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Review Article

Eye on Advances in Parasitology Diagnostics

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Abstract

Parasitic infections cause significant morbidity and mortality in tropical and subtropical regions. Accurate and rapid diagnosis is mandatory for effective clinical management. However, the diagnosis of parasitic diseases is defective, especially in developing countries. Researchers have developed new advanced parasitology diagnostics such as smartphones, digital PCR, internet-based bio-surveillance, DNA barcoding, and geometric morphometric analysis. They revealed better sensitivity and specificity, fewer human mistakes, and lower costs. The introduction of artificial intelligence will revolutionize diagnostics when used with these new approaches. Some limitations may be present in developing countries such as internet access and steady Wi-Fi coverage. Hence, combining conventional and advanced methods may decrease limitations and improve diagnosis.

Keywords: Diagnosis, Parasitology, Advances, DNA barcoding, Artificial intelligence

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Introduction

In medical parasitology, parasites are classified into six major groups. These include the Platyhelminthes, the Nematodes, the Protozoa, the Pentastomids (tongue worms), the Acanthocephala and the Arthropods (1). Parasitic infections cause significant morbidity and mortality in tropical and subtropical regions. Globally, around 3.5 billion people are affected and more than 200 000 deaths are reported annually (2). Accurate and rapid diagnosis is mandatory for effective clinical management. However, the diagnosis of parasitic diseases is defective, especially in developing countries, due to the scarcity of trained personnel and a lack of specialized equipment. Recently, great advanced new technologies, in methods of diagnosing parasitic diseases have been developed with inexpensive tools that can be applied in developing countries, such as mobile phones and smartphones, digital molecular techniques such as digital polymerase chain reaction (PCR), digital microscopic imaging, internet-based bio-surveillance and data collection in vector-borne parasitic infections. This review aims to describe the details of some emerging technologies with a glance at their cost-effective budget benefits.

Conventional Diagnostic Methods

Microscopy

Microscopic examination is the basic method for the detection of parasites. It has been used for parasitological diagnosis for several hundred years (3). Direct smear with saline or iodine, concentration techniques, and staining are used to detect ova and parasite stages (4) as shown in Figure 1. Regarding malaria diagnoses, thick and thin Giemsa-stained blood films are the gold standard in practice due to their very low cost. However, several approaches have emerged, such as the immune chromatographic technique and PCR. In arthropod identification, the skin-snip technique is used for scabies diagnosis, while microscopy is used for pediculosis and fleas diagnoses (5). Although, these methods are labor-intensive and time-consuming, their sensitivity and specificity are of low accuracy (6).

Culture Techniques

Mostly, culture is not a routine identification tool in parasitology. However, it is useful for diagnosing some protozoan infections, e.g. free-living amoebae (7). In fact, cultivation is an attractive method for research, but it needs experience and knowledge of all factors that affect its success (8). The complex life cycles of various parasites with different stages and hosts often make parasite cultivation a hard trophy. We have two types of cultivation, *in vivo* and *in vitro*. Although *in vitro* culture techniques are used more frequently than *in vivo* techniques, the latter techniques are sometimes used to diagnose certain parasitic infections, such as trypanosomiasis and toxoplasmosis (7). Some examples of the *in vitro* cultures include Novy, McNeal, and Nicole (NNN) medium for *Trypanosoma* and *Leishmania*, made by Novy and McNeal in 1904 and modified by Nicole in 1908 (9,10), Peptone-Yeast Extract-Glucose (PYG), and Nelson's media for *Acanthamoeba spp.* and *Nagleria fowleri*, respectively.

