

Review Article

Situation and Outcome of Multiple Parasitic Infections in Iran: A Narrative Review

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ABSTRACT

Multiple parasitic infections are common throughout the world. This phenomenon is particularly prevalent in the developing countries, where a combination of environmental and socioeconomic parameters factors creates an environment conducive to the proliferation of a range of parasitic agents. This phenomenon complicates the diagnosis, treatment, and control of parasitic diseases in endemic communities, causing significant adverse clinical and epidemiological outcomes. This study aimed to explore different aspects of multi-parasitism in humans and tried to present a conceptual outline and a comprehensive outlook on the outcomes of multi-parasitism and missed infections in Iran, where this issue has been understudied. Concomitant parasitic infections present several challenges, including misdiagnosis and underdiagnosis of human parasitic diseases, which represent significant shortcomings in clinical parasitology laboratories in Iran. A reliable diagnosis is essential for the proper and effective treatment of parasitic infections, and this process could be more complicated and challenging in the case of multiple parasitic infections. In immunocompromised individuals, inaccurate diagnosis could have severe consequences. Moreover, the transmission dynamics, distribution patterns, and control programs of parasitic diseases are significantly affected by underdiagnoses of parasitic infections. Polyparasitism is a prevalent phenomenon in the epidemiology of parasitic infections in Iran. The confluence of environmental and sanitary conditions, coupled with socioeconomic parameters that predispose communities to infection, contribute to this phenomenon. The use of complementary diagnostic methods has the potential to detect low-intensity infections that would otherwise remain undiagnosed in a conventional clinical laboratory. In the majority of laboratories located in the endemic countries, the utilization of these methods is neither feasible nor cost-effective. The successful implementation of national reference laboratories for the diagnosis of parasitic infections is essential for the reliable diagnosis, effective treatment, and alleviation of the burden of disease.

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1. Context

The co-evolution of *Homo sapiens* and parasites has spanned millions of years, with over 400 species of organisms parasitizing human hosts, including protozoans, helminths, and arthropods. It is possible for humans to be infected by one or more species of parasites (1). Polyparasitism, defined as co-infection by multiple parasite species, is a prevalent phenomenon globally, particularly in the developing countries. The prevalence of polyparasitism in these regions is attributed to a complex interplay of factors, including inadequate sanitation, limited access to safe water supplies, sewage system, and a convergence of environmental and socioeconomic parameters that predispose communities to infection (2, 3). Despite some advances in the control of parasitic diseases worldwide, they remain important health challenges, especially in developing countries. The remarkable socioeconomic burden of parasitic infections and related diversity is more prevalent among humans and animals in rural areas of these countries (4-7). The presence of parasites of various species in a single host could complicate the diagnosis, potentially leading to underdiagnosis of some parasites. Subsequently, this can result in an underestimation of prevalence data in endemic areas. Furthermore, the use of different protocols for the management of multiple parasitic infections may result in concomitant infections being left untreated, which could have serious and potentially life-threatening consequences (2). This presents a significant challenge to the control of diseases and affects the epidemiology of parasitic infections in human societies. However, the public health consequences and socioeconomic implications of this issue remain largely uninvestigated in Iran (8). On the other hand, underdiagnosed Parasitic Infections (UPI) could have confounding effects on the illustration of the burden of human parasitic infections in endemic areas. The actual prevalence of parasitic infections in a certain endemic region may be considerably higher than expected when precise diagnostic techniques are applied in the monitoring of endemic communities (9). This study aimed to explore different aspects of multiple parasitic infections in human and tries to present a conceptual outline and a comprehensive outlook on the outcomes of multi-parasitism and missed infections in Iran.

2. Acquisition of Evidence

In order to conduct a comprehensive analysis of this topic, extensive research was conducted by collecting scientific papers from various databases such as Google Scholar, PubMed, Scopus, Web of Science, Medline, and Cochrane Library. Relevant studies were carefully selected for data extraction. Since the aim of this study was to assess the situation and consequences of polyparasitism in Iran, special attention was given to include papers related to this region to compile this article.

