

Review article

Molecular diagnosis of parasitic diseases in Korea

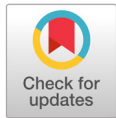
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Abstract

The aim of this review is to provide practical guidance for the molecular diagnosis of parasitic diseases in Korea in 2024. Specifically, the prevalence of parasitic diseases, commercially available molecular diagnostic kits, and reference laboratories for molecular diagnosis are presented. It is based on the literature and the medical diagnosis device database of the Korea Disease Control and Prevention Agency. In Korea, molecular diagnostic kits are available for intestinal protozoa (*Giardia lamblia*, *Entamoeba histolytica*, *Cryptosporidium hominis*, and *Cryptosporidium parvum*), *Trichomonas vaginalis*, and malarial parasites. Molecular diagnosis of other parasites is also possible; however, there is no commercially available kit. Therefore, parasite samples or derivatives for molecular diagnosis should be sent to specific laboratories, the parasitology departments of medical schools, or the Division of Vectors and Parasitic Diseases, Bureau of Infectious Disease Diagnosis Control at the Korea Disease Control and Prevention Agency. In commercial diagnostic kits, multiplex real-time polymerase chain reaction (PCR) is used to rapidly and easily detect the amplified parasitic DNA. The loop-mediated isothermal amplification (LAMP) was developed to diagnose *T. vaginalis* and *Acanthamoeba* infections. Its merits are that it does not require a PCR machine and has a short test time of approximately 60 min. Although LAMP is not commercially available, it may be widely used to screen for parasitic diseases. Commercial molecular diagnostic kits for parasitic diseases are limited to the clinical setting in Korea. Available kits are used to diagnose certain intestinal protozoa, *T. vaginalis*, and to differentiate *Plasmodium* species using multiplex PCR.

Keywords: Malaria; Multiplex polymerase chain reaction; Real-time polymerase chain reaction; Republic of Korea; *Trichomonas vaginalis*



OPEN ACCESS

pISSN : 2288-0585
eISSN : 2288-6850

Ann Clin Microbiol 2024 June, 27(2): 69-79
<https://doi.org/10.5145/ACM.2024.27.2.4>

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Received: April 03, 2024

Revised: May 08, 2024

Accepted: May 09, 2024

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Introduction

Background

The incidence and prevalence of parasitic infections exhibited several notable characteristics in Korea in the 2020s. First, although intestinal nematode infections have declined, *Ascaris lumbricoides*, *Trichuris trichiura*, and *Enterobius vermicularis* persist [1]. Amid the tissue-invading nematodes, the seroprevalence of toxocariasis is expected to remain undiminished as an increasing number of families embrace dogs as domesticated companions [1]. Trematode infection maintains its foothold, with clonorchiasis outbreaks

persisting along the tributaries of the Nakdong and Seomjin Rivers. Although reduced in prevalence, other intestinal trematode infections continue to manifest in endemic regions. Intestinal caestodiasis is a rare condition. Tissue-invading helminthiasis, such as paragonomiasis, cysticercosis, and sparganosis, are seldom reported. Sentinel surveillance for paragonomiasis detected one case in 2021, two in 2022, and three in 2023 (Table 1) [2]. In 2023, the designated sentinel surveillance institutions for parasitic diseases comprised three public health centers, seven public hospitals, and 17 branches of the Korean Association of Health Promotion, totaling 27 institutions. Sentinel surveillance institutions for imported parasitic diseases include one general hospital and 26 advanced general hospitals, amounting to 27 designated institutions [3].

Table 1. Common parasitic diseases in Korea, according to the sentinel surveillance by the Korea Disease Control and Prevention Agency

Year	Al	Tt	Ev	Cs	Pw	Intesttrema
2020	9	184	165	643	0	444
2021	1	133	83	466	1	483
2022	4	83	21	266	2	265
2023	4	110	6	214	3	349

Abbreviations: Al, *Ascaris lumbricoides*; Tt, *Trichuris trichiura*; Ev, *Enterobius vermicularis*; Cs, *Clonorchis sinensis*; Pw, *Paragonimus westermani*; Intesttrema, intestinal trematodes.

