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Al- Quds University

Molecular and phylogenetic analysis of *Enterobius-vermicularis* from appendectomy specimens in the West Bank, Palestine.

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Thesis

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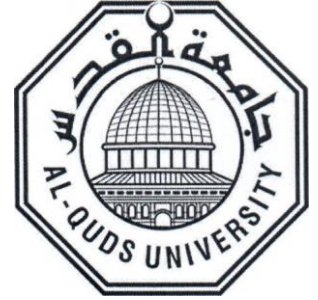
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Thesis Approval

Molecular and phylogenetic analysis of *Enterobius- vermicularis* from
appendectomy specimens in the West Bank, Palestine

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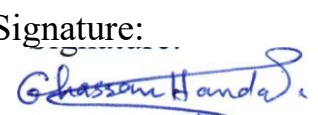
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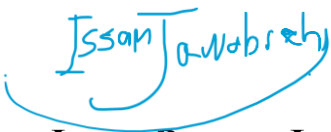
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Declaration

I certify that this thesis submitted for the degree of Master is the result of my research, except where otherwise acknowledged, and that this study or any part of it has not been submitted for a higher degree to any other university or institution.

Signature:



Issam Bassam Jawabreh

Date: 22/012/2024

Dedication

This thesis is dedicated

To Palestine, my homeland

To Gaza, the resilient heart beats with unwavering strength.

To the soul of my great father who dedicated this life to us.

To my devoted mother, who paved the way for my success.

To my patient wife, who was beside me in every moment.

To my brothers, my sister, and my family.

To my teachers

To my friends and colleagues.

To all my relatives who encouraged me to complete this work.

Thank you, and may Allah grant you blessings.

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Abstract

Background A parasitic nematode known as *Enterobius vermicularis* (human pinworm) frequently causes gastrointestinal symptoms and, in some cases, acute appendicitis. Despite its widespread prevalence, Palestinian populations have limited molecular data on *E. vermicularis*. This study aims to investigate the molecular and phylogenetic characteristics of *E. vermicularis* in appendectomy specimens across Palestine. The study will concentrate on the *COXI* gene to comprehend the genetic diversity and transmission patterns.

Methods A number of 55 formalin-fixed, paraffin-embedded (FFPE) appendectomy specimens previously collected from 2018-2023, were confirmed for *E. vermicularis* infection. Samples originated from three government hospitals: Queen Alia Hospital in Hebron, Rafidia Hospital in Nablus, and Beit Jala Hospital in Bethlehem. DNA extraction using FFPE sections was carried out, followed by polymerase chain reaction (PCR) targeting the *COXI* gene. Phylogenetic, sequence, and haplotype analysis were performed...

Results The male-to-female ratio was 1.2:1, with 54.5% of patients being male and 45.5% female. The average age of patients was 13.3 years. There are no sequence variations among the sequenced samples, suggesting the conservation of this population's genetic structure. The phylogenetic analysis revealed the existence of Type B *E. vermicularis* in the Palestinian samples, which is exclusively associated with human hosts. Similarly, the haplotype analysis using median-joining network group all Palestinian samples in Group B. Statistical analysis of demographic and clinical variables; including age, gender, WBC, and neutrophil counts, showed no significant correlations with the infection

Conclusion The study revealed a strong genetic uniformity among *E. vermicularis* samples from appendectomy samples in Palestine, suggesting limited transmission dynamics. All samples were identified as Type B, exclusive to human hosts when compared with global patterns. No significant associations were found between demographic or clinical manifestations and infection, indicating stable infection patterns across the population. The findings of the current research provide baseline molecular information about *E. vermicularis* infection in Palestine and may direct public health efforts by the Palestinian Ministry of Health in future research on the transmission and epidemiology of Enterobius.

LIST OF CONTENTS

Branch	Content	Page
	Declaration	I
	Dedication	II
	Abstract – English	III
	LIST OF CONTENTS	V-VII
	List of Table	VIII
	List Of Figure	IX
	List of abbreviations	X
Chapter (1) Introduction		
1.1	Background	1-5
1.2	Problem statement	5
1.3	Research objectives	6
1.4	Hypothesis	6
1.5	Significance of the study	6 -7
Chapter (2) Literature Review		
2.1	Overview of <i>E. vermicularis</i>	7
2.2	Epidemiology of <i>E. vermicularis</i>	7- 9
2.3	Life cycle	10
2.3.1	Routes of Transmission	10-11
2.3.2	Global prevalence and patterns	11

2.3.3	Factors Influencing the Parasite's Life Cycle	12
2.3.4	Clinical Manifestations and Complications	12
2.4	Diagnosis of <i>E. vermicularis</i> infection	13 -14
2.5	Parasitic Infections and Their Role in Appendicitis	15
2.5.1	<i>E. vermicular</i> and Appendicitis -Relationship	15
2.6	Treatment and Management	15
2.7	Molecular Characteristics of <i>E. vermicularis</i>	15 – 17
Chapter (3) MATERIAL & METHODS		
3.1	Study Design and Population	17
3.2	Sample Collection	18
3.2.1	Sample Preparation	18
3.3	DNA Extraction	18
3.4	PCR Amplification of the Cox1 Gene	19
3.5	DNA Sequencing	19
3.6	Phylogenetic Analysis	20
3.7	Haplotype Network Analysis	20
3.8	Collection and analysis of epidemiological data	21
3.8.1	Statistical analysis and interpretation	21
3.9	Ethical consideration	21

Chapter (4) Results		
4.1	Prevalence of <i>E. vermicularis</i> in Appendectomy spacemen	22
4.2	Demographics characteristic result	22 -23
4.3	Statistical analysis	23
4.4	Clinical Presentation of Symptoms	24
4.4.1	Association between Gender and Clinical Symptoms	25
4.5	DNA extraction of paraffine tissue	25-28
4.5.1	PCR amplification and Gel electrophoresis result	28-29
4.6	Sequence and phylogenetic analysis	29-30
4.7	Median-joining analysis	30 - 33
Chapter (5)		
Discussion		34-39
Conclusion		40
Reference		41 – 45
Appendixes		46 – 55
الملخص		56

LIST OF TABLES

No	Name of table	Page
1	Frequency Distribution of <i>E. vermicularis</i> in Appendectomy Samples	26
2	The annual Distribution in three Hospitals versus the geographical distribution	27
3	The association between gender and clinical symptoms in patients infected with <i>E. vermicularis</i> .	28
4	DNA Analysis Results: Measuring the concentration and Purity of DNA samples using Nanodrop Instrument.	29
5	Haplotype frequencies and geographical distribution	32
6	. Alignment of Variant Nucleotide Positions for the 333-bp (COX1) Gene.	33

List of Figure

NO	Name of Figure	Page
1	Geographic location of study areas in the West Bank of Palestine.	20
2	The patient's appendix Lumina under 400× magnification	27
4	Gel amplification of the <i>E. vermicularis</i> Cox1 gene in appendectomy samples.	30
5	A phylogenetic tree	31
6	Median-joining network for haplotype	34

List of abbreviation

E. vermicularis: *Enterobius Vermicularis*

NGS: Next-generation sequencing

rRNA: ribosomal RNA

ITS: Internal Transcribed Spacers

COX1: Cytochrome c oxidase subunit I

APC: Appendix

FFPE: Formalin-fixed, paraffin-embedded

MEGA 11: Molecular Evolutionary Genetics Analysis Version 11

PS: Palestine

DEN: Denmark

JAP: Japan

GRE: Greece

KOL: Kotlarska

CHI: China

POL: Poland

IRN: Iran

SPSS: Statistical Package for the Social Sciences

WBC: white blood cells

P-value: Pearson Chi-Square test

Chapter one

Introduction

1.1 | Background

Enterobius vermicularis, commonly known as the human pinworm, is a ubiquitous intestinal parasite infecting millions of individuals worldwide, particularly children (Hotez, Brindley et al. 2008). This intestinal parasite belongs to the family *Oxyuridae* and it is one of the most prevalent helminth infections globally (Pullan, Smith et al. 2014). Depending on population density and hygienic conditions, prevalence rates are projected to range from 5.8 % to 61% (Taghipour, Olfatifar et al. 2020). For instance, the incidence among students in a recent study in Thailand was 5.79%; however, rates have sometimes been substantially higher in earlier investigations, especially in poorer countries and areas with insufficient sanitation (Laoraksawong, Pansuwan et al. 2020). Research in Ethiopia discovered a prevalence incidence of 61% among children, especially in regions with restricted access to sanitary facilities and clean water (Lashaki, Mizani et al. 2023). Comparably, studies conducted in Kenya's rural areas revealed a prevalence of 39%, linking the high infection rates to crowded living arrangements and a lack of awareness about proper hygiene (Janthu, Dumidae et al. 2022). Based on data from the Palestinian Ministry of Health, the prevalence

of *E. vermicularis* infection in the West Bank and Gaza Strip was examined from 2008 to 2018. A total of 29,390 instances were documented, with 329 cases (1.1%) in the Gaza Strip and 29,061 cases (98.9%) in the West Bank. This demonstrates that *E. vermicularis* infection is more common in the West Bank and less common in the Gaza Strip. (Hamarsheh 2021). Generally, the infection rates vary between areas depending on many factors such as socioeconomic level and hygiene standards. Transmission of the disease occurs through the fecal-oral route, primarily via contaminated hands, bedding, or clothing. The major route for transmission is ingestion of embryonated eggs, which are usually spread through the fecal-oral pathway. After ingestion, the eggs hatch in the small intestine, and the larvae develop into adult worms in the colon. Female worms move to the perianal regions to lay their eggs, causing excruciating itching (Cook 1994).

Clinical manifestations of the disease are characterized by perianal itching, especially at night, (Moussavi, Houssaini et al. 2023). While enterobiasis is generally considered a mild condition, chronic or heavy infections can lead to complications such as secondary bacterial infections, urinary tract infections, and, notably one of the most significant complications associated with *E. vermicularis* is appendicitis (Leder 2009).

The etiology of acute appendicitis is multifactorial, involving complex interactions between inherited susceptibility, luminal obstruction, mucosal inflammation, and microbial infections. While fecaliths and lymphoid hyperplasia have been traditionally implicated as primary causes of appendiceal obstruction leading to acute appendicitis, the role of parasitic infestations, including *E. vermicularis* (Oh, Pimentel et al. 2020).

Recent studies have highlighted the role of *E. vermicularis* in appendicitis, demonstrating how its presence may exacerbate blockage and inflammation, which may result in severe appendicitis. Approximately 2.89% of appendicitis cases worldwide were linked to an *E. vermicularis* infection, according to a comprehensive study and meta-analysis. The appendix's helminth infection may cause inflammation and reactive lymphoid hyperplasia, both of which increase the risk of appendiceal blockage. Its presence is thought to have a contributing role in some cases of appendicitis, while its significance as a direct cause is still up for discussion. (Efared, Atsame-Ebang et al. 2017, Habashi and Lisi 2019, Taghipour, Olfatifar et al. 2020).

Appendicitis was seen to be becoming more common in newly industrialized nations at the start of the twenty-first century. Furthermore, reports on the economic effect, morbidity, and death related to appendicitis have been made in Western countries. (Gebre Selassie, Tekle Selassie et al. 2021). Between 1979 and 1984; 561,000 appendectomies were performed annually in the United States, indicating that several etiological factors may have contributed to this condition. (Addiss, Shaffer et al. 1990).

There is an association between *E. vermicularis* infestation and acute appendicitis; a common surgical emergency with significant morbidity and mortality rates worldwide. Acute appendicitis is characterized by inflammation of the vermiform appendix, often necessitating prompt surgical intervention to prevent complications such as perforation and peritonitis. (Chhetri, Al Mamari et al. 2023).

Studies in the Middle East, including Palestine, have reported varying prevalence rates of pinworm infection among appendectomy specimens, in Palestine enterobiasis is highly prevalent, especially among children. (Hamarsheh and Amro 2020, Hamarsheh 2021). The prevalence rate of enterobiasis in the Gaza Strip is 14.3% among pediatric appendectomy cases, highlighting the clinical significance of pinworm infection in the region (Hamdona, Lubbad et al. 2016). Jawabreh et al. (2024) conducted a new study in the West Bank of Palestine and confirmed using molecular techniques the presence of *E. vermicularis* worms or eggs in clinical samples collected from an appendectomy sample, the study describes two pediatric cases in which an *E. vermicularis* infestation presented with symptoms similar to acute appendicitis. Both children experienced abdominal pain, which is a classic symptom of appendicitis. However, during appendectomy, histopathological examination revealed pinworm infestations in the appendix rather than actual appendicitis. This study is consistent with previous studies describing *E. vermicularis* infestation can mimic or exacerbate appendicitis. (Jawabreh, Amro et al. 2024).

Global studies on *E. vermicularis* have contributed significantly to the understanding of the genetic makeup and diversity and the epidemiology of the infection. In a study conducted by Zhang et al. (2015) in China, a phylogenetic analysis of Internal Transcribed Spacers (ITS) sequences was conducted to investigate the genetic structure of *E. vermicularis* populations in different regions. The study identified distinct genetic clusters associated with

specific geographic locations, highlighting the importance of local transmission dynamics (Zhang, Xu et al. 2015).

Despite the clinical importance of pinworm infection, molecular studies investigating the genetic diversity and phylogenetic relationships of *E. vermicularis* isolates in Palestine and the Middle East are limited. Molecular techniques, including PCR amplification of specific genetic markers, coupled with phylogenetic analysis, offer a promising avenue to investigate the genetic diversity and evolutionary relationships of *E. vermicularis* strains within the context of appendiceal pathology (Ramezani and Dehghani 2007). El-Badry et al. (2018) conducted a phylogenetic analysis of *E. vermicularis* isolates from appendectomy specimens in Egypt, highlighting the genetic diversity of pinworm populations in the region. However, further research is needed to elucidate the genetic diversity and evolutionary dynamics of *E. vermicularis* isolates specifically in Palestine.

The genetic variation, phylogeographic, and host-specific characteristics of *E. vermicularis* have all been investigated using a small number of target genes. Data on mitochondrial DNA (mtDNA) sequences have been utilized as a suitable genetic marker in population genetic research (Blouin 2002).