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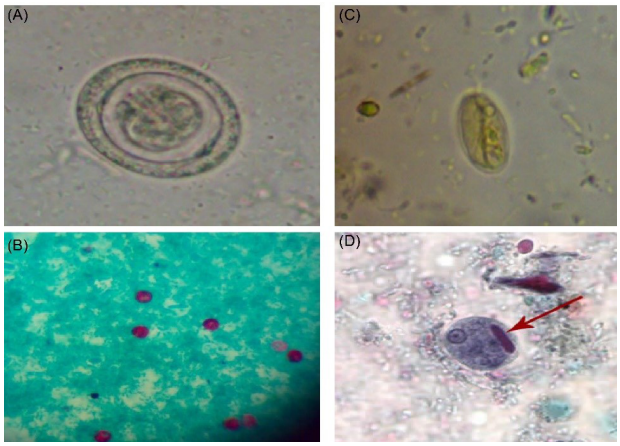

Figure 1. Detection of Ova and Parasite Stages Using Direct Smear with Saline or Iodine, Concentration Techniques, and Staining.

Immunological Techniques

Immunological/serological techniques are tools based on antigen or antibody detections to find proof of existing parasites. These antibodies, or antigens, are produced when the body is infected with a parasite and the immune system is trying to fight off the invader (11). Many tests were used; indirect hemagglutination (IHA), indirect immunofluorescence (IFAT), enzyme-linked immunosorbent assay (ELISA), immuno-chromatographic test (ICT), and Western blot (WB). They help diagnose rapidly to overcome the invasive techniques but are more expensive than the direct conventional methods.

Molecular Biology Examination

The introduction of molecular techniques in diagnosis created a promising alternative to conventional methods and helped overcome the difficulties that may be associated with these methods. PCR is a highly sensitive, rapid technique that provides alternative methods for specific detection of parasites and arthropods in combination with techniques such as restriction fragment length polymorphism (RFLP) or nested PCR. They have been used for genotyping organisms (11). Compared with microscopic and immunological examinations, PCR displays more advantages in terms of sensitivity and specificity, and multiplexed PCR can detect several parasite-specific sequences in the same reaction. However, inhibitors from stool samples and cross-contamination problems still have the most impact on the sensitivity and specificity of this method. Moreover, other techniques can be applied for parasite and arthropod identifications, such as loop-mediated isothermal amplification (LAMP), the Luminex xMAP assay, RFLP, next-generation sequencing (NGS) and mass spectrometry (MS) (3,6,12).

Advanced Digital Approaches

Smartphones in the Diagnosis of Parasitic Diseases

Smartphones have had a significant impact on cosmopolitan society, as shown by the broad spectrum of users worldwide. They possess fully functional computing capabilities such as applications for managing personal information, compact digital cameras, global positioning system (GPS) navigation, and internet accessibility (13). As a result of these robust built-in sensors, smartphones are making inroads into the medical field as a cost-effective alternative to expensive laboratory instruments for a variety of diagnostic purposes, particularly in regions with limited resources (14).

Standalone Smartphone Technology

Due to the utilization of high-magnification lenses and powerful image processors, smartphone applications offer an additional tool as an independent instrument in the detection of parasitic diseases, such as the interpretation of malaria rapid diagnostic tests (15,16). Consequently, modifications are necessary to enhance the sensitivity of this method. These mobile device-based diagnostic techniques enable automated identification, secure record-keeping, and quality assurance, thereby holding immense potential for utilization in malaria surveillance programs (13).

Lens-Mounted Smartphone "Microscopy"

Utilizing a basic, portable lens on a smartphone camera has the potential to provide a highly efficient handheld microscope for parasite detections. The size of the lens determines the spatial resolution and field of view of the microscope (FOV). Smaller lenses offer greater spatial resolution but a smaller FOV, while larger lenses offer a larger FOV but lower spatial resolution (17). A handheld microscope was formed by attaching a 3-mm ball lens to a smartphone camera, which was successfully used for the identification of soil-transmitted helminths (STH) and Schistosoma eggs in urine and stool samples of children (18,19). It should be noted that this device had limitations, including low to moderate sensitivities and specificities and a small FOV that resulted in lower-quality images. However, it is cost-effective and portable, and with further improvements in sensitivity, it could become a valuable tool for the field diagnosis of STH infection in developing countries.