Tertian malaria (*Plasmodium vivax* malaria) is an endemic disease. From 2018 to 2022, 2,234 malaria cases were reported, including 576 imported cases in 2018, 559 in 2019, 385 in 2020, 294 in 2021, and 420 in 2022. A total of 747 cases are reported by 2023, including both domestic and imported cases [4]. Infections with intestinal protozoa such as *Entamoeba histolytica*, *Giardia lamblia*, and *Cryptosporidium* spp. are rare, but continue to cause diarrhea [5]. Trichomoniasis is a persistent sexually transmitted disease. After 123,735 infections were reported in 2012, 84,671 cases were confirmed in 2020 according to the Health Insurance Review & Assessment Service of Korea [6]. Trichomoniasis is easily detectable because its molecular diagnosis has been included in the multiplex polymerase chain reaction (PCR) for sexually transmitted diseases. Toxoplasmosis was reported in 2023, with 32 outbreaks and 26 domestic and six imported cases [3]. *Kudoa septempunctata*, amyxosporean protozoan parasite, has been reported to cause diarrhea in humans who consume halibut from Jeju Island. However, further research is needed to determine its pathogenicity [7]. Scabies continues to be prevalent in long-term care hospitals [8,9]. The number of scabies cases in Korea in 2021 is 29,693, and the age-standard incidence rate is 43.4 per 100,000 people [10].

Leishmaniasis, babesiosis, African sleeping sickness, schistosomiasis, Chagas disease, angiostrongyliasis, gnathostomiasis, filariasis, and echinococcosis have been monitored and reported as imported parasitic diseases. However, the number of infections was small, with only one case leishmaniasis reported in 2021 from 2020 to 2023. Because these data were from sentinel surveillance, there may be other unreported cases of infection [11]. In summary, intestinal nematode infections have a low prevalence, and toxocariasis has a high positive serology rate. Clonorchiasis, an intestinal trematode infection, and trichomoniasis continue to occur in the endemic areas. Malaria has not yet been eradicated, and the number of infections is increasing. However, reports on imported parasitic diseases are scarce.

Diagnosis of parasitic diseases in Korea

Most intestinal parasitic diseases are diagnosed through stool examinations, and medical institutions use fecal concentration techniques. These included roundworms (*A. lumbricoides*), whipworms (*T. trichiura*), *Clonorchis sinensis*, intestinal flukes, and tapeworms. The perianal swab method was used to detect enterobiasis (pinworm infection). For tissue-invading helminthiasis, such as toxocariasis, paragonimiasis, sparganosis, and cysticercosis, imaging tests are performed along with serological tests using ELISA. Blood smears and rapid diagnostic kits for antigen detection are primarily used to diagnose malaria. Diagnosis of parasitic diseases using molecular biology techniques is rare in Korean clinics and hospitals. However, with the recent development of various technologies, molecular diagnostic kits have become available. Although not commonly used for diagnosing patients, they are available for exceptional cases.

Molecular diagnosis of parasitic diseases

Molecular diagnosis involves extracting DNA from parasitic tissues or secretions as a template, designing primers specific to known parasite sequences, and primarily using PCR to detect the parasite, or employing techniques such as next-generation whole-genome sequencing for diagnosis. Unlike other methods, molecular diagnosis has the advantage of increased sensitivity and specificity; however, it is not a gold standard diagnostic method, so its sensitivity and specificity may not be 100%. They can provide a diagnosis in a shorter time than culture tests. However, it can be more expensive and time-consuming than other methods such as microscopic diagnosis or rapid diagnostic kits. Molecular diagnosis can be helpful when other diagnostic methods are complicated, or for large-scale screening purposes.

Objectives

In this article, we introduce commercially available molecular diagnostic devices in Korea, summarize how molecular diagnostic methods can be utilized in clinical settings, guide where to request diagnosis when direct molecular diagnosis is unavailable, and introduce various molecular diagnostic methods for parasitic diseases. The contents are based on the literature in PubMed, Web of Science Core Collection (Clarivate), and the medical diagnosis devices database of the Korea Disease Control and Prevention Agency.

