Title: A SYSTEM FOR DIAGNOSING INTESTINAL PARASITES BY COMPUTERIZED IMAGE ANALYSIS

Abstract: This present invention discloses a system for diagnosing intestinal parasites in humans and animals by computerized analysis of microscopic images obtained from fecal samples. The system uses commercially available equipment, a suitable parasitological kit, and computer and parasitological techniques especially developed to solve the problem. The advantages of the system proposed in relation to procedures currently adopted in clinical analysis laboratories are: greater productivity; greater diagnostic sensitivity; lower manpower expenses; reduction of utensils and physical space in the parasitology laboratory; speed, practicality and diagnostic security; decreased risk of contact and contamination for laboratory technician; disposal of analyzed material into a recipient suitable for toxic material, in accordance with biosafety regulations; innovation in delivery of results through images; restoration of credibility in diagnosing intestinal parasites by medical class, avoiding the empirical, indiscriminate and ineffective use of drugs.
Specification

“A SYSTEM FOR DIAGNOSING INTESTINAL PARASITES BY COMPUTERIZED IMAGE ANALYSIS”.

Field of the Invention

The present invention discloses a system for diagnosing intestinal parasites in humans and animals by computerized analysis of microscopic images, which are obtained from fecal samples. The system employs commercially available equipment, a suitable parasitological kit, and computer and parasitological techniques, specially developed to solve the problem.

The system considerably improves the diagnostic sensitivity achieved by clinical analysis laboratories, principally taking into account the human errors occurring in the processing and identification of parasitic species, in addition to eliminating the use of conventional methods and/or commercial kits with technical limitations. In this sense, the system contributes to improve diagnostic productivity and credibility, and to reduce and streamline the use of human resources, and the physical space and laboratory utensils required for carrying out parasitological tests on feces.

Background of the Invention

Human intestinal parasitoses are still rife worldwide, especially in tropical regions, including Brazil. The
World Health Organization (WHO) estimates that over 3.5 billion of people are infected with some kind of intestinal parasite in the world. Some suggest that these figures could reach 1/3 of the world’s population. According to WHO, infectious and parasitic diseases continue to rank among the main causes of death, being responsible for 2 to 3 million fatalities per year worldwide. To illustrate this picture, one in ten people in the world suffers from infection of one or more of the ten main parasitoses, which include: ascariasis, ancylostomiasis, thricuriasis, amebiasis, schistosomiasis, giardiasis, malaria etc. Further according to WHO, approximately 450 million of the infected people in the world are sick. In many parts of Latin America and Africa, parasitic diseases rank top as the cause of death, in other areas they come second behind diseases of the circulatory system. In Brazil, in mid 2001, the Organization of South American Countries (OPAS), carried out a fact-finding survey and discovered that over half the population of Brazil was infected by one or more species of intestinal parasite. Certain regions are highly endemic, to the extent that in Brazil alone over 7 million suffer from schistosomiasis.

The current widespread occurrence of infections caused by protozoarian parasites and helminths in the digestive tract justify the agent’s precise diagnosis in order to implement suitable measures to solve this public health problem. According to information supplied by DATASUS, from the source IBGE, in
Brazilian public laboratories alone over 19 millions feces tests are carried out annually.

The laboratory diagnosis of intestinal parasites is performed manually by optical microscopy, through the visual identification of the parasitic species existing in fecal samples. This procedure is not ideal, and often leaves much to be desired because: a) the diagnostic sensitivity is low to average; b) the fecal matter analyzed is small (50μl); c) the high level of fecal residues present on the microscopy slides often provides a misrepresentative result; and d) the microscopy technician must know how to diagnose all species of intestinal parasites, and such manpower is rare. An assessment performed by INMETRO in the year 1997, involving various parasitological tests on feces in major laboratories of Rio de Janeiro and São Paulo concluded that the analysis performed by these laboratories was unsatisfactory, because 3 out of 4 laboratories in São Paulo showed a significant number of diagnostic errors, and 7 out of 10 laboratories in Rio de Janeiro also committed significant mistakes. Accordingly, it is obvious that there is a need for techniques, methods, kits or systems that have good diagnostic performance, with high-sensitivity, reproductibility and practicality.