Smartphone-Assisted Manual Microscopy

In an effort to create a compact microscope, Tseng et al introduced a lens-free microscope for identifying *Giardia lamblia* cysts. The sample was illuminated vertically using an incoherent LED light. The scattered light interacted with un-scattered LED light, resulting in a hologram of each cell, which was captured by a smartphone camera (20). In another study, *G. lamblia* cysts were identified using a smartphone-based fluorescence microscope. This involved using an LED light to excite the sample and detecting the emitted fluorescent light with an external

lens placed in front of a smartphone camera (21). An inexpensive color filter was used to create a dark-field background to achieve fluorescent imaging. Smartphone-assisted microscopes have also been employed to diagnose *Plasmodium* spp. (22,23). This system achieved moderate sensitivity and high specificity, making it potentially valuable for large-scale malaria screening programs. Additionally, other malarial biomarkers, such as hemozoin, have been identified in blood smears using a low-cost and high-resolution smartphone-assisted polarized microscope (24). However, this system requires sufficient lens resolution to distinguish the presence of hemozoin within an infected blood smear.

Smartphone-Assisted Automated Microscopy

A potential solution would be to use a dedicated smartphone app or algorithm for automated parasite detection. For example, the acquisition of two pattern-recognition algorithms for identifying *S. haematobium* eggs in images was enabled by a smartphone or a webcam (25). This method demonstrated high specificity and moderate sensitivity. Another study introduced a smartphone-based technique for counting fecal eggs in animals. The eggs were stained with a fluorescent chitin-binding protein and captured using a smartphone. Subsequently, automated egg counting was conducted (26,27). Smartphone-assisted automated microscopy expands beyond egg identification, as exemplified by a recently developed smartphone-based fluorescence microscopy technique for quantifying DNA from *Trypanosoma cruzi*. Polymerase chain reaction (PCR) was performed by controlling the heating/cooling cycles using computer software (28). The products were then exposed to UV light and imaged by a smartphone with a low-cost filter. Koydemir et al designed a method that involved capturing fluorescently labeled *Giardia* cysts on a membrane using a smartphone and transferring the images to a remote processing system for automatic detection and counting of cysts within a short period (27). Smartphone-assisted microscopy goes beyond still imaging, as demonstrated by the use of smartphone video microscopy for the quantification of *Loa loa* microfilariae. The device utilized a smartphone to perform video imaging of an unprocessed blood sample, which was then analyzed using an algorithm for automatic quantification of microfilariae. The final result was displayed in less than 2 minutes (29). The device exhibited high sensitivity and specificity compared to manual counts in thick blood smears.

Smartphone-Assisted Microfluidic Technology

The utilization of microfluidic lab-on-a-chip devices (LOCDs) has been significantly increased in medical diagnostics due to their advantages such as high throughput, easy handling, parallelism, and sensitivity [30]. The potential of smartphones in the measurement of biochemical reactions in LOCDs has been widely recognized as shown in Figure 2. For example, a handheld smartphone-assisted LOCD for the detection of a specific protein, HRP-2, in *P. falciparum* was developed (30). In this approach, anti-HRP-2-conjugated sub-microbeads were combined with a 10% whole blood sample in a microfluidic LOCD. Illumination and detection of scattered light were achieved using a smartphone. By utilizing the scattering/absorption characteristics of the sample, the system demonstrated the capability to detect HRP-2 levels as low as 1 pg/mL in blood within 10 minutes [30]. In a separate investigation, Liu et al. presented an integrated microfluidic chip equipped with a smartphone recorder for the identification of *Anopheles* spp. (31).

Such an advanced smartphone-based LOCD holds immense value not only in the on-site diagnosis of parasites but also in the efficient recording of test results and geographic location for quality control purposes. In 2024, a study was done with a completely lab-constructed device to measure the fluorescence of liquids using microfluidic technology. The device was completely automated using a smartphone as a data logger. The results were satisfactory and the device can measure over 200 samples per hour (32).