Intestinal helminthiasis

The following intestinal nematode species are commonly encountered in clinical settings in Korea: *A. lumbricoides*, *T. trichiura*, *E. vermicularis*, *Strongyloides stercoralis* (nematodes), *C. sinensis*, *Metagonimus yokogawai*, *Echinostoma* spp., *Gymnophalloides seoi* (trematodes), *Taenia* spp., *D. latum* (cestodes). Except for *E. vermicularis*, which is diagnosed using perianal swabs, all can be diagnosed by detecting eggs in the stool, which is a highly sensitive and specific gold standard diagnostic method. Although there are research papers on the molecular diagnosis of intestinal nematode infections, molecular diagnosis is not used in clinical settings. Molecular analysis is used to differentiate species, such as *Metagonimus* spp. [12], *Taenia* spp. [13], and *Diphyllobothrium* spp. [14]; however, this species-level identification is irrelevant to clinical

symptoms or treatment. Therefore, there is no need to perform this method in clinical practice. If needed, it would be sufficient to provide the recovered worms to a parasitology laboratory for species confirmation.

Tissue-invading nematode infection

Tissue-invading nematodes include *Capillaria hepatica*, *Anisakis* spp., *Toxocara canis*, *Toxocara cati*, *Trichinella spiralis*, *Gnathostoma spinigerum*; trematodes such as *P. westermani*, *Fasciola hepatica*; and metacestodes of *T. solium* and *Spirometra* spp. Tissue-invading nematodes can be diagnosed by identifying the worms, histopathological findings, or imaging and serological tests, leaving little room for molecular diagnostic applications. Species differentiation of anisakids by PCR [15] is possible, but irrelevant for treatment. Fascioliasis has been reported in a few recent human infections in Korea, making molecular diagnosis unnecessary. Paragonimiasis, cysticercosis, and sparganosis are mainly diagnosed by imaging, serology, and histopathology and rarely require molecular diagnosis. Although research using PCR for species identification in paragonimiasis and sparganosis exists, identification of the exact species is clinically irrelevant for lesions or treatment in practice.

Intestinal protozoa infection

Intestinal protozoan diseases primarily cause diarrhea. Methods have been developed to extract DNA from protozoa in feces and perform molecular diagnoses. Several companies in Korea have developed various diagnostic kits; however, only a limited number are available for purchase and use in clinical settings. Many kits have received approval from the Ministry of Food and Drug Safety for export purposes, likely because of low domestic demand.

The BD MAX Enteric Parasite Panel (imported by the Becton Dickinson Korea Co.) is commercially available for the diagnosis of the intestinal protozoa *G. lamblia*, *Cryptosporidium parvum/hominis*, *E. histolytica*. Stool samples were treated with a non-preservative or 10% formalin fixative to extract protozoan nucleic acids and analyzed using real-time PCR. Real-time PCR eliminates the need for post-PCR analyses such as gel electrophoresis because amplification and detection co-occur in a closed system. The amplified products were detected using fluorophore-labeled TaqMan probes. The combined sensitivity and specificity for prospective and retrospective specimens were 98.2% and 99.5% for *G. duodenalis*, 95.5% and 99.6% for *C. parvum/hominis*, and 100% and 100% for *E. histolytica*, respectively [16].

In addition to this product, a domestic company has developed a multiplex PCR panel for various intestinal protozoa (*Cryptosporidium* spp., *E. histolytica*, *G. lamblia*, *Blastocystis hominis*, *Dientamoeba fragilis*, and *Cyclospora cayatanensis*) and has published research papers; however, it is not commercially available [17]. Since there are few other intestinal protozoan diseases that cause diarrhea in Korea, besides those covered by the BD MAX Enteric Parasite Panel, the lack of market demand likely explains why other kits are not commercialized.

