COMPARATIVE EFFICACY OF DIFFERENT PRESERVATIVES FOR INTESTINAL PARASITES PRESERVATION

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE, SCHOOL OF BASIC MEDICAL SCIENCES, UNIVERSITY OF BENIN, BENIN, EDO STATE

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SUPERVISED BY:

DR. (MRS) Z. OMORUYI

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CERTIFICATION

This is to certify that the project titled "Comparative efficacy of different preservatives for intestinal parasite preservation" was carried out by AKANDE GODWIN OBALOLUWA, with matriculation number BMS2001147, under the supervision of DR. (MRS) Z.OMORUYI in partial fulfillment of the requirements for the award of Bachelor in Medical Laboratory Science. (B.MLS) Degree

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DEDICATION

This project is dedicated to Almighty God who is the source of all knowledge and wisdom and to my lovely parents and siblings for their unwavering love and support

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I wish to express my heartfelt gratitude to all those who contributed to the successful completion of this research project, titled *Comparative Efficacy of Different Preservatives for Intestinal Parasite Preservation*.

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ABSTRACT

Intestinal parasitic infections are a major public health concern in tropical regions, where accurate diagnosis depends on proper preservation of stool specimens. Fresh samples degrade quickly, making effective preservatives essential for maintaining parasite morphology.This study aimed to evaluate and compare the efficacy of three preservatives 10% formalin, sodium acetate–acetic acid–formalin (SAF), and low-viscosity polyvinyl alcohol (LV-PVA) in preserving intestinal parasites. A total of 20 stool samples were collected and examined fresh to confirm the presence of parasites. Identified organisms included Trichostrongylus eggs, Coccidia, Paramphistomum cervi, Trichuris trichiura, and Strongyloides larvae. Each sample was then divided into three aliquots and preserved separately in the three fixatives. Preserved specimens were re-examined microscopically to assess morphological clarity and diagnostic suitability over time.Results showed that 10% formalin gave the most consistent preservation of helminth eggs and larvae. SAF also maintained good morphological integrity across parasite types and was nearly as effective as formalin, with the added advantage of being mercury-free and safer to handle. LV-PVA was less reliable for helminths but provided better detail for protozoan structures, though further research is needed to optimize LV-PVA for consistent use.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the study

Intestinal parasitic infections, caused by protozoa and helminths, remain a significant global health burden, particularly in tropical and resource-limited regions, where they contribute to morbidity and mortality (World Health Organization, 2020). Accurate diagnosis through stool examination is important for identifying pathogens such as *Entamoeba histolytica, Giardia intestinalis,* and *Ascaris lumbricoides,* enabling timely treatment and disease control (Utzinger *et al*., 2020). Stool preservation is a cornerstone of parasitological diagnostics, as fresh samples degrade rapidly, distortingg parasite morphology and detection (Centers for Disease Control and Prevention, 2019). Challenges in stool preservation include maintaining structural integrity, ensuring compatibility with diagnostic techniques, and addressing logistical constraints in field settings, which underscore the need for effective preservatives (Papaiakovou *et al*., 2021).

Three widely used stool preservatives 10% formalin, sodium acetate-acetic acid-formalin (SAF), and low viscosity polyvinyl alcohol (LV-PVA), have been used for decades in parasitological diagnostics. Formalin (10%) is valued for its ability to preserve helminth eggs, larvae, and protozoan cysts, making it suitable for concentration techniques like formalin-ether and UV fluorescence microscopy (Agarwal *et al*., 2025). SAF combines sodium acetate, acetic acid, and formalin to support both concentration and permanent staining, such as iron hematoxylin, and is compatible with immunoassay kits (Utzinger *et al*., 2020). LV-PVA, containing mercuric chloride, excels in preserving protozoan trophozoites and cysts for trichrome staining but is less effective for concentration methods (Maneesha *et al*., 2019). Historically, these preservatives have been adopted based on their accessibility and

effectiveness, yet each has limitations, such as formalin's reduced staining compatibility, SAF's need for additives, and LV-PVA's environmental concerns due to mercury (Centers for Disease Control and Prevention, 2019).