Digital PCR

The third generation of PCR methodology has brought new molecular techniques, including digital PCR (33). Digital PCR (dPCR), as a term, was first mentioned in the 1990s (34). In dPCR, the reaction mixture contains specific primers and fluorescent-labeled probes. The idea is to divide the mixture into thousands of partitions; each is amplified to an endpoint. Absolute quantification is provided using Poisson statistics. The dPCR has two types droplet digital (ddPCR) and chip digital PCR (cdPCR). The dPCR workflow is shown in Figure 3. In ddPCR, the sample is partitioned into ~20 000 separate replicate

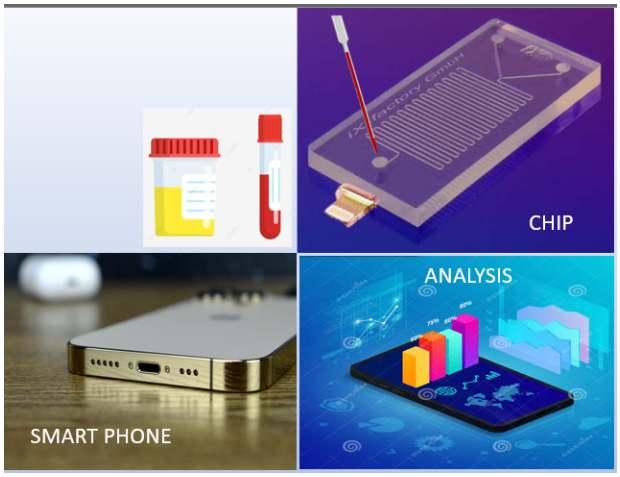

Figure 2. Potential of Smartphones in Measuring Biochemical Reactions in LOCDs

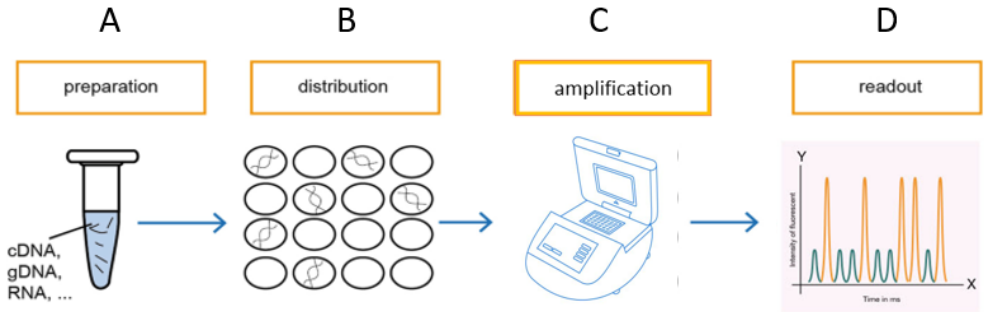

Figure 3. PCR Process

oil droplets; each one is read as independent PCR. Each droplet may have one or zero copies of targets. Positive and negative reactions can be detected and counted through their fluorescence amplitude. The starting concentration of the target is calculated by Poisson statistical analysis without a standard curve (35).

Regarding cdPCR, partitioning of the reaction is done in chambers by a microfluidic device. The dPCR reaction mix is divided into 10 000 to 45 000 partitions on a chip. Endpoint amplifications were done in a PCR thermocycler. Fluorescence detection is achieved by multi-colored detection optics or multi-channel filters. Calculations and quantifications are shown by a specific software (36). It is an expensive technique, but it offers several benefits, such as endpoint analysis of specific partitioned targets, absolute quantification, reduced susceptibility to inhibition, and the elimination of the need for standard curve analysis.