Trichomoniasis

Because *T. vaginalis* dwells in the vagina or prostate, sample collection differs from that of intestinal protozoa and requires separate testing. It is commonly diagnosed by direct smear examination, immunochromatographic assays, or culture; however, a molecular diagnosis is beneficial. Rapid diagnostic kits, such as JTVAG-H-02 and JTVAG-02 (Checkncare Co.), are commercially available to detect *T. vaginalis* antigens in vaginal discharge and urine samples using immunochromatographic assays in women. The careGENETM STD-12 detection kit (Wells Bio Co.) includes *T. vaginalis* among the 12 sexually transmitted disease pathogens that can be detected using multiplex real-time PCR [18], enabling diagnosis along with other STD pathogens. However, a standalone PCR test kit for *T. vaginalis* is not commercially available. The Allplex™ Vaginitis Screening Assay [SD9750Y, SD9750X] (Seegene) has received approval for multiplex real-time PCR diagnosis of bacteria, *Candida* spp., and *T. vaginalis* causing sexually transmitted diseases [19], but it is only for export and not sold domestically. Similarly, the Allplex™ STI Essential Assay (SD9801X, SD9801Y, and SD10245Z) is also for export only [20].

Researchers in Korea have developed loop-mediated isothermal amplification (LAMP) to diagnose *T. vaginalis* infection, a relatively simple and sensitive molecular technique that amplifies DNA rapidly under constant temperature conditions. This method eliminates the need for the sophisticated and expensive thermal cyclers used in traditional PCR. In LAMP, the naked eye can easily identify a positive reaction, either turbidity (cloudiness) or fluorescence, by incorporating fluorescent dyes such as SYBR green or hydroxynaphthol blue. The fluorescent signals were visualized under UV light illumination (Fig. 1). The LAMP assay is a straightforward approach for DNA amplification and detection without complex instrumentation. LAMP shows higher sensitivity and specificity than PCR and does not cross-react with bacteria or *Candida* spp. Detection was confirmed within 60 min [21]. However, they are not commercially available in Korea.

PCR can be performed directly in the obstetrics, gynecology, and urology departments of hospitals with enough test subjects. This is beneficial for the detection of asymptomatic cases. *T. vaginalis* positivity in men has been considered benign and consequently overlooked. Men with this infection may develop urethritis, prostatitis, and reduced fertility. Active treatment of asymptomatic but positive men can also reduce the morbidity of their sexual partners [22]. As no commercially available molecular diagnostic kits specifically for *T. vaginalis* are available in Korea, samples must be sent to testing laboratories or parasitology departments capable of performing PCR assays.

