NOVEL RESOURCES FOR LEARNING THE IDENTIFICATION OF HUMAN-RELATED PARASITES

A. Peña-Fernández[1], M.C. Lobo-Bedmar[2], L. Acosta[3], F. Izquierdo[4]

[1]*De Montfort University, School of Allied Health Sciences (UNITED KINGDOM)*
[2]*IMIDRA, Departamento de Investigación Agroambiental (SPAIN)*
[3]*Universidad Miguel Hernández de Elche (SPAIN)*
[4]*Universidad San Pablo CEU, Facultad de Farmacia (SPAIN)*

Abstract

Microscopic detection of human-related parasites in a range of clinical samples remains the cornerstone of parasitological diagnosis despite recent advances in technology and molecular sciences. However, the use of the light microscope for diagnostic purposes requires comprehensive training, skills and parasitology knowledge that is difficult to appropriately provide to future health professionals due to different challenges including shortages of health science academics, resources, time and specimens for delivering appropriate training. An international teaching innovation team from different European universities, led by De Montfort University (UK), is building a novel resource for learning and teaching parasitology, which is equipped with a Virtual Laboratory and Microscope. In the Virtual Laboratory (http://parasitology.dmu.ac.uk/learn/laboratory.htm), we are building a complete sub-section with a series of engaging units for learning different parasitological staining/fresh preparation techniques for detecting common and rare (emerging and re-emerging) human parasites from several taxa: protozoa (mostly cysts and oocysts) and helminths (eggs and organs for parasitological differentiation such as scolex or proglottids for *Taenia* spp.) and fungi (spores), which will be publicly available in 2019. Examples of staining techniques included are Kinyoun, Trichrome and Modified Trichrome stain and fresh preparations for investigating eggs as well as more recent techniques such as immunofluorescence. The Virtual Laboratory will also provide resources to undertake appropriate sample (faeces, blood and urine) collection, management and preparation for parasitological diagnosis and the use of different microscopes including the light microscope for parasite analysis. These units will be equipped with short videos of academics and technicians performing the different techniques, which will include audio and subtitles in English, and will be supported by photos, artworks, designs and self-assessment mini-quizzes and exercises, to provide students with the most practical experience possible. Finally, a complete library of digitised clinical slides of different specimens and parasites is provided here: http://parasitology.dmu.ac.uk/learn/microscope.htm. Each virtual slide is provided with the functionality of a microscope, so the user will be able to zoom in and out and explore all of the clinical sample to learn the morphological characteristics of cysts, oocysts, eggs, spores and other important structures for parasitological diagnosis. When relevant, a variety of virtual slides for the different species for the same parasite will be provided to enhance the identification of parasites to species level in conjunction with a short description and tips for easy identification. The resources that are being created will cover the theoretical foundation and current scientific information so they will be suitable for undergraduate/postgraduate students as well as for more professional training. This paper will present a complete overview of these novel resources that are aimed at training future professionals in parasitic disease diagnosis with microscopic identification of parasites; these web-based resources could help to overcome current limitations that are eroding the teaching status of parasitology. Finally, different strategies will be presented to facilitate the introduction and use of this novel resource in any human health programme.

Keywords: DMU e-Parasitology, staining techniques, specimen identification, parasitology diagnosis.

1 INTRODUCTION

Parasitological knowledge is of paramount importance for facing current outbreaks due to emerging and re-emerging parasites affecting humans in Europe such as *Leishmania* in Italy [1], as well as affecting food [2] or food producing animal species that could, in turn, impact human health, *e.g. Fasciola hepatica* in rumiants [3]. Moreover, these outbreaks and public health risks can have a severe impact not only in society but also in their economies; thus, for example the costs of a waterborne outbreak due to *Cryptosporidium hominis* in Ireland has been estimated to be higher than

€19 million [4]. However, the reduction in student contact hours for teaching parasitology due to the changes in curricula to align them to the Bologna process [5] in conjunction with a decrease in the parasitology departments and other factors such as the exponential increasing of parasitological knowledge and diversity of subjects that require to be taught [6], have impacted negatively in the teaching status of parasitology.