Internet-Based Bio-surveillance

Internet-based bio-surveillance was first reported in 1994, when 40 subscribers had established the ProMed system, via email. The sources of the reports were the media, government, and clinicians who communicated them to public health officials (37). Internet-based bio-surveillance means that it is based on internet-derived data, does not always require an infected person to present to healthcare to obtain communicable surveillance data, and often uses nonclinical or nonlaboratory proxies for disease activity (38). Communicable diseases became the initial application of Internet-based bio-surveillance. These diseases are a major cause of death in low-income countries (39). Furthermore, these disease data were collected through laboratory tests or sentinel systems over many years and such information now forms the database that can be used to validate the findings (40). In 2009, "digital epidemiology" was established by launching Google Flu Trends, which predicted the epidemic peek in two weeks by collecting data from Google and comparing it with that of Centers for Disease Control (CDC) (41). Many studies have surveyed web information sources to upgrade communicable illness surveillance, such as Google and similar engines, Wikipedia, Twitter, and web newswires (38). Real-time internet-based bio-surveillance strategies for vector-borne diseases (VBDs) such as dengue fever, other arthropod-borne infections (arboviruses), malaria, and Kinetoplastida have been examined in a few tropical countries (42,43). These VBDs influence low and middle-income nations of the world with quickly rising web access but limited surveillance infrastructure (44). In 2013, a study demonstrated the success of malaria surveillance using real-time Google search query data between 2005 and 2010 that matched the traditional epidemiological methods in Thailand according to official malaria case counts reported by the World Health Organization (WHO) (43). A WHO information retrieval system (45) is shown in Figure 4.

Facebook is one of the most visited websites in the world; its data are generally less trusted to form infectious disease surveillance because of the lack of public access to much of the data (38). As a commonly used open-access application, extraction and content analysis from YouTube videos are really hard. Consequently, these two social servers have the potential to disseminate false information instead of serving as an effective tool for epidemic surveillance (46).

DNA Barcoding

New approaches, especially the DNA barcoding technique, were invented to be used for parasite and arthropod identifications during the last decade (47).

This technique depends on the analysis of (~800 bases) DNA fragments called "DNA barcode". The best barcode region is the mitochondrial cytochrome c oxidase subunit I (COI) (48,49). Lack of introns and limited exposure to recombination are the main reasons for choosing that region (50). The steps of parasite detection using DNA barcoding are shown in Figure 5. Extracted DNA from different samples is amplified using the universally specific primers for the COI gene in most organisms (51). The amplicon is then sequenced and analyzed for homology to other recorded sequences in international reference databases, such as the GenBank and Barcode of Life Data (BOLD) system.

As a result, unknown parasites and arthropod samples can be identified accurately at the level of genus and species by efficient algorithms without human intervention. DNA barcoding benefits entomology by