The molecular and phylogenetic analysis of *E. vermicularis* in appendectomy specimens holds several implications for clinical practice and public health. Elucidating the genetic diversity and phylogenetic relationships of *E. vermicularis* strains associated with acute appendicitis may provide insights into the pathogenesis of pinworm-related appendiceal inflammation and inform diagnostic and therapeutic strategies (Shafiei, Jafarzadeh et al. 2023).

Understanding the connection between *E. vermicularis* and appendicitis is crucial in the Palestinian healthcare system, where parasitic infections are common. Addressing this gap could improve diagnostic accuracy, differentiate between infection-induced appendicitis and other forms, and improve therapeutic strategies. Greater awareness of *E. vermicularis* as a potential cause of appendicitis-like symptoms could lead to more efficient patient management in Palestinian healthcare settings. Additionally, exploring molecular characteristics, emergence, and development of *E. vermicularis* infection in the context of appendicitis is necessary to contribute to a broader understanding of parasitic infections and

their impact on human health, especially in Palestinian society, due to the limited studies related to this topic.

1.2 | Problem Statement

There is an extensive gap in the genetic epidemiology of *E. vermicularis* throughout the Middle East, particularly in Palestine. There are no studies concerning the genetic diversity of *E. vermicularis* isolates from Palestinian communities, even though the parasite is known to be common in this area. Significant genetic heterogeneity has been found in *E. vermicularis* strains in various locations, which may impact their virulence and transmission pattern. The lack of genetic information on *E. vermicularis* from Palestine hinders the attempts to control *Enterobius* infection and understanding of the parasite's epidemiology in this area.

This proposed study aims to conduct a comprehensive molecular and phylogenetic analysis of *E. vermicularis* from appendectomy specimens to understand its genetic diversity, transmission dynamics, evolutionary relationships, and clinical implications of pinworm infestation in acute appendicitis cases.

1.3 | Research objectives

The primary objective of this study is to investigate the molecular and phylogenetic characteristics of *E. vermicularis* in appendectomy specimens from Palestine. Therefore, the main objectives include the following:

1. To assess and confirm the prevalence of *E. vermicularis* in appendectomy specimens from Palestine.
2. To collect appendectomy specimens from pathology departments of the Palestinian hospitals suspected to have *E. vermicularis* pinworms.
3. To carry on molecular investigation using PCR to genotype the samples based on the mitochondrial *COXI* gene.
4. To perform phylogenetic analysis using obtained sequences to elucidate the genetic diversity and evolutionary relationships of *E. vermicularis* isolates.
5. To compare the phylogenetic analysis results using the sequences of this study and the other global sequences deposited in the GenBank, to understand the genetic makeup.

6. To investigate any potential correlations between genetic variants of *E. vermicularis* and clinical parameters of the host.

1.4 | Hypothesis

This study hypothesizes that isolates of *E. vermicularis* from Palestine have genetic properties different from those of isolates from other places. Additionally, it suggests a potential connection between genetic variety and the pathogenicity of *E. vermicularis* by speculating that these genetic variants could be correlated with the clinical presentation of appendicitis.

1.5 | Significance of the Study

The findings of this study will have significant implications:

1. Contribution to Molecular Epidemiology: This study will add significant information to the global understanding of the genetic diversity of *E. vermicularis*, which is crucial for comprehending the parasite's evolution, transmission, and potential for treatment resistance (Ahmed, Kotepui et al. 2023). On the Palestinian side, this is the first genetic and phylogenetic analysis of Palestinian *E. vermicularis* samples.

2. Improving Diagnostic Accuracy: By exploring the connection between *Enterobius vermicularis* and appendicitis, the study could help healthcare providers in Palestine differentiate between appendicitis caused by *E. vermicularis* and other forms, leading to more accurate diagnoses.

3. Implications for Public Health: By providing molecular information on the role of *E. vermicularis* in appendicitis, the study may be impacted positively on clinical practice and public health initiatives in Palestine by raising awareness of the role of *E. vermicularis* in the pathogenicity and infectivity to the appendix, which may help raise more effective diagnostic and treatment strategies (Chhetri, Al Mamari et al. 2023).

4. Platform for Future Research: The results of this molecular and phylogenetic study will provide a framework for further large-scale investigations, including all Palestinian hospitals

and health centers and other countries in the Middle East. This will help in understanding *E. vermicularis* infection epidemiology.

Chapter two

Literature Review

2.1 | Overview of *E. vermicularis* and Enterobius's

Enterobius vermicularis, commonly known as the human pinworm parasite, is a member of the Secernentea subclass and the Oxyuridae family. These worms are distinguished by their tiny size and unique tails that point posteriorly. Male worms are around 2–5 mm long, while adult females measure approximately 8–13 mm. Due to its great prevalence in both tropical and temperate regions, this parasite poses a threat to world health. *E. vermicularis* is the main member of the intestinal parasites family that infects various animals, including humans. (Gardner 2023) René Descartes, a French physician and philosopher, investigated what led to the first observation and recording of *E. vermicularis*, also known as the pinworm, in the 17th century. (Blasczyk and Füchsel 1991).

2.2 | Epidemiology of *E. vermicularis* infection

Geographical and socioeconomic factors influence the prevalence rates of *E. vermicularis* worldwide. The cosmopolitan parasite *E. vermicularis* is one of many countries most common parasitic infections (Dutto, Montu et al. 2012). According to the World Health Organization, the prevalence of the disease in children ranges from 4 to 28%. Estimates suggest that the infection affects approximately 200 million people worldwide, with children aged 5 to 10 accounting for over 30% of cases (Ali 2022). Reports indicate that the prevalence of oxyuriasis among children ranges from 2.5% to 45% in Latin America, 18% in Norway, 18.5% in the Republic of Korea, 17.2%, in Iran, and 2.9% in north-central Ethiopia (Bisetegn, Debash et al. 2023, Lashaki, Mizani et al. 2023).

Previous studies conducted in Palestine have consistently shown the incidence of *E. vermicularis* infection in the country, highlighting an ongoing public health concern. An initial study in Gaza City revealed that 31.9% of school children had *E. vermicularis* infection. Poor sanitation and hygiene practices in the region contributed to the high prevalence, underscoring the need for public health interventions to address this issue. (Yassin, Shubair et al. 1999). Recent research conducted by Hamarsheh and Amro (2020) revealed a higher incidence of *E. vermicularis* among children in both rural and urban areas of the Palestinian West Bank. According to their research, there was a higher incidence (24.6%), researchers identified socioeconomic differences as a significant factor in their findings, in which children from lower-income communities were more vulnerable to illness. To reduce the risk of *E. vermicularis* infection in these populations, the authors advocated for frequent health education programs and coordinated deworming initiatives. (Hamarsheh and Amro 2020, Hamarsheh 2021).

2.3 | Life Cycle of *E. vermicularis*

The host consumes eggs to initiate the life cycle of *E. vermicularis*, which subsequently hatches in the small intestine. Within the colon, the larvae develop into adult worms. When females relocate to the perianal area to lay eggs, it causes excruciating itching and aids in the parasite's spread. (Cook 1994).

2.3.1| Routes of Transmission

I. Direct transmission: The fecal-oral pathway, which involves the passage of eggs from contaminated hands into the mouth, is the main route of direct transmission of *E. vermicularis*. Children are susceptible to infection, especially in nurseries, childcare centers, and schools. One of the main causes of the parasite's spread is contaminated hands with eggs just after using the restroom or right before eating. Research has shown that schoolchildren, especially those between the ages of 5 and 10, have the highest transmission rates (Burkhart and Burkhart 2005, Yen, Chen et al. 2018).

Burkhart and Burkhart (2005) conducted a comparative study and found that implementing hand hygiene education programs significantly reduced the transmission rates of *E. vermicularis* by approximately 40% when compared to areas without any intervention. This study highlights the critical role of hygiene promotion in reducing direct transmission, particularly in schools (Burkhart and Burkhart 2005).

II. Environmental Transmission: Environmental transmission significantly influences the duration and spread of infections caused by *E. vermicularis*. For up to two weeks, *E. vermicularis* eggs may survive in the environment on a variety of surfaces, including toys, beds, clothes, and furniture. If these eggs contaminate food or beverages, or if they spread from infected surfaces to hands, they may easily get into the mouth. Hasegawa et al. (2020) found that the survival of infectious eggs on fomites for up to 14 days, particularly in dry and dusty conditions, significantly increases the risk of infection (Medkour, Amona et al. 2020).

Unlike direct transmission, environmental contamination poses a challenge in controlling *E. vermicularis* due to its ability to adhere to frequently touched surfaces. An investigation by Bloomfield et al. (2011) compared households with high levels of environmental contamination (dust, bedding, clothing) to those with regular cleaning and disinfection, showing that the former had infection rates three times higher than the latter. This suggests that maintaining a clean environment, particularly in areas where children play, is essential to preventing the spread of the parasite (Bloomfield, Exner et al. 2011). Studies conducted by Fürnkranz & Walochnik et al. (2021) suggest that highly polluted environments, particularly crowded settings like schools and hospitals, may aerosolize eggs. This raises concerns about the wider

public health effects of *E. vermicularis* in densely populated areas, where maintaining cleanliness would be more difficult (Fürnkranz and Walochnik 2021).

2.3.2 | Global Prevalence and Patterns:

Numerous studies in different regions have reported varying prevalence rates of *E. vermicularis* in appendectomy specimens. Many cases identify the parasite incidentally, while others believe it to be a potential cause of appendicitis due to obstruction or irritation. Akbulut et al. (2011) conducted a study in Turkey that reviewed over 2,000 appendectomy cases, identifying *E. vermicularis* in approximately 2-3% of the specimens (Akbulut, Tas et al. 2011). They emphasized that the parasite was usually incidental and did not directly cause inflammation. Similarly, Da Silva et al. (2007) in Brazil documented cases where they found *E. vermicularis* in inflamed appendices. Still, it remained unclear whether the parasite was the primary cause of appendicitis or if bacterial infections played a more significant role (da Silva, da Silva et al. 2007). In the Middle East, where parasitic infections are prevalent due to socioeconomic and environmental conditions, several studies have highlighted the role of *E. vermicularis* in appendicitis cases.

Hamdona et al. (2016) focused their study on children presenting with gastrointestinal symptoms in the Gaza Strip. They found that *E. vermicularis* was present in 15% of the appendectomy cases examined. Overcrowded living conditions and limited access to clean water contributed to the region's high prevalence of *E. vermicularis* (Hamdona, Lubbad et al. 2016). Despite the availability of numerous studies, that documented *E. vermicularis* role in appendicitis, the role of the parasite remained uncertain. Luminal blockage, inflammation, and bacterial infection have possibly explained the connection between the parasite and appendicitis. In Palestine, there is currently no comprehensive epidemiological or molecular study documenting the relationship between *E. vermicularis* and appendicitis, highlighting the need for further research in the country.

2.3.3 | Factors Influencing the Parasite's Life Cycle

Environmental factors and personal hygiene have a significant impact on whether *E. vermicularis* completes its life cycle. Infection rates are much greater in communities with inadequate sanitation measures. Socioeconomic factors like living in cramped quarters, not

having access to clean water, and having inadequate public health infrastructure further facilitate the parasite's transmission (Muliawati et al., 2020; Vargas-Arzola et al., 2024). In Palestine, the healthcare system has been harmed by ongoing political unrest, and the sanitary conditions in rural areas and refugee camps are often deficient. *E. vermicularis* is more likely to proliferate under these conditions, particularly in young persons and low-income households (Hamarshah and Amro 2019, Hamarshah 2021).

2.3.4 | Clinical Manifestations and Complications

Besides the most common manifestations of enterobiasis, the most significant complication is association with appendicitis. Several studies have reported the presence of *E. vermicularis* in the appendix, with some suggesting the parasite may play a role in the pathogenesis of appendicitis (Sinniah, Leopairut et al. 1991, Mansueto, De Simone et al. 2021). There is uncertainty about the precise mechanism via which *E. vermicularis* causes appendicitis. Acute appendicitis may result from inflammation, blockage, and subsequent bacterial infection brought on by the worm's presence in the appendix (Akbulut, Tas et al. 2011, Kartal, Kalayci et al. 2023). However, the connection between *E. vermicularis* and appendicitis is still debatable; some research contends that the parasite is just a coincidental discovery and not the actual cause of the infection (da Silva, da Silva et al. 2007).

2.4 | Diagnosis of *E. vermicularis* infection

Diagnosing *E. vermicularis* infection is challenging due to the sporadic nature of symptoms and the limitations of conventional diagnostic techniques. For an accurate diagnosis, clinical signs knowledge and proper diagnostic test utilization are necessary (Wendt, Trawinski et al. 2019). The "Scotch tape" test, which involves applying adhesive tape to the perianal area to collect eggs for microscopic examination, is a widely used diagnostic method. However, its limitations include a sensitivity of approximately 70% and the potential for false negatives, especially in cases of incorrect performance (Wendt, Trawinski et al. 2019).

2.4.1 | Histopathological Findings:

After appendectomy operations, *E. vermicularis* is often accidentally found during the histological examination of the appendix tissue. Patients suspected of appendicitis often discover *E. vermicularis* by mistake during appendicectomies, as many infections are asymptomatic or only produce moderate symptoms. A histological examination may reveal the worm's lodgment in the mucosa or appendix lumen, even in cases where appendicitis symptoms are absent. Although it is still unclear whether appendiceal enterobiasis; a condition where the appendix becomes inflamed is a direct cause of appendicitis, or appendicitis is not related to the presence of the parasite (Aydin 2007). Though, it may not be the main cause of the illness, the parasite's presence often results in minor inflammation that might resemble appendicitis and need surgery. Although the parasite may induce an inflammatory reaction, it does not necessarily result in noticeable clinical signs. Pathologically, the adult worms, eggs, or larvae are evident in the appendix. Antiparasitic therapy should be given to all households due to the parasite's high transmission. The fact that *E. vermicularis* is often reported accidentally after an appendectomy indicates the high transmissibility of the parasite in the community, often without noticeable symptoms. During appendectomy operations, histopathological studies of appendiceal tissue often result in the accidental discovery of *E. vermicularis* (Zaghlool, Hassan et al. 2015, Hasan, Nafie et al. 2020). A study by Jawabreh et al. (2024) in the West Bank, Palestine found that two pediatric cases showed symptoms similar to acute appendicitis due to an *E. vermicularis* infestation. Despite abdominal pain, the histopathological examination revealed pinworm infestations in the appendix during appendectomy, confirming previous research that *E. vermicularis* infestations can mimic or worsen appendicitis (Jawabreh, Amro et al. 2024).