To address this, an international teaching innovation team from different European universities, led by De Montfort University (DMU, Leicester, UK), is building a novel resource for learning and teaching parasitology, named DMU e-Parasitology [7-8], which will be available on the DMU website in 2019. Owing to the fact that microscopic detection of parasites in human, animal or environmental samples remains the cornerstone of parasitological diagnosis despite advances in technology and molecular sciences [9], DMU e-Parasitology is equipped with the following resources for appropriate learning of this technique:

a) **Virtual Biomedical Laboratory**, which will be equipped with different web-based units for learning how to perform different staining techniques for parasites. This resource is briefly explained below.

b) **Virtual Microscope**, which is provided with a collection of virtual slides of real clinical specimens displaying human parasites for their study. This resource is accessible at the DMU website here http://parasitology.dmu.ac.uk/learn/microscope.htm; and the user with be able to explore any part of the slide/specimen at any magnification, using the functionality of the virtual microscope. Thus, the user can learn the morphological features and the characteristics of the different infective forms of human parasites including cysts, oocysts, eggs and spores as well as the adult forms of helminths (cestodes, nematodes and trematodes) and hematic phases of some parasites. A more detailed description of this resource has been depicted in Peña-Fernández et al. (2018) [10].

1.1 Virtual laboratory

We are following the same methods and structure used to build the other DMU e-Parasitology modules to develop the Virtual Laboratory section, which will be equipped with different sub-sections for learning how to detect, identify and study common and emerging human parasites [11]. Sub-sections currently under development are the following, which present different e-learning units: microscopy; molecular biology; cell and parasite culture; biochemical and immunological techniques; staining techniques.

2 MICROSCOPIC DETECTION TECHNIQUES

As described previously, the virtual laboratory module has a series of sub-sections to facilitate navigation and enhance their use and comprehension. To date, we have initially prepared the following two sub-sections, as they are relevant for detecting parasites using microscopy techniques: staining and immunological techniques. The e-learning units included in these sub-sections followed a similar scaffold, which consisted of providing a complete description of (Figure 1): a) detection technique and their applicability; b) consumables needed; and c) short videos of an academic demonstrating each of the steps of the procedure in real conditions in the laboratory (*i.e.* step-by-step). Units are also enhanced with slides depicting a full description of the protocol with photographs and/or graphic designs to facilitate acquisition of knowledge. The videos were recorded following previous experiences from the literature (Sowan and Idhail, 2014). To promote student engagement and inclusivity, as well as the full immersion of the student when playing the different videos, videos are provided with audio and subtitles. Finally, to enhance self-learning, these units will be provided with some formative quizzes and/or short exercises, using the applicability of the Virtual Microscope.

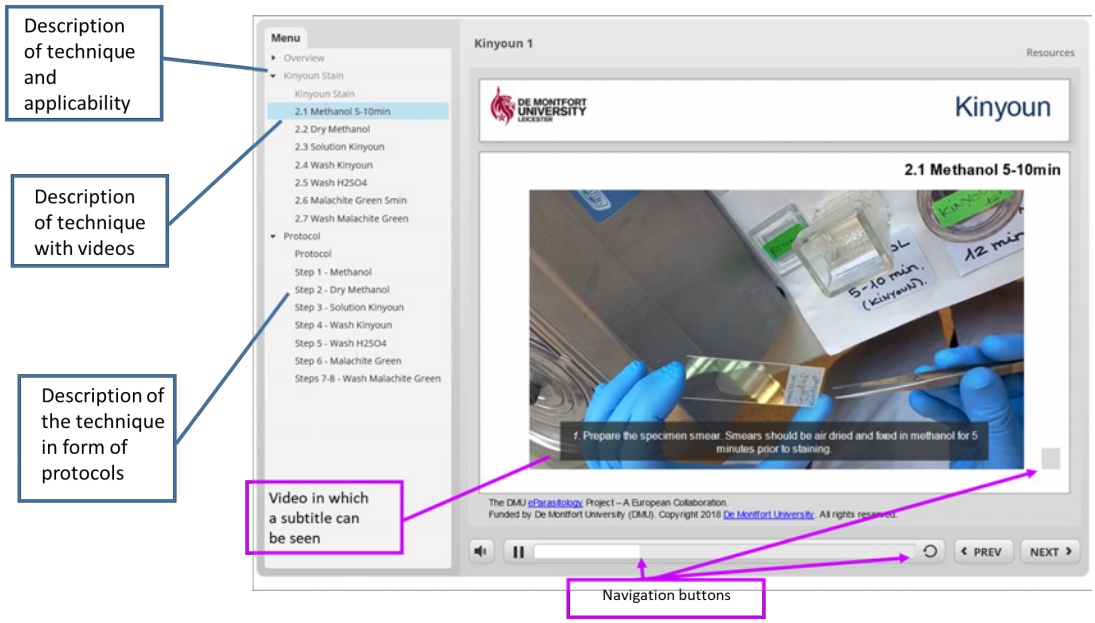

Figure 1: *Overview of one of the DMU e-Parasitology staining units (Kinyoun stain) displaying the structure (Image courtesy of DMU). Available at: http://parasitology.dmu.ac.uk/learn/lab/Kinyoun/story_html5.html*

2.1 Staining techniques sub-section

This sub-section will be equipped with the most relevant staining techniques for detecting parasites in a human, animal and/or environmental samples. Detailed information will be provided about the different staining techniques as, in contrast with bacteria and viruses, parasites are usually detected and identified by these methods. Other detection methods such as culture is only used for a few parasites when it is available, and under no circumstances would be the primary diagnostic technique. Antigen testing techniques are also used for a limited number of parasites, and molecular tests are restricted to reference and/or research laboratories [12].

