS involvement; however, it may resolve with therapy.

**Laboratory diagnosis**

Diagnosis of FLA infection, particularly systemic disease, is challenging: it may masquerade as bacterial or viral infection, exposure events may not be apparent and specialised diagnostic testing availability is limited. Unfortunately as a result, many CNS infections are diagnosed post-mortem. Successful early diagnosis depends on appropriate clinical suspicion and collection of suitable diagnostic material, usually tissue or CSF.

**Microscopy**

CSF samples in cases of PAM appear purulent, with no bacteria evident on gram stain, a polymorphonuclear pleocytosis, elevated protein and decreased glucose. Wet preparations may show motile *Naegleria fowleri* trophozoites (Figure 1a), as there are usually large numbers of organisms in the CSF. These findings contrast with those of GAE caused by *Acanthamoeba* or *Balamuthia*. While CSF from cases of GAE also demonstrates elevated protein and lowered glucose, these changes are more modest, and a mononuclear, rather than polymorphonuclear inflammatory response is seen. Furthermore, *Acanthamoeba* or *Balamuthia* trophozoites are not typically seen in CSF preparations.

Trophozoites from all pathogenic FLA species can be difficult to differentiate from host inflammatory cells, especially in stained tissue sections. The nuclear characteristics of amoeoba can be helpful in differentiating these parasites from host cells, with *Naegleria fowleri* possessing a nucleus with a large, round, central nucleolus and *Acanthamoeba* and *Balamuthia* (Figure 1b) a rounded nucleus with large, central nucleolus forming a halo. Polyclonal and monoclonal antibodies, with a secondary detecting fluorescent anti-IgG antibody (such as FITC), may be used to identify and
differentiate each of these amoebae in tissue specimens (as can molecular methods), though availability is limited to the CDC, Atlanta, USA.

In *Acanthamoeba* keratitis, a diagnosis may be made by demonstrating trophozoites and/or cysts in corneal samples. It is possible to directly identify *Acanthamoeba* trophozoites within the cornea using confocal microscopy and in experienced hands this technique is sensitive and specific. Trophozoites and cysts may be revealed by staining the smear with H&E or Giemsa, while cysts are also readily identified using PAS and fluorescent stains, such as calcofluor white and acridine orange. On occasion, non-specific fluorescence or binding to fungi in mixed infections can lead to diagnostic errors, especially when used by inexperienced microscopists.

**Culture**

Samples intended for amoebic culture should be kept at room temperature and processed as quickly as possible. Freezing should be avoided, particularly for samples where *Naegleria* is suspected (the cyst stage is more fragile), as this compromises organism viability. *Naegleria fowleri* and *Acanthamoeba* sp. (Figure 1c) can be readily cultured using non-nutrient media containing live or killed non-mucoid bacteria (usually *E. coli* or *Enterobacter* sp.) as a food source. *Acanthamoeba* and *Naegleria* will cover the agar surface in 1–2 days when incubated at 37°C and their presence can be confirmed by examination of the plate with a plate microscope, or by performing microscopy of a wet mount from the agar plate. *Balamuthia* do not appear to use bacteria as a food source and therefore cannot be cultivated in the same fashion. They may be successfully cultured using axenic and tissue culture methods, however with generation times of around 25 h, culture is a lengthy process and not part of routine diagnostic testing.

**Nucleic acid testing**

The use of molecular testing to diagnose and confirm infections with FLA has transformed diagnostics in this area. It allows more rapid diagnosis, with greater sensitivity than other methods and reduces the requirement for specialist, experienced staff to discern subtle microscopic features. Most molecular assays use ribosomal genes, such as the 18S rRNA or ITS repeat regions, as targets for PCR. However, as requests are infrequent, these tests are generally only offered by reference or research laboratories. Of particular note is a multiplex PCR, described by Qvarnstrom *et al*. This assay uses 18S rRNA primer/probe sets to accurately identify *Acanthamoeba* to the genus level and *Naegleria fowleri* and *Balamuthia mandrillaris* species. The sensitivity is reported at one amoeba per sample. Sullivan Nicolaides Pathology instituted

![Figure 1. (a) Naegleria trophozoite in CSF wet prep (x400). (b) Balamuthia trophozoite in brain tissue (haematoxylin and eosin). (c) Acanthamoeba cysts in culture, wet prep (x400).](image)

<table>
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*aDuplicates excluded (same patient and site, within 2 months). CNS, central nervous system.*
this test in 2010 and our experience is summarised in Table 1. As a significant number of ocular specimens have been tested against all three targets, a large number of negatives for *Naegleria fowleri* and *Balamuthia mandrillaris* are expected. It is also notable that we have recently reported a probable false negative *Acanthamoeba* result. The referred CSF specimen tested negative at our laboratory, but positive at the CDC (using the same multiplex PCR). It is possible the small volume of CSF received at our laboratory contributed to this result (only 100 μL was received, where 200 μL is the standard volume for extraction).

**Treatment and prognosis**

**Primary amoebic meningitis**

There have been few survivors of primary amoebic meningitis and the factors that have resulted in successful therapy are poorly defined, though early diagnosis and institution of therapy appears critical. The treatment of choice is the antifungal amphotericin B, which is often used both intravenously and intrathecally. Other agents used in survivors include miltefosine, sulfisoxazole, fluconazole, miconazole and rifampicin.

**Granulomatous amoebic encephalitis**

*Acanthamoeba*

Suboptimal efficacy of antimicrobial agents, the high morbidity of patients affected and tendency to late diagnosis contribute to a poor prognosis in *Acanthamoeba* GAE, with a mortality rate of >90%.

In the few reported survivors of GAE or cutaneous infection, most have received combination therapy. The agents used have included trimethoprim-sulfamethoxazole, miltefosine, thioridazine, amphotericin B, albendazole and trimethoprim-sulfamethoxazole. Miltefosine, in particular, has demonstrated amoebicidal activity *in vitro* and its inclusion in combination regimens may offer a survival advantage.

**Balamuthia**

Early recognition of cutaneous disease is critical to allow early therapy and prevent progression to CNS infection. Unfortunately, the overall prognosis in *Balamuthia* CNS infection is extremely poor. However, there are several case reports of survival in the literature, including an Australian case.

These cases have received varied combination therapy regimens, with agents including pentamidine, flucytosine, fluconazole, macrodides, sulfadiazine, miltefosine, thioridazine, amphotericin B, albendazole and trimethoprim-sulfamethoxazole. Miltefosine, in particular, has demonstrated amoebicidal activity *in vitro* and its inclusion in combination regimens may offer a survival advantage.

**Acanthamoeba keratitis**

Like disseminated disease, success in treatment is dependent on early diagnosis and institution of therapy. Fortunately, success rates in treating *Acanthamoeba* keratitis are more promising, with cure rates in the literature generally greater than 75–85%.

Topical chlorhexidine and polyhexamethylenebiguanide (PHMB) are effective against trophozoites and cysts and form the mainstay of therapy. These are usually used in combination with diamidine, propamidine or hexamidine, though other agents including ketoconazole, itraconazole, voriconazole and topical imidazoles have been used. Surgical intervention, including enucleation, is sometimes required in severe cases.

**References**


**Biographies**

**Dr Evan Burse**, BSc, MBBS, is a microbiology and infectious diseases registrar based at Sullivan Nicolaides Pathology. Although his microbiological tastes are broad and still being refined, he has a keen interest in parasitology.

**Dr Jenny Robson**, MBBS (Hons I); FRACP, FRCPA, FACTM, is an infectious disease physician and microbiologist who has worked for the past 26 years at Sullivan Nicolaides Pathology. She has a broad range of interests, which includes travel and tropical medicine.

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**Future issues of Microbiology Australia**

**May 2016**: Education to enhance microbiology graduate employability

**Guest Editor**: Danilla Grando

**September 2016**: Diseases of Aquaculture

**Guest Editor**: Nicky Buller

**November 2016**: Microbiology of Travel

**Guest Editor**: Ipek Kurtböke; joint issue with the Society for General Microbiology

**March 2017**: Bat-associated diseases

**Guest Editor**: Glenn Marsh
In former times of central and South America, Africa and east and south-east Asia, the disease did not happen in New York. However, it does happen across large areas where pigs roam freely. This does not happen in New York because the parasite is not, and has never been known to be, endemic here. Nevertheless, a research program at the University of Melbourne with an original genesis way back in the 1970s led to the development of both the first effective non-living vaccine against a eukaryotic parasite and eventually also to a vaccine that can stop pigs being infected with T. solium. The vaccine uses a recombinant antigen known as TSOL18. Several independent experimental trials of TSOL18 have confirmed that it is extraordinarily effective. A field trial of the vaccine was undertaken in which pairs of young piglets were distributed to families living in a T. solium endemic region of north-east Cameroon. One animal from each pair was vaccinated and one acted as a control. When the animals were of normal eating-age (~12 months), they were recovered from the farmers and assessed for T. solium infection. About 20% of the controls were infected but not a single parasite was found in any of the 110 vaccinated animals.

For those living in poor countries in which T. solium is endemic, help is at hand from, of all places, Australia. I say ‘of all places’ because the parasite is not, and has never been known to be, endemic here. Nevertheless, a research program at the University of Melbourne with an original genesis way back in the 1970s led to the development of both the first effective non-living vaccine against a eukaryotic parasite and eventually also to a vaccine that can stop pigs being infected with T. solium. The vaccine uses a recombinant antigen known as TSOL18. Several independent experimental trials of TSOL18 have confirmed that it is extraordinarily effective. A field trial of the vaccine was undertaken in which pairs of young piglets were distributed to families living in a T. solium endemic region of north-east Cameroon. One animal from each pair was vaccinated and one acted as a control. When the animals were of normal eating-age (~12 months), they were recovered from the farmers and assessed for T. solium infection. About 20% of the controls were infected but not a single parasite was found in any of the 110 vaccinated animals.

So far, so good. However the field trial involved a single cohort of animals that was vaccinated. In a real life situation, new disease susceptible piglets are born into the community more-or-less every day. Difficulties arise when we try to work out a feasible and sustainable program to deliver vaccination regularly enough to prevent pigs becoming infected on an on-going basis. The ‘standard’ vaccination protocol calls for two immunizations about a month apart. It is difficult to give any veterinary care to pigs in the communities where T. solium is transmitted; however having to deliver two vaccines a month apart would be close to impossible.

Imagine the consternation; you are a member of an orthodox Jewish family and you and another family member are diagnosed with larvae of a pork tapeworm in your brain. You have recurrent seizures as a result. Ridiculous? Not for members of a Jewish community in New York where a Mexican domestic worker harbouring a Taenia solium tapeworm had apparently contaminated the family’s food with eggs from her tapeworm. T. solium is a cestode parasite that is transmitted between pigs and people. Pigs may harbour the parasite’s larval stage in their muscles (Figure 1). If we accidentally eat one of these larvae in poorly cooked pig meat, the adult tapeworm will develop in our small intestine. Humans are the only definitive host (the host in which the parasite undergoes sexual reproduction) for this species of tapeworm. A person with the tapeworm releases the parasite’s eggs in their faeces, and the lifecycle is completed if foods contaminated with these eggs, or indeed the faeces themselves, are consumed by a pig.

