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Letter to the Editor

Identification of gaps in the performance of routine microscopy for the diagnosis of parasitic infections revealed by the Dutch laboratory quality assessment scheme

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To the Editor,

Microscopic examination of clinical material is a widely used, all-round method, which is globally considered to be the reference test for the laboratory diagnosis of parasitic infections. This, despite the fact that alternative diagnostic procedures, such as immune-chromatographic tests and nucleic acid amplification methods (e.g. real-time PCR), are increasingly introduced in medical microbiology laboratories [1]. A major advantage of microscopic examination of clinical samples is the fact that it is an all-round technique, detecting a wide range of parasite species, whereas alternative procedures are generally designed to detect a single or small set of predefined parasites only. In addition, microscopic examination requires non-expensive equipment and cheap consumables. Consequently, well-trained technicians are still needed to perform adequate examination of clinical samples such as blood and stool.

External quality assessment schemes (EQAS) support clinical laboratories in improving their laboratory diagnostics by offering regular distributions of blinded samples. The Dutch Foundation for Quality Assessment in Medical Laboratories (SKML) organizes EQAS for a range of laboratory disciplines, including parasitology.

In this study, the results of the Dutch SKML-EQAS for microscopic examination of blood and intestinal parasites over an 8-year period were examined to determine the gaps in the performance of routine microscopy, which will indicate focus points for training of technicians. During the period 2013–2020, approximately 90 and 75 clinical laboratories participated in the SKML-EQAS for blood parasites and intestinal parasites, respectively. Their findings were examined for overall trends in the level of performance and possible associations with parasite species or types of specimens (see Supplementary documentation for applied methods).

The evaluation of the results of the Dutch EQAS showed better performance scores in microscopic identification of blood parasites than intestinal parasites (Fig. 1(a) and (b)). Concerning the blood parasites, more than 80% of participating laboratories correctly identified 90–100% of the *Plasmodium falciparum* ($n = 28$) and *P. malariae* ($n = 5$) samples (Fig. 1(a)). The best scores were found in samples with *P. falciparum* and the poorest scores in samples with *P. ovale*, *P. vivax*, *Loa loa*, and *Trypanosoma brucei* species. *P. falciparum* samples with parasite densities below 1% of infected erythrocytes were more frequently misidentified compared with *P. falciparum* samples with higher parasite densities (see Supplementary Results and Table S4). Concerning the intestinal parasites, better performance scores were observed for samples with helminths than with protozoa (Fig. 1(b)). The five intestinal parasites

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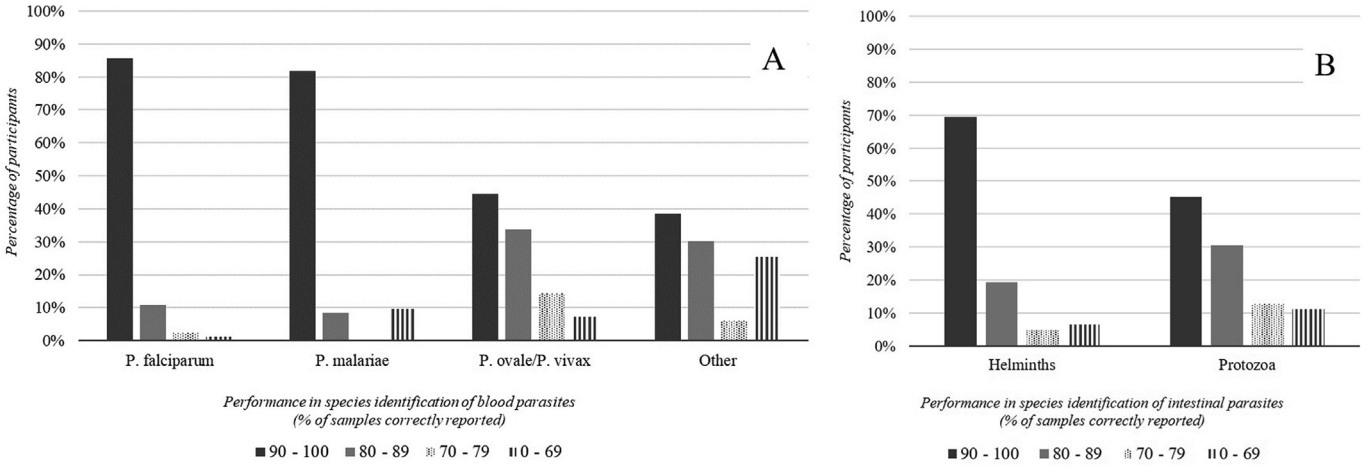

Fig. 1. Performance of participating laboratories on identification of (A) blood and (B) intestinal parasites. 'Other' refers to *Loa loa* and *Trypanosoma brucei* species.

for which the most mistakes were reported were *Dientamoeba fragilis* (56/171, 33%); *Cryptosporidium* species (33/160, 21%); *Schistosoma mansoni* (33/161, 20%); hookworm (16/55, 29%); and *Schistosoma haematobium* (6/59, 10%) (see Supplementary Results and Tables S4 and S5). Participating laboratories that performed poorly in the EQAS for blood parasites also performed poorly in the EQAS for intestinal parasites (Fig. S1).

Reasons for poor performance are unknown, but a few causes can be postulated. First, some laboratories might use non-optimal techniques (e.g. specific staining for *Dientamoeba fragilis* and *Cryptosporidium* species) for microscopic examinations, which is supported by recent surveys that revealed an astonishing diversity in methodology used for the detection of blood and intestinal parasites [2–5]. Second, some laboratories may have relatively little experience with microscopic examination for parasites, because some laboratories examine relatively few samples or perform diagnostics for a population with few travellers or migrants in which parasitic infections occur more frequently. Furthermore, it has been suggested that the lack of expertise in microscopic detection of parasites has been exacerbated by the increased use of non-microscopy-based diagnostics, such as PCR and rapid diagnostic tests. These novel techniques are replacing the microscopy-based methods and thereby contribute to the progressive, widespread loss of morphology expertise for parasite identification [1]. This problem argues for further concentration of expertise centres of microscopy-based methods by either regional collaboration or outsourcing of testing. A possible reason why laboratories performed better at identifying blood parasites than intestinal parasites is that there may be more focus and training on blood malaria because of its potentially fatal outcome if mistakes are made.

The results of this study demonstrated the several gaps in the performance of routine microscopy. Concerning training of microscopists on the detection of blood parasites, the focus should be on (a) proper determination of *Plasmodium* species in case of low parasitaemia and (b) detection and species determination of microfilaria and trypanosomes. For the detection of intestinal parasites in stool, it should focus on discrimination (a) between *Rodentolepis nana* (previously known as *Hymenolepis nana*) and *Hymenolepis diminuta*, (b) between larvae of *Strongyloides stercoralis* and those of hookworms, and (c) between the protozoan cysts of *Entamoeba histolytica/E. dispar* and other protozoan cysts.

This study demonstrated that the overall performance of participating laboratories for the detection of blood and intestinal parasites is quite variable. Participation in an EQAS is an essential tool for laboratories to monitor their quality of microscopic detection and identification of parasites and can be used to identify the focus points for training of their technicians.

Author contributions

MBB and RK curated and analysed the data. MBB wrote the draft version of the manuscript and prepared all figures and tables. JJvH conceived and supervised the study. All authors were involved in the organization of SKML-EQAS for blood and intestinal parasites, design of the study, revision of draft manuscript, and they have read and approved the final manuscript.

Transparency declaration

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2024.02.018.

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