3. Results

3.1. Multiple Parasitic Infections in Human

Concomitant infections with different species of protozoa and/or helminths have already been demonstrated in humans worldwide, particularly in developing countries (10). Causes and consequences of multiple infections with endoparasites (protozoans and helminths) and ectoparasites (e.g. lice, fleas and ticks) have been poorly studied in endemic regions such as Iran (8). Coexistence of pathogenic and non-pathogenic organisms, especially protozoa, has been frequently reported in human populations throughout Iran (11-14). Mixed infection of endoparasites is mostly reported for intestinal parasites; however, extraintestinal multiparasitism has also been reported (15-17). This issue could have important implications especially in rural areas where multiple infections are common (18, 19). The exact co-infection profile of multi-parasites has not been clearly documented in Iran as an endemic region for several anthroponotic and zoonotic parasites.

3.2. Diagnostic Challenges of Multiple Parasitic Infection

Multiple parasitic infections with co-infection patterns in humans present several challenges and obstacles (8). First, misdiagnosis of morphologically similar parasites is a common pitfall in clinical parasitology laboratories. Parasite similarity is a major challenge for laboratory practitioners in diagnosing and differentiating of co-infecting parasites. Due to the same transmission route, co-infection of different amoeboflagellate is frequently observed in Iran (20). High morphological similarity between pathogenic and non-pathogenic amoeboflagellates in humans is a major problem in the reliable diagnosis of these species. In helminths, there are obvious challenges for the identification of eggs, larvae and adult stages of these parasites. Based on the morphology alone, different *Taenia* species could not be differentiated by stool microscopy and further studies on the helminth segments are needed (21). Among the human flukes, differentiating *Fasciola* / *Fasciolopsis* and eggs is a problem, especially in the regions where both parasites are co-endemic. Eggs of the human nematodes, *Trichuris trichiura* and *Aonchotheca philippinensis* (formerly *Capillaria philippinensis*) can be easily misdiagnosed in the laboratory (22). Furthermore, there is an inherent risk of misdiagnosis when examining helminth and protozoan parasites from different taxonomic groups (23). Another significant challenge is underdiagnosis of certain parasitic organisms. This may be attributed to the inefficient performance of conventional diagnostic methods. In many developing countries the common technique for diagnosing intestinal parasites is preparing a wet smear and conducting a direct microscopic examination of fecal specimens. However, due to the small size of the specimen, this method may result in the overlooking of the parasites, leading to false negative reports and underdiagnosis (24). It is important to note that no single diagnostic method is universally applicable to all parasites. To ensure a reliable diagnosis of all parasites in stool samples, a variety of techniques have to be considered

including direct smear, a concentration method (formalin-ether, Kato-Katz, etc.), a culture method (Harada and Mori, agar plate, etc.), trichrome stain, and modified Ziehl-Neelsen staining (10). Furthermore, additional paraclinical examinations, including imaging, serodiagnostic and molecular techniques, must be considered for the diagnosis of certain extraintestinal parasitic infections, such as ascariasis, fascioliasis, schistosomiasis, trichinellosis and visceral leishmaniasis. It is evident that the simultaneous utilization of these methods is neither feasible nor cost-effective in most laboratories situated in the endemic countries. In Europe, national and international reference laboratories for the diagnosis of parasitic infections have been established for the purpose of developing standard methods and providing assistance in the reliable diagnosis and effective treatment of human and animal parasites. The two most successful models are the European Union Reference Laboratory for Parasites and the Public Health England's National Parasitology Reference Laboratory. The former operates at the international and level while the latter focuses on national level (25, 26) (25, 26) (24, 25) (24, 25). Unfortunately, there is no established "best practice" model for such reference laboratories in Iran. The reliability of a diagnosis of human intestinal coccidia depends on the use of appropriate concentration and staining methods. *Cryptosporidium* and *Cyclospora* species present a significant challenge in diagnostic parasitology laboratories because of the minute size of their oocysts and the lack of distinctive morphological features, which makes differentiation from spores and other fecal contaminants particularly difficult. The use of Sheather's flotation method and modified Ziehl-Neelsen staining is essential for the accurate identification of oocysts. A similar approach is required for the diagnosis of opportunistic intestinal microsporidians, for which a specific trichrome staining technique is necessary for definitive diagnosis of the infection. The diagnosis of mixed infections of malaria parasites and sub-microscopic malaria infections represents another significant challenge in tropical and subtropical regions, as well as in immigrants and travelers returning from malaria-endemic areas (16, 17, 27). Among helminth infections, accurate diagnosis of strongyloidiasis is consistently a challenge. A number of studies have demonstrated that *Strongyloides stercoralis* infection is frequently overlooked in both endemic and non-endemic areas. It is of particular importance to avoid misdiagnosis of the infection in immunocompromised individuals, as the parasite can cause hyperinfection syndrome and disseminated strongyloidiasis (28). The negative binomial distribution of soil-transmitted helminths and schistosomiasis indicates that a few number of infected people harbor the majority of adult worms in endemic communities. Consequently, supplementary parasitological examinations could detect many individuals with low-intensity infections who would otherwise remain undiagnosed using routine diagnostic methods (23). The conventional methods currently employed in Iran and the