The disease is fully transmitted only where pigs roam freely and humans defecate in areas where the pigs are free roaming. This does not happen in New York. However, it does happen across large areas of central and South America, Africa and east and south-east Asia.

In former times T. solium and the human brain disease that the parasite causes, neurocysticercosis, were endemic throughout Europe and other parts of the current-day First World; however, improved public sanitation and hygienic standards for raising pigs have seen the disease in pigs eliminated without any efforts directed specifically to the disease. Likely the same will happen, in time, through economic development in those areas of the world where the disease remains endemic today. Unfortunately, this is unlikely to occur in our lifetimes. Meanwhile, those living in the T. solium endemic areas of the world continue to suffer epilepsy and death due to the presence of T. solium cysts in their brain. Keep in mind that people from poor countries can travel anywhere with their intestinal residents and deliver their tapeworm eggs to you or me, just as happened in the New York community referred to above. No point turning vego. Consider this: I have a worm and I am not particularly hygienic when I go to the toilet. I make your salad; bingo – see you in the neurology clinic.

T. solium is not an obscure parasite. The Food and Agriculture Organization of the United Nations considers it to be the most important foodborne parasitic infection from a global perspective. T. solium is the most frequent preventable cause of seizure disorders, being associated with 29% of people with epilepsy. The World Health Organization list T. solium as one of 16 Neglected Tropical Diseases, and is actively promoting efforts to reduce the parasite’s transmission. For those living in poor countries in which T. solium is endemic, help is at hand from, of all places, Australia. I say ‘of all places’ because the parasite is not, and has never been known to be, endemic here. Nevertheless, a research program at the University of Melbourne with an original genesis way back in the 1970s led to the development of both the first effective non-living vaccine against a eukaryotic parasite and eventually also to a vaccine that can stop pigs being infected with T. solium. The vaccine uses a recombinant antigen known as TSOL18. Several independent experimental trials of TSOL18 have confirmed that it is extraordinarily effective. A field trial of the vaccine was undertaken in which pairs of young piglets were distributed to families living in a T. solium endemic region of north-east Cameroon. One animal from each pair was vaccinated and one acted as a control. When the animals were of normal eating-age (~12 months), they were recovered from the farmers and assessed for T. solium infection. About 20% of the controls were infected but not a single parasite was found in any of the 110 vaccinated animals.

So far, so good. However the field trial involved a single cohort of animals that was vaccinated. In a real life situation, new disease susceptible piglets are born into the community more-or-less every day. Difficulties arise when we try to work out a feasible and sustainable program to deliver vaccination regularly enough to prevent pigs becoming infected on an on-going basis. The ‘standard’ vaccination protocol calls for two immunizations about a month apart. It is difficult to give any veterinary care to pigs in the communities where T. solium is transmitted; however having to deliver two vaccines a month apart would be close to impossible.
Recently we have completed an experiment in which pigs received their secondary immunization with TSOL18 at 4, 8, 12, 16 or 20 weeks after the first injection. The results were very promising. Antibody responses to the vaccine generally increased beyond the ‘standard’ 4-week interval. Responses seen in the animals vaccinated at a 12 week interval were the best, and field evaluation of T. solium interventions are about to begin in several endemic regions of Africa that will involve vaccinations at 3 or 4 monthly intervals.

Despite the solid progress that has been made so far, much remains to be achieved before we would be likely to see pig vaccination contribute to reducing the incidence of neurocysticercosis in people. One major difficulty with implementation of pig vaccination to prevent transmission of T. solium is that the owners of the animals often have little incentive to undertake control measures because the infection does not often directly cause illness or death in pigs. In the future, combination vaccines that include TSOL18 and also antigens that can provide protection against pig deaths caused by Classical Swine Fever (CSF) in the Americas, or African Swine Fever (ASF) in African countries, would improve the acceptability of pig vaccination by providing an economic incentive for the animal owners to vaccinate. While the potential for a combination with CSF is something that can be explored immediately because commercial vaccines already exist, there is yet to be a commercial vaccine for ASF.

As for the Jewish community in New York who use domestic staff sourced from T. solium endemic countries, and of course all the people who live permanently in those endemic countries, the risk of exposure to T. solium remains. Hopefully we will be able to overcome the practical difficulties around working with pigs in T. solium endemic areas so that implementation of pig vaccination can reduce T. solium transmission and decrease the incidence of human neurocysticercosis as a result.

**References**


**Biography**

**Marshall Lightowlers** has been a full-time research scientist supported by medical research funding for more-or-less all of his working life. He currently holds appointments as Laureate Professor at The University of Melbourne’s Faculty of Veterinary and Agricultural Sciences, and Principal Research Fellow with the NHMRC.
Fish are host to many parasites, some of which can cause disease in humans. With the increase in cultural and culinary diversity and the increased popularity of eating raw or slightly cooked seafood dishes in Australia it is speculated that seafood-borne parasitic infections in Australian consumers may rise. Seafood-borne zoonotic parasites are recognised as a significant public health concern worldwide. In Australia there are few reports of infection in humans in the medical literature. Australian Government enforcement agencies rate the risk of seafood-borne zoonosis as low; however, the prevalence of seafood-borne zoonoses may be under-reported in Australia due to misdiagnosis. Although food safety regulations and import controls for seafood in Australia are strict, the focus is more on the control of food-borne bacterial, viral and chemical contaminant related illnesses rather than parasitic diseases.

Increasing demand for raw and exotic seafood has significantly expanded the geographical and demographic limits of fish-borne parasitic infections. Medical literature on the regular consumption of seafood is plentiful. It is heavily promoted for prolonging life, aiding in childhood development, increasing brain stimulation and even to help our pets. However, there is an increasing risk of contracting parasitic zoonoses from consuming raw and undercooked seafood, which should be of concern in Australia. Zoonotic parasites are common in Australian seafood, and can pose a major health risk to people who consume seafood or work in a fishing industry. Among zoonotic parasites in seafood, Anisakid nematodes are of the greatest significance due to their high prevalence in wild caught fish and also due to the severity of the disease they cause, which is known as anisakidosis. Other nematodes such as Gnatostoma and Angiostrongylus as well as platyhelminths such as Diphyllolotrium, Clonorchis and Paragonimus, can occasionally cause seafood-borne zoonotic infections.

**Infection in humans (zoonosis)**

Anisakidosis results from accidental infection with one or more larvae of species of certain genera of anisakids, including Anisakis spp, Contraacaeum spp and Pseudoterranova spp. Infection with larvae of Anisakis and Pseudoterranova is of common occurrence, whereas infection with other genera, such as Contraacaeum has been reported less frequently. Humans usually become infected with these parasites after eating raw, undercooked or improperly processed fish or seafood (Figure 1). Clinically, several different types of human anisakidosis have been described based on the location of the parasite. This includes gastro-intestinal, visceral, oopharyngeal and transient luminal anisakidosis. The first two types are associated with severe symptoms such as vomiting, severe pain in lower abdomen and fever.

In recent years, it has been recognised that an allergic response can occur in humans due to live anisakids or food in which worms have been killed by cooking or pasteurisation. Other allergic disorders, such as chronic urticaria may also result from parasitism. There is anecdotal evidence in other countries (e.g. Spain) that some allergic reactions to seafood are indeed allergic reaction to anisakids in seafood.

**The Australian scenario**

In Australia our knowledge about these important parasites is poor. Although anisakid nematodes are commonly found in Australian marine fish (Figure 2), such as mackerel, flathead, snapper and whiting, all forms of seafood are available and popular in Australia and 40% of fish consumed raw in Australia have been considered as infected with Anisakis at the time of processing for trade. There are only a handful of documented confirmed cases acquired in Australia.

Case 1: There has been only one documented confirmed case of infection with anisakid nematodes acquired in Australia. This case involved a 41-year-old South Australian woman of Tongan descent who became ill after eating raw mackerel that had been caught locally. She was hospitalised with severe gastrointestinal pain, diarrhoea and vomiting that progressively worsened for 3 weeks. The diagnosis only occurred after a worm was passed in her faeces.

The initial presumptive identification of the larva was an intestinal nematode, possibly a species of the Trichonstrongylus or Ascaris genera. However, on further detailed microscopic examination, the larva was identified as a species of Contraacaeum. Had the worm not been found, or properly examined by a taxonomist,
misdiagnosis would have occurred. It is very rare for anisakid larvae to be found after passing through the gastro-intestinal tract as was the case in this Australian patient.

Since then the author has come across several suspected cases of anisakidosis, mostly among Australian travellers after returning home (unpublished cases). The cases could not be confirmed due to the lack of knowledge, clinical suspicion and a reliable diagnostic technique in Australia. In many countries, the disease is usually diagnosed by endoscopy, radiography, or surgery if the worm has embedded within the gastro-intestinal tract in patients with symptoms and a history of consuming raw seafood. Serological tests to detect parasite allergens have also been recently developed10. However, in Australia there is no standard test available for diagnosis of these important parasites. General health practitioners are not aware of the presence and high abundance of these parasites in Australian fish and diagnostic laboratory staff are not trained to
identify these worms. As a result, the full extent of hidden cases of the disease in Australia remains unknown.

Case 2: Another confirmed case report of fish-borne parasitic disease in humans in Australia occurred in 2011. In this case, a couple became infected with Gnatostoma species after consumption of ‘Black Bream’ (possibly Acanthopagrus berda or Hoplaeustus jenkinsi), which they caught in the Calder River in Western Australia. They presented with recurring skin swellings. The fish had been cooked on a camp fire, but the cooking time was unclear. The diagnosis was based on eosinophilia and a positive serology. Humans become infected accidentally by consuming third-stage larvae. Gnatostoma larvae are unable to mature inside the human body, and instead migrate through visceral and cutaneous tissues, causing a variety of generalised, non-specific symptoms. Gnathostomiasis is generally endemic in parts of the world where seafood is consumed raw, such as in South-East Asia and Japan, but also more recently in Latin America, India and Africa, and in travellers returning from these areas.

Another aspect of Australia’s seafood consumption that must be considered is the large volume of imported seafood that Australians consume. The most important sources of seafood products are Thailand, New Zealand, Vietnam and China (frdc.com.au/knowl-edge/Factsheets/Factsheet_Imported_Seafood_in_Australia.pdf). The literature describes several zoonotic parasites affecting fish endemic to these countries. CSIRO conducted a comprehensive review of AQIS’s imported seafood testing protocols and concluded that imported seafood does not pose any greater health risk to the consumer than locally produced seafood. Nevertheless proper precautions when preparing seafood in order to reduce the risk of illness or disease has been recommended. However, recommendations to eliminate or reduce parasites have not been dealt with in details.