One alternative for solving this problem is the automation of the parasitological diagnosis of feces by computer image analysis. Today there is no similar commercially available solution, and the laboratories are entirely dependent on visual
diagnosis (performed by a microscopy technician). In most cases, the diagnosis is performed using decennial techniques, on a lesser scale with commercial kits and, in smaller number using immunological techniques.

Automatic biological sample analysis techniques are known. Patent US6005964 describes a system for the automatic detection of protozoarian parasites in water samples, with the use of a computer-controlled microscope. This patent differs from the present invention in that it only detects protozoarian parasites in matter free of impurities (water), it does not detect intestinal helminths, and it does not have an automatic procedure for retrieving and discarding fecal sediment, which greatly facilitates the diagnosis in this condition. Detecting intestinal parasites in fecal material is a hard task to accomplish, because it requires a concentration of parasites present in the fecal sample, and the elimination of a large part of the fecal impurities that could misrepresent the result. Nevertheless, many micro-impurities remain in the fecal sediment processed on the microscope slide, which requires the use of precise computer techniques and the use of innovative reagents in order to favor a good diagnosis. What is more, the parasitic concentration technique applied to water samples is different when compared to feces samples, and much more laborious compared to the parasitological technique used in the current invention. Another discordant factor in relation to the above-mentioned patent with
the current one is that although it has components in common such as the microscope, digital camera and computer, it differs from the proposed system in terms of its main objective, processes and the components, because the present invention consists of an automated system for diagnosing intestinal parasitoses, including helminths and intestinal protozoarian parasites, by computerized image analysis.

WO03102210 describes techniques for identifying anomalies in cells by using a computer, microscope and digital camera. The concentrate material (blood) for analysis is placed into a contaminant-free medium with the assistance of coloring techniques, achieving the diagnosis by way of computer analysis. These techniques do not apply to the diagnosis of intestinal parasites. The same comment applies to patent FR2572528, which describes a system for counting the cells in blood and bacteria in water and milk, also with the use of a microscope and computer.

Patent RU2123682 discloses a method of detecting parasitic agents with dyestuff and a fluorescent microscope, using a video camera and a computer. Detecting parasite species with fluorescent dyestuff is an indirect detection method; that is to say, it uses antibodies and antigens in the antigenic variability study. Various methods are used for such purposes, but they are limited in that they only detect a small number of species of intestinal parasites, and are also restricted in
terms of sensitivity and specificity, and these factors may vary from laboratory to laboratory. As a routine basis, the method is also financially unfeasible on a large scale.

Patents EP0774734 and US20060133657 disclose systems for the visual analysis of generic objects in images, with the use of a microscope, camera and computer. However, they do not apply to the automatic diagnosis of intestinal parasites.

Summary of the Invention

The present invention consists of the following elements, which combined with certain procedures in a given order, form a system for diagnosing intestinal parasites in humans and animals by computerized image analysis: parasitological kit for multiple collections and double-filtering of fecal material; a peristaltic pump with support for various centrifuge tubes, a tubular slide and a robot to control the pump suction shaft; a microscope; digital camera attached to the microscope; motorized stage attached to the microscope; a driver to calibrate the microscope focus; and a computer (software) to control the devices (stage, focus driver, camera, pump and robot), perform the automatic analysis of the images, and generate the results of the diagnosis.

The fecal samples are collected and processed in a laboratory in accordance with the operational protocol of the parasitological kit until the fecal sediment is obtained in a
centrifuge tube. The processed sediment must be homogenized in the tube with the physiological saline solution, the dyestuff solution, and clearing solution. The centrifuge tubes containing processed and homogenized sediment are placed in the pump supports. The laboratory technician types in the data on each tube (test), the respective number and position of each on the support, thus starting the diagnosis process.

Next, the computer sends a sequence of commands for the robot to position the pump shaft on the first tube (or on the corresponding tube of the sample processing sequence); to move the stage to the initial sweeping position; to establish the initial focus; for the pump to retrieve fecal sediment; and for the camera to acquire an image.

