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Performance analysis of software for identification of intestinal parasites

Análise de desempenho de software *para identificação de parasitas intestinais*

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ABSTRACT

Introduction: Intestinal parasites are among the most frequent diagnoses worldwide. An accurate clinical diagnosis of human parasitic infections depends on laboratory confirmation for specific differentiation of the infectious agent. **Objectives:** To create technological solutions to help parasitological diagnosis, through construction and use of specific software. **Material and method:** From the images obtained from the sediment, the software compares the morphometry, area, perimeter and circularity, and uses the information on specific morphological and staining characteristics of parasites and allows the potential identification of parasites. **Results:** Our results demonstrate satisfactory performance, from a total of 204 images analyzed, 81.86% had the parasite correctly identified by the computer system, and 18.13% could not be identified, due to the large amount of fecal debris in the sample evaluated. **Discussion:** Currently the techniques used in Parasitology area are predominantly manual, probably being affected by variables, such as attention and experience of the professional. Therefore, the use of computerization in this sector can improve the performance of parasitological analysis. **Conclusions:** This work contributes to the computerization of healthcare area, and benefits both health professionals and their patients, in addition to provide a more efficient, accurate and secure diagnosis.

Key words: software; images; parasitological diagnosis; HPJ; *Trichuris trichiura*; *Ascaris lumbricoides*.

INTRODUCTION

The presence and the advancement of technology in health care are due to the recognition that processing and analysis of experimental data, whether clinical or epidemiological, require constant evolution(9). In clinical laboratories the process is the same, each day they are demanding from their information systems, allowing for improvement to meet their needs.

The information system has numerous applications for clinical laboratory. A good example is a database with information on all exams, where the key elements of test are defined, such as name, identification code, units of measurement, reference values and critical values, among others. Fast and preparation information provided to customers are based on them, as well as the examination price and locations where it is performed(14). Information technology is also present in the customer care process from the registration, which assists in phonetic search, eliminating possible registration duplicity errors. The utilities of this system are also present in the collection process, by using barcode for sample identification, increasing efficiency, resulting in significant errors decrease, and reducing costs(15). Otherwise, electronic receiving of tests result, through automatic interface with the equipment that performed the analysis, and compiling patient's previous data to create a delta check, assist in the approval of data obtained in the analysis(2). The latest developments in the laboratories management applications facilitated the access to the results of tests released by the technical area over the Internet, or automatically sent by email to the end user, either the patient or their physician(4).

According to the World Health Organization (WHO)(5), it is estimated that 3.5 billion people worldwide are infected with one or more types of parasites. In Brazil, it is performed about 20 million parasitological examinations per year in public bodies, and large portion of infected population is in rural and urban poverty areas(6).

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An accurate clinical diagnosis of parasitic infections in human depends on laboratory confirmation to differentiate the specific infectious agent. Clinical examination is the first step in the diagnosis, and the stool ova and parasites (O&P) test is the standard test for this diagnosis[7, 8]. Such test may be carried out by several techniques standardized in clinical laboratories. Although different sensibilities and methodologies, all consist of two fundamental steps: microscopic and macroscopic analysis of samples[8]. Macroscopic examination allows observing stool consistency, smelling, presence of abnormal elements, such as mucus and blood, and adult worms or parts of them. The microscopic examination allows observing helminth eggs or larvae, cysts, protozoan trophozoites or oocysts.

Currently, exclusively manual techniques are used in Parasitology area, and are likely to be affected by variables, such as professional attention and experience. Furthermore, the sensitivity of conventional diagnostic ranges from 48% to 76%, since all the final analysis process depends exclusively on human evaluation, automated protocols for Parasitology are not found, so far[9].

Knowing these limitations and seeking to bring greater reliability to O&P test, this study aimed to develop an information system to assist in the parasitological diagnosis, which would be able to meet specificity, sensitivity and speed criteria. In this paper, we chose two types of roundworms to characterize the parasitological diagnosis aid system: *Ascaris lumbricoides* and *Trichuris trichiura*. The choice of the parasites was based on eggs morphology, since they are significantly different, and also because they are commonly found in O&P test.