Prevention

Gastro-intestinal anisakidosis can be prevented by properly cooking fish to an internal temperature of approximately 63°C or freezing at or below –20°C for 7 days. This will most likely prevent most other parasites as well.

Consuming raw seafood in reputable restaurants only, where chefs are trained to recognise infected seafood.

Conclusion

In the absence of standard diagnostic techniques it is difficult to have a realistic estimation of occurrence of seafood borne parasitic diseases in the country. As Chai et al. stated it appears that fish borne parasitic disease in Australia remains a ‘public health orphan’ with very little research having been carried out on what the real risks are. The economic value of the fishing industry and health of consumers must be protected. Therefore further research is required to fill in the current knowledge gaps of the biology and ecology of these parasites and the risk they pose to consumers and workers.

References


Biography

Dr Shokoofeh Shamsi is a senior lecturer in veterinary Parasitology. After completing a Masters Degree in Medical Parasitology (Tehran University of Medical Sciences) she gained her PhD in Veterinary Parasitology from The University of Melbourne. She is a taxonomist who couples conventional morphological techniques with modern molecular technologies to diagnose infections and identify parasite species, especially aquatic parasites. This has resulted in the discovery of several new pathogenic species and strains, and their recognition as disease causing agents in humans and animals in Australia and worldwide.
Brachylaimids are parasitic trematode fluke worms that have a terrestrial life cycle involving land snails and slugs as the first and/or second intermediate hosts for the cercarial and metacercarial larval stages. A wide range of mammals, birds, reptiles and amphibians are the definitive hosts for the adult worm. Brachylaima spp. have been reported from most continents including Europe, Africa, Asia, North and South America and Australia. There are over 70 described species in the genus with seven species indigenous to Australia. Although Brachylaima spp. are a cosmopolitan terrestrial trematode they have not been recorded to infect humans other than the three Brachylaima cribbi infections reported in two children and an adult from South Australia.

The publication of a new species of brachylaimid, Brachylaima cribbi by Butcher and Grove in 2001 was the culmination of a 12-year scientific journey that would not have been possible without a broad scientific network of colleagues and some ‘scientific luck’, which enabled the establishment of a laboratory life cycle using parasite eggs recovered from the stool of an infected human. My long-term association, as an active member of the Australian Society for Microbiology and the special interest groups played a significant role in having a network of colleagues to help find techniques and data to assist in the study of this parasite.

The publication of the first human Brachylaima sp. infections followed the detection of a fluke worm egg, in the stool of two South Australian children, who had never travelled overseas. The eggs were identified as belonging to a genus of trematode worm endemic in the local area. After conversations with many local and interstate colleagues, a veterinary parasitology colleague Michael O’Callaghan remembered the post-doctoral work of Thomas Cribb in South Australia. He had studied and published work on a brachylaimid in South Australia that infected mice. This provided the data on a local trematode that had an egg morphology matching the eggs detected in the children’s stools.

However, was this a true human infection that resulted in the establishment of mature gravid worms producing the eggs detected in their stool or was it a spurious infection? The answer to this question was resolved 18 months later when an elderly lady from the mid-north of South Australia presented with chronic diarrhoea. Microscopic examination of her stool detected Brachylaima eggs, which matched the morphology of the eggs detected in the two children. In addition, on the first day following treatment with praziquantel a gravid degenerate adult Brachylaima worm was recovered from her stool. This provided the evidence that a Brachylaima sp. was infecting humans and completing its life cycle.

Figure 1. (a) European helicid and hygromiid land snails aestivate over summer to escape the ground heat by attaching to fence posts, which presents an easy meal for natural definitive hosts like birds. (b) Fertile Brachylaima cribbi egg being smooth shelled with an inconspicuous operculum, an abopercular knob or thickening and measuring 26–32 μm (29.1 μm) long and 16–17.5 μm (16.6 μm) wide.
However, due to the advanced state of decomposition of the worm there was insufficient morphological detail to establish the species identification.

Previous to the finding of the third case described above I had commenced the cultivation of introduced European helicid and hygromiid land snails (Figure 1a) in homemade terrariums using techniques published for snail farming6. My office in the diagnostic Microbiology laboratory had now become part snail culture laboratory. These snails were used to study the larval stages in an attempt to understand how humans could have become infected. At the same time I had obtained a copy of an article by Mas-Coma and Montoliu detailing the life cycle of *Brachylaima ruminae* from the duodenum of a rodent from the Mediterranean island of Formentera (Spain)7. This article provided methods for the establishment of a laboratory life cycle. With these methods in hand and my snail cultures thriving I set myself the task to attempt to establish a laboratory life cycle using eggs recovered from the stool of the third patient. Faecal sediment of the patient’s stool containing washed concentrated eggs was smeared on wet filter paper and was fed to laboratory snails in Petri dishes overnight. Eight weeks later, when the snails were exposed to moisture in a Petri dish, cercariae by the thousands were observed emerging from the snails (Figure 2). Cercariae were collected in water and used to infect the common brown garden snails *Cornu aspersum* (*Helix aspersa*) via the respiratory orifice. After 8–10 weeks mature metacercariae were harvested from kidneys of the snails. Mice were inoculated orally with metacercariae. Seven weeks later eggs were detected in mouse

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**Figure 2.** Scanning electron microscopy ventral view of a *Brachylaima cribbi* cercaria showing oral (os), ventral suckers (vs) and elongated sensory papilla (sp). Bar = 50 μm.

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**Figure 3.** *Brachylaima cribbi* adult worm. (a) Oral (os) and ventral suckers (vs) and genital pore (gp). Bar = 500 μm. (b) Ventral view of the oral and ventral suckers showing rows of tegumental spines. Bar = 100 μm. (c) Genital pore showing extended cirrus (c) releasing sperm (s). Bar = 100 μm.
faeces. Gravid adult *Brachylaima* worms were recovered from the small intestine for characterisation and morphological identification (Figure 3). Eggs from mouse faeces and dissected worms were used to continue the life cycle (Figure 4) in the laboratory to study the life cycle kinetics.

At the same time as I was using my spare time to tinker with this life cycle experiment the former Institute of Medical and Veterinary Science (IMVS) was undergoing amalgamation with the Queen Elizabeth Hospital (TQEH) Laboratories. Professor David Grove was the Clinical Microbiologist Head of TQEH Microbiology Department and a world recognised expert clinical parasitologist. At that time Professor Grove offered me the opportunity to join the TQEH Microbiology team under the amalgamation program to continue the *Brachylaima* study and commence a PhD under his supervision. That is scientific luck, right place at the right time and the rest is now history, with the following years being the most simulating and productive science of my career.

Figure 4. Life cycle of *Brachylaima cribbi*. The definitive host sheds eggs (a) in their faeces. The egg is eaten by introduced European land snails (b), which hatch in the snail’s gut to release the miracidium. The miracidium develops into a sporocyst in the snail’s digestive gland. Mature cercariae (c) released from the sporocyst emerge from snail passing into the environment. Cercariae infect other land snails and develop into metacercariae (d) in the snail’s kidney. The definitive host (mammals, birds and reptiles) (e) ingest infected snails, releasing metacercariae, which migrate to the small intestine, attach and develop into mature adult worms. Humans are incidental definitive hosts being infected after eating raw snails containing metacercariae.
Human cases
Since the publication of the three human cases to my knowledge there have been a further 12 laboratory confirmed human infections (A.R. Butcher and D.I. Grove, unpublished observations). Part of my PhD studies was the follow-up of all human infections with a standard questionnaire to obtain clinical history. The main presenting symptoms were mucoid, watery diarrhoea, abdominal pain, anorexia and weight loss or poor weight gain in children. All identified cases were in patients who lived in rural or semi-rural districts where helicid and hygromiid land snails infected with *B. cribbi* metacercariae were abundant. The likely infection source was the ingestion of raw snails. The majority of infections (80%) were in children under the age of two years. At this age the mouthing of objects is a common behavioural characteristic with parents of the infected children detailing their child’s consumption of snacks. For the three adults infected they had all eaten home-grown garden vegetables contaminated with snails. The duration of symptoms ranged from 1 month to 2 years with the majority of infections diagnosed within 1–2 months of the first signs of symptoms. All patients were treated successfully with praziquantel with no adverse side-effects. The most commonly used dose in both adults and children was 20 mg/kg once per day for 3 days.

Laboratory diagnosis
Diagnosis of the infection relies on the detection of typical eggs in the faeces which are asymmetrical, being ovoid, with one side slightly flattened. They have a smooth shell, inconspicuous operculum, an abopercular knob or thickening and measure 26–32 μm by 16–17.5 μm. The eggs generally contain a well-developed miracidium (Fig. 1b). However, in chronic infections many of the eggs may be infertile, being of similar morphology but smaller size and lacking a developed miracidium. Infected land snails have been reported from coastal and inland Western Australia, South Australia and western Victoria. Therefore, the potential exists for further human infections, especially in children living in these regional districts.

References

Biography
Dr Andrew Butcher is a retired medical scientist with 38 years experience as a diagnostic medical microbiologist with a special interest in parasitology. He has been an active member of the ASM Parasitology and Tropical Medicine Special Interest Group for many years being national convenor for 7 years. He has been involved in teaching diagnostic medical parasitology and one of the founding committee members of the ASM Parasitology Master Class.
The enormous decline in the annual morbidity and mortality from malaria is the spectacular global public health success of the past decade. This achievement results largely from increased finance for investment in measures known to prevent malaria: bednets treated with long-lasting insecticides, chemoprophylaxis, and rapid access to effective treatment. Such has been the success of these measures that plans are being put in place to achieve the vision of a malaria-free world within the next three decades. Large financial and political commitments and ongoing research will be required to maintain the gains, overcome known and unknown challenges such as drug and insecticide resistance, and to achieve those goals. Effective vaccines or methods for reducing mosquito vectorial capacity would add enormously to the chance of achieving this goal. The aim of this article is to summarise the current status of malaria control, the recent research successes, the challenges being addressed, and the plan for progress to elimination of malaria in the longer term.

Spectacular progress in reducing the burden of malaria

Funding for major efforts in implementation of known effective preventive measures in the past 15 years has caused an almost 40% reduction in incidence of malaria disease episodes. These measures, together with increased access to effective treatment, have led to a reduction in malaria death rates of 60% globally, and 71% in children under five. It is estimated that between 2001 and 2015, ~6.2 million lives were saved by increasing the provision of these services. Approximately 110 countries are free from malaria, ~40 have committed to an elimination timetable, while 70 focus predominantly on malaria control. Many factors contributed to this success from the many constituencies that make up the Roll Back Malaria Partnership, but most of the progress in Africa is usually attributed to widespread distribution of bednets treated with long-lasting insecticides, combined with increased access to highly-effective drugs.