2.4.2| Molecular Diagnostics

Polymerase chain reaction (PCR) methods have made it much easier for *E. vermicularis* in clinical samples, such as tissues from the appendix obtained during appendectomy operations. PCR is a sensitive and specific method for the detection of small amounts of target DNA. The *COXI*; a mitochondrial gene commonly used in molecular diagnostics and phylogenetic studies. It is a reliable marker for distinguishing *E. vermicularis* from other organisms (Rivera-Perez 2017, Schaumberg 2020). Comparing traditional histology, and PCR; the PCR method was able to detect *Enterobius* parasites in appendiceal tissues with

high specificity even if histopathology results were negative. According to their research, in certain situations where the histological diagnosis is negative, PCR was able to detect *E. vermicularis* DNA in 15% of appendectomy samples, this demonstrates the sensitivity of molecular methods in detecting latent or low-level infections. Moreover, *E. vermicularis* eggs and larval stages can be detected by PCR, which is typically not easily detected in conventional techniques. (Zhang, Xu et al. 2015).

Furthermore, to enhance the detection and quantification of *E. vermicularis* DNA, researchers can employ PCR in conjunction with other advanced molecular diagnostics like real-time PCR (qPCR) and next-generation sequencing (NGS). Real-time PCR allows the quantification of the parasite's load, in infected tissues. This quantitative approach is crucial in cases where the burden of infection is correlated with disease severity or clinical outcomes. Next-generation sequencing, on the other hand, provides full genomic analysis, enabling fine detection of genetic differences that might affect the pathogenicity of the parasite and its drug resistance. Together, these molecular techniques provide a robust framework for the detection, analysis, and understanding of the genetic makeup of *E. vermicularis* in clinical and research settings (Mohy, Al-Hadraawy et al. 2022, Shafiei, Jafarzadeh et al. 2023).

2.5 | Parasitic Infections and Their Role in Appendicitis

Enterobius vermicularis is a well-known parasitic infection that has been linked to appendicitis-like symptoms in clinical cases. However, other parasitic infections, such as *Entamoeba histolytica*, *Giardia lamblia*, and *Ascaris lumbricoides*, can also cause similar gastrointestinal symptoms and potentially contribute to appendicitis or mimic its clinical presentation. *Entamoeba histolytica* is known to cause amoebic dysentery and can lead to inflammation of the appendix in some cases *Giardia lamblia*, a common intestinal protozoan parasite, may cause chronic diarrhea, which could complicate the diagnosis of appendicitis (Eslahi, Aida Vafae, et al. 2022). *Ascaris lumbricoides*, the large roundworm, can lead to appendicitis through mechanical obstruction or inflammatory responses in the appendix

(Vafae Eslahi, Aida, et al. 2018). Given that parasitic infections are common in regions like Palestine, it is important to consider these other pathogens when diagnosing appendicitis to avoid misdiagnosis or overlooked infections.

2.5.1 | Pinworm-related Appendicitis

Clinicians have been interested in the connection between appendicitis and *E. vermicularis* (pinworm) for many years. Throughout the world, especially in unsanitary settings, *E. vermicularis* is a widespread parasitic nematode. Most commonly found in the large intestine, the parasite's connection to appendicitis raises some concerns. There is still disagreement about whether *E. vermicularis* causes appendicitis directly or whether it is just coincidental, even though several studies have examined the connection between the parasite and the illness (Nordstrand and Jayasekera 2004, Akbulut, Tas et al. 2011). Beginning in the early 1800s, there has been documentation linking *E. vermicularis* to appendicitis. Pilliet and Brumpt (1898) were the first to report findings of *E. vermicularis* in inflamed appendices and speculate about its possible connection to appendicitis. The connection between *E. vermicularis* and appendicitis is complex and not fully understood, but several mechanisms have been proposed. These include mechanical obstruction of the appendix by the worms, triggering local immune responses that cause inflammation, and potential disruption of the local microbiota, which may lead to secondary infections. Additionally, the worms can cause direct tissue damage through their movement and feeding, promoting inflammation. Chronic low-grade inflammation, allergic reactions, and molecular mimicry, where the worms' antigens resemble host tissues, may also contribute to appendicitis-like symptoms. These mechanisms highlight the potential for *E. vermicularis* to exacerbate inflammation in the appendix and mimic appendicitis (Hussen et al., 2020; Vavrinec et al., 2015) This preliminary finding established the basis for further research that would investigate the possible connection between the parasite and appendix inflammation (Dahlstrom and Macarthur 1994).

2.6 | Treatment and Management of *E. vermicularis* Infection

Anthelmintic medications, hygiene improvements, and public health interventions are the primary ways to manage *E. vermicularis* infection and reduce reinfection rates. The most

commonly prescribed drugs are Mebendazole, Albendazole, and Pyrantel Pamoate. These medications disrupt the parasite's metabolic processes, which interferes with the parasite's absorption of glucose; a vital molecule for the parasite's survival (Ng'etich, Amoah et al. 2024). Mebendazole and Albendazole are commonly used and preferred to be prescribed due to their high efficacy and minimal side effects (Albonico, Bickle et al. 2002).

2.7 | Molecular Characteristics of *E. vermicularis*:

The study of parasitic infections has been revolutionized by molecular methods, which make it possible to get a deeper insight into the genetic diversity and evolutionary links of parasites such as *E. vermicularis* (Shafiei, Jafarzadeh et al. 2023). Studies use various molecular markers to identify *E. vermicularis* and explore its evolutionary relationships. Among these, mitochondrial genes like Cytochrome c Oxidase Subunit 1 (*COXI*) are considered particularly effective. (Piperaki, Spanakos et al. 2011, Hagh, Oskouei et al. 2014, Shafiei, Jafarzadeh et al. 2023, Shilanabadi, Derakhshan et al. 2023).

1. rRNA (ribosomal RNA) genes

Since the 18S and 28S ribosomal RNA (rRNA) genes are substantially conserved in various species, they are a good choice for extensive taxonomic research. These genes identify genetic variability within a species and are valuable for higher-level phylogenetic analysis and species identification. For example, researchers have used rRNA genes to investigate the taxonomic placement of *E. vermicularis* within the Oxyuridae family (Zelck, Bialek et al. 2011)..(Zelck, Bialek et al. 2011, Kiene 2021).

2. Internal Transcribed Spacers (ITS)

The more variable ITS sections between rRNA genes are often used in population-level research to distinguish closely related species. The more quickly evolving ITS1 and ITS2 sequences may be used to identify minute genetic differences across *E. vermicularis* populations (Nakano, Okamoto et al. 2006). ITS sequences have been used in studies to distinguish *E. vermicularis* from similar species, providing crucial information on the genetic composition of the parasite (Tomanakan, Sanpool et al. 2020).

3. Cytochrome c oxidase subunit I (*COXI*)

At the molecular level, *E. vermicularis* may best be studied using mitochondrial genes, especially *COXI*. Despite being largely conserved across species, *COXI* is a component of the mitochondrial genome that varies sufficiently for population-level studies, which has been extensively used to examine the genetic diversity, evolutionary history, and phylogenetic relationships of *E. vermicularis* (Hagh, Oskouei et al. 2014, Haghshenas, Koosha et al. 2023). The *COXI* gene is one of the mitochondrial genes that have the highest diversity useful for phylogenetic analysis of *E. vermicularis*. When compared to other genes such as ribosomal DNA, it is superior for *E. vermicularis* evolutionary history. On the other hand, *E. vermicularis COXI* gene sequences in human and captive chimpanzee eggs and adult Japanese worms have shown three different genetic types, denoted as type A, B, and C, respectively (Nakano, Okamoto et al. 2006, Traversa, Kuzmina et al. 2008). While type C has only been found in chimpanzees, types B and A have been found in the human species. In 2011, Piperaki et al. studied *E. vermicularis* genotypes in scotch samples from children in Greece, identifying 11 haplotypes. These sequences belong to a type known as type B, previously documented solely in chimpanzees (Piperaki, Spanakos et al. 2011). In contrast, the *COXI* sequencing of *E. vermicularis* eggs from people in Greece, Denmark, Poland, Germany, and Iran grouped all the isolates in type B (Piperaki, Spanakos et al. 2011, Ferrero, Röser et al. 2013, Tavan, Mikaeili et al. 2020). Types A and B were identified from individuals using an adhesive tape perianal swab method in a recent study conducted in Thailand. This finding suggests that type A of the infection may only be found in eastern Asia (Tomanakan, Sanpool et al. 2020).

Chapter Three

Methodology

3.1 | Study Design and Population

The cross-sectional design of this study investigates the molecular and phylogenetic characteristics of *E. vermicularis* in appendectomy specimens from Palestine. We retrospectively identified appendectomy specimens containing *E. vermicularis* from patient records in the pathology departments of governmental hospitals in the West Bank, Palestine (Queen Alia Hospital, Rafidia Hospital, and Al-Hussein Hospital). These hospitals are in Hebron, Nablus, and Bethlehem, Figure 1 illustrates the geographic locations of the study. The study encompasses patients who underwent appendectomy at the selected Palestinian hospitals between the years 2019-2023. A total of 4930 samples from patients admitted to government hospitals with appendicitis. Only 55 samples contained *E. vermicularis*, confirmed by histopathology examination.

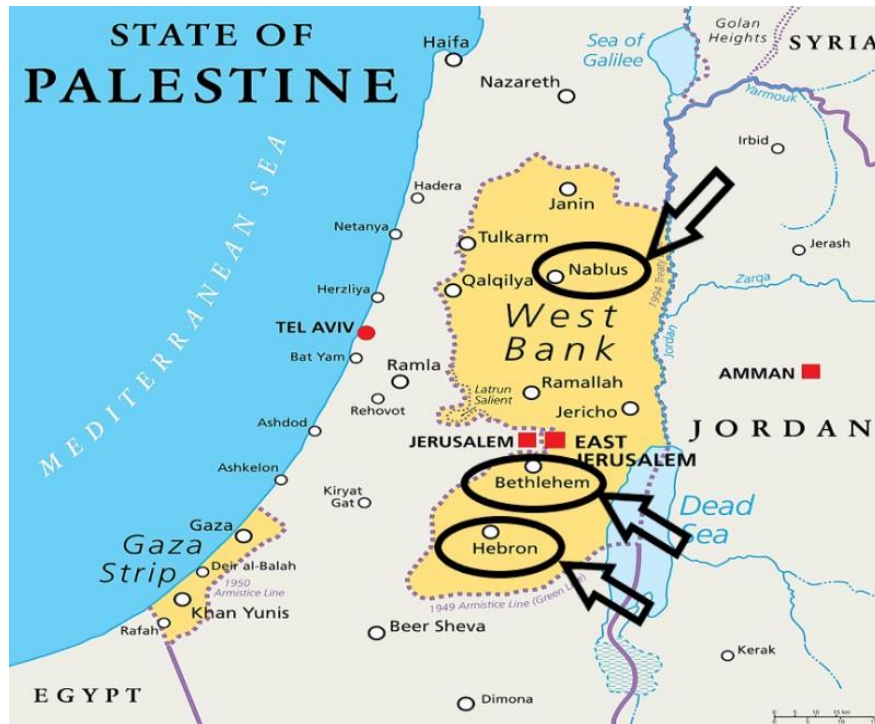


Figure 1: Geographic location of study areas in the West Bank of Palestine

3.2 | Sample Collection

This study utilized 55 formalin-fixed, paraffin-embedded (FFPE) appendectomy specimens from 2019-2023, which present challenges in DNA extraction due to formalin fixation, causing DNA fragmentation and degradation, which can hinder high-quality genomic DNA extraction and complicate gene amplification through PCR. After confirming the diagnosis of appendicitis and identifying *E. vermicularis* in the appendix, we selected a section of the pathology block containing the parasite for further analysis. Samples were handled aseptically and stored at -80°C until processed for DNA extraction (Al-Taei and Al-Quraishi 2022). The inclusion criteria included patients who underwent an appendectomy procedure at governmental hospitals and specimens with confirmed *E. vermicularis* presence. The exclusion criteria encompassed specimens without confirmation or insufficient data for molecular analysis, as well as patients diagnosed with any specific type of malignancy.

3.2.1 | Sample Preparation

The paraffin blocks containing the appendectomy samples were sectioned using microtome at a thickness of 15 micrometers (μm). Each section was transferred to a 1.5 ml microfuge tube and stored at -80 C until further processed.

To remove paraffin wax from sections and overcome the formalin challenge the following steps were performed; samples were treated with xylene, and washed with normal saline to eliminate the formalin; formalin cross-links proteins and nucleic acids, which halts PCR amplification and subsequent processes. Therefore, samples were heated at $90\text{ }^{\circ}\text{C}$ for three hours to undo the effects of formalin fixation on DNA.

3.3 | DNA Extraction

DNA extraction was carried out using NucleoSpin Tissue Kit XS (Germany) following the manufacturer's instructions. Sections in 1.5 ml microfuge tubes were smashed against the walls of the tube using a sterile micro pestle, then $80\text{ }\mu\text{l}$ of pre-lysis buffer T1, and $8\text{ }\mu\text{l}$ of proteinase K were added to each tube. All samples were incubated at $56\text{ }^{\circ}\text{C}$ overnight, about $80\text{ }\mu\text{l}$ of DNA was eluted from each extracted sample, and stored at -20 until used for PCR amplification. A Nanodrop spectrophotometer (Thermofisher, USA) was used to assess the DNA integrity. This method will measure the DNA concentration and purity (A260/A280 ratio), confirming its suitability for PCR amplification and ensuring the accuracy of subsequent analyses.

3.4 | PCR Amplification

The *E. vermicularis COXI* gene was amplified using specific primers; Forward: (5-TTTTGGTCATCCTGAGGTTTATATTC-3) and reverse: (5-CACATTATCCAAAATAGGATTAGCC-3), which designed to target conserved regions of the gene (Shafiei, Jafarzadeh et al. 2023).