Figure 2. Overview of the Staining Techniques' subsection in the DMU e-Parasitology virtual laboratory main page (Image courtesy of DMU and USP-CEU University). Available at: http://parasitology.dmu.ac.uk/learn/laboratory.htm

This sub-section is equipped with the following units so far (Figure 2):

a) **Acid-fast Kinyoun stain:** also known as modified Ziehl-Neelsen stain as it does not require a heating step. This staining involves the application of basic carbolfuchsin (stain), a decoloriser (acid-alcohol) and the use of methylene blue as a counterstain. Kinyoun stain has been described as the best cost-effective technique for detecting coccidian parasites including *Cryptosporidium* spp., *Cyclospora* spp. or *Cystoisospora* spp. in faecal matter [13-14] and as a reliable screening tool for detecting their oocysts in these types of sample [15].

b) **Modified Trichrome or Chromotrope 2R stain:** has been described as a useful stain for demonstrating human-related microsporidia in smears [16-17]. This staining method uses 2R stain and fast green as a counterstain [18].

2.2 Immunological technique sub-section

Although differential staining techniques can be considered as appropriate detection and/or screening tools, detection of parasites in clinical samples such as faeces, bodily fluids and/or tissues can be difficult and require highly skilled personnel to be performed. Thus, immunological techniques such as immunofluorescence have become emerging useful methods to diagnose different human parasitic infections such as microsporidiosis [19]. Therefore, we are building a complete sub-section on relevant immunological techniques for the study of parasites, which to date includes a unit related to indirect immunofluorescence antibody test (IFAT) for microsporidia (Figure 3). We have used the same methods and structure to develop the IFAT unit, which is also equipped with videos and detailed information of the procedure (Figure 4).

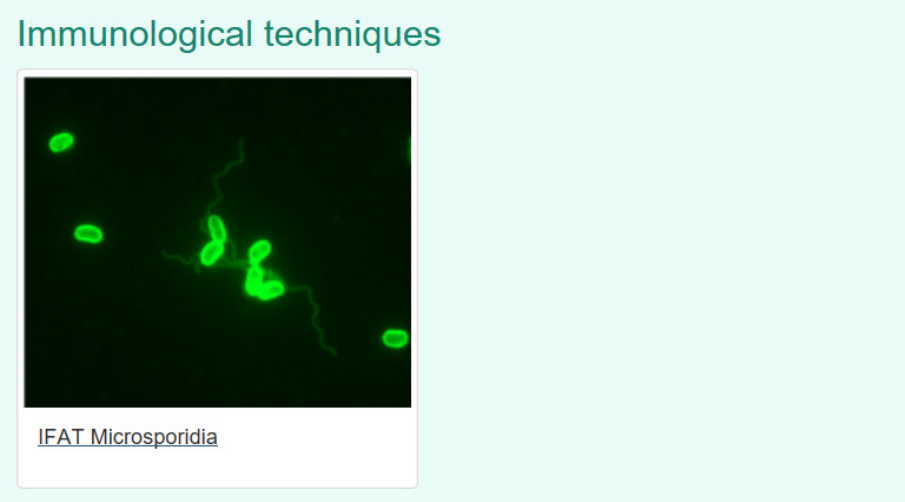

Figure 3. Overview of the Immunological Techniques' subsection in the DMU e-Parasitology virtual laboratory main page (Image courtesy of DMU and USP-CEU University). Available at: http://parasitology.dmu.ac.uk/learn/laboratory.htm