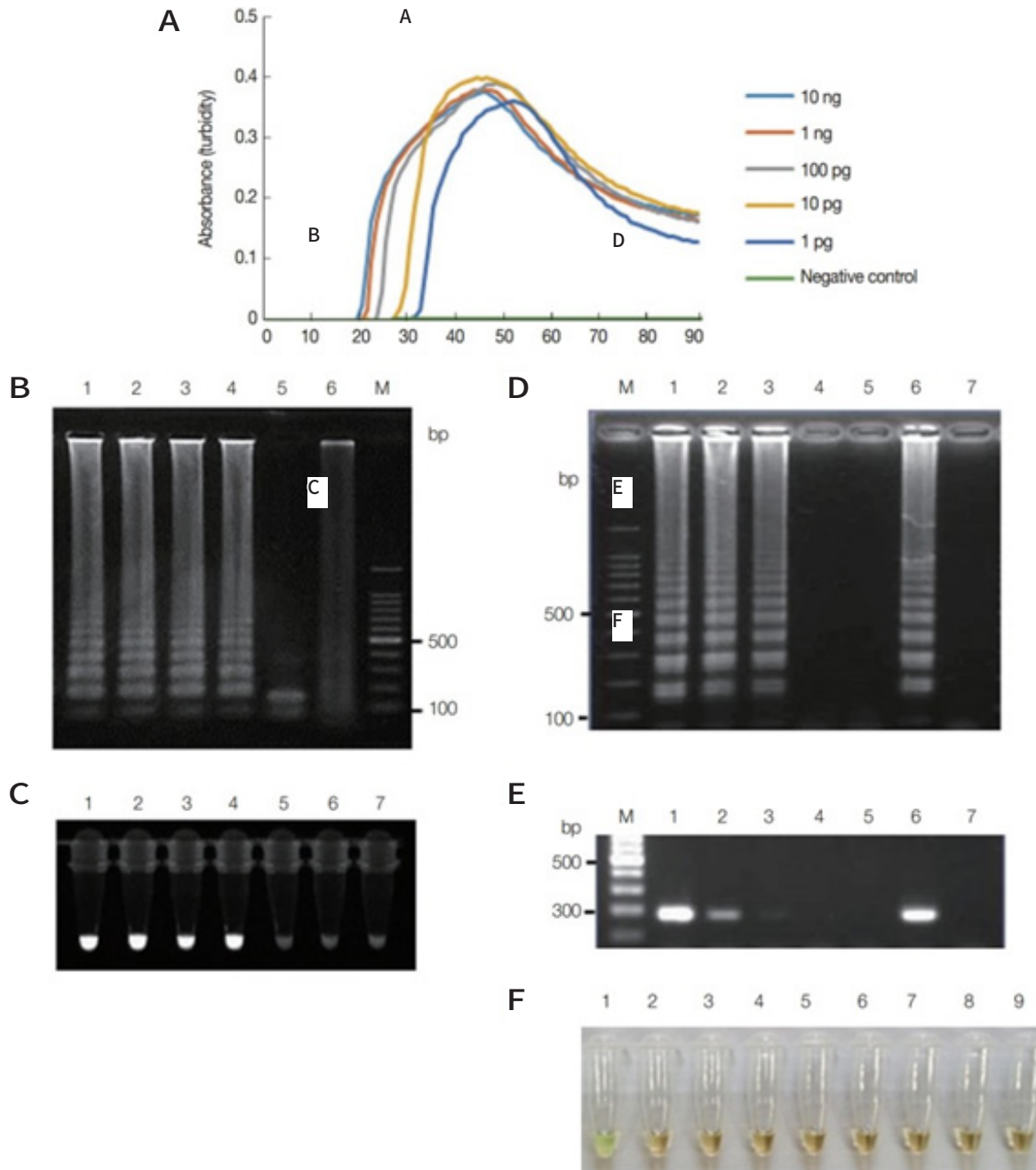


Fig. 1. Functionality of *Trichomonas vaginalis* actin LAMP assays. (A) LAMP on 10-fold serial dilutions of *T. vaginalis* genomic DNA (10 ng to 1 pg per reaction) monitored by measuring absorbance. Distilled water was used as a negative control. (B) LAMP products were visualized by gel electrophoresis. Lane 1, 1 ng; Lane 2, 100 pg; Lane 3, 10 pg; Lane 4, 1 pg of *T. vaginalis* genomic DNA; Lane 5, LAMP product after HindIII digestion; Lane 6, distilled water; and Lane M, 100-bp DNA marker. (C) LAMP products were visualized under UV light using Loopamp fluorescent detection reagent. Lane 1, 1 ng; Lane 2, 100 pg; Lane 3, 10 pg; Lane 4, 1 pg; Lane 5, 100 fg; Lane 6, 10 fg of *T. vaginalis* genomic DNA; and Lane 7, distilled water. (D and E) *T. vaginalis* at a density of 1×10^2 parasites/ μ L was serially diluted and tested using LAMP assay (D) and PCR (E) with F3 and B3 primers (Table 1). Lane M, 100-bp DNA marker; Lane 1, 100; Lane 2, 10; Lane 3, 1; Lane 4, 0.1; Lane 5, 0.01 parasite (s) per reaction; Lane 6, positive control, 100 pg of plasmid DNA containing LAMP targeting regions of actin gene; and Lane 7, distilled water. (F) Specificity of LAMP primers for detection of *T. vaginalis* assessed using template DNA from other microbial species. Lane 1, *T. vaginalis*; Lane 2, *Candida albicans*; Lane 3, *Chlamydia trachomatis*; Lane 4, *Neisseria gonorrhoeae*; Lane 5, *Cryptosporidium parvum*; Lane 6, *Entamoeba histolytica*; Lane 7, *Giardia lamblia*; Lane 8, *Escherichia coli*; and Lane 9, human genomic DNA. LAMP products were visualized via color change that was also observable by the naked eye under normal visible light reproduced under the CC-BY-NC license from the original article [19].