For PCR amplification, which was carried out in a $25\text{ }\mu\text{l}$ volume and included the following ingredients: $10\text{ }\mu\text{l}$ of genomic DNA, $12.5\text{ }\mu\text{l}$ of *Taq* Mix Red, $2\text{ }\mu\text{l}$ of Ultra-Pure double-distilled water (ddH₂O), and $0.25\text{ }\mu\text{l}$ of each primer. The negative control, contains only PCR mix without DNA subjected to the same conditions as the other PCR reactions. The positive control includes DNA extracted from a pinworm previously isolated from a stool

sample. The PCR conditions comprised an initial denaturation at 95°C for 3 minutes, followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing at 56°C for 30 seconds, and extension at 72°C for 1 minute, with a final extension at 72°C for 5 minutes. Amplified products were analyzed on a 2% agarose gel, stained with ethidium bromide, and visualized and photographed using a gel documentation system.

3.5 | DNA Sequencing and Sequence Analysis

Ten samples were directly sequenced in both directions using the primers already used for PCR amplification. The sequences were confirmed of *COXI E. vermicularis* sequences using BLAST search (NCBI), trimmed and edited, and aligned with other sequences from the GenBank using MEGA 11 software (Version 11.0.10) and MultAlin (Kumar, Stecher et al. 2018, Tamura, Stecher et al. 2021).

3.6 | Phylogenetic Analysis:

The *COXI* sequences were alignment with reference sequences from other isolates of *E. vermicularis* available in the GenBank (Thompson, Higgins et al. 1994) using MultAlin software (Version 5.1), and ClustalW multiple sequence alignment (Thompson et al., 1994) have been conducted and the alignment file has been imported to MEGA 11 software which was converted to MEGA input file for phylogenetic analysis. The evolutionary relationships between the Palestinian *E. vermicularis* sequences and others from the GenBank were conducted and a a maximum parsimony condensed tree was generated with the following parameters; bootstrap analysis using 1000 iterations, and missing data and gaps were not included in the analysis. Based on the genetic divergence, samples were grouped into three main types: Type A, Type B, and Type C (Kumar, Stecher et al. 2018, Tamura, Stecher et al. 2021).

3.7 | Haplotype Network Analysis:

Haplotype analysis was conducted using DNAsP software (Version: 6.12.03) and a median-joining haplotype network was constructed using NETWORK software, Fluxus (v10.2) to examine genetic diversity and population structure. This network analysis included 100 haplotypes representing diverse geographic isolates from different countries. Each circle in

the network denotes a haplotype, with the circle size proportional to the haplotype's frequency. Previous *E. vermicularis* studies supported the color-coding of geographic origins to facilitate visualization of the region-specific clusters. Geographical regions were color-coded as follows: PS (Palestine), DEN (Denmark), JAP (Japan), GRE (Greece), KOL (Kotlarska_Poland), CHI (China), POL (Poland), and IRN (Iran).

3.8 Collection and analysis of epidemiological data:

The relevant data of patients diagnosed with appendicitis and subjected to appendectomy were collected from their medical records maintained at governmental hospitals in the West Bank, Palestine. These data include patient name, age, gender, clinical history, laboratory tests and results, and histopathological findings confirming the presence of *E. vermicularis*.

3.8.1 Statistical Analysis and Interpretation:

Statistical analysis of the data using Statistical Package for Social Sciences (SPSS) version 23.0 (IBM Corp), correlation between demographic and clinical data have been tested using descriptive statistics. The chi-square test has been conducted to analyze categorical variables, and the student's t-test to analyze continuous variables.

3.9 Ethical Considerations:

The Research Ethics Committee at Al-Quds University reviewed and approved the study protocol. Informed consent was obtained from the participating governmental hospitals through the Palestinian Ministry of Health before using the appendectomy specimens for research purposes. Without any intimidation, every hospital was free to accept or refuse participation in the study.

Chapter Four

Results

4.1 | The prevalence of *E. vermicularis* in appendectomy specimens.

The prevalence of *E. vermicularis* in appendectomy specimens collected between 2019 and 2023 from three governmental hospitals in Palestine confirmed the presence of the parasite in 55 out of the total 4930 (1.12%) appendectomy samples examined. All appendectomy specimens examined in this study were collected in the past five years (Table 1).

Table 1: Frequency of *E. vermicularis* in appendectomy samples collected from three hospitals in the West Bank, Palestine.

Category	Frequency	Percent
Positive for (<i>E. vermicularis</i>)	55	1.1%
Negative	4875	98.9%
Total	4930	100%

4.2 | Demographic characteristics of the study participants

This study analyzed 55 appendectomy specimens. The average age of the patients is 13.3 years, ranging from 5 to 42 years. The study included 55 cases, of which 54.5% were male and 45.5% were female, a male-to-female ratio of [1.2:1]. The histopathological examination

of the samples confirmed the presence of *E. vermicularis* worm and /or eggs in all examined specimens (Figure 2).

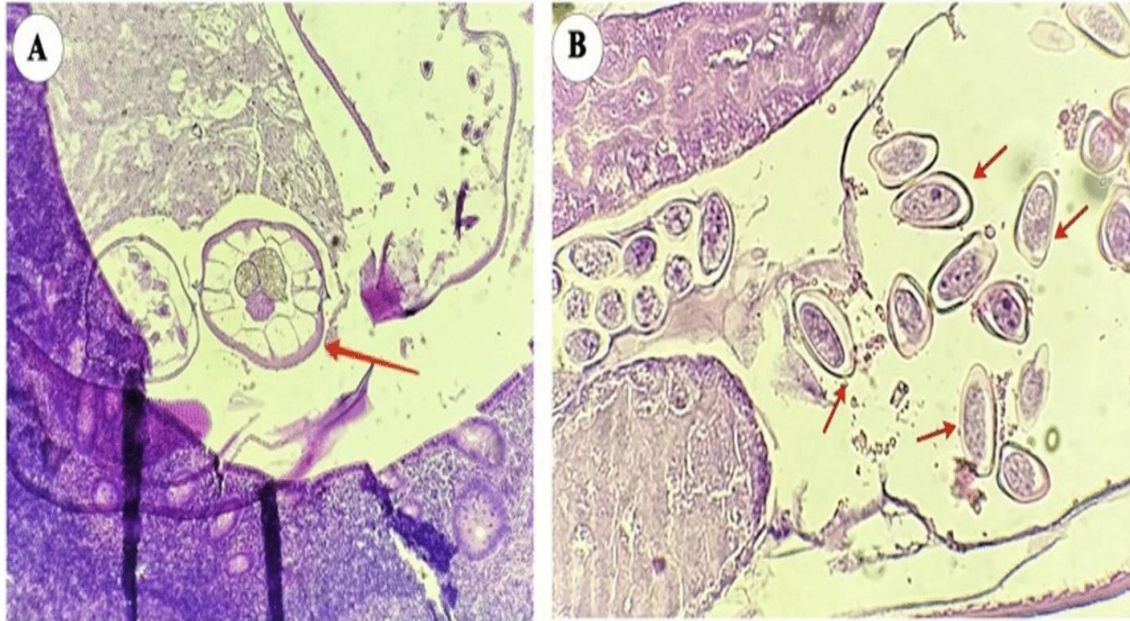


Figure 2: The appendix of the patient had an *E. vermicularis* pinworm in its lumen, as seen in histopathological sections stained with hematoxylin-eosin. The appendix's cross-section, magnified 400 times, shows the pinworm (red arrow). A longitudinal section demonstrates the presence of a gravid female pinworm with eggs (red arrows) in 400× magnification.

4.3 | Statistical analysis

The distribution of samples collected from the West Bank hospitals was as follows; 26 (47.3%), were from Hebron, 22 samples (40%) were from Nablus, and 7 samples (12.7%) were from Bethlehem. Table 2 shows the Annual Distribution (2019-2023) of *E. vermicularis* cases used in this study; 55 positive samples were collected over five years from three Palestinian governmental hospitals Hebron, Nablus, and Bethlehem.

Table 2: Annual Distribution (2019-2023) of *E. vermicularis* Cases from Three Palestinian Governmental Hospitals (Hebron, Nablus, and Bethlehem)

Distribution al Geographic	2019	2020	2021	2022	2023	Total
Hebron (Queen Alia)	6 (22%)	7 (25%)	3 (18%)	7 (24%)	2 (11%)	26
Nablus (Rafedia)	5 (22%)	5 (25%)	6 (18%)	5 (24%)	2 (11%)	22
Beit Jala (Bethlehem)	1 (22%)	2 (25%)	1 (18%)	1 (24%)	2 (11%)	7
Total	12 (22%)	14 (25%)	10 (18%)	13 (24%)	6 (11%)	55

4.4. | Clinical Presentation of Symptoms

The study involved 55 patients with *E. vermicularis* infection, all reported gastrointestinal discomfort. The most common symptom was abdominal pain, with 100% of the patients experiencing it. Nausea was common but not as prevalent as abdominal pain. Vomiting was less frequent, with only 12.7% of patients reporting it. Diarrhea was uncommon, with the majority not showing any signs. 14.5% of patients reported fever, The majority of individuals did not exhibit any signs of distress.

4.4.1|Association between gender and clinical symptoms

The relationship between gender and the presence of clinical symptoms was analyzed out of 55 patients, 78.2% reported experiencing common symptoms like nausea, 22 out of 30 males, and 8 out of 25 females. A Pearson Chi-Square test (*P*-value) showed no significant association between gender and nausea, with a *P*-value of 0.514. All patients experienced abdominal pain. A chi-square test was not carried out since this symptom was present in all patients. Seven males out of 30 (23.3%) reported vomiting, four out of 25 (16%) females reported vomiting or results showed no

statistically significant association between gender and diarrhea (P -value = 0.202), out of 5 (16.7%) of males experienced fever (P -value = 0.102) (Table 3).

Table 3: The association between gender and clinical symptoms in patients infected with *E. vermicularis*.

Clinical Symptom	Total patient	Males (M)	Female	Males	Females	* P -value
Nausea	55	30	2	22 (73.3%)	21 (84%)	0.34
Abdominal Pain	55	30	2	30 (100%)	25 (100%)	-
Vomiting	55	30	2	7 (23.3%)	4 (16%)	0.498
Diarrhea	55	30	2	0 (0%)	2 (8%)	0.115
Fever	55	30	2	5 (16.7%)	5 (20%)	0.75

* The Chi-square test used to compare the group's P -value < 0.05 was considered significant.

4.5 | DNA extraction

Nanodrop ND-1000 was used to estimate the DNA concentration and purity of the genomic DNA. High-quality genomic DNA was obtained from all 55 samples, with concentrations ranging from 7 ng/ μ L to - 400 ng/ μ L. The purity of the DNA, as measured by the A260/A280 ratio, was within the acceptable range of 1.8 - 2.0. (Table 4 A, B, and C).

Table(4-A): DNA Analysis Results: Measuring the concentration and Purity of DNA samples using Nanodrop Instrument.

Sample No.	DNA Conc. (ng/μl)	260/280	260/230
1	99	1.96	1.38
2	137	1.81	1.48
3	7	1.76	0.38
4	123	1.95	1.64
5	118.7	1.77	0.94
6	70.2	2.04	1.44
7	21.6	1.84	0.77
8	41.6	1.78	0.84
9	272.4	1.84	0.67
10	140.34	1.72	1.02
11	25.9	1.88	0.71
12	38.9	1.65	0.91
13	45.2	1.81	0.73
14	49.1	1.86	0.69
15	17.8	1.86	0.92
16	40.31	1.79	0.69
17	21.56	1.35	0.47
18	85	1.59	0.54
19	7	1.73	0.52
20	67.55	1.50	0.51

Table(4-B): DNA Analysis Results: Measuring the concentration and Purity of DNA samples using Nanodrop Instrument.

21	272	1.75	0.66
22	31.8	1.80	0.77
23	39	1.78	0.67
24	39.7	1.74	0.68
25	44.2	1.63	0.71
26	44	1.67	0.83
27	44	1.67	0.83
28	88.8	1.67	0.86
29	60.9	1.86	1.17
30	69.1	2.00	1.16
31	182	2.00	1.17
32	50.4	1.83	1.06
33	135.5	1.84	0.79
34	94.5	1.93	0.91
35	421.3	1.87	0.89
36	43.5	1.47	0.75
37	147	1.83	1.01
38	90.7	1.78	0.77
39	85.9	1.81	0.79
40	99.4	1.84	0.91
41	100.3	1.89	0.67
42	177.3	1.89	0.55

Table(4-C): DNA Analysis Results: Measuring the concentration and Purity of DNA samples using Nanodrop Instrument.

43	157	1.77	0.62
44	107	1.76	0.59
45	46.1	1.88	0.71
46	71.7	1.65	0.86
47	400.6	1.81	0.71
48	171	1.86	0.68
49	184	1.88	0.92
50	440.6	1.79	0.69
51	40.3	1.35	0.49
52	122	1.73	0.58
53	36.3	1.50	0.54
54	88.3	1.50	0.50
55	66.9	1.52	0.51
Mean	109.84	1.74	0.76

4.5.1 | PCR amplification

The majority of the samples;54 out of 55 samples were successfully amplified using *COXI* primers, the PCR band size (380 bp) is diagnostic for *E. vermicularis* along with positive control from the worm isolated from the stool of a patient (Figure 3). Only one sample did not amplify probably due to low DNA quantity.