MATERIAL AND METHOD

The analytical data were initially obtained by selecting microscopic images in the Laboratório de Biomedicina da Universidade Feevale, which were captured from pools known positive for the selected parasites (*Ascaris lumbricoides* and *Trichuris trichiura*). The resources used were a color CCD camera with resolution of 410,000 pixels, connected to the microscope Nikon Eclipse E200, from which the images were subsequently, transferred to a computer, and thus stroing the images. From the images obtained, the software Diagnosis Support System (DSS), specially developed for the system, made the identification of parasites eggs through morphometric analysis, area, perimeter and circularity, and use information on specific morphology and coloration characteristics of the parasites in question.

The system was developed in partnership with the Computer Course of Universidade Feevale, so that it is free to use without patent or paid registration. This system was created using C# language, with Microsoft Visual Studio 2005 platform and MATrix LABoratory (MATLAB) tool. C# is a object-oriented programming language designed by Microsoft, it is part of.NET platform, and is based on C++ and Java languages. According to Microsoft, Visual Studio 2005 is included in the Software Development Kit. Mostly, this platform is used for development in VB.NET and C#. MATLAB is a tool originally designed to perform matrix calculations. Today, it has several features in relation to image processing. According to Mosmann (2007), it is an interactive easy handling system that allows formulating solutions to the most diverse problems, especially those involving matrices. Therefore, specific solutions can develop quickly, compared with C or FORTRAN languages; also it has several libraries to work in different areas of scientific computing. In academic environments, MATLAB is often used for research and development[10].

The analysis of images obtained from the slides prepared with sediment acquired by spontaneous sedimentation technique, initially the system obtained the sample image through a camera connected to a microscope. At first, the prototype produced a new image from the original, but in grayscale. This was done because grayscale image is easier to manipulate. In preprocessing, and the image already in grayscale, a filter was applied to reduce image noise level by blurring it, thus, causing blurry, so the software could distinguish the edges of objects contained in the image more easily. In the segmentation process, an edge detector was applied in the filtered image; with this procedure a new image with the contours of objects was obtained. Subsequently, the image was scanned for the region of interest, that is, objects similar to a parasite, generating a new image. Then, the software analyzed the characteristics of the images and its objects, checking color, size, shape and texture. The *Ascaris lumbricoides* and *Trichuris trichiura* eggs were identified by eggs area, perimeter, and circularity characteristics; the area was defined as the number of black pixels in the segmented image. Then, to obtain the perimeter, an edge detector was applied in the image to identify the number of pixels in the edge. With the area and perimeter, the calculation was applied to determine the circularity (**Figure**).

In this study were analysed 204 images obtained through the camera connected to the microscope, 85 *Ascaris lumbricoides*, 54 *Trichuris trichiura*, and also 65 artifacts, to verify the reliability of the DSS software. Each image was subjected to the software, which processed it and indicated the type of parasite eggs in the sample.

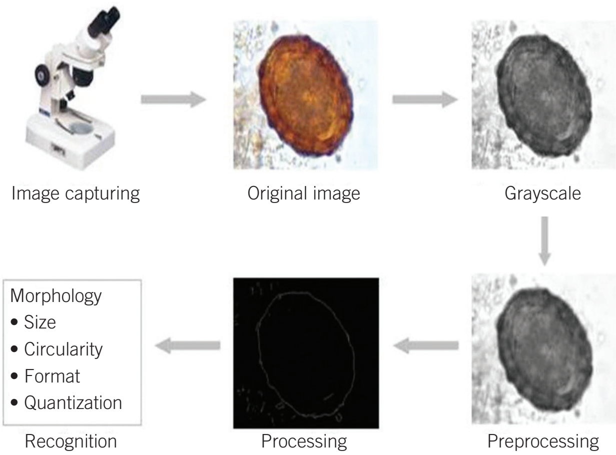


FIGURE – *Flow chart showing the steps of parasite eggs identification using Parasitology software DSS*

DSS: Diagnosis Support System.