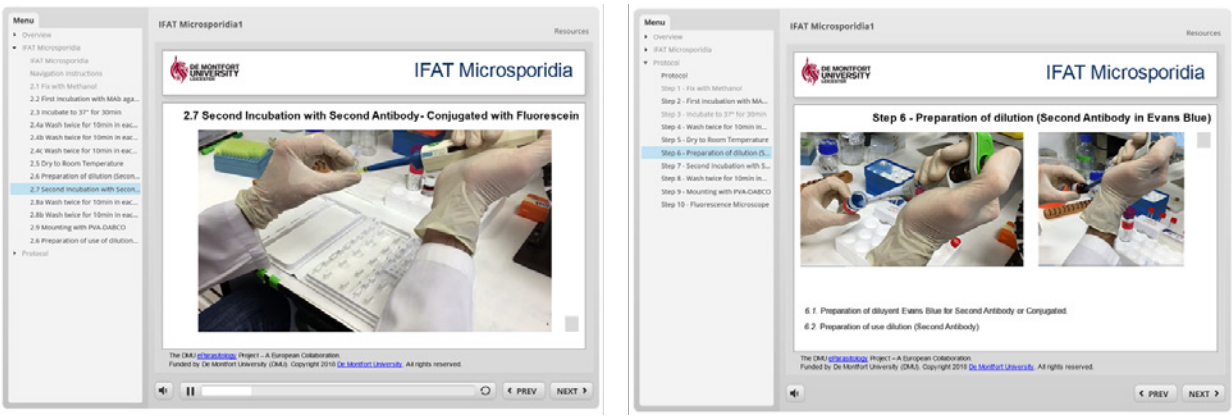

Figure 4. Overview of the IFAT technique, displaying video (left) and procedure protocol (right), in the DMU e-Parasitology virtual laboratory main page (Images courtesy of DMU). Available at: http://parasitology.dmu.ac.uk/learn/lab/IFAT%20Microsporidia/story_html5.html

2.3 Future work

The development of the detection sub-sections within the Virtual Laboratory started in early summer 2018 and some have been created so far. Units in development include normal Trichrome for the analysis of protozoa, including *Entamoeba* spp. and *Giardia* spp., and methods for identification of helminth eggs. Additionally, sampling and management preparation of samples for parasitological

analysis will be also appropriately covered, which will include coproscopy (including flotation and sedimentation) methods to provide the user with the key competences for diagnostic parasitology as recently described by Joachim et al. (2018) [6]. Additionally, a sub-section on microscopy, to cover the use and applicabilities of different microscopes including the scanning electron microscopy (SEM), due to its significance to study parasitic protozoa [20], is being built. Finally, the user will be provided with short quizzes and exercises for learning result interpretation, to facilitate self-learning and cement knowledge.

3 CONCLUSIONS

This paper described the preliminary development of a series of sub-sections and units that will cover the theoretical and practical foundation and emerging methods for the detection of human parasites, which are suitable for undergraduate/postgraduate students as well as for more professional training. The units are being prepared by academics and professionally recognised parasitologists working in the Research Support Service (SAI) and Testing Laboratory of “Análisis Parasitológicos” (SAI-ANP, 2018 [21]), which is integrated into the Network of Laboratories and Infrastructures of the Community of Madrid. Detection units are aimed at providing the user with a comprehensive insight for each diagnostic method, specifically traditional and new staining and immunological methods. These units and sub-sections within the DMU e-Parasitology will have a myriad of applicabilities once completed, from creating courses to enhance the teaching status of parasitology by overcoming barriers such as time and resources. Finally, these sub-sections will be appropriately validated with students from the participating universities in due course, prior to launching them in 2019.

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REFERENCES

[1] Cesinaro AM., Nosseir S., Mataca E., Mengoli MC., Cavatorta C., Gennari W. An outbreak of cutaneous leishmaniasis in Modena province (Northern Italy): report of 35 cases. Pathologica 2017; 109(4):363-367.

[2] Stentiford GD., Becnel J., Weiss LM., Keeling PJ., Didier ES., Williams BP., Bjornson S., Kent ML., Freeman MA., Brown MJF., Troemel ER., Roesel K., Sokolova Y., Snowden KF., Solter L. Microsporidia - emergent pathogens in the global food chain. Trends Parasitol 2016; 32(4):336-348.

[3] Beesley NJ., Caminade C., Charlier J., Flynn RJ., Hodgkinson JE., Martinez-Moreno A., Martinez-Valladares M., Perez J., Rinaldi L., Williams DJL. Fasciola and fasciolosis in ruminants in Europe: identifying research needs. Transbound Emerg Dis 2018; 65 Suppl 1:199-216.

[4] Chyzheuskaya A., Cormican M., Srivinas R., O'Donovan D., Prendergast M., O'Donoghue C., Morris D. Economic assessment of waterborne outbreak of cryptosporidiosis. Emerg Infect Dis 2017; 23(10):1650-1656.

[5] van Doorn DCK., Nijsse ER., Ploeger HW. Pitfalls and opportunities of teaching veterinary parasitology within an integrated curriculum. Vet Parasitol 2018; 252:85-88.

