Background

Definitive diagnosis of malaria infection requires identification of the parasite on a stained blood film. The ICHS recommendation is to report their presence and identification of the species should be made and reported. There are currently 5 species of the malaria parasite and each species has a unique set of morphological characteristics. RCPAQAP provides an external quality assurance (EQA) program for malaria parasite identification. This paper reviews the performance of the program in its 11 years of providing proficiency testing in malaria parasite morphology.

Method

The EQA program consists of 2 surveys per year and 2 case studies per survey. Each case study consists of a thick and thin blood film, stained at pH 7.2. A small expert committee, using a similar format for morphological diagnosis as the participants, conducts selection of case studies. In each survey, participants receive two case studies, brief clinical details and limited full blood count results. Participants report the morphological feature(s) and a diagnosis using a predetermined list of morphological features and diagnoses. A graphical report including results, peer comparisons and educational commentary is then provided to the participants. The results of 44 cases over 11 years from 2008 to 2018 have been reviewed.

Results

Case studies submitted for assessment include: Plasmodium falciparum (1), Plasmodium vivax (13), Plasmodium malariae (6), Plasmodium ovale (3), mixed infections (6) and a normal blood film (1). Of the 15 cases of P. falciparum, 2 cases returned <50% acceptable response (figure 1). In contrast the other three species (figure 2 and figure 3) returned acceptable response ranges from 52% to 97%. Figure 4 outlines the case studies with mixed infection and a case study with no malaria parasites on the thick or thin film. The 2 case studies with P. falciparum > P. malariae returned >70% acceptable responses, whereas the 2 case studies with P. falciparum > P. ovale returned acceptable responses of <50%. Acceptable responses for 2 cases with P. falciparum > P. vivax infection varied, with one returning 21% and the other 36%. The normal blood film returned a 76% acceptable response.

Discussion

The great standard for detecting and identifying Plasmodium species remains the visualisation of the parasite on light microscopy. Review (figure 1-4) of the Malaria Parasite EQA proficiency program by RCPAQAP indicates varied performance on the identification and differentiation of the 5 species of plasmodium. The current review presents data on the acceptable responses submitted by participants. To align the current review to previous published reports, the discussion will focus on the unacceptable responses by participating laboratories.

In this retrospective review, the rate of unacceptable response for samples containing P. falciparum was an average of 22%, a result that is similar to 2 previous studies. A report from Ontario, Canada and the British EQA indicated unacceptable response rates of 27% and 21% respectively. In contrast a report by the American Proficiency Institutes (API) Parasitology program and Hong Kong EQA program indicated much lower unacceptable rates of 3.4% and 5% respectively. Of the other species, case studies containing P. malariae demonstrated the lowest unacceptable response rate of 9%. In contrast, P. ovale and P. vivax had unacceptable response rates of 28% and 25%, respectively. Case studies with mixed infections (P. falciparum plus other species) had the highest unacceptable rate of 48%.

The difference in the rate of acceptable response across the different EQA was in the design of the EQA schemes. In the API scheme all slides were evaluated by a reference laboratory and the evaluation criteria of acceptability includes “Plasmodium sp parasites seen, referred and Plasmodium, not falciparum”. These responses indicate that participants refer their final or definitive diagnosis to a reference laboratory. On the other hand, the Hong Kong participants use a central reference laboratory (CMRL) to cross-check all case studies, and the assessment was based on a comparison between the report from CMRL and those from the participants. The assessment criteria utilised by UK-NEQAS, Canada and the current review by RCPAQAP, might have contributed to the high unacceptable rate for the identification of Plasmodium species. The EQA by RCPAQAP requires participants to recognise and report both the morphological features of the parasites as well as identifying the species of the malaria parasite.

The high unacceptable response for P. falciparum infection as a mixed infection can be due to morphological variations in the stages of development of the parasites. In the early trophozoite stage of P. falciparum the parasites are smaller in size and have a tiny nucleus and delicate cytoplasm, in contrast with mature trophozoites which are relatively bigger in size, more compact and pigmented. This stage of development may have directed participants to the diagnosis of other malaria species. In contrast the misclassification of P. ovale infections as P. vivax and vice versa could be attributed to the close morphological similarities between the two species (i.e. inclusions, host cell enlargement, rounded gametocytes).

Although microscopic examination remains the ‘gold standard’ for the identification of the malaria infection, laboratories which are not proficient in the accurate identification and speciation of Plasmodium species should have access to alternative testing, such as referral of samples for verification of speciation at a reference laboratory, or molecular amplification.

Conclusions

The identification and differentiation of the malaria parasite species through light microscopy remains clinically relevant and challenging. The review highlights the need for continued professional development. Participation in an EQA program may facilitate the identification of areas requiring improvement, ultimately reducing misclassification or misdiagnosis of malaria infection.

References