supplementary techniques recommended for the reliable diagnosis of parasitic infections are summarized in table 1. Finally, the important limitation of previous studies is the consideration of pseudoparasites as a significant challenge in the laboratory diagnosis of multiple parasitic infections in Iran.

3.3. Undertreatment of Multiparasitism

A reliable diagnosis is of the utmost importance in the proper and effective treatment of parasitic infections. The situation is further complicated in the case of multiple parasitic infections. A variety of antiparasitic agents are available for the treatment of parasitic diseases. However it should be noted that the majority of these drugs are specific to protozoan and helminth parasites, and few therapeutic agents are capable of treating a wide spectrum of parasitic infections. Examples of such agents include praziquantel, albendazole, ivermectin and metronidazole. Praziquantel has been demonstrated to be an effective drug against most of the diseases caused by trematode and cestode parasites, including various species of *Schistosoma*, *Hymenolepis* and *Taenia* species. Albendazole has been demonstrated to be effective against a wide variety of nematode parasites, those transmitted via soil. Among anti-protozoal agents, metronidazole has the capacity to treat people infected with anaerobic / microaerophilic bacterial and protozoan organisms. This drug has been utilized for the treatment of trichomoniasis, giardiasis and amoebiasis. Nonetheless, metronidazole has been observed to have no significant effect against other protozoa that infect the human intestine, blood, and tissues. In recent years, there has been a growing body of evidence in the literature indicating the efficacy of Ivermectin against a wide range of parasitic infections are growing in the literature (29). The majority of the people living in endemic countries are affected by polyparasitism, a condition characterized by the co-existence of multiple parasitic infections. Failure to diagnose and treat one or more of these infections can result in inadequate patient management (9).

3.4. The Clinical and Epidemiological Consequences of Misdiagnosis - or and Undertreatment of Parasitic Infections

In both endemic and non-endemic countries, failure to diagnose parasites correctly can have significant clinical epidemiological consequences. The presentation of various outcomes of multiple parasitic infections in Iran is illustrated in Figure 1. In individuals with compromised immune systems, inaccurate diagnosis could have severe consequences. The literature contains numerous reports of fatal cases of disseminated strongyloidiasis. It is evident that the use of conventional direct fecal smears is inadequate for the diagnosis of *S. stercoralis* infections, which can result in serious clinical outcomes. There have been numerous cases of death due to the underdiagnosis of strongyloidiasis (30). Among protozoal infections, cryptosporidiosis and microsporidiosis have been frequently left undiagnosed in clinical laboratories (31). Both diseases are important opportunistic infections in

immunocompromised individuals suffering from acquired and congenital immunodeficiencies, severe diabetes, chronic renal failure, and malignancies. The use of appropriate concentration and staining techniques is crucial for the reliable diagnosis of these infections (32, 33). The transmission dynamics and distribution pattern of parasitic diseases are significantly influenced by UPI (34). The successful control of parasitic diseases in endemic communities has been achieved through proper diagnosis and treatment of the infections. The introduction of Rapid Diagnostic Tests (RDTs) facilitated the reliable and fast

detection of malaria cases in the endemic regions, thereby markedly improving the efficiency of case finding and disease surveillance. The utilization of portable ultrasound devices used in community screening for cystic echinococcosis has facilitated a deeper comprehension of the epidemiological and transmission dynamics of the disease in endemic countries (35). The development of new tools for reliable and cost-effective diagnosis of other parasitic diseases represents a fundamental factor in reducing the burden of these infections in poor endemic communities.