RESULTS

After the initial development and testing of Parasitology DSS software, it was possible to define that the area of *Ascaris lumbricoides* eggs was in a range from 60,000-100,000 pixels; since the area of *Trichuris trichiura* was 20,000-41,000. It was also determined that *Ascaris lumbricoides* eggs had circularity between 0.79-1.1 pixels and *Trichuris trichiura*, between 0.9-1.2 pixels, these data used for comparing the images (**Table 1**). These values were essential for the identifications, because the system initially conducted a comparative study verifying if the values found were in a possible range of area and circularity of the *Trichuris trichiura* eggs, otherwise, the same comparative study would be done for the *Ascaris lumbricoides* eggs.

For system tests, we obtained 54 *Trichuris trichiura*, 85 *Ascaris lumbricoides* and 65 artifacts, totaling 204 images. **Table 2** shows the percentages of errors and accuracy for each egg of the evaluated parasites, as well as to artifacts, after selection and analysis of the images by the software.

By analyzing the images, it was realized that we obtained clear images and with good lighting, as well as bad images, i.e., dark, deformed and plenty of dirt, which affected the analysis by the software. Then, representative data for errors that occurred for both the artifacts identification (**Table 3**) and *Trichuris trichiura* (**Table 4**) and *Ascaris lumbricoides* eggs (**Table 5**) were identified.

TABLE 1 – Range of area, perimeter, and circularity values of each parasite (expressed in pixels)

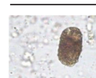
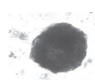
	Ascaris lumbricoides (pixel)	*Trichuris trichiura* (pixel)
Area	60,000-100,000	20,000-41,000
Perimeter	800-1,095	591-791
Circularity	0.79-1.1	0.9-1.2

TABLE 2 – Evaluation of Parasitology DSS software performance

Type	Total	Error	%Error	%Accuracy
Trichuris trichiura	54	17	31.5	68.5
Ascaris lumbricoides	85	14	16.5	83.5
Artifacts	65	6	9	90.1
Total	204	37	18.1	81.9

DSS: Diagnosis Support System.

TABLE 3 – Representative data of erroneous identification of artifacts found Parasitology DSS software. Area, perimeter and circularity data are expressed in pixels

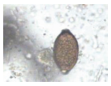
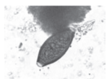
Original	Area	Perimeter	Circularity	Result reported by SAD	Actual result
	36,198	687	1.04	*Trichuris trichiura*	Artifact
	99,065	1,027	0.84	*Ascaris lumbricoides*	Artifact
	90,635	1,007	0.89	*Ascaris lumbricoides*	Artifact
	35,968	669	0.99	*Trichuris trichiura*	Artifact

DSS: Diagnosis Support System.

DISCUSSION

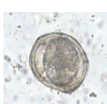
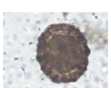
Usually, the identification and quantification of intestinal parasites, protozoa or helminths, are performed through microscopic inspection and manual sample, requiring technical expertise, as well as more time[9]. However, advances in technology and lower costs for implementation of computerized methods have opened opportunity to the emergence of new techniques, such as computerized image analysis. This can be used in different sectors of clinical laboratory, especially in the Parasitology sector routine, allowing the identification of parasites by an automated mode and not subjective and overcoming some of the disadvantages of manual techniques[11].

TABLE 4 – Misidentification of "*Trichuris trichiura* eggs" as "artifacts" by Parasitology DSS software. Area, perimeter, and circularity data are expressed in pixels

Original	Segmented	Result	Area	Perimeter	Circularity	Observations
		Negative	66,678	1,837	4.03	Excessive dirt on slide damaged image segmentation
		Negative	69,195	891	0.91	Excessive dirt on slide damaged image segmentation
		Negative	37,226	338	0.24	Identified area bigger than the actual, due to dirtiness inclusion in egg image
		Negative	33,508	793	1.49	Identified area bigger than the actual, due to dirtiness inclusion in egg image

DSS: Diagnosis Support System.

TABLE 5 – Misidentification of "*Ascaris lumbricoides* eggs" as "artifacts" by Parasitology DSS software. Area, perimeter and circularity data are expressed in pixels

Original	Segmented	Result	Area	Perimeter	Circularity	Observations
		Negative	79,546	1,600	2.56	Excessive brightness on slide damaged image segmentation
		Negative	80,722	1,176	1.36	Excessive dirt on slide damaged image segmentation
		Negative	62,566	1,567	3.12	Object coloring very similar to sample background damaged image segmentation
		Negative	97,250	1,238	1.25	Excessive dirt on slide damaged image segmentation

DSS: Diagnosis Support System.