The image is analyzed by the computer to test the focus. If the image is out of focus, the computer checks whether the focus driver has run its course and, if not, it sends a command to calibrate the focus and a command to the camera to snap a new image. This process is repeated for as long as the images are out of focus. When it detects an image in focus, the computer stores this image and checks whether the focus driver has run its course along the path. If not, the focus driver is calibrated and the process continues snapping more images. All images found to be in focus are stored for computerized analysis. As soon as the focus driver has run its course along the path, the computer begins analyzing these images.
The image analysis consists of detection, segmentation and recognition of the parasite species present in the image. After the analysis, the computer checks whether the slide was fully photographed (i.e., whether the stage sweeping path ran its course) and, if not, it sends a command to position the stage in a next field of view of the slide not visited and restarts the focus driver. The movements of the stage are programmed to cover all the fields of view of the tubular slide. The process of obtaining images, focus calibration, storing images in focus and analysis of images in focus is repeated for each of the fields of view.

After analyzing the entire slide, the computer drives the pump to discard the sample into an appropriate recipient and cleans the tubes to start analyzing the next retrieval of sediment. The computer checks whether the whole sediment was analyzed (i.e., if a certain number of retrievals, that guarantees this task, was achieved) and, if not, reactivates the stage and the focus driver, and drives the pump to carry out a fresh retrieval of the same sediment. The whole process is repeated until finalizing the sediment in the centrifuge tube. At this point, the computer generates the result of the test diagnosis (images of parasites identified and report).

The computer checks whether all the tubes were analyzed and, if not, it activates the robot to move the pump shaft to begin the next test, and carries out all the other procedures for the start of diagnosis. The process is repeated until all the tubes are analyzed, finalizing the diagnoses.
Optionally, the system may be operated by manual control (by the microscopy technician) of the peristaltic pump (without the robot), stage, focus driver, and digital camera. It can also be used without the stage and focus driver. In this case, however, images would be obtained on a completely manual basis and only the image analysis would be performed automatically by the computer (i.e., the diagnosis is still automatic). The system may also be simplified to detect the presence or not of intestinal parasites, in which case a microscopy technician would be in charge of determining the species.

All these variations of the system, included within the scope of this invention, reveal the inventive character of this technology, which is unprecedented in the state of the art. In other words, although there are patent documents that have elements in common with this present invention (microscope, digital camera and computer), they differ in terms of the main objective, in the processes, and in the other system elements. The advantages of the system proposed in relation to the procedures currently used in clinical analysis laboratories are: greater productivity; greater diagnostic sensitivity; less manpower expenses; reduction in utensils and physical space of the parasitology laboratory; speed, practicality and diagnosis security; decreased risk of contact and contamination of laboratory personnel; disposal of analyzed material in an appropriate recipient for toxic material, in accordance with biosafety
regulations; innovation in the delivery of results through images; restoration of the credibility in diagnosing intestinal parasitoses by the medical profession, avoiding the empirical, indiscriminate and ineffective use of drugs.

5 Brief Description of the Drawings

Figure 1 presents a chart showing: System components. (a) Peristaltic pump; (b) Computer; (c) Microscope; (d) Motorized stage; (e) Focus driver; (f) Digital camera. The dotted lines indicate coupling and/or data transfer (images, samples). The solid lines with arrows indicate transfer of control commands.

Figure 2 presents a diagram of the system processes.

Figure 3 presents the phases of the image analysis module.

Detailed Description of the Invention

The invention consists of the following elements, which combined with certain procedures in a given order create a system for diagnosing intestinal parasites by computerized image analysis (see Figure 1).

1) Parasitological kit for multiple collections and double-filtering of fecal material. This kit is used to carry out field collection procedures, homogenization, preservation, transport and laboratory processing of fecal samples. The conditions of the multiple collections and double-filtering of fecal
material are fundamental to increase the diagnostic sensitivity and reduce the quantity of impurities.