The current situation

According to the latest information provided by the WHO, ~1.2 billion people are at risk of malaria with the major burden of disease carried by young children and pregnant women living in endemic areas. The burden of malaria is not evenly distributed across the world. About 90% of malaria deaths occur in sub-Saharan Africa and recent estimates suggest that 80% of malaria deaths occur in only 15 countries. The large morbidity resulting from P. vivax is seen mainly in the Asia Pacific region. Further general information on malaria is available in the most recent World Malaria Report from WHO. There has been great recent interest in the finding that infections with the simian malaria P. knowlesi have become increasingly important in Malaysia and surrounding countries.

The strategy for malaria control leading to elimination

The overall guiding strategy for addressing malaria is summarised in the Global Technical Strategy for Malaria Elimination 2016–2030, which was approved and adopted by the World Health Assembly in May 2015. The strategy calls for accelerated action towards malaria elimination in countries and regions, but does not set a time frame for global eradication. Notably this document recognises the continuum of activities from malaria control to elimination, the
importance of a multi-disciplinary approach, and the need for tailor-made strategies for different situations, even within one country, in other words, ‘One size does not fit all’. One area, for example a mountainous region, may have only the problem of introduced malaria occurring in people of all ages, whereas another highly endemic area may need to focus on children and pregnant women who remain at risk and suffer the consequences all year round.

The Global Malaria Programme of WHO provides support to countries, compiles the Annual Global Malaria Report, and through the Malaria Policy Advisory Committee provides a normative role in reviewing new data or new problems to provide advice for policy implementation at country level.

This WHO strategy is complemented by the Roll Back Malaria advocacy plan, Action and Investment to Defeat Malaria 2016–2030, that was approved in 2015. The publication outlines both the health and economic benefits that would flow from malaria elimination, and like the Global Technical Strategy was the result of extensive consultative processes involving the participation of more than 400 malaria experts from 70 countries. Ambitious but achievable global targets were established, including:

- reducing malaria case incidence by at least 90% by 2030
- reducing malaria mortality rates by at least 90% by 2050
- eliminating malaria in at least 35 countries by 2050
- preventing a resurgence of malaria in all countries that are malaria-free.

The timeline of 2016–2030 is aligned with the 2030 Agenda for Sustainable Development, the new global development framework adopted by all UN Member States in September.

Malaria elimination

The major progress already referred to is the result of implementation of known effective preventive measures, including vector control with long-lasting insecticide-treated nets and indoor residual spraying; chemoprophylaxis including intermittent preventive treatment of malaria in pregnancy (IPTp); intermittent preventive treatment of malaria in infants (IPTi) or malaria chemoprevention during the season of malaria transmission (SMC); and provision of ready access to case management with rapidly effective drugs, primarily artemisinin combination treatments (ACTs).

As countries, or areas within countries, move towards elimination, the emphasis changes from population level data and disease control to intense surveillance for identification of individual infections, followed by planning and, most importantly, implementation of a response. For example, teams might test everyone in a compound or a village surrounding a person presenting with illness, apply control measures locally, and search for the source of introduced cases.

MalERA (Malaria Eradication Research Agenda)

Following the call for malaria eradication, the Bill & Melinda Gates Foundation recognised the need for new and better tools for elimination, and new and better systems to guide delivery of services to achieve those goals. Following extensive discussions by working groups, a research agenda was developed (MalERA) that included:

- Better and safer drugs for ease of administration to populations. The ‘dream’ product profile is ‘SERCAP,’ (Single Encounter Radical Cure And Prophylaxis), i.e. a single dose treatment to kill not only asexual blood-stage parasites but also liver and sexual stages. An intermediate goal would be an alternative and more effective drug to kill hypnozoites, the dormant liver stage responsible for relapse in P. vivax, but without the troublesome side-effects of primaquine, the only drug currently available for that purpose.
- Basic research enabling culture of all life cycle stages of all parasites infecting humans would be required for development of vaccines and for production of better drugs for eradication of hypnozoites. This would entail investment in culture of hepatic stages of P. vivax, and achieving the dream of laboratory culture of sporozoites from gametocytes.
- The emphasis on elimination draws attention to P. vivax, common outside Africa, and with separate challenges such as long-term relapses from hypnozoites, and transmission by vectors biting outdoors and during the daytime, which are therefore not amenable to the standard interventions for vector control.

Many considered that elimination would not be achieved without a major breakthrough such as effective vaccines to reduce morbidity and transmission, or a technology for vast reduction in vectorial capacity of Anopheles gambiae, the most efficient and important vector of P. falciparum in Africa.

It is a source of optimism that since the MalERA agenda was proposed, funding was directed to address important gaps, a Malaria Eradication Scientific Alliance (MESA) was established, and now through its MESA Track database tool can capture relevant research and development projects. By the end of October 2015, 685 projects from 647 institutions and 84 countries had been documented. MESA is currently coordinating several expert groups to perform a 5-year ‘refresh’ of the MalERA agenda.

Progress is being made on many other fronts, including conference reports of the in vitro culture of sporozoites from gametocytes; successful culture of hepatic stages of plasmodia; and development of new antimalarial drugs, through ‘Medicines for Malaria Venture’ (MMV), a highly successful not-for-profit public-private partnership. The timeframe for initial testing of new drugs (or vaccines) has been dramatically shortened by the development of a blood stage challenge model by Queensland-based researchers.
The challenges for malaria control and elimination

See Table 1 for a more comprehensive list. Some of the biological, societal and economic requirements for elimination are listed in Table 2.

Artemisinin resistance

The time and mode of action of artemisinin are not well understood, and in vitro tests are not sufficiently developed and standardised to replace the requirement for in vivo testing for the presence of resistance. A warning sign, before emergence of clinical resistance, is the detection of delayed parasite clearance from the blood over the first few days of treatment. Because of the fear of emergence of resistance, combination therapy is essential, for example with piperaquine or lumefantrine, so the efficacy of partner drugs is also of great importance to ensure complete parasite clearance. Recently, drug resistance has been associated with some but not all mutations of the kelch gene in P. falciparum that will probably assist in mapping resistance. Melbourne-based researchers have developed synthetic artemisinin-like compounds, which are probably not susceptible to kelch-based resistance.

Insecticide resistance

Chemical resistance to insecticides is a well known threat. Strong movements against DDT that is cheap and effective for indoor residual spraying (because of past evidence of harmful environmental effects when very widely distributed in agriculture) mean that there are few suppliers globally, and alternatives are very expensive. Behavioural resistance is another well known complication of the introduction of effective vector control directed at mosquitoes that bite indoors in the evening. Repeated studies have documented the gradual increase in proportion of mosquitoes biting earlier in the evening or later in the morning and resting outdoors where they are not susceptible.

Another huge challenge, particularly for control of P. vivax is that the majority of vectors for this parasite bite outdoors and during the daytime. No satisfactory method for long-term control of these vectors is available.

Elimination as an approach to drug resistance in the Mekong region

Where artemisinin has been widely used in the Mekong region, delayed parasite clearance has been noted in many countries, and it is felt that in the absence of alternative drugs, the only appropriate approach is to eliminate malaria in the area where resistance has already been detected. Pilot studies have been initiated to test the feasibility of this approach.

Fortunately, delayed parasite clearance due to artemisinin resistance has not yet been seen in parasites from India and Africa, but in the recent past, development of resistance to chloroquine or sulphadoxine/pyrimethamine accompanied by ongoing use of those drugs led to many unnecessary deaths.
Funding shortfall
The major advances of the past two decades are at great risk from lack of funding. Not only could progress stall, but major epidemics of malaria could return to cleared areas, as has been seen so often in the past, with serious effects on people of all ages whose immunity has declined through lack of boosting by recurrent sub-clinical infections.

The case is now strong for the economic benefits that will follow from the elimination of malaria. This message needs to reach the highest levels of government to ensure financial and political commitment and the engagement not just of the health system, but all sectors of society.

Strengthened Health Services
As the malaria map shrinks, the last pockets of transmission are often found in marginalised poor or rural populations with least access to services. As episodes become infrequent, success will depend on a general health service with the capacity to recognise and diagnose infections, then respond with appropriate increased surveillance to detect the origin of the infection and interventions to prevent further spread.

Regional initiatives
APMEN Recognising the need for a partnership across regions and across borders, the Asia Pacific Malaria Elimination Network was established with the support of the Australian government in 2008 to facilitate collaboration amongst nations of the region that had made commitments to achieve elimination within a defined time period. This network enables regional collaboration and facilitates capacity building and knowledge transfer among nations of the region that share similar challenges such as artemisinin resistance, inadequate finance for malaria, or a vast market penetration of counterfeit drugs.

AICEM, the Australian Initiative for Control and Elimination of Malaria, is funded by the Australian government to strengthen malaria control in the Solomon Islands and Vanuatu, and at the same time establish pilot projects for elimination of malaria in certain island populations.

APLMA (www.aplma.org), the Asia Pacific Leaders Malaria Alliance, co-chaired by the Heads of Government of Australia and Vietnam, modelled loosely on the African Leaders Malaria Alliance, aims to bring together Heads of State, politicians, and opinion leaders of civil society to advocate for political and financial commitment to the goals of elimination of malaria. Special task forces are designed to address critical issues that could be barriers to achievement of elimination such as financing, or the safety and quality of medicines (to tackle the problem of counterfeit drugs and ensure that only certified effective products are available in the public and private sectors). Recently, the Alliance endorsed a Malaria Elimination Roadmap towards a malaria-free Asia Pacific by 2030.

Elimination to eradication
The malaria eradication campaign of 50 years ago succeeded in eliminating malaria from many countries but did not achieve its goals in areas of most intense transmission such as heartland Africa. However, many important lessons were learned about the need for community engagement, political will, intensive surveillance as a response, and the need for continued vigilance to prevent return of malaria to a now susceptible entire population that has benefited from a few years of interrupted transmission. As transmission declines and malaria-specific services are phased out, it is critical for awareness of the disease in all its manifestations to be retained at a high level in general services for detection, management, surveillance and response to any new episodes.

Lessons of the eradication campaign are being refreshed, for example on mass drug administration to entire populations (MDA), and comparing this strategy with treatment only of those found with highly sensitive tests to be infected, thus avoiding the unnecessary use of drugs that could have side-effects, so called Mass Screening and Treatment, or MSAT. Research on application of more modern tools such as rapid diagnostic tests, including nucleic acid amplification or data transmission by mobile phones are providing further data to guide strategy. For example, it is important to decide whether the cost of a more sensitive but more expensive and technically difficult nucleic acid detection test adds significant benefit in elimination campaigns, or how PCR-based parasite ‘barcoding’ (genetic epidemiology) can be used to provide rapid analysis of the source of emergent infections, the spread of drug resistance, or the risk of the parasite’s appearance in susceptible areas.