Image analysis is not restricted to the process of analyzing, and yes, capturing the image, and to process it are also important. This technique allows improvement in the images obtained, and the automatic recognition and identification of patterns using certain characteristics, and resulting in time and work reduction. This analysis has proven to be a potential alternative tool to overcome the disadvantages associated with visual identification of microorganisms and parasites[9, 11, 12].

In this paper, the image analysis procedure allowed the identification and differentiation of *Trichuris trichiura* and *Ascaris lumbricoides* eggs, and artifacts. During the evaluation of *Trichuris trichiura* eggs image, from 54 truly positive images, 17 were not detected by the software, which corresponds to a percentage of 31.5% error and 68.5% accuracy. The lack of correct identification could be justified due to the interference of fecal debris present in large numbers in the slides. We emphasize the importance and the professional responsibility to be careful in preparing the slides, minimizing interferences that could be a confounding factor when detected by the system, and still opting to use an O&P preparation method that produces fewer waste[13, 14]. When the images of *Ascaris lumbricoides* eggs were evaluated, we could observe that the correct identification percentage was higher, since from the total of 85 images, 14 had problems in identifying, which corresponds to 83.5% accuracy and 16.5% error. The main source of error can be attributed to too much light at the time of image capture, undermining the segmentation by software and subsequent identification of parasite. This fact calls our attention to the need for standardization of equipment, such as the microscope and the camera, the intensity of light and objectives magnification. In addition to the factors reported, care during obtaining preparations is crucial to achieve good images, since the fecal waste is an important confounding factor for appropriate analysis by the software. Regarding the use of automation in the analytical phase of Parasitology, difficulties are still experienced in its implementation, justified due to the lack of commercially available equipment, plus the cost of implementation of automation, particularly in small to medium-sized laboratories. The search for standardization of a software dedicated to the identification of eggs and parasite cysts, some studies have been conducted, both in Brazil and abroad. An important differentiation in our study compared to other studies found in the literature refers to the means of comparing images, since the developed Parasitology DSS software differs from the others for allowing the identification of parasite species based on morphometric analysis, involving area, perimeter, circularity and also typical morphological structures of each species, and not only in a comparison of selected images in file database. Two other parasites identification systems were found in the literature, both are based on the comparison of images stored in image banks. Such systems acquire images automatically, scanning slide in different focuses, 400× increase, sending them to the computer analysis module, where the presence of parasites in samples is detected. In a similar study, Dogantekin *et al*. (2008), using a parasite identification software, could identify 16 different species of parasites with an accuracy rate of about 95%[15]. In the study by Falcão *et al*. (2008), the developed software proved to be able to report the presence or absence of parasites with an index of 93% accuracy, but without performing the their identification[13]. It should be noted that in this latest work important improvements have been made, such as the use of more concentrated Lugol-based dye, followed by alkaline digestion of fecal micro debris, which led to greater clarity on parasitic structures and improved the contrast obtained.

Based on the results and implementing improvements to obtain samples with less debris, identification of eggs of other parasites is presented as a perspective for using this software.

CONCLUSION

We conclude that the use of automation in the analytical phase of Parasitology is also possible, and, thus, therefore it is necessary to increase mechanical functions associated with the software, such as automatic prepare of sediment and slides, as well as scanning it through specialized microscopy. This system may bring important gains for laboratories and patients, as it may allows, for example, that the reports issued, today presented only in text form, they can be accompanied by confirmatory images, and allows a reducing the time spent for samples analysis.