Table 1. An overview of the conventional methods currently in use in Iran and the supplementary techniques recommended for reliable diagnosis of parasitic infections

Disease	Etiology	Conventional method(s)	Supplementary method(s)
Amebiasis and other non-pathogenic amoeba	<i>Entamoeba histolytica/dispar</i> , other commensal amoeba	Direct stool exam	Concentration techniques, permanent staining, PCR-based methods
Acanthamoeba keratitis and amebic meningoencephalitis	<i>Acanthamoeba spp.</i> , <i>Naegleria fowleri</i> , other free-living amoeba	Clinical suspicion	Non-nutrient agar plate, Wright or Giemsa staining, PCR-based methods
Malaria	<i>Plasmodium falciparum</i> , <i>P. vivax</i> , <i>p. malaria</i> , <i>p. ovale</i> .	Thick and thin blood smears	Rapid Diagnostic Test (RDT), PCR-based methods
Cryptosporidiosis, cyclosporiasis	<i>Cryptosporidium spp.</i> , <i>Cyclospora cayetanensis</i>	None	Concentration technique (sheather's sugar flotation), modified acid-fast staining, PCR-based methods, immunoassay
Blastocystosis	<i>Blastocystis hominis</i>	Direct stool exam	Concentration techniques, permanent staining, immunoassay, routine histology, PCR-based methods
Microsporidiosis	<i>Enterocytozoon bienersi</i> , <i>Encephalitozoon hellem</i> , <i>Encephalitozoon intestinalis</i> , <i>Encephalitozoon cuniculi</i>	None	Modified Trichrome staining, routine histology, hematoxylin and eosin, PAS, modified gram staining, electron microscopy, serology antibody detection, culture, PCR-based methods
Trichomoniasis	<i>Trichomonas vaginalis</i>	Direct wet mount of vaginal discharges, Pap smear and Giemsa staining	Culture, serological techniques, PCR-based methods
Visceral leishmaniasis	<i>Leishmania infantum</i>	Bone marrow aspiration and Giemsa staining, serologic testing	Culture, animal inoculation, buffy coat preparation, PCR-based methods
Strongyloidiasis	<i>Strongyloides stercoralis</i>	Direct stool exam	Harada-Mori, agar plate, Baerman concentrate, duodenal contents, skin test, serologic test, PCR-based methods
Trichinellosis	<i>Trichinella Spp.</i>	Clinical suspicion, muscle biopsy	Serologic test, direct stool exam, PCR-based methods
Toxocariasis and visceral larvae migrans	<i>Toxocara canis</i> , <i>T. cati</i> , <i>Toxascaris leonina</i>	Serologic tests	Biopsy, PCR-based methods
Soil-transmitted helminthiasis	<i>Ascaris lumbricoides</i> , <i>Trichuris trichiura</i> , hookworms.	Macroscopy, direct stool exam	Concentration techniques, PCR-based methods
Anisakiasis	<i>Anisakis spp.</i>	Clinical suspicion, larval recovery or histologic examination	Serologic tests, PCR-based methods
Echinococcosis	<i>Echinococcus granulosus s.l.</i> , <i>E. multilocularis</i> .	Imaging techniques (X-ray, CT scan, ultrasonography)	Serological techniques, MRI, microscopic examination, fine-needle aspiration, cytology
Cysticercosis	<i>Taenia solium</i>	Clinical suspicion, biopsy, imaging techniques	Serologic testing, fine-needle aspiration
Hymenolepiasis	<i>Hymenolepis nana</i> , <i>H. diminuta</i>	Macroscopy, direct stool exam	Concentration techniques, PCR-based methods
Fascioliasis and other food-borne trematodiasis	<i>Fasciola hepatica</i> , <i>Dicrocoelium dendriticum</i> , <i>Heterophyes heterophyes</i>	Direct stool exam	Sedimentation concentration, duodenal contents (Entro-test), serologic tests, imaging techniques, PCR-based methods
Schistosomiasis	<i>Schistosoma spp.</i>	Direct stool/urine exam	Kato Katz method, serologic tests, rapid diagnostic tests, PCR-based methods