Other major lessons of the eradication campaign were that research must continue throughout the programs to deal with new challenges, and that every country needs to develop the human resource capacity to deal with the multi-disciplinary approach to this grand challenge.

Recent vaccine breakthroughs; progress with P. falciparum but little progress with other species
Successful Phase 3 field trials of RTS,S vaccine in African children. Development of the RTS,S vaccine, which provides
35% efficacy against clinical episodes of *P. falciparum* malaria, was an important milestone for the malaria vaccine field. The vaccine includes a fusion protein containing the immunogenic repeat regions of the circumsporozoite protein, T-cell epitopes of the same molecule and the hepatitis B surface antigen that is co-expressed with free hepatitis B virus surface antigen leading to formation of a viral-like particle known as RTS,S. In recent very large trials, participants had good access to malaria prevention and rapid access to treatment, so mortality was low and no effect of vaccination on mortality could be detected. There was a concerning, but unexplained, slight increase in episodes of meningitis in the RTS,S arm, requiring further evaluation. A recent review of RTS,S by WHO recommended that the next step should be to initiate 3–5 pilot implementation studies12. Further analysis of trial data showed that the vaccine had higher efficacy against strains homologous to the vaccine, suggesting both a strain-specific as well as a strain-independent mechanism13. This vaccine is a very important first step but on its own, is unlikely to be the solution that is required.

**Attenuated sporozoite vaccines**

The most impressive of all malaria vaccine trial results are those achieved with intravenous inoculation of radiation-attenuated whole sporozoites in which up to 100% protection has been achieved in Phase 1 studies using multiple doses of vaccine14. Field studies in Mali and Tanzania have also demonstrated efficacy and further trials are planned to optimise dose and delivery schedule14.

**Conclusion**

With the spectacular reduction of the burden of malaria in the past 15 years, the time is ripe to re-double efforts both to prevent resurgence and to increase resources with the goal of achieving the health and economic benefits that would result from disease elimination. Major progress is being reported in developing the tools to add to current successful interventions from basic research into biology of hypnozoites and transition of gametocytes to sporozoites, to field trials of vaccines and innovations in surveillance and epidemiology. A multi-disciplinary response coupled with strong community engagement, and ongoing political and financial support will be required to maintain the current rate of decline in malaria, and hopefully achieve the ambitious goal of elimination.

**References**


**Biographies**

**Graham Brown**, AM Professor Emeritus at The University of Melbourne, currently serves as the Deputy Chair of the Board, and Chair of the Executive Committee of Roll Back Malaria. As a clinician-researcher, his interests include malaria vaccine development, antigenic variation and clinical infectious diseases.

**Stephen Rogerson** is a Professor at The University of Melbourne. His research interests include the pathogenesis of malaria in pregnant women and young children, and tools for prevention of malaria in these high-risk groups.

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Plasmodium knowlesi: an update

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There were only four species of Plasmodium that were thought to cause malaria in humans until a large number of human infections by Plasmodium knowlesi, a malaria parasite typically found in long-tailed and pig-tailed macaques, were reported in 2004 in Malaysian Borneo. Since then, cases of knowlesi malaria have been reported throughout South-east Asia and also in travellers returning from the region. This article describes the molecular, entomological and epidemiological data which indicate that P. knowlesi is an ancient parasite that is primarily zoonotic, and there are three highly divergent sub-populations. It also describes the detection methods for P. knowlesi, which is morphologically similar to P. malariae, and the clinical features and treatment of this malaria parasite that is potentially fatal.

Malaria parasites and discovery of large focus of human knowlesi malaria cases

Malaria is caused by parasites that belong to the genus Plasmodium and there are more than 150 species of Plasmodium that infect reptiles, birds and mammals1. These parasites, in general, tend to be host-specific. Long-tailed and pig-tailed macaques (Macaca fascicularis and M. nemestrina respectively) are hosts to five species (P. knowlesi, P. inui, P. cynomolgi, P. fieldi and P. coatneyi). Only four species of Plasmodium, namely P. falciparum, P. vivax, P. malariae and P. ovale, were thought to cause malaria in humans until a large number of human cases due to P. knowlesi were reported in Sarawak, Malaysian Borneo over 11 years ago2. The study in Kapit was prompted by observations that cases diagnosed by microscopy as P. malariae had high parasitaemias, required hospitalization and that 95% of patients were adults. This was in contrast to P. malariae infections which typically are asymptomatic with low parasitaemia and occur in all age groups. When blood samples from 208 malaria patients at Kapit Hospital were analysed by PCR assays, none were identified as P. malariae, although 141 had been diagnosed as P. malariae by microscopy. Fifty-eight percent (120) were either single P. knowlesi infections or mixed infections of P. knowlesi with P. falciparum and P. vivax. Misdiagnosis had occurred because the blood stages of P. knowlesi and P. malariae are morphologically indistinguishable3.

Epidemiology and risk factors of acquiring knowlesi malaria

Human infections with P. knowlesi have been reported throughout Malaysia and in Thailand, Singapore, the Philippines, Vietnam, Cambodia, Indonesia, Brunei, Myanmar and in the Nicobar and Andaman Islands of India4,5. In Malaysia, P. falciparum and P. vivax cases have declined over the past five years and P. knowlesi has now become the most common cause of human malaria6,7. The true incidence of knowlesi malaria is not known in other parts of South-east Asia since not many large-scale studies have been undertaken with molecular detection assays.

The geographical distribution of human P. knowlesi infections is similar to that of the natural hosts of P. knowlesi, the long-tailed and pig-tailed macaques8. Reports from 1931 to 1970 identified macaques as hosts of P. knowlesi in Peninsular Malaysia, Singapore and the Philippines9, and a banded leaf monkey (Presbytis melalophos) in Peninsular Malaysia10. Since 2007, P. knowlesi infections detected by molecular methods have been described in macaques in Peninsular Malaysia, Malaysian Borneo, Singapore and Thailand11.

The transmission of P. knowlesi in nature has been shown to be restricted to mosquitoes belonging to the Anopheles leucosphyrus group10. The members of this forest-dwelling group of mosquitoes that have been identified as vectors include An. latens (in Sarawak, Malaysian Borneo)11, An. balabacensis balabacensis (in Sabah, Malaysian Borneo), An. dirus (in Vietnam)12 and An. backeri and An. cracens (in Peninsular Malaysia)13,14. People that are at risk of acquiring knowlesi malaria are those that enter the habitat of the macaque reservoir hosts and the Anopheline vectors at dusk or later as this coincides with the peak biting time of the vectors14,15. These include subsistence farmers, timber camp workers, hunters, army personnel and also travelers to forests or forest-fringe areas. Visitors to South-east Asia from Australia, USA, Finland, Sweden, Germany, France, New Zealand, Taiwan and Japan have acquired knowlesi malaria following holidays or working visits.
to Malaysian Borneo, Peninsular Malaysia, Brunei, Thailand, Indonesia and the Philippines.

**Molecular and whole genome studies**

In order to understand the molecular epidemiology and demographic history of knowlesi malaria, the mitochondrial (mt) genome sequences of *P. knowlesi* were initially studied. Certain mt haplotypes were shared between humans and macaques and there were no haplotypes that were associated exclusively with either host; further evidence supporting *P. knowlesi* as a zoonotic parasite. Additional analyses indicated that *P. knowlesi* was as old as, if not older than, *P. falciparum* and *P. vivax*, and that it underwent a population expansion between 30,000 to 40,000 years ago. Macaques colonized Asia over 5 million years ago and are probably the original hosts for *P. knowlesi*. A recent study, where 599 *P. knowlesi* samples from Peninsular Malaysia and Malaysian Borneo were analysed by a panel of ten microsatellite markers, showed there are two highly divergent sub-populations of *P. knowlesi*, and each of these subpopulations correspond with parasites from either long-tailed or pig-tailed macaques. More recently, genome-wide sequence analysis of clinical *P. knowlesi* isolates from Malaysian Borneo shows sub-population structure that matches the analysis using microsatellite markers and also demonstrate there is a third sub-population of parasites, corresponding to laboratory strains isolated over 50 years ago from Peninsular Malaysia and the Philippines. No signals of positive selection were observed in *P. knowlesi* around five orthologues of known *P. falciparum* drug resistance genes, indicating that the parasites in the reservoir macaque hosts have not been under antimalarial drug selection, thereby providing further evidence that knowlesi malaria is a zoonosis.

**Diagnosis**

In laboratories in malaria-endemic countries, malaria is diagnosed by examination of blood films by microscopy. Under the microscope, the early blood forms of *P. knowlesi* are identical to those of *P. falciparum*, while the other developmental stages, including the ‘band forms’, are similar to those of *P. malariae*. There are minor morphological differences between these two species. The mature schizonts of *P. knowlesi* can contain up to 16 merozoites, whereas those of *P. malariae* have between 6–12. However, mature schizonts are not found in all blood films examined and in diagnostic laboratories, where technologists are only trained to recognise *P. falciparum, P. vivax, P. ovale* and *P. malariae*, most *P. knowlesi* infections have been identified by microscopy as *P. malariae*. Although morphologically similar, *P. knowlesi* parasites multiply every 24 h in the blood while this erythrocytic cycle is 72 h for *P. malariae*.

Molecular detection methods are the most sensitive and accurate techniques for identification of *P. knowlesi*. These include single and nested PCR assays, real-time PCR assays and loop-mediated isothermal assays. However, these assays are relatively expensive, not rapid and are not readily available in resource-poor laboratories where the majority of *P. knowlesi* infections are detected. Rapid diagnostic tests (RDTs) for malaria are available, but the overall sensitivity of detection of a small number of RDTs that have been evaluated against knowlesi malaria cases varied between 26–74% and was even lower (0–45%) for parasitaemias below 1000 parasites/µL. Due to the rapid multiplication rate of *P. knowlesi* in the blood of 24 h, sensitive RDTs capable of detecting knowlesi malaria at the early phase of infection are urgently required for rural laboratories.