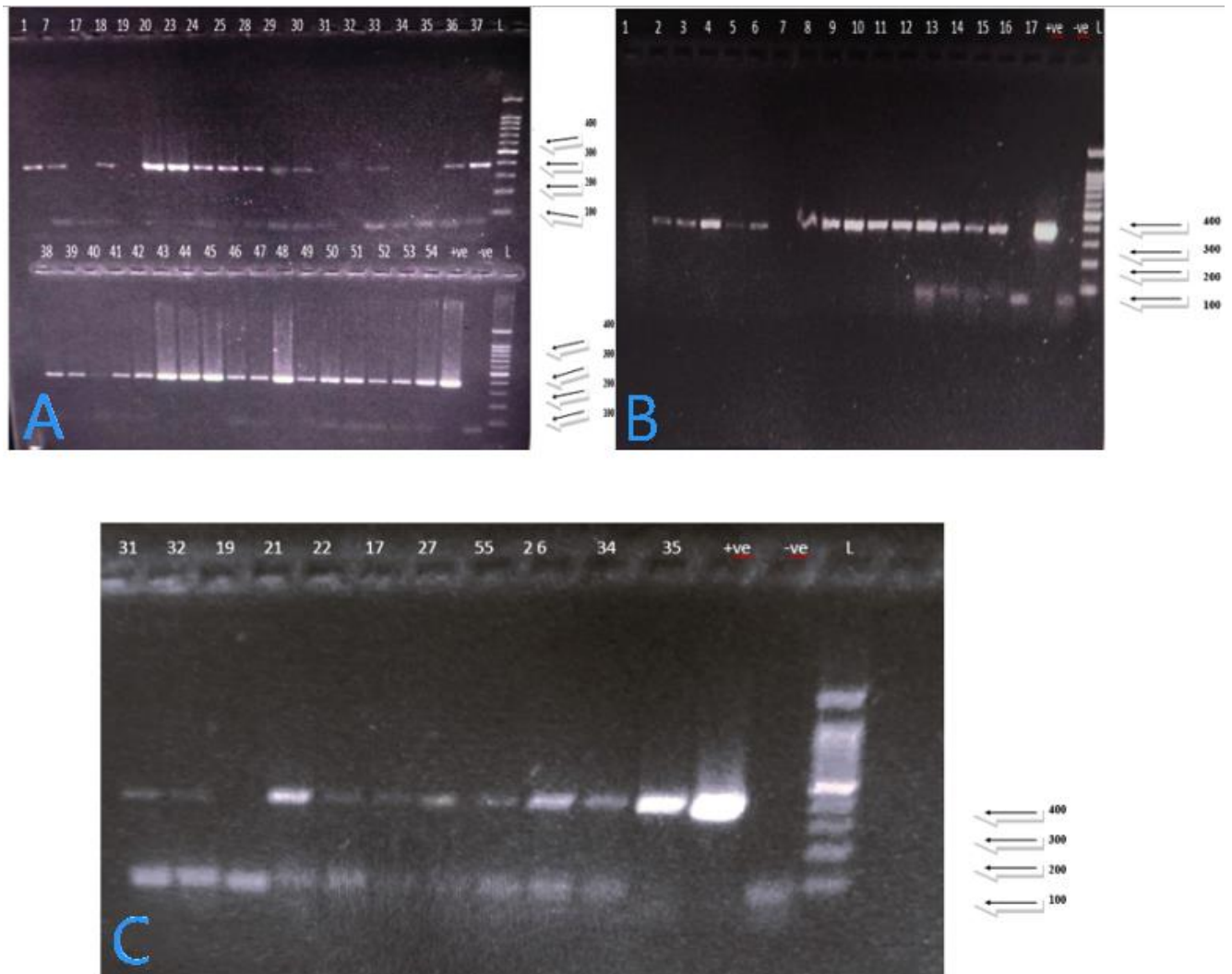


Figure 3: Pictures A, B, and C show successful *E. vermicularis* *Cox1* gene amplification in appendectomy samples. Clear bands at the 380 bp marker indicate positive samples with successful gene amplification, except for sample 19 as shown in Figures 3 A and C. The experiment included both positive and negative controls to ensure validity. The molecular ladder serves as a size reference for amplified products.

4.6 | Sequence and phylogenetic analysis

A maximum parsimony phylogenetic tree was constructed for all *COX1* data sets; 10 sequences from Palestinian samples and 90 sequences from GenBank from different countries. The phylogenetic tree revealed the existence of three distinct groups: A, B, and C. Group A contains sequences from many areas, particularly from countries like Japan, Greece, Denmark, and Poland. Group B, include all Palestinian sequences, Iran, Greece, and Denmark. This clustering implies a close genetic connection between the Palestinian isolates and those from Greece and Denmark, despite the geographical

distance. Group C includes sequences from Japan and China, suggesting a distinct evolutionary lineage. Group C includes unique sequences from 15 non-human primates.

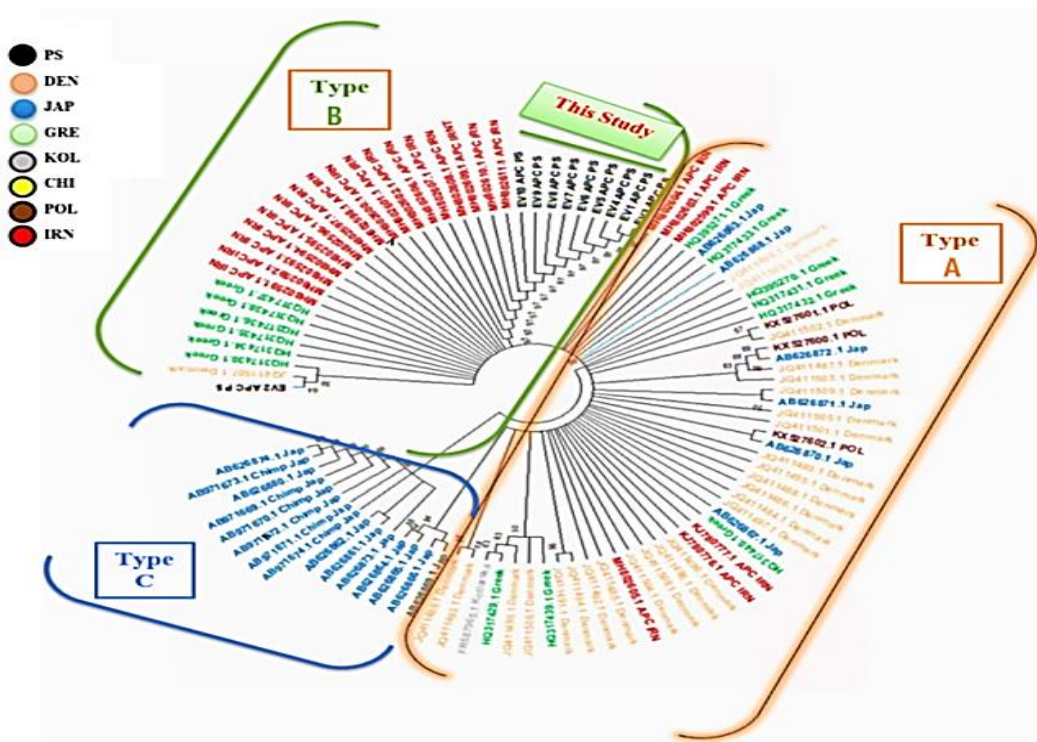


Figure 4: A phylogenetic tree is constructed using the maximum parsimony method. Bold letters indicate the GenBank accession numbers of the sequences obtained in the present study. **PS** (Palestine), **DEN** (Denmark), **JAP** (Japan), **GRE** (Greece), **KOL** (Kotlarska_Poland), **CHI** (China), **POL** (Poland), and **IRN** (Iran) contain 380 bp of the *COXI* gene. Numbers on the branches indicate bootstrap values. Only values over 50% are included. The symbols A, B, and C represent the appendectomy human samples, while the symbol Chimp represents samples derived from chimpanzees.

4.7 | Median-joining analysis

A median-joining network based on 15 haplotypes from 100 *COXI* sequences of *E. vermicularis* from this study and from the GenBank, in which each circle denotes a haplotype with sizes reflecting their frequency. The network reveals unique haplotypes; haplotypes Hap1 – Hap 15 (Table 5). Analysis of variant nucleotide positions in the *COXI* gene revealed minor variations among the haplotypes (Table 6). The network topology revealed the existence of three groups; A, B, and C. The Palestinian samples are primarily grouped among group B, dominated by haplotypes from Iran, Greece, and Denmark. (Figure 5).

Table 5: Haplotype Frequencies and Geographical Distribution Based on COX1 Sequences of *E. vermicularis*.

Haplotype	Frequency	Sample IDs	Regions
Hap_1	34	EV1_APC_PS, EV2_APC_PS, EV3_APC_PS, EV4_APC_PS, EV5_APC_PS, EV6_APC_PS, EV7_APC_PS, EV8_APC_PS, EV9_APC_PS, EV10_APC_PS, MH8026101_APC_IRN, ...	PS (Palestine), IRN (Iran), POL (Poland), GRE (Greece), DEN (Denmark)
Hap_2	35	MH8026051_APC_IRN, MH8026091_APC_IRN, MH8026031_APC_IRN, MH8025991_APC_IRN, KX5276021_POL, HQ3174401_Greek, ...	IRN (Iran), POL (Poland), GRE (Greece), DEN (Denmark), JAP (Japan), KOL (Kotlarska)
Hap_3	3	KX5276001_POL, JQ4114871_Denmark, AB6268721_Jap	POL (Poland), DEN (Denmark), JAP (Japan)
Hap_4	1	HQ3174381_Greek	GRE (Greece)
Hap_5	1	HQ3174371_Greek	GRE (Greece)
Hap_6	9	AB9716731_Chimp_Jap, AB9716721_Chimp_Jap, AB9716711_Chimp_Jap, AB9716701_Chimp_Jap, AB9716691_Chimp_Jap, AB6268801_Jap, AB6268741_Jap	JAP (Japan)
Hap_7	6	JQ4115091_Denmark, JQ4115001_Denmark, AB6268711_Jap, AB6268691_Jap, AB6268661_Jap, ...	DEN (Denmark), JAP (Japan)
Hap_8	1	JQ4115031_Denmark	DEN (Denmark)
Hap_9	1	JQ4114951_Denmark	DEN (Denmark)
Hap_10	2	JQ4114941_Denmark, JQ4114911_Denmark	DEN (Denmark)
Hap_11	2	JQ4114931_Denmark, JQ4114891_Denmark	DEN (Denmark)
Hap_12	1	JQ4114921_Denmark	DEN (Denmark)
Hap_13	1	KY3107231_China	CHI (China)
Hap_14	1	AB6268651_Jap	JAP (Japan)
Hap_15	2	AB6268621_Jap, AB6268611_Jap	JAP (Japan)

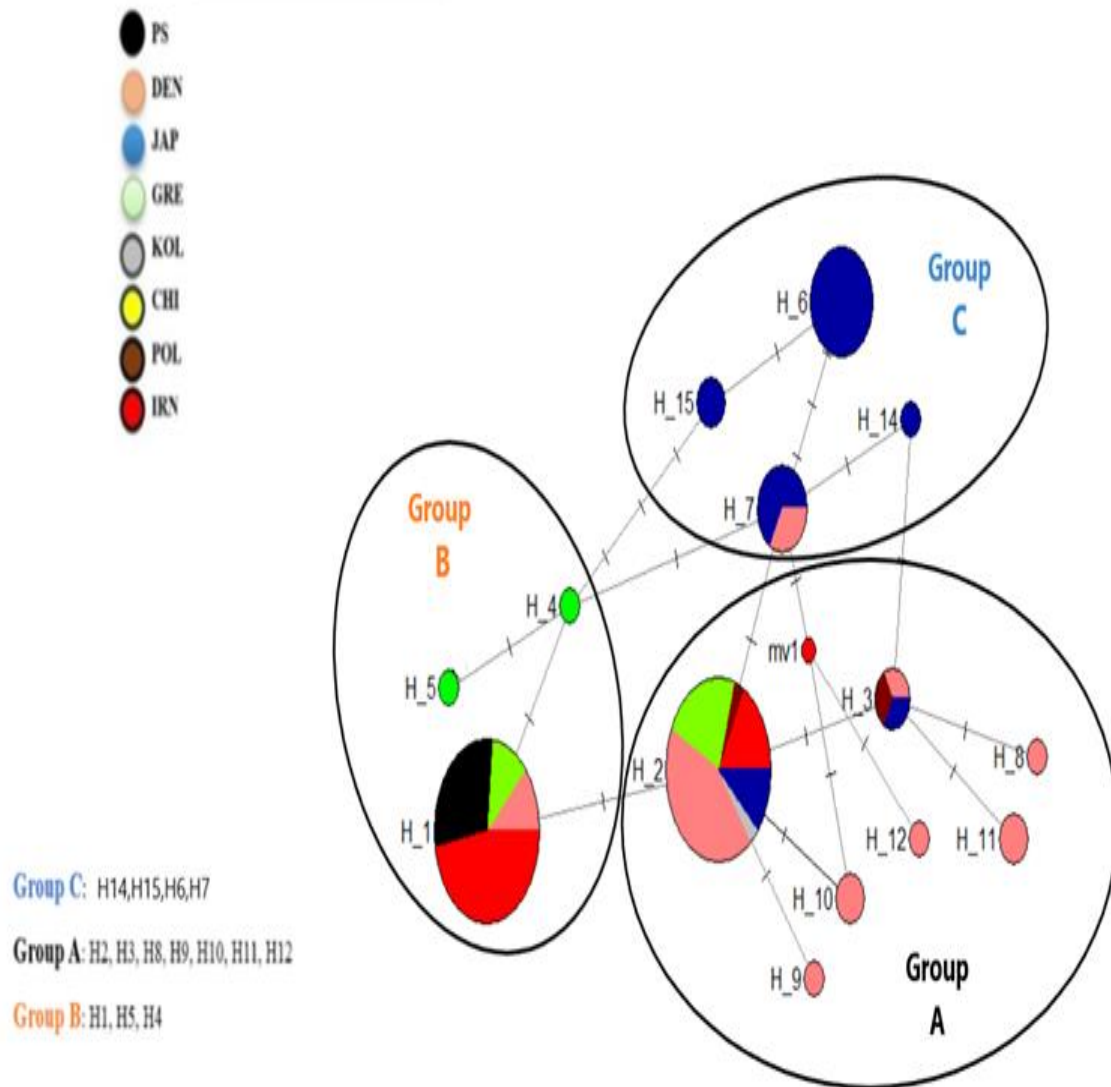


Figure 5: Median-joining network for 15 haplotypes from 100 *COX1* sequences of *E. vermicularis*. A circle represents each haplotype. The sizes of the circles are proportional to the frequency of the haplotypes. Circles of the same color represent haplotypes from the same geographical region. The numbers beside the circles represent haplotype numbers. The underlined numbers between circles indicate nucleotide substitutions. Divergent haplotype groupings A, B, and C exist. PS, Palestine; DEN, Denmark; JAP, Japan; GRE, Greek; KOL, Kotlarska; CHI, China; POL, Poland; IRN, Iran.