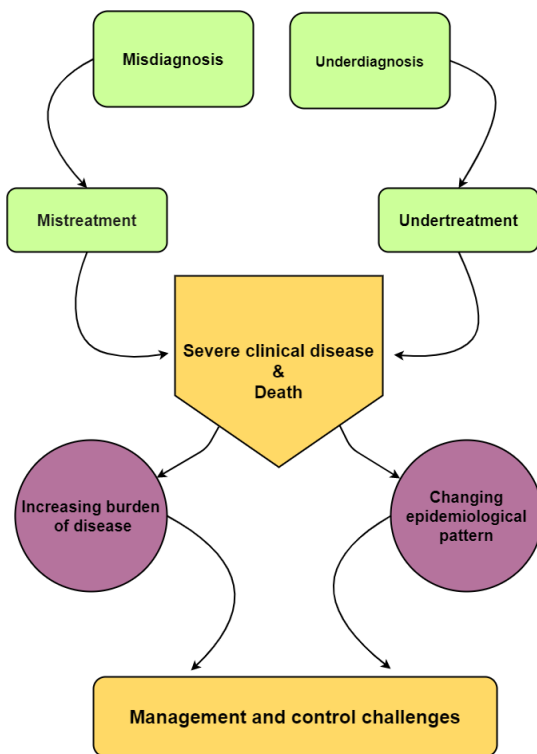


Figure 1. Schematic presentation of various outcomes of multiple parasitic infections challenging the management and control of the infections in Iran.

4. Conclusions

Polyparasitism represents a prevalent phenomenon within the epidemiology of parasitic infections in Iran. The confluence of environmental and sanitary conditions, coupled with socioeconomic parameters, predispose communities to these infections. It remains as an important challenge for the diagnosis, treatment, and control of parasitic diseases particularly in rural areas. Misdiagnosis and underdiagnosis of parasitic infections are prevalent issues in clinical parasitology laboratories in Iran. A reliable diagnosis is of paramount importance for the proper and effective treatment of parasitic infections. Incorrect morphological identification of similar co-infecting parasites and the inefficiency of routine diagnostic methods result in false negative reports. Additional parasitological examinations may facilitate the diagnosis of individuals with low-intensity infections who would otherwise remain undiagnosed using conventional diagnostic methods. In most laboratories in Iran, the utilization of these methods is neither feasible nor cost-effective. The successful implementation of national reference laboratories for the diagnosis of parasitic infections is essential for the reliable diagnosis and effective treatment of the infections, as well as for the alleviation of the disease burden. Failure to diagnose one or more of the infections can result in inadequate treatment of the patients, which may have serious clinical and epidemiological consequences. Consequently, the effective surveillance and control of

parasitic diseases can only be achieved when appropriate diagnostic tools are available in reference central laboratories in endemic regions. In the case of zoonotic parasites of animals, the use of reliable diagnostic methods is of equally importance. This could be achieved through an integrated One Health approach for the management and control of parasitic diseases.

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Authors' Contribution

Study concept and design: M. E. and M. F. H.

Acquisition of data: M. E.

Data analysis and interpretation: M. E., M. F. H., and M. N.

Manuscript preparation: M. E., M. F. H., and M. N.

Critical revision of the manuscript for important intellectual content: M. E., M. F. H., and M. N.

All authors approved the submitted version and agreed to be personally accountable for the integrity of any part of the work.

Ethics

As no human or animal subjects were involved in this study, and the data were collected from previous studies conducted in Iran, ethical committee approval was not required.

Conflict of Interest

The authors declare that they have no conflict of interests.

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Data Availability

The data that support the findings of this study are available on request from the corresponding author.

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