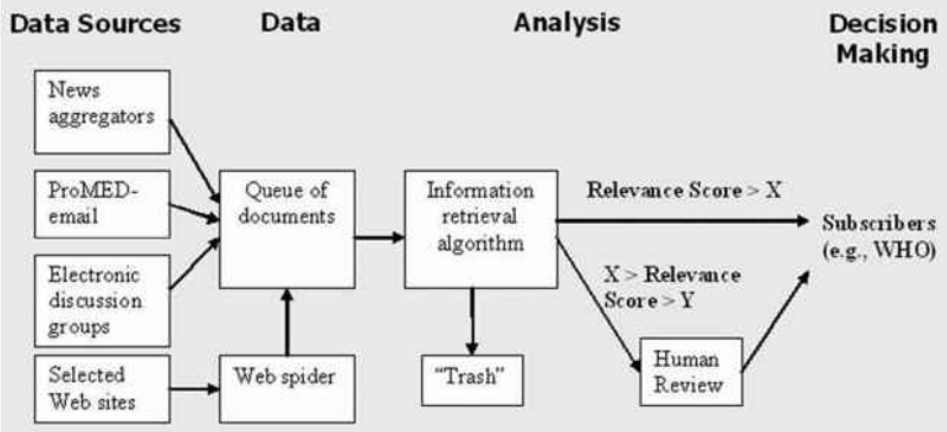

Figure 4. A WHO Information Retrieval System

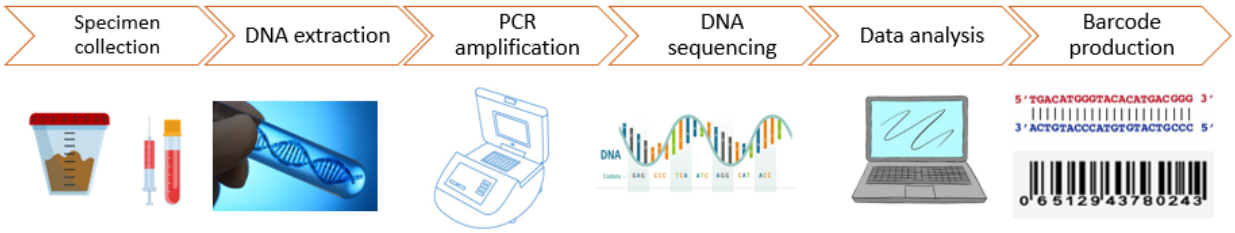

Figure 5. Digital PCR Process

identifying genera and species of different arthropods in the absence of a skilled entomologist bypassing the problem of morphological misidentification. DNA barcoding of members in class Insecta such as Culicidae mosquitoes, Psychodidae sandflies, Simuliidae black flies, and class Arachnida, such as Buthidae scorpions, Ixodidae ticks, and Trombiculidae mites, have been reported (48,52-54). However, this technique cannot be utilized to detect intestinal protozoa lacking mitochondria, such as *Blastocystis* spp., *Cryptosporidium* spp., *Entamoeba* spp., and *Giardia* spp. (55).

Some advantages of the DNA barcoding technique were revealed such as the usage of minimal volume specimens because it only needs a small amount of DNA, diagnoses of many samples can be performed simultaneously, indicating its potential as a high-throughput technology, and rare wrong results due to analyses and interpretations are processed via a computer program unless a wrong nucleotide sequence was introduced from the start. Unfortunately, there were limited disadvantages related to high-cost issues and unintentional amplification of nuclear mitochondrial pseudogenes (49,56).

Geometric Morphometric Analysis

In 1993, Rolf and Marcus started the revolution of morphometric methodology. This technique has usually been applied to arthropod identification (57). The basic concept implies multivariate analysis of landmarks located on the surface of an object, following certain rules. As a result, morphological differences between organisms or parts of them can be revealed. Geometric Morphometric Analysis is based on the concept of Kendall's space, which is defined as a hypersphere with points distributed on its surface. These points are defined as aligned landmark configurations (58). Sets of morphological variables such as linear length, height, and width of digitalized anatomical landmarks undergo statistical software analysis (58). Species identification of unknown specimens is processed by comparing them with reference data sets or a digital bank of known individuals. This technique can be performed by computer software, so there is no need for skilled workers or expensive tools to accomplish it (59). Geometric morphometric analysis is divided into two methods: landmark-based and outline-based. Recently, this technique has been used more in medical parasite diagnoses. In 2020, the improvement of modern Geometric morphometrics, including both approaches, was explored. This helped with the non-molecular identification of *Fasciola gigantica, Fasciola hepatica,* and *Fasciola intermediate* forms. The results were satisfactory when using pseudo landmarks (outlines); and less satisfactory when using the landmarks method (60). In the same year, geometric morphometric analysis was used in the detection of 88 non-human *Trichuris* spp. eggs. Mahalanobis distance (used to measure how distant a point is from the center of a multivariate normal distribution), principal component analysis and canonical variate analysis were used to analyze the shape pattern (61). Recently, in 2022, a study was conducted using the outline-based approach to distinguish the eggs of 12