Chapter Five

Discussions

Discussions

This study is the first unique cross-sectional study in Palestine that discussed the molecular diagnosis and phylogenetic analysis of the pinworm *E. vermicularis* in appendectomy samples in the West Bank of Palestine which have been collected from three Palestinian government hospitals. The histopathological studies performed over five years confirmed *E. vermicularis* in 55 out of the 4930 appendectomy samples, representing a 1.12% prevalence rate. Despite recognizing *E. vermicularis* infection as a cause of appendicitis, it is suggested that pinworm infection accounts for a minor percentage of appendectomy cases. The prevalence of this infection is in agreement with other studies (Ariyathenam, Nachimuthu et al. 2010, Kocaman, Anadolulu et al. 2022). In Palestine, a previous study in the Gaza Strip documented a higher prevalence rate of 15% in appendectomy samples collected from several hospitals in the Gaza Strip (Hamdona, Lubbad et al. 2016). This suggested a difference in prevalence between Gaza and the West Bank, Palestine. Additionally, socioeconomic status affects the number of patients between Gaza and The West Bank. For example, places with higher poverty tend to have higher rates of *E. vermicularis* due to limited access to clean drinking water and highly populated areas with low hygiene. Environmental conditions such as temperature (humidity) and living conditions can also impact an individual's quality of life and vulnerability to infection.

The demographic study revealed that the mean age of patients diagnosed with *E. vermicularis* in our study ranged from 5 to 42 years. This observation is consistent with the epidemiology of *E.*

vermicularis in the country, which states that children are the most frequently affected by this infection. According to the research, the ratio of males to females was 1.2:1, which indicates a slight male predominance. Several previous studies have reported this pattern in surrounding countries; in Egypt, similar findings were reported, with a similar male predominance, which could reflect variations in exposure between the two genders (Elmonir, Elaadli et al. 2021). This is particularly evident in scenarios where boys engage in more outdoor activities, thereby increasing the likelihood of parasites. Global research has also observed similar age patterns (Sočan, Štromajer et al. 2022). Other factors include close contact in schools and the tendency of children to participate in activities that create an increased risk of infection, such as not practicing proper hand hygiene, which may explain the high frequency among children.

The geographical distribution of confirmed infected samples per year is different in the studied districts; it is dominated by Hebron (47.3%), Bethlehem (12.7%), and Nablus (40%). This variation in the distribution of cases in the studied districts may be due to various factors including; population density variations, socioeconomic conditions, and differences in the availability and quality of primary healthcare in each district. These factors play a critical role in the spread of pinworm infection. The distribution of the disease from 2019-2023 changed and shifted toward higher levels in 2020 and 2022, possibly as a result of changes in healthcare access and personal hygiene practices (Fan, Chuang et al. 2019) besides changes in environmental and social conditions.

The immunological response of 65.5% of patients had elevated white blood cell (WBC) counts (mean 13.01×10^3 cells/L), significantly higher than the normal range of 4.5 to 11.0×10^3 cells/L, indicating an immunological response common in parasitic infections and appendicitis. (Wakelin 1996). Patients with pronounced appendicitis had neutrophil counts exceeding 80%, indicating that the parasitic infection may exacerbate the inflammatory condition of the appendix (MacDonald, Araujo et al. 2002). On the other hand, the significant elevation of neutrophils correlated with more severe symptoms of appendicitis, suggesting a severity of inflammation and the immune response (Jawabreh, Amro et al. 2024). Moreover, The Pearson correlation analysis between WBC and neutrophil counts indicates an important positive correlation ($r = 0.746$, $P < 0.01$). This significant relationship indicates a coordinated and efficient immunological response and is manifested as increased WBC and neutrophil counts.

In cases of suspected appendicitis, differentiating between parasitic infections, such as those caused by *E. vermicularis*, and acute appendicitis is critical to avoid unnecessary surgical intervention (Dunphy, L., Clark, Z. and Raja, M.H., 2017). A thorough differential diagnosis should incorporate patient history, particularly signs and symptoms related to parasitic infections, along with imaging studies (Abdominal Ultrasound and CT). Additionally, stool analysis, perianal tape tests, and blood tests, including a complete blood count (CBC) and white blood cell (WBC) count, are essential for accurately identifying parasitic infestations. The WBC count differential, which measures the different types of white blood cells, can indicate whether the patient is dealing with a bacterial or parasitic infection. For example, an elevated eosinophil count could suggest a parasitic infection, while an increase in neutrophils may indicate a bacterial infection. Early administration of antiparasitic treatment (e.g., albendazole or mebendazole) should be considered for patients with confirmed *E. vermicularis* infection. This approach ensures targeted medical management and reduces surgical intervention.

All patients reported abdominal pain as the most prominent symptom, which is not surprising since abdominal pain frequently serves as the primary symptom of parasitic appendicitis or other infections involving the gastrointestinal tract (Zouari, Louati et al. 2018). Surprisingly, Diarrhea and fever were uncommon symptoms in this study, probably these symptoms are less common in *E. vermicularis* infection unless there is a secondary bacterial infection or a severe inflammatory response (Braseth, Elliott et al. 2021).

There is no statistically significant correlation between gender and common parasitic symptoms like fever, nausea, vomiting, and diarrhea, The Pearson Chi-Square test ($P = 0.514$) indicates no difference in clinical symptoms presented in both men and women.

The extracted DNA from clinical samples yielded adequate quality and concentration for further molecular investigations such as PCR amplification and sequencing. The DNA concentration across the samples exhibited considerable variation, with values ranging from 7 ng/L to 421.3 ng/μl. This variation is a feature of DNA extraction from paraffin-embedded tissue, due to differences in tissue amounts, fixation durations, and the efficacy of the extraction process. Most of the DNA samples were within the acceptable concentration range of 20 to 400 ng/μl. The amplification of the *COXI* gene by PCR and consequent gel electrophoresis gave clear bands of size at about 380 base pairs (bp). Most samples were positive with clear diagnostic bands at 380 bp. The negative control did

not show any amplification, which validates the PCR reaction by indicating the absence of contamination. However, samples;1, 7, 17, 19, 31, 33, and 34, failed to amplify (Figure 3 A, B). When the negative samples were tried to reamplify, the diagnostic bands at 380 bp showed up, except for only one sample (sample 19), which may suggest low DNA quantity, likely caused by DNA degradation or insufficient extraction, particularly from formalin-preserved tissue. Although this did not significantly affect the overall results, as 54 out of 55 samples yielded sufficient DNA, it highlights the importance of ensuring DNA quality (Figure 3 C). The successful amplification of the *COXI* gene in 54 out of 55 samples confirms the robustness of the PCR method. The successful diagnosis of *E. vermicularis* in the appendectomy samples indicated the high specificity and sensitivity of the molecular methods (Ummarino, Caputo et al. 2022).

Sequence analysis of the Palestinian *COXI* sequences using multiple sequence alignment revealed no sequence variation, indicating that all samples are genetically identical and related. The subsequent phylogenetic analysis of all sequences from Palestine and the GenBank ended up with clear groups. These groups are especially related to specific pathotypes; Types A and B, which have evolved together and can both be found in humans. Notably, none of our sequences clustered with Type C, which is exclusively reported only in chimpanzee populations. This result backs up the idea that Type C is host-restricted (chimpanzees) and that it evolved separately from Groups A and B. It also backs up earlier studies that found similar host specificity (Nakano et al., 2006). This further underscores the fact that chimpanzees possess this specific trait. The fact that there is no evidence of crossover to humans indicates a significant evolutionary gap between the human and chimpanzee populations (Figure 4).

Pathotypes A, B, and C are shown in Figure 4, all Palestinian samples grouped among pathotype B, which is a human-related group indicating a clear genetic separation from primates-only lineages and in agreement with previous Iranian study (Shafiei, Reza, et al. 2023). Group B is the predominant group, which includes sequences from Denmark and Greece, indicating that group B is not geographically restricted and has a broad geographical distribution across different countries. In Denmark, both Type A and Type B were identified, suggesting potential co-circulation or mixed infection patterns. In Greece, a dominant presence of Type B may indicate regional variations or environmental influences (Nakano, Okamoto et al. 2006, Traversa, Kuzmina et al. 2008). Group B has successfully evolved traits that support *E. vermicularis* survival and persistence in human

populations, possibly due to genetic compatibility or immune factors unique to humans. In contrast, group C appears exclusive to chimpanzees, with no evidence of its presence in humans, yet this clade's grouping of *E. vermicularis* sequences from Japan and China suggests that these populations may have a unique evolutionary history, known only from chimpanzee isolates and not from humans, supporting the idea of host-specific adaptation.

The median-joining analysis of all datasets revealed 15 haplotypes indicating different levels of genetic variation, unique haplotypes in this analysis. The study of the 333-bp *COXI* gene identified 15 unique haplotypes defined by 28 polymorphic sites (Table 6). The topology of the median-joining network is in agreement with the phylogenetic tree indicating the reliability of the results obtained in this study. This study may indicate that some human populations, especially those in the Middle East, have unique genetic variants. This is due to localized evolutionary pressures or demographic history (Piperaki, Spanakos et al. 2011, Ferrero, Röser et al. 2013, Tavan, Mikaeili et al. 2020). Group A, which is made up of haplotypes H2, H3, H8, H9, H10, H11, and H12, has a more compact structure, with fewer nucleotide substitutions and shorter branches between haplotypes. This suggests that these haplotypes share a recent common ancestor. This group contains samples from diverse geographic regions, including Denmark, Japan, and Greece, suggesting gene flow or close evolutionary connections between *E. vermicularis* populations in these areas (Piperaki, Spanakos et al. 2011). Group A likely represents an ancestral lineage of *E. vermicularis* adapted to humans, which may spread across different regions over time. Group C, which includes haplotypes H14, H15, H6, and H7, distinguishes itself from Groups A and B and is exclusive to chimpanzee samples from Japan and China. The long branches in this group show that there is a huge genetic variation. This is plausible because chimpanzees are host-specific species that have evolved differently, which suggests that *E. vermicularis* has evolved in a way that is separate from the human-associated lineages. The discovery of Group C exclusively in chimpanzees reinforces the notion that distinct *E. vermicularis* lineages have simultaneously evolved with various hosts, resulting in their increasing divergence over time.

The grouping of the Palestinian samples in group B is expected to be closely related to other human samples from the Middle East, further research is recommended using other human samples from different ages and various populations in Palestine.

While the findings of this study provide valuable insights, there are several limitations that should be considered when interpreting the results.

First, the study was conducted at three hospitals in the West Bank—Queen Alia Hospital, Rafidia Hospital, and Beit Jala Hospital. This limited geographic scope may affect the generalizability of the findings to other regions of Palestine. Different geographic areas may have varying epidemiological characteristics, and expanding the study to include hospitals from other parts of Palestine could enhance the representativeness of the results.

Second, the exclusion criteria in this study were not explicitly defined for certain patient demographics or medical conditions, other than malignancy. Factors such as age, gender, and comorbidities (e.g., chronic diseases or immunocompromised states) may influence the study's outcomes, and their absence as exclusion criteria could introduce potential biases. Future studies should define these exclusion criteria more clearly to ensure a more homogeneous sample, which would help in minimizing confounding variables.

Lastly, the sample size of 55 cases, while adequate for initial findings, may limit the statistical power of the study, particularly when analyzing subgroups or rare associations. A larger sample size would improve the robustness and precision of the results, and future research could consider increasing the sample size to enhance the reliability and generalizability of the findings.

Conclusion

This molecular study using haplotype network and phylogenetic analysis confirmed three evolutionary paths within *E. vermicularis*: one chimpanzee-adapted lineage (Group C) and two human-adapted lineages (Groups A and B). Samples from Palestine primarily comprise Group B. The genetic structure within Groups A and B suggests recent divergence and potential gene flow among human populations, while Group C reflects long-term adaptation in chimpanzees. These findings support the role of host-specific evolutionary pressures in shaping the genetic structure of *E. vermicularis*. Further research with expanded sampling could deepen our understanding of the evolutionary dynamics and host-parasite relationships of *E. vermicularis* across various host populations. The immunological responses of humans and chimpanzees are distinct, and *E. vermicularis* presumably developed particular adaptations to evade the immune defenses of each host. In conclusion, hospitals and internists should utilize a comprehensive diagnostic approach for suspected appendicitis, including imaging studies, stool analysis, perianal tape tests, and blood tests such as CBC with WBC count differential, CRP, and other immune markers. This approach helps differentiate between parasitic infections like *E. vermicularis* and acute appendicitis, emphasizing that physicians should not rely solely on clinical symptoms, as they may overlap between conditions.