RESUMO

Introdução: *As parasitoses intestinais figuram entre os diagnósticos mais frequentes no mundo. Um diagnóstico clínico acurado das infecções parasitárias humanas depende da confirmação laboratorial para diferenciação específica do agente infeccioso.* ***Objetivos:*** *Criar formas tecnológicas para auxiliar no diagnóstico parasitológico por meio da construção e da utilização de um* software *específico.* ***Material e método:*** *A partir das imagens obtidas do sedimento, o* software *compara a morfometria, a área, o perímetro e a circularidade, além de utilizar informações sobre características específicas de morfologia e coloração dos parasitos e permitir a identificação dos possíveis parasitas.* ***Resultados:*** *Nossos resultados apontam desempenho satisfatório, sendo que do total de 204 imagens analisadas, 81,86% tiveram o parasita identificado corretamente pelo sistema computacional e 18,13% não puderam ser identificados, em função da grande quantidade de detritos fecais na amostra avaliada.* ***Discussão:*** *Atualmente, as técnicas realizadas no setor de Parasitologia são predominantemente manuais, sendo afetadas possivelmente por variáveis como atenção e experiência do profissional. Portanto, a utilização da informatização deste setor pode melhorar a performance das análises parasitológicas.* ***Conclusão:*** *O presente trabalho contribui para a informatização da área da saúde e beneficia tanto os profissionais da saúde como também seus clientes, além de proporcionar um diagnóstico mais eficiente, preciso e seguro.*

Unitermos: software*; imagens; diagnóstico parasitológico; HPJ;* Trichuris trichiura*;* Ascaris lumbricoides.

REFERENCES

1. Jacob R. A importância da informática na administração hospitalar. Manaus: Centro Universitário Nilton Lins, Especialização em Administração Hospitalar e Serviços de Saúde; 2001.

2. Saccheta TEP. Informatização em laboratório clínico. Rev Soc Cardiol Est SP. 2003; 13(6): 1.

3. Schumacher HR, Anderson PF, Riggs C. Efficiency gains with bar coding and interfaced instruments Medical Laboratory Observer [Internet]. 1997. Available at: http://www.findarticles.com/p/articles/mi_m3230/is_n8_v29/ai_19773631.

4. Fraser HSF, Kohane IS, Long WJ. Using the technology of the world wide web to manage clinical information. BMJ. 1997; 314: 1600.

5. Organização Mundial de Saúde. Working to overcome the global impact of neglected tropical diseases. First WHO report on neglected tropical diseases; 2010.

6. Alves filho M. 'Olho eletrônico' obtém detecção automática de parasitos intestinais. Jornal da Unicamp. 2007; 361: 5.

7. De Carli GA. Parasitologia clínica: seleção de métodos e técnicas de laboratório para o diagnóstico das parasitoses humanas. São Paulo: Atheneu; 2001.

8. Neves DP. Parasitologia humana. 10 ed. São Paulo: Atheneu; 2000. 428 p.

9. Castanon CAB, Fraga JS, Fernandez S, Gruber A, Costa LF. Biological shape characterization for automatic image recognition and diagnosis of protozoan parasites of the genus Eimeria. Pattern Recognition. 2007; 40: 1899-1910.

10. Mossmann JB. Estudo de técnicas de processamento de imagens aplicadas ao apoio do diagnóstico de derrames serosos de etiologia benigna e maligna [Internet]. Feevale, Brasil. Available at: http://ead.feevale.br/tc/index.php?codcurso=1.

11. Ginoris YP, Amaral AL, Nicolau A, Coelho MAZ, Ferreira EC. Development of an image analysis procedure for identifying protozoa and metazoa typical of activated sludge system. Water Res. 2007; 41(12): 2581-99.

12. Suzuki CTN, Gomes JF, Falcão AX, Papa JP, Hoshino-Shimizu S. Automatic segmentation and classification of human intestinal parasites from microscopy images. Biomedical Engineering, IEEE Transactions ON. 2012; 60(3): 803-12.

13. Falcão AX, Shimizu SH. Recentes avanços tecnológicos no exame parasitológico de amostras de fezes. Biofarma – Rev Tec Cient Farm Bioquim Anal Clin Toxicol. 2008; 3(6): 44-53.

14. Centers for Disease Control & Prevention (CDC). Diagnostic procedures - microscopic examination [Internet]. 2012. Available at: www.dpd.cdc.gov/dpdx/HTML/Frames/DiagnosticProcedures/body-dp-stoolexamin.htm>.

15. Dogantekin E, Yilmaz M, Dogantekin A, Avci E, Sengur A. A robust technique based on invariant moments – anfis for recognition of human parasite eggs in microscopic images. Expert Syst Appl. 2008; 35(3): 728-38.

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