common human parasites, including *Ascaris lumbricoides, Trichuris trichiura, Enterobius vermicularis, hookworm, Capillaria philippinensis, Opisthorchis* spp., *Fasciola* spp., *Paragonimus spp., Schistosoma mekongi, Taenia* spp., *Hymenolepis diminuta,* and *Hymenolepis nana.* Results revealed that shape analysis was much better to depend on than size analysis, with 84% overall accuracy. This can support copro-microscopic analysis, to effectively screen helminth eggs (62). Regarding medical arthropods, a wide application of Geometric Morphometric analysis was used to identify different groups. The wings of mosquitoes were a rich source and a good example to study this technique. This was shown in a study conducted in 2015 to differentiate three species of *Aedes* mosquitoes (*Aedes aegypti, Aedes. Albopictus,* and *Aedes pseudotaeniatus*) using landmark-based geometric morphometric analysis. The results were powerful and conclusive, based on 20 landmarks of female left-wing veins (63). In 2018 and 2019, two studies on ticks and mites revealed the ability to use this technique in the identification of different species. The first study investigated the variation in the morphological components of Haller's organ to differentiate three medically important tick species: *Ixodes scapularis* the black-legged tick, *Amblyomma americanum* the lone star tick, and *Dermacentor variabilis* the American dog tick (64). The second one was done on the chiggers, the larval stage of trombiculid mites, which are the vectors of scrub typhus. Both, landmark-based and outline-based geometric morphometric techniques were applied to differentiate *Walchia* species using scutum measurements (65). Recently, a study of triatomine bugs using multivariate geometric morphometry succeeded in identifying variants through the female external genitalia (66). The steps of arthropod diagnoses using geometric morphometric analysis are shown in Figure 6.

Future Viewpoints

Artificial Intelligence

Artificial intelligence (AI); is a new and attractive trend that is still in its childhood era. It is an advanced computer science technology that has been tried many times during the 20th century in many sectors. However, its application to parasitology only occurred recently in the 21st century. It attempted to replicate human tasks, such as recognition and interpretation skills, using computer programs. It is administered by a complex computerized algorithm that is done via a machine-learning process (67). Analyzing large amounts of data; enables the algorithm to take experts' decisions and apply what it learns from the upcoming samples. Experts can correct some errors that may occur during the algorithm's initial use. One of its hilarious advantages is that the algorithm can learn what is correct from registered errors. The main difference between AI and other software or computer-based technologies is that AI can learn and improve (68). One example of the development of AI and deep-learning algorithms is the convolutional neural networks (CNNs) that have been extensively applied to medicine, especially for medical image processing.

Regarding the parasitology field, AI was applied in 2017 to detect the ova of STH and trematodes in a total of 17 stool and urine samples. *Ascaris lumbricoides, Trichuris trichiura*, hookworm and *Schistosoma haematobium* eggs were digitized using a mobile microscopy scanner. A deep learning-based image analysis algorithm was used to analyze samples. Results showed an 83.3%–100.0% sensitivity (69). Torres et al. (2018) reported the use of a convolutional neural network algorithm to detect *Plasmodium spp.* from 700 samples of Giemsa-stained blood smears with a 72.0% sensitivity and an 85.0% specificity (70). Also, intestinal protozoa were detected in trichrome-stained stool specimens using a deep convolutional neural network in 2020. A Web interface was used for data labeling. Accuracy was calculated as slide-level agreement with microscopy. Positive agreement was 98.88% and negative agreement was 98.11% (71). In 2021, a study used the well-known algorithm "you only_look_once" (YOLO) on 1585 biological samples to recognize different strains of mosquitoes. Thirteen classes were detected with a mean average precision and sensitivity of 99% and 92.4%, respectively (72-76). By the year 2022, a new algorithm "The Viola-Jones," was designed and used for *Leishmania* detection. Three steps were taken in the algorithm, feature extraction, integral