2) Peristaltic pump (Figure 1a): This equipment has a support for various centrifuge tubes (where the processed fecal samples are sedimented) and a peristaltic pump to draw up and send samples of fecal sediment through a plastic tube to the tubular glass slide. The slide has a depth and therefore the sediment must be diluted and retrieved from a liquid medium (physiological saline solution). The tubular slide provides images with far less impurities than conventional slides. Besides avoiding manual retrieval and reducing the use of laboratory utensils, the equipment enables the retrieval of all the fecal sediment in discontinuous form, automatically disposes of the analyzed material into an appropriate recipient for toxic liquids, in accordance with biosafety regulations, and then automatically washes the plastic tubes for the next analysis with physiological solutions. The passage of the pump suction shaft from one tube to another is performed by a robot. The robot and the other functions of the pump are controlled by the computer.

3) Microscope (Figure 1b): An optical microscope.

4) Digital camera attached to the microscope (Figure 1c): A digital camera is used to generate images of different fields of view of the tubular slide, including horizontal and vertical sweeps on the plane of the microscope base, and in
depth (focus calibration).

5) Motorized stage attached to the microscope (Figure 1 d): The tubular slide is fixed onto the stage that moves horizontally and vertically over the base of the microscope.

6) Microscope focus control (Figure 1 e): A driver to calibrate the microscope focus is used for in-depth sweeping of the tubular slide.

7) Computer (Figure 1 f): A computer (software) is used to control the motorized stage, the focus driver, the digital camera, the peristaltic pump and its robot; and to analyze the images captured, generating the diagnosis results. The objectives of the analysis are to detect the presence of parasites in the images, isolate (segment) these parasites from the other components of the image, and recognize the parasite species, concluding the diagnosis.

The fecal samples collected are, therefore, processed in the laboratory in accordance with the operational protocol of the parasitological kit until the fecal sediment is obtained in a centrifuge tube (Figure 2 a).

The processed samples of each patient (test) are prepared for analysis as follows (Figure 2 b).

The processed sediment must be homogenized with a physiological saline solution at 0.85% and dyestuff solution. The system requires a standardized dilution of saline solution, and homogenous and strong dyestuff for all species of
parasites. This condition is not obtained with commercial coloring solutions (iodin-based), since the sample is in a liquid medium inside a tubular slide (not conventional). The homogenized sediment is set to rest for a certain period. Next, a KOH-based clearing solution is added to the sediment, finalizing the sediment preparation phase for analysis. This solution was developed for this project so as to avoid impurities, which depending on their nature, end up absorbing the color solution and become the same color as the parasites. Some of these impurities adhere to the parasite membrane generating false negative results, and others are shaped similar to the parasites, generating false positive results. The clearing solution reduces and modifies the dyestuff and the shape of the impurities, facilitating segmentation of the parasites in the images. Another benefit of the clearing solution is that it avoids clogging in the pump tubes, which often occurs when conventional techniques and other commercial kits are used.

Each centrifuge tube contains homogenized fecal sediment with saline, dyestuff and clearing solution of a given patient. After placing the tubes on the pump support, the laboratory technician types in the data relating to each (test) on the computer, its respective number and position on the support, thus starting the automatic diagnosis process (Figure 2 c).

Next, the computer sends the following sequence of commands (Figure 2 d): a command for the robot to
position the pump shaft on the first tube (or on the corresponding tube of the processing sequence of the samples); a command to move the stage to the initial sweeping position; a command to establish initial focus; a command for the pump to retrieve fecal sediment (Figure 2 e); and a command for the camera to snap an image (Figure 2 f).

The image is analyzed by the computer to check the focus. If the image is out of focus, the computer checks whether the focus path ran its course and, if not, it sends a command to calibrate the focus and a command for the camera to snap a fresh image (Figure 2 f-g). This process is repeated for as long as the images remain out of focus. When it detects an image in focus, the computer stores this image (Figure 2 h) and checks whether the focus driver has run its full course. If not, the focus driver is calibrated (Figure 2 g) and the process continues to snap more images (Figure 2 f-h). All images in focus are stored for computerized analysis. As soon as the focus driver path is completed, the computer begins analyzing these images (Figure 2 i).

