**A PhD Proposal in Parasitology**

**A comparative analysis of microscopically and molecular marking for human intestinal helminthiasis in Erbil Province**

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 **SUMMERY**

 In this study, examination and detecting of human intestinal helminthiasis in Erbil province will be done using a traditional microscopy method and molecular technique (DNA marking from stool samples) for detection. A comparison of the diagnosing results of microscopy and conventional PCR or molecular techniques will be reveal according to their sensitivity, cost and quantitative infections. As well as the effects of patient’s sex, age, and hygiene level, geographical areas (Rural and Urban areas) will be reveal.

**INTRODUCTION**

Intestinal helminth parasites are responsible for a significant amount of pathology in both humans and farmed animals, resulting in a reduction in the quality of life, and occasionally lethal infection as well as causing severe infections among children, also decreases livestock productivity. The importance of these parasites globally has resulted in efforts to understand the mechanisms of the host–parasite interaction epidemiology, and to develop methods for diagnosis and control (Aramendial et al., 2020).

The prevalence of and morbidity from intestinal helminthiasis is enormous. Many parasitic infections, especially those of helminthic origin are asymptomatic, could produce mild or, in a typical cases, confusing symptoms and are often neglected until, serious or chronic clinical pictures are present. In most rural communities low bizarre standard of sanitation and poor socio-economic conditions are obvious predisposing factors to high prevalence of human intestinal helminthiasis (Anosike et al., 2006).

These parasites are transmitted by eggs released in human feces that contaminate the soil, water and food in areas where sanitation is poor (Vaz Nery et al. , 2019).

Intestinal helminthes infections occur worldwide even in developed countries. Causative species infections differ significantly depending on the geographical areas and globalization has further complicated the epidemiology of such infections. Nonetheless, recent epidemiological data on some of them, like cestode infections, are limited (Tsuboi et al., 2018).

Helminth parasites show a variety of transmission patterns determined by their life cycle characteristics and ecological requirements. As a result, their prevalence and abundance has been correlated with both life history characteristics of the host as well as environmental factors that act on helminth development (Seasonal variation in temperature and humidity and host features such as feeding habits, habitat preference, age, gender and body size can regulate the host-parasitism dynamic and are often considered in ecological studies of many parasites (Gomesa et al., 2019).

Intestinal helminths are diagnosed by using a conventional method like simple microscopy, egg numeration assay, serology based assay and egg culture. Polymerase Chain Reaction (PCR) has advantages over the traditional microscopy method as they are highly sensitive and specific (O'Connell and Nutman, 2016).

**OBJECTIVES OF THE STUDY**

 The study aims to reveal these below:

1**-**Diagnosing human intestinal helminthes in random stool samples from different point of Erbil Province by using traditional microscopy method and molecularly by PCR technique, and screening of the distribution, abundance and prevalence of these parasites in Erbil population.

2 –Comparing the sensitivity of microscopy and conventional PCR techniques to detect some intestinal helminths by using a modified DNA extraction technique in stool samples.

3- Investigation of the effects of host sex, age, geographical distribution and hygiene level on infection.

**Methodology:**

**1-Sample collection and preparation**

Stool samples will be collect randomly and weekly for a year (from May 2021-May 2022) from patients in different points of Erbil Province (Urban and Rural regions) for diagnosing of intestinal helminthes by simple microscopy, then processing by PCR technique (DNA marking) for identification of the helminthic infections. The stool samples will preserve by adding of preservative (100 mM EDTA buffer) to be usable for nest molecular study (Gawor et al., 2017).

* **Eggs isolation by using flotation and sedimentation techniques:**

1. **Microscopy examination:**
2. **For sedimentation method:**

Formal Ether Sedimentation Technique will be use:

1. In a suitable container, thoroughly mix a portion of stool specimen about the size of a walnut into 10mL of saline solution. Mix thoroughly.
2. Filter the emulsion through fine mesh gauze into a conical centrifuge tube.
3. Centrifuge the suspension at relative centrifugal force (RCF) of 600 g (about 2000 rpm) for no less than 10 minutes. The suspension should yield about 0.75mL of sediment for fresh specimens and 0.5 mL for formalinized feces.
4. Decant the supernatant and wash the sediment with 10 mL of saline solution. Centrifuge again and repeat washing until supernatant is clear.
5. After the last wash, decant the supernatant and add 10 mL of 10% formalin to the sediment. Mix and let stand for 5 minutes to effect fixation.
6. Add 1 to 2 mL of ethyl acetate, Stopper the tube and shake vigorously.
7. Centrifuge at 450 g RCF (about 1500 rpm) for 10 minutes. Four layers should result as follows
	1. a top layer of ethyl acetate;
	2. plug of debris;
	3. layer of formalin; and
	4. sediment
8. Free the plug of debris from the side of the tube by ringing with an applicator stick. Carefully decant the top three layers.
9. With a pipette, mix the remaining sediment with the small amount or remaining fluid and transfer one drop each to a drop of saline and iodine on a glass slide. Cover with a coverslip and examine microscopically for the presence of parasitic forms.
10. **For floatation technique**:

***Mini-FLOTAC Basic technique –***

 **From fresh stool samples** 5g will be add to 15 ml of 5% formalin (proportion 1:4). Homogenize the sample. To analyze in a laboratory, homogenize the sample and filter for a 250 μm sieve. Take 2 ml (0.5g) of the fecal suspension for each of 2 tubes (4 ml/1g total). Centrifuge at 1500 rpm (170 RCF) for three minutes. Supernatant pour off and discard, leaving only a pellet in the tube. Add flotation solution (one tube until 5 ml of FS1 for the 1:10 dilution and second tube until 10 ml of FS7 for the 1:20 dilution). Homogenize the suspension and fill with 1 ml (0.1g) each chamber of Mini-FLOTAC using the filling holes, until a small meniscus is formed. Fill the two chambers with each FS, using two Mini-FLOTAC (one for FS1 and one for FS7). In order to avoid the formation of air bubbles, the chambers should be filled with the Mini-FLOTAC held at a slope. After 10 minutes, translate the reading disc and put the Mini-FLOTAC under the microscope, using the microscope adaptor. Examine and measure under a microscope with objectives 10 and 40 X for quantification of parasitic elements. This technique has the highest analytical sensitivity for Mini-FLOTAC procedure. The analytical sensitivity is 5 EPG for FS1 at 1:10 dilution and 10 EPG for FS7 at 1:20 dilution ( Nisha et al, 2020).

1. **Conventional PCR examination:**

The stool samples from flotation techniques efficiently, the will subjected to conventional PCR procedure. PCR is increasingly used to confirm parasite species when morphologic detection is a challenge.

There are 2 basic approaches: species-specific assays and universal assays. In order to extract the DNA eggs undergone a disruption method to lyse the wall of the eggs was used for nucleic acids isolation. For DNA extraction commercial mini kit was used to extract the DNA from the eggs with universal primers for each group of helminthes (Gawor et al., 2017).

**TIME TABLE:**

|  |  |  |  |
| --- | --- | --- | --- |
| Activity | Place | Period | Notes |
| Sample Collection | Erbil Province cities | May 2021- May 2022 | Weekly Samples.  |
| Morphological Study | Adv. Parasitology Lab + Private Lab. | May 2021- May 2022 |  |
| Molecular Study | Adv. Parasitology Lab + Private Lab. | May 2021- May 2022 | PCR technique by DNA marking techniques with Universal Primers.  |
| Ecological Study  | Field + Adv. Parasitology Lab + Private Lab. | May 2021- May 2022 | Effects of Sex, Age, Geographical distribution.  |

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