[6] Joachim A., Hinney B., Duscher G., Preusche I., Winter P. Teaching parasitology in a modular veterinary curriculum - The Vienna experience. Vet Parasitol 2018; 252:101-106.

[7] Peña-Fernández A., Ollero MD., Fenoy S., Magnet A., Izquierdo F., Peña MÁ., Bornay F., Acosta L., Parker LA., Sgamma T., Del Águila C. Creating a model module for the novel resource DMU e-Parasitology. ICERI Proceedings 2017; 1599-1604. ISBN: 978-84-697-6957-7.

[8] Peña-Fernández A., Acosta L., Fenoy S., Magnet A., Izquierdo F., Bornay FJ., Ollero MD., Hurtado C., del Águila C. Evaluation of a novel digital environment for learning medical parasitology. Higher Education Pedagogies 2018; *submitted for publication*.

[9] Penzhorn BL. A century of teaching veterinary parasitology in South Africa - Lessons learnt. Vet Parasitol 2018; 252:62-66.

[10] Peña-Fernández A., Fenoy S., Halliwell R., Izquierdo F., Magnet A., Hurtado C., Bornay F., Acosta L., Ollero MD., Armstrong M., Evans MD., Del Águila C. Development of a virtual library of clinical samples for medical parasitology diagnosis. INTED2018 Proceedings 2018b; pp. 7599-7604. ISBN 978-84-697-9480-7.

[11] Peña-Fernández A., Fenoy S., Evans MD., Sgamma T., Hurtado C., Izquierdo F., Randles M., Young C., Acosta L., del Aguila C. Development of a virtual environment for teaching and learning biomedical techniques and equipment for the study of human pathogens. EDULEARN18 Proceedings; pp. 7249-7253. ISBN: 978-84-09-02709-5.

[12] Murray PR. The clinician and the microbiology laboratory. In: Mandell, Douglas, and Bennett's principles and practice of infectious diseases. Edited by John E. Bennett, Raphael Dolin, Martin J. Blaser. Elsevier Saunders, Eighth edition, 2015; pp. 3047-3058. ISBN: 978-1-4557-4801-3.

[13] Ribes JA., Seabolt JP., Overman SB. Point prevalence of *Cryptosporidium*, *Cyclospora*, and *Isospora* infections in patients being evaluated for diarrhea. Am J Clin Pathol 2004; 122(1):28-32.

[14] Magi B., Canocchi V., Tordini G., Cellesi C., Barberi A. *Cryptosporidium* infection: diagnostic techniques. Parasitol Res 2006; 98(2):150-2.

[15] Ezzaty Mirhashemi M., Zintl A., Grant T., Lucy FE., Mulcahy G., De Waal T. Comparison of diagnostic techniques for the detection of *Cryptosporidium* oocysts in animal samples. Exp Parasitol 2015; 151-152:14-20.

[16] Weber R., Bryan RT., Owen RL., Wilcox CM., Gorelkin L., Visvesvara GS. Improved light-microscopical detection of microsporidia spores in stool and duodenal aspirates. The Enteric Opportunistic Infections Working Group. N Engl J Med 1992; 326(3):161-6.

[17] Moura H., Da Silva JL., Sodré FC., Brasil P., Wallmo K., Wahlquist S., Wallace S., Croppo GP., Visvesvara GS. Gram-chromotrope: a new technique that enhances detection of microsporidial spores in clinical samples. J Eukaryot Microbiol1996; 43(5):94S-95S.

[18] Garcia LS. Laboratory identification of the microsporidia. J Clin Microbiol 2002; 40(6):1892-901.

[19] Izquierdo F., Moura H., Bornay-Llinares FJ., Sriram R., Hurtado C., Magnet Á., Fenoy S., Visvesvara G., Del Aguila C. Production and characterization of monoclonal antibodies against *Encephalitozoon intestinalis* and *Encephalitozoon* sp. spores and their developmental stages. Parasit Vectors 2017; 10(1):560. doi: 10.1186/s13071-017-2503-z.

[20] de Souza W. and Attias M. New advances in scanning microscopy and its application to study parasitic protozoa. Exp Parasitol 2018; 190:10-33.

[21] SAI-ANP (Análisis Parasitológicos Laboratory), Research Support Services (SAIs) and Testing Laboratories integrated into the Network of Laboratories and Infrastructures of the Community of Madrid, 2018. Available at: http://www.uspceu.es/investigacion/servicios-apoyo and at: https://view.genial.ly/5aaa4a1bc1bd1b55e30fd538