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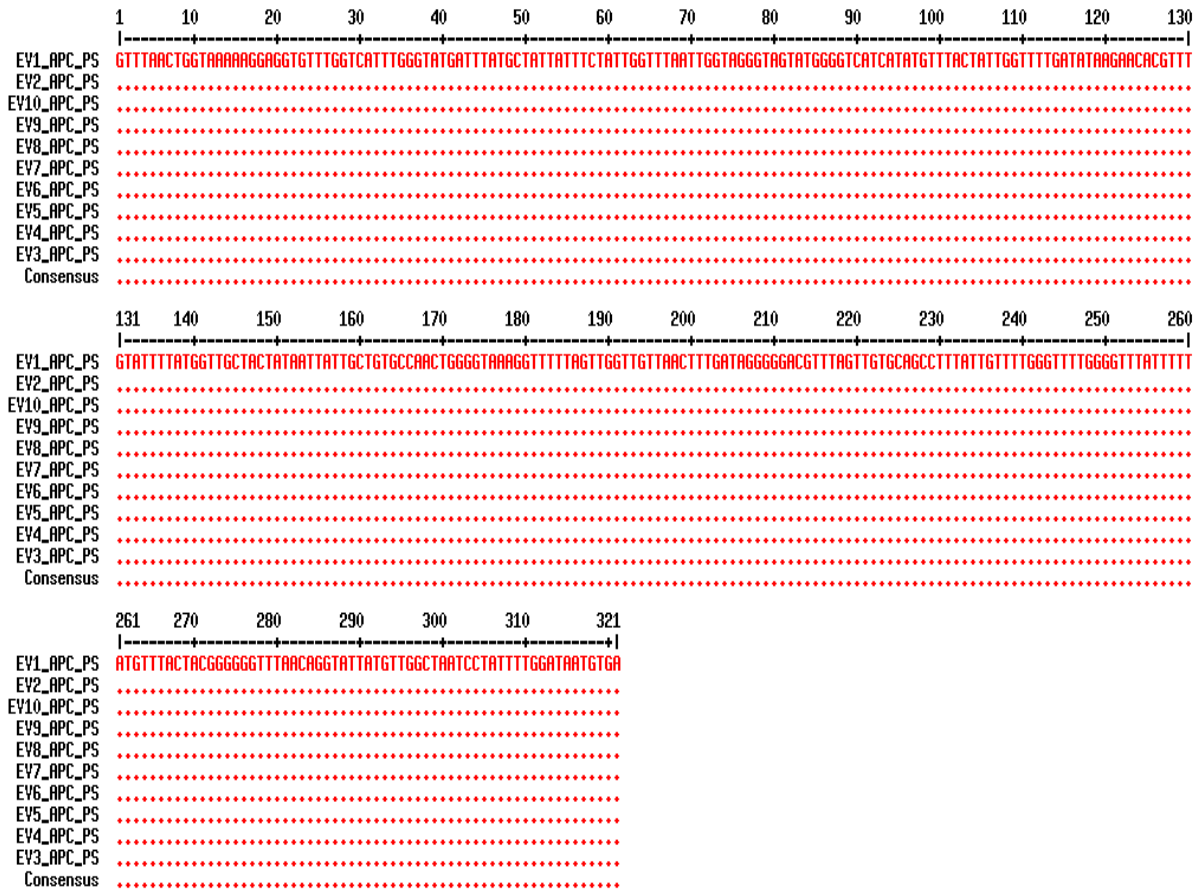
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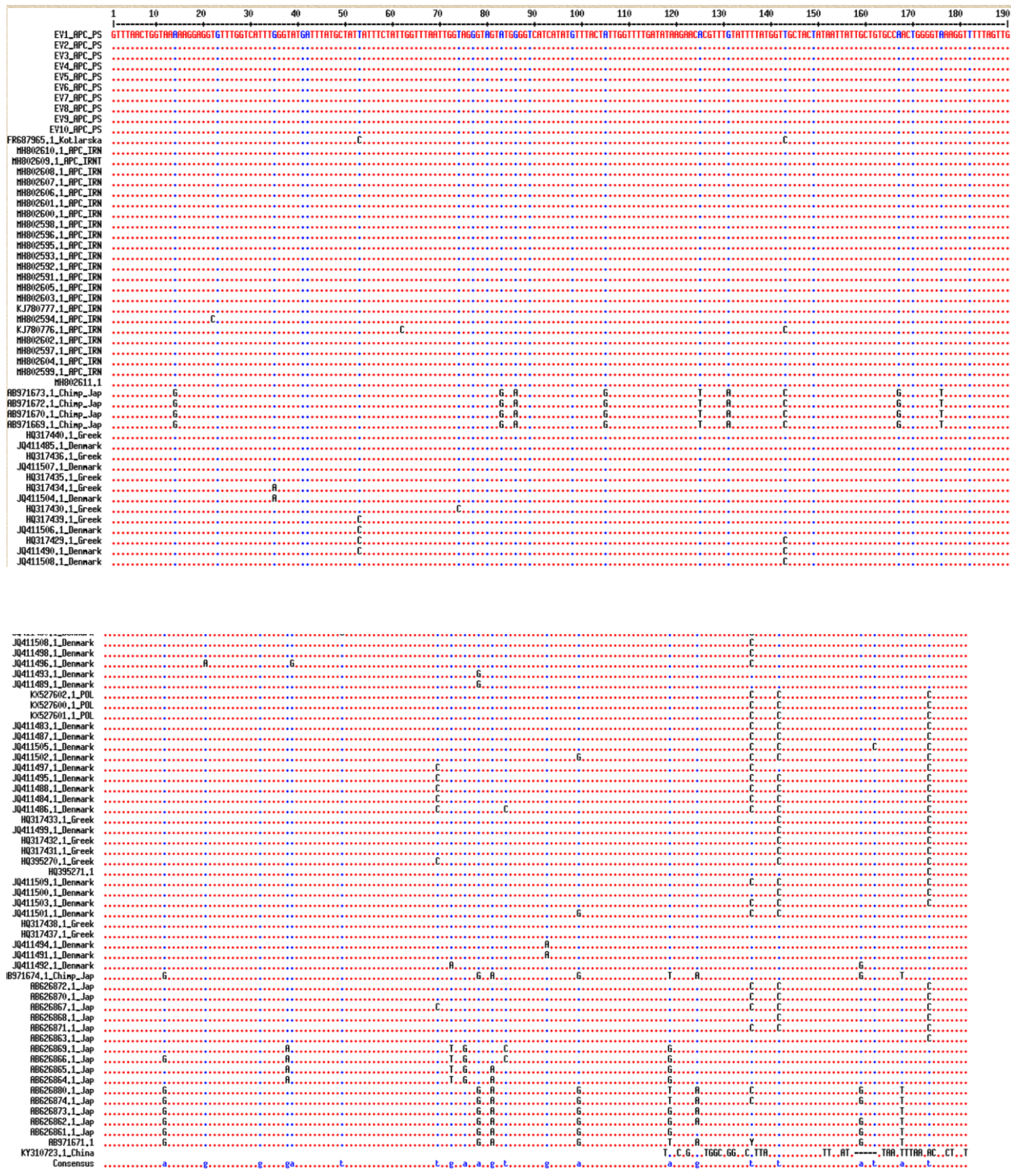
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Appendixes

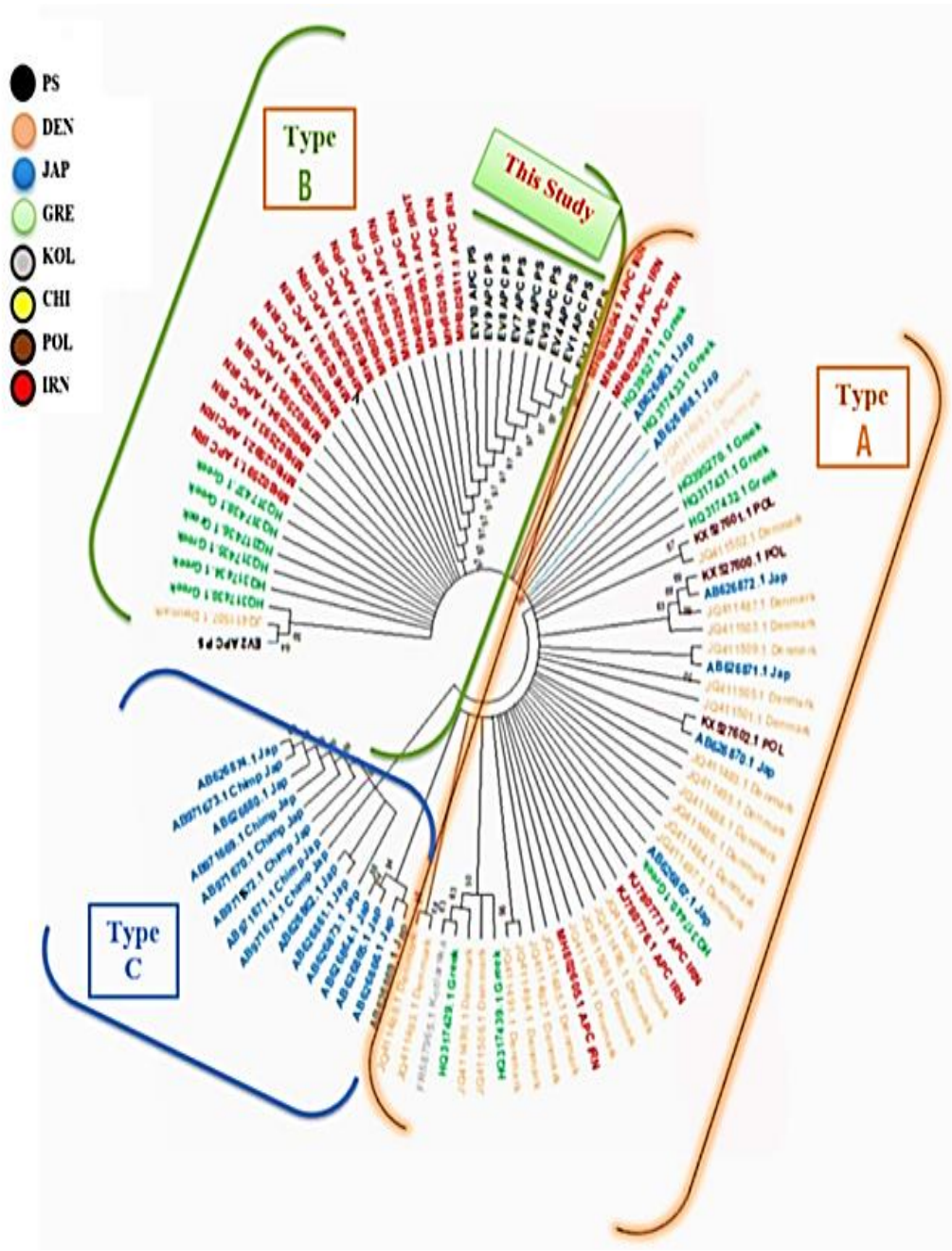
1.1 Appendix: the alignment result of our *COXI* sequences of *E. vermicularis* using the Multi Alin program, PS – Palestine.



1.2 Appendix: the alignment result of *COX1* sequences of *E. vermicularis* from Palestine and other sequences from the GenBank using the Multi Alin software.



1.3 phylogenetic tree



1.4 Appendix: The article that has been published in this study

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CASE REPORT **Open Access**

Enterobius vermicularis (pinworm) infestation mimicking acute appendicitis in two children from Palestine: a case report

Issam Jawabreh¹, Ahmad Amro², Kifaya Azmi^{1,4}, Hamza Batran³, Ziad Abdeen⁴ and Omar Hamarshah^{5*}

Abstract

Background Pinworm, *Enterobius vermicularis*, is a common parasitic illness, particularly among children in Palestine. On rare occasions, it can cause serious consequences such as acute appendicitis, which can still present a challenging diagnosis especially in children.

Case presentation This report describes two cases (a 9 year old male and a 11 year old female, both Palestinian children from Hebron district) of acute appendicitis referred to Queen Alia Hebron Governmental Hospital in Palestine. The clinical examination revealed a lower abdominal pain, and they were diagnosed with appendicitis. The pathological examination after appendectomy showed the presence of eggs and pinworms. Anthelmintic medication was provided, and they were followed up for 6 weeks after the operation without any incidents.

Conclusion These cases highlight the importance of considering *Enterobius vermicularis* infestation in children with abdominal pain, as the parasites can mimic appendicitis. Prompt recognition and cautious laparoscopic appendectomy are crucial to prevent unnecessary surgery and peritoneal contamination, with the infestation being easily treatable with anthelmintic medication.

Keywords *Enterobius vermicularis*, Appendicitis, Pinworm, Palestine

Introduction

Across the world, suspected acute appendicitis is one of the most common emergency abdominal procedures. This condition, often presents as abdominal pain, results from an infection and obstruction of the appendix. Abdominal imaging, laboratory testing, physical examination, and medical history of the patient are all important for diagnosis. A low-grade temperature, nausea, sporadic vomiting, loss of appetite, migrating discomfort to the right lower abdomen, and nonspecific periumbilical pain are examples of classic symptoms. In almost 90% of instances, these symptoms result in the diagnosis of acute appendicitis [1]. A laparoscopic appendectomy is the most typical course of therapy. Appendicitis can be caused by many different factors; in rare instances, it can be brought on by intestinal parasites, low-grade appendicular mucinous neoplasms, neuroendocrine tumors, and serrated adenomas.

Pinworms, *Enterobius vermicularis*, are the most common human parasite, accounting for 40% of all helminth infections reported in the USA. From 2008 to 2018, the Palestinian Ministry of Health recorded 29,390 cases of *E. vermicularis* infection, with 1.1% (329 cases) in the Gaza Strip and 98.9% (29,061 cases) in the West Bank.

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Recent studies demonstrate that the West Bank and, to a lesser extent, the Gaza Strip have high rates of *E. vermicularis* infection [2, 3]. Pinworm infection is a self-limiting disease, because adult worms have a very short life expectancy unless they autoinfect the patient, and the most common symptom of this infection is pruritus, which usually rises at night while the infected person is asleep [4].

Pinworms can migrate to the vaginal region and cause discomfort or inflammation, a condition known as vulvovaginitis, which is a rare scenario. *Escherichia coli* and other bacteria may stick to this inflamed region and cause an infection of the urinary system [5]. Additionally, pinworm infestation has been connected to acute appendicitis, and it is the most common helminthic infection in the appendiceal lumen.

Pinworms were initially found in the appendix by Fabrius in 1634, but a clear causal connection has not yet been shown. For many years, there has been discussion on *E. vermicularis* potential role in the development of acute appendicitis [6].

Case presentation

Case-1

A 9-year-old Palestinian Arab boy, accompanied by his parents, was referred by his general practitioner to the emergency department of Hebron Governmental Hospital (Queen Alia Hospital, Hebron, Palestine). He presented with 10 hour history of predominant abdominal pain radiating from his umbilicus to the right iliac fossa (RIF). The medical history of the patient obtained from the family. The patient had no history of hospital admission and no previous medical surgery or known drug allergies. On arrival at the hospital, the patient

presented with no vomiting, fever, diarrhea, or seizures. Upon examination, the patient's vital signs were within normal limits, alert conscious, mildly dehydrated, with no cyanosis or jaundice.

The radiating pain in the lower abdomen mainly in the RIF area was progressive. These manifestations raises suspicions of acute appendicitis. Biochemical laboratory analysis showed an increased C-reactive protein (23 mg/L, $N=0-6$ mg/L) and a normal liver function test. Hematological investigations revealed a normal eosinophil ($0.09 \times 10^9/L$, $N=0.02-0.62 \times 10^9/L$), mild leukocytosis ($15.7 \times 10^9/L$, $N=4.5-10.6 \times 10^9/L$) and neutrophils (89%, $N=50-70\%$). Microscopic examination of urine was normal.

Ultrasonography showed an enlarged appendix, 6–7 mm in diameter, without echogenic fat plans and no free fluids. After diagnosis confirmation of appendicitis, the patient was kept nothing by mouth (NPO) for laparoscopic appendectomy operation and was given intravenously (IV) normal saline and Perfalgan® (10 mg/mL paracetamol, IV). Surgical intervention and histopathological examination revealed acute suppurative appendicitis, was negative for malignancy, and the presence of *E. Vermicularis* parasite was found within the lumen of the appendix (Fig. 1A). One day postoperation, the patient looked well, with soft abdomen, passed urine, passed gases, and wound dry. After 2 days, the patient was discharged from the hospital on Augmentin (amoxicillin/clavulanate) 400 mg suspension (dose 5cc 1 × 3), Flagyl (metronidazole) 300 mg (dose 5cc 1 × 2), and paracetamol (dose 5cc 1 × 3). The patient gradually improved after 1 week and was fully recovered after 6 weeks.