**Clinical and laboratory features of knowlesi malaria**

*P. knowlesi* causes a wide spectrum of disease, from asymptomatic infections to fatal ones. The most common presenting signs and symptoms reported are fever with chills, followed by headache, myalgia, poor appetite, arthralgia, cough, abdominal pain and diarrhoea. These are not significantly different to those observed in patients with vivax and falciparum malaria. The majority of cases (93.5% and 84.5%) at district hospitals in Sarawak had uncomplicated malaria with a fatality rate of 2%, whereas in a retrospective study in a referral hospital in Sabah, 61% of 56 cases were uncomplicated and the fatality rate was 27%. However, subsequently at the same referral hospital, the use of intravenous artesunate for severe malaria cases and artemisinin combination therapy for non-severe cases, resulted in no deaths among 130 knowlesi malaria patients. Typical complications of severe knowlesi malaria in adults include jaundice, acute kidney injury, hypotension, acute respiratory distress syndrome and metabolic acidosis. In adults, severe anaemia has not been observed and neither has cerebral malaria, while severe disease has not been noted in the relatively small number of children with knowlesi malaria. Thrombocytopenia is very common, occurring in 97.3 to 100% of knowlesi malaria patients, and together with parasitaemia, correlates with severity of disease. Following a case control study, it was recommended that any patient with a platelet count of <45,000/µL or parasitaemia of >35,000 parasites/µL should be regarded at risk of developing complications and should be treated for severe malaria.
**Treatment of knowlesi malaria**

Since knowlesi malaria is primarily a zoonosis, the parasites have been under no antimarial drug pressure and should be susceptible to all antimalarias. This has been observed in hospital-based studies as well as case reports where several antimalarias have been used successfully to treat knowlesi malaria patients. *P. knowlesi* parasites are highly sensitive to chloroquine but following an informal consultation on the public health importance of knowlesi malaria organised by the WHO in 2011, it was recommended that in areas where knowlesi malaria has been detected, all infections diagnosed as *P. malariae* by microscopy should be treated and managed as for falciparum malaria. Therefore, for uncomplicated knowlesi malaria cases in South-east Asia, artesunate in a tertiary care facility should be administered and the use of artesunate in a tertiary care facility recommended. For severe knowlesi malaria, intravenous antimalarials are recommended. For severe knowlesi malaria, intravenous antimalarials should be administered and the use of artesunate in a tertiary care facility recommended. For severe knowlesi malaria, intravenous antimalarials should be administered and the use of artesunate in a tertiary care facility recommended. For severe knowlesi malaria, intravenous antimalarials should be administered and the use of artesunate in a tertiary care facility recommended. For severe knowlesi malaria, intravenous antimalarials should be administered and the use of artesunate in a tertiary care facility recommended. For severe knowlesi malaria, intravenous antimalarials should be administered and the use of artesunate in a tertiary care facility recommended.

**Future directions**

The available molecular, entomological and epidemiological data strongly indicate that knowlesi malaria is primarily a zoonosis. However, human-to-human transmission has been demonstrated under experimental conditions and it is not known whether it is currently occurring. The reasons for the increase in the number of knowlesi malaria cases, particularly in Malaysian Borneo, are also unknown. Whether the increase is due to increased awareness, changes in the feeding habits of the vectors, the destruction of the natural habitats of the macaque reservoir, human migration to areas close to macaque habitats, a recent adaptation of knowlesi malaria parasites to humans, or to some other factors needs to be investigated. In addition, currently available methods of control of human malaria involving the use of insecticide treated bednets and residual spraying of houses are ineffective against knowlesi malaria, where transmission primarily occurs outdoors. Therefore, effective methods of prevention and control need to be found and implemented, in order to prevent *P. knowlesi* from establishing itself in the human population.

**References**


**Biography**

**Professor Balbir Singh** is the Director of the Malaria Research Centre at University Malaysia Sarawak. His research interests include the epidemiology, pathogenesis and evolution of malaria parasites.
Diagnosis of human taeniasis

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*Taenia solium*, *T. saginata* and *T. asiatica* are taeniid tapeworms that cause taeniasis in humans and cysticercosis in intermediate host animals. *T. solium* can also cause cysticercosis in humans. A number of diagnostic methods have been developed to diagnose *Taenia* species that infect humans. This article is aimed at providing an overview of currently available diagnostic methods for human taeniasis.

Human taeniasis is an important zoonotic health problem and is caused by adult stages of taeniid cestodes, including *Taenia solium*, *T. saginata*, and *T. asiatica*. These parasites have indirect life cycles where humans act as definitive hosts whereas pigs (for *T. solium* and *T. asiatica*) and cattle (*T. saginata*) serve as intermediate hosts. Humans become infected by ingesting parasite cysts present in raw or undercooked infected meat/liver; when the cyst reaches the human intestine, it develops into an adult tapeworm, releasing segments and/or eggs in the stools or motile segments (e.g. *T. saginata*) are expelled actively. Intermediate hosts such as cattle and pigs become infected when they ingest taeniid eggs via contaminated feed or water and the larval stage (cysticercus) forms in muscular and sometimes other tissues. The adult tapeworm stage of *Taenia* spp. is relatively innocuous and does not cause pathogenic effects in humans; however, the intermediate stage of *T. solium* can also develop in human brains causing neurocysticercosis, a major cause of neurological disease in many developing countries as well as other organs causing intramuscular, ocular, subcutaneous and spinal cysticercoses. *T. saginata* is endemic in Australia, whereas *T. solium* and *T. asiatica* are exotic. However, people coming and/or returning to Australia from endemic countries can be infected with *T. solium*, leading to the possibility that infected individuals may pass segments of the parasite in their stools, which can serve as a source of infection for human cysticercosis.

Therefore, differentiation of *Taenia* species becomes significant for surveillance and control of human taeniasis.

A number of diagnostic methods have been used to differentiate the common human cestodes, *T. saginata* and *T. solium*; however, each method has its advantages and disadvantages, and careful attention should be paid to determining which particular test is best to use for differentiation of the two species. The following sections provide a quick rundown on various diagnostic methods available to differentiate *Taenia* spp. that infect humans.

**Microscopic diagnosis**

Traditionally, diagnosis of taeniasis has been based on the detection of eggs by microscopic examination but this method lacks sensitivity and specificity as *T. solium* and *T. saginata* eggs are morphologically identical, making species identification impossible. However, morphological examination of gravid proglottids can allow the differentiation of *T. solium* and *T. saginata* provided the internal structures (i.e. uterine branches) are intact. Sometimes, even the morphological examination of proglottids does not allow the differentiation of *T. solium* and *T. saginata*, thus requiring alternate methods for the differentiation of human *Taenia* spp.

**Immunodiagnosis**

The first method of coproantigen (parasite antigens in human stool) detection using enzyme-linked immunosorbent assay (ELISA) was developed by Allan et al. Although the test displayed a higher sensitivity and specificity than microscopic diagnosis for the detection of *Taenia* spp., it did not allow differentiation of *T. saginata* and *T. solium*. Recently, Guezala et al. developed another coproantigen ELISA and successfully differentiated *T. solium* from *T. saginata*. These tests were developed in individual labs using...
in-house reagents and have not been independently validated in different laboratories nor have they been widely used in diagnostic laboratories.

The first serological assay to detect specific antibodies against *T. solium* infection in humans was developed by Wilkins *et al.*9 Subsequently, a number of studies reported various immunocassays for the diagnosis of human taeniasis, primarily caused by *T. solium*10,11 and these studies used either native excretory-secretory products collected from adult tapeworms9 or cloned and expressed excretory-secretory products of adult *T. solium*.10,11 Like coproantigen ELISAs, the detection of *Taenia* species-specific antibodies are also more specific and sensitive than microscopic techniques. However, these tests have been found to have some degree of cross-reactivity in sera from patients with cystic echinococcosis, ascariasis, and schistosomiasis.11 Furthermore, currently available immunodiagnostic tests may give false positive results as specific circulating antibodies in taeniasis patients could possibly remain detectable for some time either after treatment and recent past infections4.

### Molecular diagnosis

A number of molecular methods using PCR-based technologies have been developed to either determine the presence of *Taenia* species-specific DNA in human stools or differentiate *Taenia* spp. (*T. solium, T. saginata* or *T. asiatica*) based on the analysis of DNA extracted directly from tapeworm.12 PCR-based methods have higher sensitivity in the detection of taeniasis cases (i.e. the detection of parasite DNA in human stools) than microscopy alone. In addition, a combined use of PCR and microscopy have been found to improve diagnostic sensitivity where Yamasaki *et al.*13 showed that some proven egg-positive cases were negative by PCR. Specificity of PCR is high with control faecal samples, including samples from patients with other parasitic infections, being almost always negative in PCR.

To date, predominantly conventional and multiplex PCR, and PCR-restriction fragment length polymorphism (PCR-RFLP) have utilized various markers, including internal transcribed spacer, mitochondrial cytochrome c oxidase subunit I gene as well as 12S rDNA, cathepsin L-like cysteine peptidase, *T. saginata*-specific repetitive sequence (HDP1) and cestode-specific sequence (HDP2) to discriminate between human *Taenia* spp.19–21 (Table 1). Mayta *et al.*19 developed a nested PCR utilizing two rounds of PCR amplification of the Tso31 gene, which is more sensitive than conventional PCR but pose technical difficulties. A field DNA-based test known as loop mediated isothermal amplification (LAMP) was developed by Nkouawa *et al.*, which amplifies the *cox1* gene. To date, only one real-time PCR to discriminate *T. solium* and *T. saginata* has been developed which targets the internal transcribed spacer 1 of the nuclear ribosomal RNA. The majority of DNA-based methods have utilised DNA isolated from proglottids for *Taenia* species identification while relatively fewer studies isolated DNA from stool samples (Table 1). In addition, almost all studies have been tested only on small numbers of usually known positive and negative samples.

### Conclusions and future perspectives

Human taeniasis is a worldwide parasitic disease and detection and discrimination of *T. solium, T. saginata* and *T. asiatica* remains a

<table>
<thead>
<tr>
<th><em>Taenia</em> species</th>
<th>Molecular method</th>
<th>Target(s)</th>
<th>Sample type(s)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. saginata</em></td>
<td>PCR Gel</td>
<td>HDP1</td>
<td>Proglottids</td>
<td>14</td>
</tr>
<tr>
<td><em>T. solium, T. saginata</em></td>
<td>Multiplex PCR</td>
<td>HDP2</td>
<td>Proglottids</td>
<td>15</td>
</tr>
<tr>
<td><em>T. solium, T. saginata</em></td>
<td>Multiplex PCR-RFLP</td>
<td>HDP2</td>
<td>Proglottids</td>
<td>16</td>
</tr>
<tr>
<td><em>T. solium, T. saginata, T. asiatica</em></td>
<td>PCR-RFLP</td>
<td>Mitochondrial 12S rDNA</td>
<td>Proglottids</td>
<td>17</td>
</tr>
<tr>
<td><em>T. solium, T. saginata, T. asiatica</em></td>
<td>Multiplex PCR</td>
<td><em>cox1</em></td>
<td>Proglottids, stool</td>
<td>13</td>
</tr>
<tr>
<td><em>T. solium, T. saginata</em></td>
<td>PCR-RFLP</td>
<td><em>cox1</em></td>
<td>Stool</td>
<td>18</td>
</tr>
<tr>
<td><em>T. solium</em></td>
<td>Nested PCR</td>
<td>Tso31</td>
<td>Stool</td>
<td>19</td>
</tr>
<tr>
<td><em>T. solium, T. saginata, T. asiatica</em></td>
<td>LAMP</td>
<td>Clp, <em>cox1</em></td>
<td>Proglottids, cysticerci, stool</td>
<td>20</td>
</tr>
<tr>
<td><em>T. solium, T. saginata</em></td>
<td>Real-time multiplex PCR</td>
<td>ITS1</td>
<td>Stool</td>
<td>21</td>
</tr>
</tbody>
</table>

RFLP, restriction fragment length polymorphism; ITS, internal transcribed spacer; *cox1*, mitochondrial cytochrome c oxidase subunit I gene; Clp, cathepsin L-like cysteine peptidase; HDP1, *T. saginata*-specific repetitive sequence; HDP2, cestode-specific sequence. Adapted from Verweij and Stensvold12.
To the best of our knowledge, no standardised PCRs are available in Australia to discriminate human *Taenia* spp. Future studies should focus on independent validation of the nested PCR and the LAMP technique as these two DNA-based tests offer higher sensitivity and a user-friendly option without sophisticated equipment, respectively. In addition, future studies should be aimed at extracting DNA from sodium acetate-acetic acid-formalin (SAF) fixed proglottids using different methods as the current DNA extraction protocols do not provide reliable and consistent DNA yield for PCRs (Jabbar, unpublished data). To date only one study has reported the extraction of DNA from long-term stored *Taenia* specimens in formalin and this has not been independently verified.