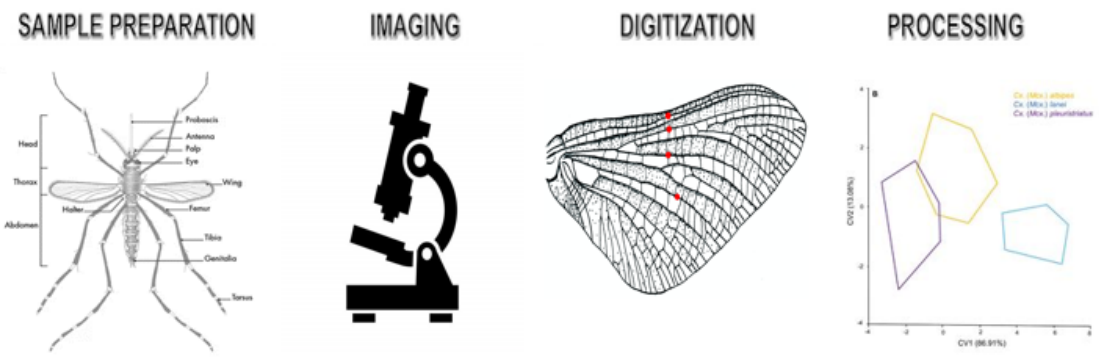

Figure 6. The Steps of Arthropod Diagnoses Using Geometric Morphometric Analysis.

image creation, and classification. A 65% recall and 50% precision were concluded in the detection of macrophages infected with the *Leishmania* parasite. The technique was easy, fast, and cost-effective (73,77). Last year, a study was conducted in Spain to assist in the diagnosis of filariasis by automatically detecting and differentiating microfilariae. Two phases were done including digitizing blood smear samples to construct the database for the development of AI algorithms. Then, the AI model used a smartphone and a pilot study was conducted to evaluate the AI's performance. Results showed an overall precision of 94.14% for the screening algorithm and 95.46%, 97.81% and 96.62% for the species differentiation algorithm, respectively (74,78). The question now arises: is AI driven by an agenda, or is it merely propaganda? Is AI a deliberate advancement aimed at reaching the pinnacle of diagnosis with ease, speed, and accuracy, or is it simply propaganda for a new technique that threatens to undermine personnel experience and quality in all fields, including medicine, after gathering data globally to establish a central hub capable of creating factual information rather than just searching for it?

Conclusion

Although microscopy is the most commonly used method for the routine diagnosis of parasites in developing countries, limitations in terms of their sensitivity, specificity, and wrong diagnoses by human technicians are recognized as major problems. Replacing microscopy with more sensitive and specific molecular methods is hampered by its cost in developing countries; Furthermore, human interpretations continue to be the primary cause of incorrect diagnoses (75,79). The hope for the future lies in the development of new smartphone and computer program-based methods that eliminate the need for expensive reagents and equipment. They revealed high sensitivity and specificity for the diagnosis of parasitic and arthropod diseases. Furthermore, they do not need to use costly reagents and machines or depend on highly skilled technicians. They are really promising in the field of parasitology diagnostics, especially with the appearance of AI technology. However, they have some limitations in developing countries, such as internet access and steady Wi-Fi coverage. Hence, combining conventional and advanced methods may decrease limitations and improve diagnosis (8,80). One last point, all these new computer-assisted, machine learning, and artificial intelligence tools must be included in all undergraduate and postgraduate curricula for healthcare personnel to prepare a generation that can deal easily with these techniques.

Authors' Contribution

Conceptualization: Manar Mahmoud El Tonsy.
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Investigation: Manar Mahmoud El Tonsy, John Talaat Nazeer.
Methodology: Manar Mahmoud El Tonsy.
Project administration: Manar Mahmoud El Tonsy, John Talaat Nazeer.
Resources: Manar Mahmoud El Tonsy, John Talaat Nazeer.
Supervision: Manar Mahmoud El Tonsy.
Validation: Manar Mahmoud El Tonsy.
Writing–original draft: Manar Mahmoud El Tonsy, John Talaat Nazeer.
Writing–review & editing: Manar Mahmoud El Tonsy, John Talaat Nazeer.

Competing Interests

None.

Ethical Approval

Not applicable.

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