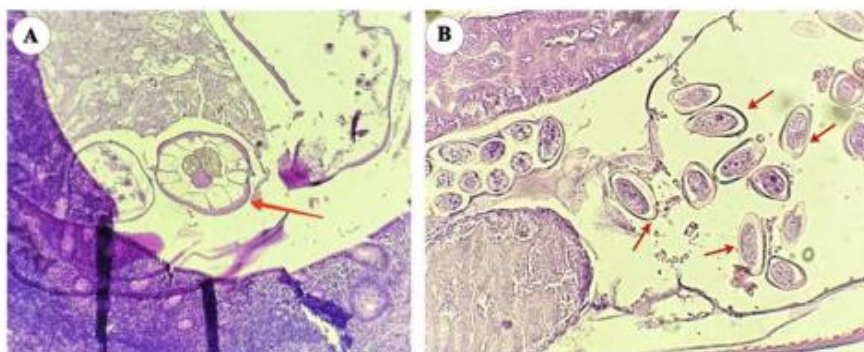


Fig. 1 Histopathological sections stained with hematoxylin–eosin stain showing *Enterobius vermicularis* pinworm in the lumen of the patients appendix. **A** Cross-section of the appendix containing the pinworm (red arrow), in 400x magnification. **B** Longitudinal section demonstrate the presence of a gravid female pinworm with eggs (red arrows) in 400x magnification

Case-2

An 11-year-old Palestinian Arab female, accompanied by her parents, was admitted to the emergency department of Hebron Governmental Hospital (Queen Alia Hospital, Hebron, Palestine). The patient complained of gradual abdominal pain, 12 hours previously with dull aching, not radiated, and associated with nausea but no vomiting and feverish sensation. The medical history obtained from the parents. The patient had no surgical history nor known drug allergies. Upon clinical examination, the abdomen showed no scar, intact hernial orifices, soft lax, localized RIF pain, and suprapubic tenderness with positive rebound and negative rovsing sign.

These symptoms raised suspicions of acute appendicitis, and ultrasonography of abdomen and pelvis revealed normal abdominal organs, minimal-to-mild pelvic free fluids, and thickened and dilated tip of the appendix.

Biochemical analysis showed negative C-reactive protein (2 mg/L, $N=0-6$ mg/L) and normal liver function test. Hematological tests revealed normal eosinophils ($0.07 \times 10^9/L$, $N=0.02-0.62 \times 10^9/L$), mild leukocytosis ($12.8 \times 10^9/L$, $N=4.5-10.6 \times 10^9/L$), and normal neutrophils (62%, $N=50-70\%$). Urine test showed hematuria. On ultrasonography, the appendix could not be visualized, with no free fluids in the surroundings. Based on the clinical examination, diagnosis with acute appendicitis was confirmed, and the patient was kept NPO and administered intravenously 1500 cc pediatric saline over 24 hours. For laparoscopic appendectomy, the following prophylactic medications were given to the patient: Zinnat (cefuroxime axetil) 500 mg (dose 5cc 1 \times 3), Flagyl (metronidazole) 300 mg (dose 5cc 1 \times 3), and Perfalgan (paracetamol IV infusion) 10 mg/mL (dose 1 \times 3). Surgical intervention and histopathological examination revealed peri appendicitis due to *E. vermicularis* parasite (Fig. 1B). After 1 day, the patient looks well with soft abdomen, dry wound, and normal urination, and she passed gases. The patient discharged from the hospital and prescribed Augmentin 600 mg syrup (dose 7cc \times 2) and paracetamol (dose 5cc 1 \times 3). The weekly follow-up of the patient showed marked improvements, and full recovery was achieved after 6 weeks.

Discussion

Acute appendicitis can be caused by *E. vermicularis*, which is a small white nematode infects around one billion people worldwide [7]. Pinworm infestation is the most prevalent helminthic infection in children, affecting up to 50% and 20% of adults [8]. The most common symptom of pinworm infection is nighttime perianal itching, caused by the female pinworm laying eggs.

Since 1898, various reports have linked pinworm to appendix inflammation. In 1919, ileocolic inflammation caused by the parasite was described. Subsequent studies noted cases of pinworm in the appendix, suggesting it can mimic appendicitis [9, 10]. Although rare, with only 1.5% of appendicitis cases involving parasites in developed countries, it is often found incidentally in children aged 7–11 years [6]. Parasitic infection can block the appendix's lumen, leading to inflammation and appendicitis. When pinworms enter the appendix, probably due to high worm burden, they disrupt mucus flow and bacterial balance, causing increased pressure and bacterial growth. This triggers an immune response, resulting in inflammation and classic appendicitis symptoms: fever, elevated white blood cell counts, and lower right abdominal pain and tenderness [11]. Although the association between *E. vermicularis* and appendicitis is debatable and unclear, the worm or its eggs can obstruct the appendiceal lumen leading to acute appendicitis [12].

The diagnosis of *E. vermicularis*-caused appendicitis is frequently made after surgery. Pinworms within the appendix may be seen by the surgeon during an appendectomy. Pathologists can confirm the presence of pinworm eggs or adults through histological examination and treatment involves both. A laparoscopic appendectomy is the main surgical intervention to remove the inflamed appendix and prevent complications such as perforation or abscesses. Antiparasitic drugs such as Flagyl (metronidazole) must be used to treat the underlying pinworm infection after surgery to prevent recurrence [13, 14].

The patient's family and close contacts may also need treatment to control the spread of *E. vermicularis* and prevent infection to the family members. They should follow strict hygiene practices to prevent reinfection. Additionally, students should avoid crowded areas and maintain good hygiene, such as washing hands with soap regularly [15]. It is important to mention that medical doctors evaluate outcomes of clinical and laboratory tests allowing them to rule out other causes of abdominal pain and confirm the diagnosis of appendicitis due to *E. vermicularis*, especially when the presence of pinworms in the appendix is confirmed through histopathological examination.

Conclusion

E. vermicularis infection can cause appendiceal pain without histological inflammation. The two mentioned cases highlight the need to consider *E. vermicularis* infestation in children presenting with right iliac fossa pain, as its clinical signs can mimic acute appendicitis. Precise evaluation of symptoms such as pruritus and eosinophilia in laboratory tests, particularly in children, can prevent

unnecessary appendectomies. This comprehensive approach reduces the risk of complications and improves overall health by effectively treating both acute appendicitis and the parasitic infection.

The learning lessons from these two reports is to consider *E. vermicularis* infestation in the differential diagnosis of right iliac fossa pain in children presenting in emergency rooms. Perianal itching in these cases, primarily nocturnal, usually results from female pinworm migration to the anus for egg laying. Moreover, exercise caution during appendectomy if *E. vermicularis* is suspected, as there is a potential risk of peritoneal contamination.

Abbreviations

NPO	Nothing by mouth
IV	Intravenous
RIF	Right iliac fossa

Acknowledgements

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Author contributions

IA collected samples and drafted the manuscript, AA finalized and improved the manuscript, KS and ZA corrected the final draft, and HB performed the pathological examination. OH revised the manuscript critically and supervised the work. All authors read and approved the final copy of the manuscript.

Funding

Not applicable.

Availability of data and materials

The datasets used during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

Ethical approval was obtained from Al-Quds University Ethical Committee (reference no: 381/REC/2024).

Consent for publication

Written informed consent was obtained from the patient's legal guardian for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Competing interests

The authors declare that they have no competing interests.

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1.5 Appendix: Ethical committee Decision letter

Al-Quds University
Jerusalem
Deanship of Scientific Research

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



جامعة القدس
القدس
عمادة البحث العلمي

**Research Ethics Committee
Committee's Decision Letter**

Date: March 16, 2024
Ref No: 381/REC/2024

Dears Dr. Omar Hamarsheh, Mr. Issam Jawabreh,

Thank you for submitting your application seeking approval for research ethics. After a thorough examination of your submission titled: "Molecular and phylogenetic analysis of *Enterobius vermicularis* in appendectomy specimens from West Bank -Palestine", the Research Ethics Committee (REC) at Al-Quds University is pleased to confirm that your application is in accordance with our research ethics guidelines. data collection procedure or modifications to any aspect of the research protocol. Please be aware that while this approval authorizes your research, however, please keep in mind that this approval does not substitute for any departmental or other approvals that may be necessary, including but not limited to sample shipment, data sharing permissions or administrative approval to distribute questionnaires.

In addition, we kindly request that you provide us with a copy of your final research report or publication once it becomes available.

Thank you once again for your commitment to conducting ethical research, and we extend our best wishes for a productive research endeavor that serves the best interests of your research subjects.

PS: Please note that this ethical approval letter will remain valid for a period of two years from the date of issuance. Should your research extend beyond this timeframe, a request for renewal will be necessary.

This ethical approval will remain valid as long as there are no alterations to the

Sincerely,

Suheir Ereqat, PhD
Associate Professor of Molecular Biology

Research Ethics Committee Chair

Cc. Prof. Imad Abu Kishek - President
Cc. Members of the committee
Cc. file



Ref.:
Date:.....

الرقم: ١٠٢٢/١٦٤
التاريخ: ٢٠٢٤/١٥/١٩

عطوفة الوكيل المساعد لشؤون المستشفيات والطوارئ المحترم،،،
الاخت ق. أ. مدير عام الادارة العامة لتكنولوجيا المعلومات المحترم،،،
تحية واحترام،،،

الموضوع: تسهيل مهمة بحث

يرجى تسهيل مهمة الطالب: عصام جوايره - ماجستير كيمياء حيوية واحياء جزيئية/
جامعة القدس، وبإشراف د. د. عمر حمارشه، في عمل بحث بعنوان:

" Trauma in Palestine: A Multicenter Retrospective Cohort"

من خلال السماح للطالب بالحصول على معلومات من خلال الاطلاع على ملفات المرضى،
والحصول على جزء من النسيج من المختبرات من العينات التي تم فحصها (بعد موافقة رئيس
قسم الباثولوجي). وذلك في:

- مستشفى بيت جالا - مستشفى رفيديا - مستشفى عاليه

على ان يتم الالتزام باساليب واخلاقيات البحث العلمي، ودون التعرض للمعلومات الشخصية
للمشاركين.

على ان يتم تزويد الوزارة بنسخة PDF من نتائج البحث، التعهد بعدم النشر لحين الحصول على
موافقة الوزارة على نتائج البحث.

مع الاحترام،،،

د. عبد الله القواسمي
رئيس وحدة التعليم الصحي والبحث العلمي

نسخة : عميد كلية الطب المحترم / جامعة القدس

المخلص

التحليل الجزيئي والتطوري لدودة الشعريه في عينات استئصال الزائدة الدودية في الضفة الغربية فلسطين.

غالبًا ما تسبب الديدان الخيطية الطفيلية المعروفة باسم (دودة الدبوس البشرية) أعراضًا معوية، وفي بعض الحالات، التهاب الزائدة الدودية الحاد. وعلى الرغم من انتشارها على نطاق واسع في فلسطين، إلا أن البيانات الجزيئية المتعلقة في الديدان الخيطية المسببه لالتهاب الزائدة الدودية غير متوفرة. لذلك، تهدف هذه الدراسة إلى دراسة الخصائص الجزيئية والتطورية للديدان الخيطية في عينات طبية على شكل خزعات ناتجة من استئصال الزائدة الدودية محفوظة في مكعبات الشمع من مستشفيات الضفة الغربية، فلسطين. ستركز الدراسة على جين وحدة أوكسيداز السيتوكروم سي الأولى (COX1) لفهم التنوع الجيني وأنماط الانتقال.

امتدت جمع العينات ما بين 2018 إلى 2023، تم جمع 55 عينة زائدة دودية مثبتة بالفورمالين ومغموسة بالبارافين تم تأكيد إصابتها بالديدان الخيطية من ثلاثة مستشفيات حكومية: مستشفى الملكة علياء في الخليل، ومستشفى رفديا في نابلس، ومستشفى بيت جالا في بيت لحم. أجرينا استخراج الحمض النووي على مقاطع الأنسجة، تلا ذلك تفاعل البوليميراز المتسلسل الذي يستهدف جين COX1. تم تحليل النيوكليوتيدات لتسلسل COX1 وبناء الشجرة التطورية وكذلك تم تحليل شبكة الانماط الوراثية التي تبين الاختلافات النيوكليوتيدية

كانت من خلال التحليل الإحصائي للعينات التي تم جمعها كانت نسبة الذكور إلى الإناث 1:1.2، حيث كان 54.5% من المرضى من الذكور و45.5% من الإناث و متوسط عمر المرضى 13.3 عامًا. تحليل التسلسل النيوكليوتيدي لم يفرضي إلى وجود اختلافات نيوكليوتيدية بين العينات، مما يشير إلى الحفاظ على البنية الجينية لمسيب المرض. صنف التحليل التطوري و تحليل شبكة الانماط الوراثية لجميع العينات التي تم عزلها وحفظها على أنها من الدودة الشعريه نوع B، المرتبط حصريًا بالمضيفين من البشر وكذلك. كما أظهر التحليل الإحصائي للمتغيرات الديموغرافية والسريية، بما في ذلك العمر والجنس وخلايا الدم البيضاء وعدد العدلات، عدم وجود ارتباطات مهمة بالعدوى..

تكشف الدراسة لأول مره عن تجانس وراثي قوي بين العينات المعزولة والمحفوظه للدودة الشعريه في فلسطين، مما يشير إلى ديناميكيات انتقال محدودة. نتائج التحليل النيوكليوتيدي وتحليل الأنماط

الوراثية تتماشى مع الأنماط الوراثية في دول أخرى. لم يتم العثور على ارتباطات مهمة بين العوامل الديموغرافية أو السريية والعدوى، مما يشير إلى أنماط عدوى مستقرة عبر السكان. تؤسس هذه النتائج خط أساس جزيئي لـ الدودة الشعريه في فلسطين، مما يدعم جهود الصحة العامة ويوجه البحوث المستقبلية حول انتقال العدوى وعلم الأوبئة.