### References


### Biographies

**Dr Abdul Jabbar** is a Senior Lecturer in Veterinary Parasitology at The University of Melbourne. His main research interests cover epidemiology and diagnosis of parasites of socioeconomic importance using next-generation molecular tools.

**Dr Charles Gauci** is a Senior Research Fellow at The University of Melbourne. He has worked with Prof Marshall Lightowlers throughout his career and his research interests focus on recombinant vaccines for prevention of transmission of the parasite causing neurocystercerosis and the related parasite that causes hydatid disease.

**Professor Marshall W Lightowlers** has been a full-time research scientist supported by medical research funding for more-or-less all of his working life. He currently holds appointments as Laureate Professor at the University of Melbourne’s Faculty of Veterinary and Agricultural Sciences, and Principal Research Fellow with the NHMRC.
Protozoa PCR: boon or bane

Parasite detection in faeces has traditionally been performed by microscopy, a procedure that is labour-intensive and highly specialised. In addition, identification by microscopy based on morphological features alone is subjective and prone to wide variability. Although enzyme immunoassays (EIA) of high sensitivity have been developed they can detect only a limited range of pathogens. Given these factors the introduction of Polymerase Chain Reaction (PCR) into the routine diagnostic laboratory has improved parasite detection rates. The ability to multiplex has enabled the detection of multiple targets from a single sample and provides an objective alternative to identification by morphology.

Results

In February 2014, St Vincent’s Pathology, Melbourne, introduced a new testing algorithm making use of a commercial multiplex PCR (LightMix® Gastroenteritis Parasite Kit, Roche Molecular Systems, Pleasanton, California, USA) for parasite detection. This assay is designed to detect *Giardia intestinalis*, *Cryptosporidium* species, *Dientamoeba fragilis* and *Entamoeba histolytica*, four human gastrointestinal protozoan pathogens. *Blastocystis* was not included as a target in this multiplex assay, as the primers do not differentiate the 17 different subtypes now known, many being of animal origin and most of doubtful clinical significance.

Since the commencement of testing by PCR, the rates of detection have improved when compared with results obtained based on the previous protocol that included microscopy and *Giardia/Cryptosporidium* antigen testing (Table 1). Comparison over a three month period showed that the range of common parasites detected in the population remains similar with *D. fragilis* accounting for the majority of the additional parasites detected (Figure 1). The reduction of *Cryptosporidium* cases from 18 (0.8%) to 4 (0.4%) was not statistically significant and is thought to relate to seasonal variation in prevalence. The additional *E. histolytica* cases were detected from microscopy-positive specimens referred from other laboratories. In addition, the assay was positive for

Table 1. Three months comparison between pre and post implementation of protozoa PCR testing.

<table>
<thead>
<tr>
<th></th>
<th>Quarter 2: 2013 Pre PCR</th>
<th>Quarter 2: 2014 Post PCR</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples</td>
<td>2198</td>
<td>1096</td>
<td><em>P</em> &lt; 0.001</td>
</tr>
<tr>
<td><em>G. intestinalis</em></td>
<td>24 (1.1%)</td>
<td>33 (3.0%)</td>
<td><em>P</em> &lt; 0.001</td>
</tr>
<tr>
<td><em>C. parvum</em></td>
<td>18 (0.8%)</td>
<td>4 (0.4%)</td>
<td><em>P</em> = 0.132</td>
</tr>
<tr>
<td><em>E. histolytica</em></td>
<td>–</td>
<td>4 (0.4%)</td>
<td><em>P</em> = 0.005</td>
</tr>
<tr>
<td><em>D. fragilis</em></td>
<td>47 (2.1%)</td>
<td>163 (14.9%)</td>
<td><em>P</em> &lt; 0.001</td>
</tr>
<tr>
<td>Total positives</td>
<td>89 (4%)</td>
<td>204 (19%)</td>
<td><em>P</em> &lt; 0.001</td>
</tr>
</tbody>
</table>
The advent of PCR has definitely become a ‘boon’ for our laboratory in providing a rapid, less labour-intensive method for detecting the three pathogenic protozoa *G. intestinalis, Cryptosporidium* species and *E. histolytica*. PCR can detect low numbers easily missed by microscopy and also can differentiate pathogenic *E. histolytica* from morphologically similar, non-pathogenic *Entamoeba dispar/ mosbokensis*. During the initial validation period, 10 *Entamoeba* species identified by microscopy were tested by PCR and only two of these samples were positive for *E. histolytica*. The ability to differentiate between pathogenic *E. histolytica* and non-pathogenic *Entamoeba* species is clinically significant. Results concur with a prevalence study of *Entamoeba* in immigrants in Spain that showed that the majority of cyst-positive samples detected by microscopy were *E. dispar* and do not require treatment.

On the other hand, PCR seemed to become a ‘bane’ by detecting a large number of *D. fragilis* in asymptomatic people, especially children. Numerous parents of ‘well’ children get anxious about this so called ‘bad bug that does not go away’. Many of these ‘worried well’ get treated with various antiparasitic drugs and combinations (some of which are known to imbalance the normal gut flora). They ‘doctor shop’ and are being referred to specialists such as paediatricians, gastroenterologists and infectious diseases physicians (communications with General Practitioners and Australia and New Zealand Paediatric Infectious Diseases Group).

The reliance on morphological diagnosis by microscopy of fixed stained-smears and the recognition that parasite shedding may be intermittent, suggests that prior to PCR diagnosis, the true prevalence of this protozoon has been underestimated. A review of *D. fragilis* carriage has shown that prevalence ranges from 0.2% to 82% in numerous geographical settings, utilising a variety of detection methods. In this review, the authors conclude that in ‘symptomatic patients who harbor *Dientamoeba* and no other pathogen, it should be considered as the etiological agent and treated’. However, they also mention that evidence is only circumstantial. There has been debate about the clinical significance of *D. fragilis* given such wide variation in prevalence estimates coupled with the observation that the protozoa may be found in symptomatic and asymptomatic individuals and variable responses to antiparasitic drugs. A recent case-controlled study by Krogsgaard *et al.* demonstrated that *D. fragilis* and *Blastocystis* were detected in a greater proportion of faecal samples from the asymptomatic background population in Denmark than from subjects with irritable bowel syndrome. Röser *et al.* presented data from four years of routine PCR testing and found that from a total of 22,484 samples, 43% were positive for *D. fragilis*. With the introduction of PCR into routine testing within our laboratory, our data suggest that the prevalence of asymptomatic *D. fragilis* carriage is higher than previously reported.

Of all *D. fragilis* detected in our study period, 40% of faeces were formed, 58% were semi-formed or unformed and only 2% were loose/liquid as observed in the laboratory. The clinical notes provided on pathology request forms ranged from diarrhoea to vague...

### Table 2. Protozoal infections detected by multiplex PCR February 2014 to May 2015.

<table>
<thead>
<tr>
<th></th>
<th>Total positive (percentage)</th>
<th>% of all positives</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. fragilis</em></td>
<td>828 (15.3%)</td>
<td>78.0</td>
</tr>
<tr>
<td><em>G. intestinalis</em></td>
<td>183 (3.4%)</td>
<td>17.2</td>
</tr>
<tr>
<td><em>Cryptosporidium spp.</em></td>
<td>34 (0.6%)</td>
<td>3.2</td>
</tr>
<tr>
<td><em>E. histolytica</em>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13 (0.24%)</td>
<td>1.22</td>
</tr>
<tr>
<td>Total positives</td>
<td>1062 (19.7%)</td>
<td>100</td>
</tr>
<tr>
<td>Total tested</td>
<td>5384</td>
<td>–</td>
</tr>
</tbody>
</table>

<sup>a</sup>Additional four liver aspirates were positive by PCR.

*E. histolytica* in liver aspirates from four cases of suspected amoebic hepatitis.

The detection of protozoa by molecular technology at St Vincent’s Pathology, Melbourne, over the 15 month period since implementation of the multiplex PCR assay is shown in Table 2. The rate of *D. fragilis* detection increased from 2.1% by microscopy to 15.3% with PCR; this represents the most significant finding, accounting for 78% of all parasites detected by this test.

### Discussion

The advent of PCR has definitely become a ‘boon’ for our laboratory in providing a rapid, less labour-intensive method for detecting the three pathogenic protozoa *G. intestinalis, Cryptosporidium* species and *E. histolytica*. PCR can detect low numbers easily missed by microscopy and also can differentiate pathogenic *E. histolytica* from morphologically similar, non-pathogenic *Entamoeba dispar/ mosbokensis*. During the initial validation period, 10 *Entamoeba* species identified by microscopy were tested by PCR and only two of these samples were positive for *E. histolytica*. The ability to differentiate between pathogenic *E. histolytica* and non-pathogenic *Entamoeba* species is clinically significant. Results concur with a prevalence study of *Entamoeba* in immigrants in Spain that showed that the majority of cyst-positive samples detected by microscopy were *E. dispar* and do not require treatment.

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IBS like symptoms such as abdominal pain or discomfort, altered bowel habit and others unrelated to gastrointestinal tract. Samples that were positive for *D. fragilis* by PCR were predominantly in children below 10 years with a second peak in young adults in age group 30–40 years (Figure 2), a similar distribution to that described by Röser *et al.*

With the more widespread adoption of faecal parasite multiplex PCR assays, the higher positive rate of *D. fragilis* by PCR reported by laboratories (especially when diagnosed in those with non-specific symptoms), is confusing and of concern to clinicians. Many patients get unnecessary courses of antiparasitic drugs with more than half not clearing the parasite. A double-blinded, placebo-controlled metronidazole study by Röser *et al.* suggests that treatment is not associated with better clinical outcomes. Apart from optimal therapy not being known and a highly variable response to treatment, the pathogenicity of *D. fragilis* has not been reliably demonstrated.