Tissue-invading protozoan infection

The family Acanthamoebidae includes *Acanthamoeba culbertsoni*, *A. castellanii*, and *Naegleria fowleri*. If *N. fowleri* enters the central nervous system through the nose while swimming in freshwater, it can cause primary amebic meningoencephalitis, which has an acute course and high mortality rates. They can also migrate to other tissues such as the lungs and cause fatal symptoms. Tissue-invasive acanthamoebiasis has an extremely high fatality rate and requires no specific treatments. Therefore, if not detected early, the amoeba can be identified in the tissues or cerebrospinal fluid (CSF). DNA extraction from CSF and PCR diagnosis are necessary. CSF PCR testing for *N. fowleri* can be requested from the Korea Disease Control and Prevention Agency (KDCA) [23].

In another typical case, *Acanthamoeba* contaminates contact lenses and causes keratitis. Ophthalmologists can detect it through eye examination and confirm it by culturing the amoeba; however, PCR enables faster diagnosis. LAMP allows for rapid molecular diagnosis without the use of a PCR instrument. The sensitivity of the LAMP assay was 100% for eight corneal scrapings and four contact lens solutions [24]. However, it is rare for ophthalmology clinics to use PCR or LAMP for diagnosis and treatment. Diagnosis is mainly made through eye examinations, and drug treatment is provided. PCR or LAMP can be performed in advanced or specialized eye hospitals. However, commercial molecular diagnostic tools are not available yet.

For *Toxoplasma gondii*, serological diagnosis is mainly performed, although biopsy is also possible. DNA can be extracted from secretions or tissues and diagnosed using PCR, which can also be used to identify strains. *Toxoplasma* is mainly diagnosed serologically; however, PCR can also be performed. Real-time PCR or conventional PCR can detect *T. gondii* in aqueous fluid [25,26]. Currently, there are no commercially available molecular diagnostic tools for acanthamoebiasis. The samples must be sent to specialized testing laboratories, the KDCA, or parasitology departments.

Malaria

Diagnosis can be made using a blood smear. Still, in cases of low parasitemia, the CareStart™ Malaria Pf/PAN (Wells Bio Co.) is commercially available and can diagnose HRP2 (*P. falciparum*) and pLDH (*P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*) in human whole blood using an immunochromatographic assay. Asan Easy Test Malaria Pf/Pan Ag (Asan Pharm. Co. Ltd.) and Malaria Pf/Pan Ag II-RUO (Genbody Inc.) detected HRP2 and dLDH in human whole blood using an immunochromatographic assay. Many products have been approved by the Ministry of Food and Drug Safety of Korea for rapid diagnostic tests using immunochromatographic assays; however, most are only for export purposes. The Veri-Q PCR 316 Malaria Multiplex Pan/Pf/Pv Detection Kit (Mico BioMed Co.) is available for clinical molecular diagnosis. Multiplex real-time PCR was performed on genomic DNA extracted from human whole blood (EDTA) to diagnose *P. falciparum* and *P. vivax*. Samples were sent to the KDCA for molecular diagnosis.

Imported tropical diseases

Owing to rare case reports, there is almost no demand for the molecular diagnosis of imported parasitic diseases, except for species identification. The KDCA can perform molecular diagnoses of imported parasitic diseases, allowing samples to be submitted for testing. The pathogens of imported parasitic diseases that can be diagnosed by PCR include *Leishmania* spp., *Babesia* spp., *Trypanosoma gambiense*, *Trypanosoma rhodesiense*, *Schistosoma* spp., *Wuchereria bancrofti*, *Brugia malayi*, *Onchocerca volvulus*, *Loa loa*, *Dirofilaria immitis*, and *Dirofilaria repens* [27].