The goal of image analysis is to isolate and classify the parasite species present in the images. Various techniques can be used in this phase. We have developed our own technique, which will be described in the example. All the parasites detected in this phase have their image stored for print-out in the final report.
After analyzing the images, the computer checks whether the slide was entirely analyzed (i.e., whether the stage sweeping path ran its course) and, if not, it sends a command to position the stage in the next slide field of view not yet visited and reinitializes the focus driver (Figure 2 j). The movements of the stage are programmed to cover all the fields of view of the tubular slide. The process of obtaining images, focus calibration, storage of images in focus and analysis thereof is repeated for each of the fields of view (Figure 2 f-j).

After analyzing the entire slide, the computer drives the pump to discard the sample into an appropriate recipient and cleans the tubes in order to start analyzing the next retrieval of sediment (Figure 2 k). The computer checks whether the entire sediment was analyzed (i.e., whether a certain number of retrievals, which guarantees this task, was achieved) and, if not, it reinitializes the stage and focus driver (Figure 2 l), and activates the pump to make a fresh retrieval of the same sediment (Figure 2 e). The entire process is repeated until all the sediment in the centrifuge tube is used up (Figure 2 l-k). At this point, the computer generates the result of the test diagnosis (images of parasites identified and report, Figure 2 m). Next, the computer checks whether all the tubes were analyzed and, if not, it activates the robot to move the pump shaft to begin the next test, and carries out all the other procedures for the start of diagnosis (Figure 2 d). The process is repeated until all the tubes are
analyzed, finalizing the diagnoses (Figure 2 d-m).

Optionally, the system may be operated by manual control (by the microscopy technician) of the peristaltic pump (without the robot), stage, focus driver, and digital camera. It can also be used without the stage and focus driver. In this case, however, images would be obtained on a completely manual basis and only the image analysis would be performed automatically by the computer (i.e., the diagnosis is still automatic). The system may also be simplified to detect the presence or not of intestinal parasites, in which case a microscopy technician would be in charge of determining the species.

The advantages of the system proposed in relation to the procedures currently adopted in clinical analysis laboratories are:

- Greater productivity;
- Greater diagnostic sensitivity;
- Less manpower expenses;
- Reduction in the physical space of the parasitology laboratory;
- Reduction in the use and washing of utensils;
- Speed, practicality and diagnosis security;
- Decreased risk of contact and contamination of laboratory personnel;
- Short-term and medium-term cost/benefit advantages;
- Disposal of analyzed material in an appropriate recipient for toxic liquids, in accordance with biosafety regulations;
- Innovation in the delivery of results through images;
- Restoration of the credibility in diagnosing intestinal parasitoses by the medical profession, avoiding the empirical, indiscriminate and ineffective use of drugs;
- Enables the creation of an image bank for reliable information gathering of the prevailing trends in different regions of the country; Allows parasites to be quantified for epidemiological survey purposes.

The system described herein has been tested for the automatic diagnosis of 18 intestinal parasites in humans (the most common in Brazil) with the following configuration:

The parasitological kit used was the TF-Test®. This kit differs from other conventionally and routinely used commercial and technical ones by presenting three collections of fecal samples, processed in a laboratory at the same time, increasing the diagnostic sensitivity by over 20%. Moreover, the kit presents a double-filtering system, by way of metallic screens measuring 400 and 200 micrometers, and a procedure to reduce impurities (waste matter, food residuals, micro-organisms, animal
and vegetable cells) by way of suitable reagents, resulting in a microscopic slide with fewer impurities than those obtained using other techniques.

The processed sediment was homogenized with a physiological saline solution at 0.85% and Lugol's iodine-based dyestuff. The clearing solution tested was developed based on potassium hydroxide (KOH).