It must be noted that the use of molecular technology is limited to the targeted screening of only a relatively small number of parasites in a low prevalence population. Microscopy still plays a key role in less common parasite identification, especially in specific patient groups where parasites other than those targeted by this multiplex are suspected. For parasite examination in populations from regions where there is high parasite endemicity, microscopy remains the ‘gold standard’ with the capacity to screen for the majority of pathogens.

In summary, molecular testing can offer accurate results for the detection of the four commonly seen parasites in low prevalence populations. It has been shown to be comparable to EIA for *G. intestinalis* and *Cryptosporidium* diagnosis and superior to microscopy for *D. fragilis* and *E. histolytica* detection. Our new testing algorithm for parasite detection utilising PCR has shown to improve sensitivity and specificity. It has significantly reduced the time taken to stain fixed smears and microscopy, allowing for a more efficient use of labour, whilst improving parasite detection rates. However, laboratories should consider the limitations of reporting parasites of doubtful clinical significance such as *D. fragilis* and *Blastocystis* species, and should alert physicians of the possibility of this being non-pathogenic in immunocompetent people.

**Summary of current faecal protozoa molecular testing:**

- Very good at detecting low numbers of known protozoan parasites such as *E. histolytica*, *Cryptosporidium* species and *Giardia intestinalis*
- Useful for discriminating between invasive *E. histolytica* and non-pathogenic *Entamoeba* species
- Detects protozoa with doubtful pathogenicity such as *D. fragilis* at a higher rate when compared to microscopy, leading to confusion, anxiety and unnecessary treatment
- Cannot differentiate animal subtypes of *Blastocystis* (non-pathogenic) from human subtypes (of debatable pathogenicity)

**References**


**Biographies**

**Colin Pham**, is a Medical Scientist with over 10 years’ experience in bacteriology, serology and molecular testing. He underwent his training at St Vincent’s Melbourne in 2003, and started his career in Microbiology at the Monash Medical Centre. Since rejoining St Vincent’s, he has specialised in molecular testing and emerging molecular technology. He completed a Masters of Applied Science (Medical Science) at RMIT in 2014, with his thesis titled ‘Impact of molecular testing on the laboratory diagnosis of *Giardia intestinalis*, *Cryptosporidium parvum*, *Dientamoeba fragilis* and *Entamoeba histolytica* from clinical stool samples’.

**Dr Harsha Sheorey**, is a Clinical Microbiologist at St Vincent’s Hospital in Melbourne. His special interest is in clinical parasitology and tropical medicine and is the author of *Clinical Parasitology – a handbook for medical practitioners and microbiologists*: a second edition has recently been published. He is an invited writer for the Nematodes chapter in ASM Manual of Clinical Microbiology. He coordinates the Victorian branch of Parasitology & Tropical Medicine SIG and is actively involved in organisation of the Australian Parasitology and Tropical Medicine Master Class.
FT020 Water Microbiology Australian Standards Committee

Robin Woodward
Email: robin.woodward@alsglobal.com

I am the Australian Society of Microbiology representative on the FT020 Water Microbiology Australian Standards Committee. This committee meets when there are Australian Standards (AS) that are up for review (approximately every 5 years) or when a new Standard has been proposed and requires work. Before I became a member on this committee I thought AS were put together by an elite group of people and that was all they did. The Committee is made up of elite members, (of course that goes without saying 😉) but we are people who work in the industry and most still work in the laboratory or have at some time in our career. There is the Standards Australia secretary who ensures the meeting and Standards follow the correct protocols, the Chair who runs the meeting, a NATA representative and members comprising people like myself who are in a supervisory role but still perform bench work in the real world. I still do bench work and that is why I enjoy the challenge of being on the Committee. It entails reading all the Standards, assessing their relevance and at meetings discussing any issues that may arise from the testing side. These issues can be industry driven changes, changes to the type of testing required, the introduction and availability of new methods or consumables, feedback from users of the Standard or personal experience when using the method in the laboratory. The Standards are reviewed and it is then the decision must be made on what to do with the Standard. They can be reconfirmed (no changes), withdrawn or revised (the Committee evaluates and revises the Standard).

When a Standard is released for public comment it is crucial that the users of the Standard read it and make any relevant comments. All of these comments are documented and must be discussed by the committee at a meeting. The discussion outcome is also documented and any changes required are made and eventually the Standard is printed. It is not an easy process. Writing an unambiguous Standard takes time and a lot of discussion. Think back to those tasks you had at TAFE or university when you had to write instructions on how to cook a piece of toast. It is often the little things that can be overlooked, the things you may take for granted. There are certain guidelines that have to be followed and are set out by Standards Australia. The method steps are there to follow so that everyone using the Standard is doing the same thing therefore creating a uniform procedure that should give the same results no matter where you are. Standard procedures are important and it is the basis of the quality systems in laboratories.

So please comment if you feel there is something you need to bring up when a Standard goes out for Public Comment. Even if it just a typographical error or something you think is wrong.-COMMENT. Your company can subscribe to StandardsWatch so you are alerted when a Standard relevant to your area is released or open for public comment.

Thank you to the ASM and my employer (ALS) for supporting me to be a committee member on FT020 Water Microbiology. It is appreciated and it is only through organisations like the ASM and generous employers that Standards Australia committees can operate. All committee members are volunteers and must have the relevant qualifications and knowledge to ensure that these Standards can continue to be developed and maintained.

2016 ASM Communication Ambassador Program

The ASM is looking for teams of student and ECR members to volunteer as part of a roster of Communication Ambassadors within the society, who will:

1. Be in charge of ASM’s online communication channels together with a team of ASM members, bolstering the society’s networks by sharing your story with scientists, industry, media members and policy makers;

2. Effectively represent the society by using these channels to showcase your work, reveal personal insights into your scientific career, and serve as a public advocate for microbiology.

We are looking for enthusiastic members who are keen to contribute. To apply for the 2016 program, please submit an Expression of Interest (maximum 200 words) to admin@theasm.com.au by 31 March 2016 and highlight the following:

- Your name, institution, qualifications, area of interest within microbiology, and ASM membership status;
- Your strategy for broadening ASM’s online presence and collaborating with other communication ambassadors;
- What conferences, scientific meetings, or events you can provide social media coverage for in 2016.

Successful applicants for the 2016 Communication Ambassador program will receive a $100 discount for registration to the 2016 ASM Annual Scientific Meeting (3–6 July 2016, Perth Convention & Exhibition Centre).
Stopping dengue: recent advances and new challenges

Gayathri Manokaran, Kirsty McPherson and Cameron P Simmons

Symposium 16 November 2015

Peter Doherty Institute for Infection and Immunity, Melbourne

Dengue remains a major problem throughout the world with an estimated 30% of the world’s population at risk of infection. In the past few years, major advances have been made across virology, clinical insights, vaccines and mosquito control strategies. The first of its kind to be ever held in Australia, the International Dengue Symposium 2015 showcased some of the best science and clinical/public health research being undertaken in the field.

Proudly organised by The Peter Doherty Institute for Infection and Immunity, under the leadership of Professor Cameron Simmons, this symposium brought together 130 national and international dengue researchers. The organising committee acknowledges the support of ASM as an important partner to this meeting, together with generous support from The University of Melbourne, The Royal Melbourne Hospital, the Oxford University Clinical Research Unit, Vietnam and the DUKE-NUS Graduate Medical School, Singapore. Industry sponsors included Sanofi Pasteur, BioTools, VWR International, In Vitro Technologies, Pacific Lab Products, Interpath Services and Alere Global.

The formal symposium was opened by Professor Cameron Simmons. This was followed by a series of 21 brief talks spanning across four main themes of dengue research: Virology, Public Health, Clinical Research and Immunology/Pathogenesis. Short breaks for networking were scheduled in between each of these four sessions.

All of the invited speakers had considerable international profiles in dengue and hence the quality of the presentations was very high. Associate Professor Sheemei Lok discussed the structural basis behind how a dengue-2-specific human monoclonal antibody effectively protects mice from dengue infection due to its ability to ‘lock’ the dengue envelope proteins while blocking the binding of enhancing antibodies. Following that, Professor Ooi Eng Eong described the novel finding of a viral RNA-host protein interaction that allows dengue virus to evade the host immune response—this work was recently published in the prestigious journal, Science.

A highlight of this symposium was the ‘Public Health’ session, which was the first of its kind in Australia, with speakers from various companies including Sanofi Pasteur and Takeda, coming together to discuss their respective dengue vaccines, thereby providing attendees with a useful summary of all the dengue vaccines currently in clinical development.

Novel and highly innovative medical technologies were next discussed in the ‘Clinical Research’ session, with Dr Jenny Low explaining how in vivo imaging technologies such as positron emission tomography are being optimised presently to track dengue virus progression in a mouse model. Dr Sophie Yacoub gave a summary on changes in microvasculature of dengue patients upon infection and how these disturbances might precede the development of severe disease.

In the next session, Dr Laura Rivino discussed their work on elucidating the human cellular responses during dengue infection and how both CD4+ and CD8+ T-cell subsets remain enriched in the skin of dengue patients up to 5 months post infection. Professor Cameron Simmons then described an interesting dengue neutralisation assay developed in Vietnam using patients’ viraemic blood and infected mosquitoes as a readout of the efficacy of monoclonal antibodies to efficiently neutralise dengue virus. Next, Dr David Muller introduced the concept of nanopatches that allow vaccine delivery in a needle-free manner.

In closing, the sponsors were wholeheartedly acknowledged for their contributions by Professor Cameron Simmons and their support was instrumental in allowing this symposium to be a success. Not only was the stellar line-up of talks highly educational but importantly, this symposium provided a networking opportunity for everyone interested in meeting the challenge of reducing dengue burden throughout the world.
14th International Conference of Culture Collections
ICCC14

1-6 November 2016
Mardan Palace Antalya / Turkey

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Prof. Dr. Bülent Güler
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www.iccc14-wfcc.info

Congress Organizer: SymCon MICE / iccc14@symcon.com.tr
Confirmed Plenary speakers

- **Professor Peter Hawkey**
  University of Birmingham
  Nosocomial infection control and antibiotic resistance

- **Professor Dan Andersson**
  Upsalla University
  Environmental pollution by antibiotics and its role in the evolution of resistance

- **Assoc Prof Susan Lynch**
  University of California San Francisco
  Colitis, Crohn’s Disease and Microbiome Research

- **Dr Brian Conlon**
  Northeastern University, Boston
  Drug discovery in soil bacteria

- **Professor Anna Durbin**
  Johns Hopkins
  Dengue and vaccines

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2016 Rubbo Oration

**Professor Anne Kelso**
CEO NHMRC

As with previous years, ASM 2016 will be co-run with EduCon 2016: Microbiology Educators’ Conference.

Watch this space for more details on the scientific and social program, speakers, ASM Public Lecture, workshops, ASM awards, student events, travel awards, abstract deadlines and much more..

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