Scabies

In 2021, Korea reported 29,693 cases of scabies, translating to an age-standardized incidence rate of 43.4 per 100,000 people. This high incidence could be attributed to the rapid proliferation of long-term care facilities and nursing homes during the 2000s coupled with various factors that make the diagnosis of scabies challenging. The increase in institutional care settings where scabies can spread more easily along with difficulties in accurately identifying cases of parasitic skin infestation may have contributed to the substantial number of scabies cases observed in 2021 [28]. The characteristic features of scabies include severe itching at night and distinctive burrows/tunnels on the skin. Diagnosis was based on clinical symptoms and skin lesions, followed by treatment. A confirmatory diagnosis can be made through microscopic examination of skin scrapings or dermoscopy [29]. The routine use of PCR for diagnosis is uncommon. Although not routinely employed, molecular techniques to detect the presence of *S. scabiei* DNA in skin scraping samples can be valuable diagnostic tools for suspected cases of scabies infestation. Studies have shown that these molecular methods demonstrate high diagnostic accuracy, with sensitivity exceeding 80% and excellent specificity of 100% [30]. Therefore, despite not being the standard diagnostic approach, the molecular detection of scabies mites in skin samples can provide helpful confirmatory evidence, especially in patients with a clinical suspicion of scabies.

Discussion

In Korea, molecular diagnosis of domestically prevalent or foreign-imported parasitic diseases is not actively utilized in clinical settings. This is because, in many cases, confirmation is possible through microscopic examination, serological tests, antigen detection tests, radiological findings, and histopathological findings without the need for molecular diagnosis. The kits available for molecular diagnosis using DNA extraction and PCR are limited to diagnosing trichomoniasis, intestinal protozoan infections causing diarrhea, and malaria. Other parasitic diseases are at the research stage for molecular diagnosis and are not routinely used because of the limited number of samples and cost-effectiveness.

Therefore, if there is a need to confirm the species responsible for a specific disease or to conduct large-scale screening projects, molecular diagnosis can be requested for research purposes from the parasitology departments of medical schools or the Division of Vectors and Parasitic Diseases, Bureau of Infectious Disease Diagnosis Control at the KDCA. The KDCA conducts molecular diagnoses of imported parasitic

diseases. When requesting a molecular diagnosis in a clinical setting, blood, stool, tissue samples, or extracts can be sent in 70% ethanol or frozen. If a sample contained blood, it was stored in an EDTA tube and refrigerated. Tissue samples fixed in 10% phosphate-buffered formalin were used for the PCR analysis. DNA extraction from the sample was also acceptable, as it was stored in 70% ethanol and sent. DNA extraction from parasites or tissues should be conducted according to specific protocols as they differ from bacteria.

While parasitic diseases should be classified according to their clinical manifestations, and diagnostic tests should be conducted equitably, there exists a propensity for diagnoses to be narrowly confined based on whether multiplex PCR testing is commercially viable. In other words, for parasitic diseases amenable to testing, asymptomatic infections may be overstated to a certain degree or, although rare, false positives may arise, particularly if multiple pathogens simultaneously test positive, rendering patient care challenging from the perspective of the practitioner.

In Korea, the number of experts capable of differentiating various parasitic diseases using microscopic findings is decreasing. As of April 2024, only 25 of the 40 medical schools (62.5%) in Korea had full-time parasitology faculty members. If medical schools cannot train parasitologists in basic medicine, another option is to separately train experts in parasitology in the laboratory medicine departments of general hospitals because they can handle other diseases and obtain salaries. However, this is not easy because it requires diagnostic skills and research capabilities in parasitology. In the future, it may be necessary to utilize molecular diagnosis for intestinal parasite eggs or judgment-type artificial intelligence, such as deep neural networks.

Conclusion

The molecular diagnostic kits available in clinical settings in Korea are limited by the number of species that can be diagnosed. They can diagnose intestinal protozoa such as *G. lamblia*, *C. hominis*, *C. parvum*, and *E. histolytica*, and *T. vaginalis*, and differentiate *Plasmodium* species using multiplex PCR. Since there are no commercially available molecular diagnostic kits for other parasitic diseases, one can request assistance is provided from the Division of Vectors and Parasitic Diseases, Bureau of Infectious Disease Diagnosis Control at the KDCA (<https://www.kdca.go.kr/>), specialized laboratories, or parasitology departments of medical schools.

Ethics statement

This was not a human population study; therefore, institutional review board approval or informed consent was not required.

Conflict of interest

No potential conflicts of interest relevant to this article were reported. The commercial diagnostic kit for parasitic diseases mentioned in this article is not for propagation, but for providing information.

Funding

None.

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