The tested methodology of analyzing the images comprising phases of pre-processing, segmentation, classification and division of components, illustrated in Figure 3. The pre-processing phase is to eliminate minor impurities and highlight image features that will facilitate segmentation. The segmentation phase is designed to detect and isolate the parasites from the other image components. In practice, the isolated components may be parasites, impurities (waste matter, food residues, micro-organisms, animal and vegetable cells), or impurities that adhered to parasites. The classification phase is designed to recognize the species of parasites and identify the isolated or parasite-adhered impurities as non-parasites. If the component is classified as a parasite species, the image of the parasite is stored for final report delivery. Components classified as non-parasites are submitted to a sub-segmentation (division of components) in the attempt to separate parasites from impurities. The subcomponents of this division are again analyzed by the classifier. Very small subcomponents are eliminated and the
CLAIMS

1. System for diagnosing intestinal parasites by computerized image analysis characterized by comprising the following components:
   a. Parasitological Kit for multiple collections and double-filtering of fecal material;
   b. Peristaltic pump;
   c. Mechanical arm (robot) for retrieving processed sediments;
   d. Optical microscope;
   e. Digital camera attached to microscope;
   f. Motorized stage attached to microscope;
   g. Microscope focus control;
   h. A computer to control items b to g;
   i. Computer program used to analyze images.

2. System for diagnosing intestinal parasites, according to claim 1, characterized by employing the following analysis procedure:
   a. The collected fecal samples are processed in a laboratory according to the operational protocol of the parasitological kit, item a, until fecal sediment is obtained in a centrifuge tube;
   b. Each centrifuge tube containing fecal sediment should be homogenized with solutions, dyestuff and a clearing solution, and placed into the pump support of item b;
c. Each tube is individually identified with the respective patient and this information is relayed to the control computer;

d. The computer sends a sequence of commands to the different elements of the system (Figure 2 d): a command for the robot to position the pump shaft; a command to move the stage to the initial sweeping position; a command to establish the initial focus; a command for the pump to retrieve fecal sediment (Figure 2 e); and a command for the camera to snap an image (Figure 2 f);

e. The image is analyzed by the computer to check the focus; when detecting an image in focus, the computer stores the image; all images in focus are stored for computerized analysis;

f. The compute begins analyzing the stored images; the purpose of this analysis is to isolate and classify the parasite species present in the images;

g. After analyzing the entire slide, the computer activates the pump to discard the sample into a suitable recipient and to clean the tubes in order to begin analyzing the next retrieval of sediment;

h. After finalizing the entire retrieval of sediment, the computer generates the test result: images of the parasites identified and report.

3. System for diagnosing intestinal parasites,
according to claim 2, characterized wherein the dyestuff and clearing solutions (item 2-b) are used to distinguish the parasite from the image background and only break up and discolor the impurities, respectively.

4. System for diagnosing intestinal parasites, according to claims 1 and 2, characterized by the parasitological kit being preferably the *TF-Test*.

5. System for diagnosing intestinal parasites, according to claim 2, characterized by the fact that the image analyzed described in item f may be comprised of the phases of pre-processing, segmentation, classification and division of components.

6. System for diagnosing intestinal parasites, according to claims 1 and 2 characterized, optionally, by the fact that the system may operate with manual control (by the microscopy technician) of the peristaltic pump (without robot), stage, focus driver, and digital camera.

7. System for diagnosing intestinal parasites, according to claims 1 and 2 characterized by the fact that it can be used without the stage and focus driver; in this case, however, obtaining the images is entirely manual and only the image analysis is performed automatically by the computer.

8. System for diagnosing intestinal parasites, according to claims 1 and 2 characterized by the fact that the system can be simplified to detect the presence or not of intestinal
parasites, the microscopy technician being in charge of determining the species.

9. System for diagnosing intestinal parasites, according to claims 1 and 2 characterized by the fact that it can analyze fecal samples from humans and animals.
Figure 1
Start

(a) Collection and processing of parasitological kit

(b) Preparation of samples for analysis

(c) Laboratory technician carries out procedures to start diagnosis

(d) Computer sends sequences of commands to start a (next) diagnosis

(e) Pump is activated to retrieve sample

(f) Camera is activated to snap image

Image in focus? No → End of focusdriver path? No → (g) Driver is activated to calibrate focus

(h) Stores image in focus → Yes

(i) Computer analyzes images in focus

End of stage path? No → (j) Stage is position in field of view not yet visited and focus driver is reinitialized

(k) Pump is activated to discard sample and washing of tube

Has entire sediment been analyzed? No → (l) Reinitialize stage and focus driver

Yes

(l) Computer generates report with diagnosis

No → All tubes analyzed?

Yes → End of diagnoses

Figure 2
Figure 3