Comparative studies of 10% formalin, SAF, and LV-PVA are essential to optimize diagnostic accuracy and inform laboratory protocols. Preservative choice directly impacts morphological integrity, diagnostic sensitivity, and compatibility with techniques like microscopy and antigen detection (Galván-Ramírez *et al*., 2020). For instance, SAF's versatility in supporting both concentration and staining makes it valuable in clinical settings, while 10% formalin's robustness suits field-based diagnostics (Agarwal *et al*., 2025). Comparative evaluations are particularly relevant in diverse contexts, such as resource-limited laboratories where cost and accessibility are critical, or field studies requiring robust preservation under variable conditions (Papaiakovou *et al*., 2021). Understanding the strengths and weaknesses of each preservative can guide improvements in diagnostic workflows and enhance global health outcomes.

Despite advances, the literature reveals significant challenges in stool preservation. Inconsistent preservation outcomes are reported, with 10% formalin outperforming SAF and LV-PVA in morphological integrity for helminths but varying in protozoan detection (Maneesha *et al*., 2019). Standardization of protocols remains a barrier, as variations in preservative concentrations and processing techniques lead to differing detection rates (Utzinger *et al*., 2020). Long-term storage data is scarce, with limited studies assessing preservative efficacy beyond a few months, particularly for LV-PVA (Centers for Disease Control and Prevention, 2019). Additionally, the use of mercury in LV-PVA raises environmental and safety concerns, prompting exploration of alternatives like ethanol or DESS, though these are understudied in comparison to traditional preservatives (Hass *et al*., 2024; Papaiakovou *et al*., 2021). The scarcity of studies directly comparing all three

preservatives under identical conditions further complicates evidence-based decision-making (Maneesha *et al.,* 2019).

This study will address these gaps by systematically comparing 10% formalin, SAF, and LV-PVA in terms of morphological integrity, diagnostic sensitivity and specificity, compatibility with staining and concentration methods, and long-term preservation efficacy. By analyzing these preservatives under standardized conditions, the research seeks to provide proof to enhance parasitological diagnostics, particularly in varied clinical and field settings. The study also explores the feasibility of safer, mercury-free alternatives to address environmental concerns associated with LV-PVA. The findings are expected to contribute to improved laboratory practices, enhance diagnostic accuracy, and inform public health strategies for managing intestinal parasitic infections globally

1.2 Statement of the Problem

The integrity of stool specimens especially the morphology of protozoan trophozoites and cysts, as well as helminth ova must be preserved from the time of collection until assessment. However, in many tropical and subtropical regions, slowdown in sample processing due to limited laboratory access, poor transport conditions, or understaffing often result in degradation of diagnostic forms and incorrect reporting (Garcia, 2021)

Although a variety of chemical preservatives are available for stool fixation such as formalin, polyvinyl alcohol (PVA), and sodium acetate-acetic acid-formalin (SAF) each has its limitations. Formalin, though inexpensive and widely available, may distort delicate protozoan structures and poses safety hazards due to its carcinogenic properties (Hass *et al.*,

2024). PVA, while superior in preserving nuclear detail in trophozoites, traditionally contains toxic mercuric chloride and is incompatible with concentration techniques (Jensen *et al.,* 2000). SAF offers a mercury-free alternative but may result in suboptimal staining of protozoa, especially under trichrome stain (Vandenberg *et al*., 2020).

Furthermore, existing literature has largely focused on comparing 10% formalin and mercury-based PVA, with limited investigation into the performance of 5% formalin, low-viscosity mercury-free PVA, and SAF under standardized, long-term evaluation protocols (Maneesha *et al.,* 2019; De Oliveira *et al.,* 2021). There is also a lack of studies that comprehensively assess fixative performance across both protozoa and helminths, using consistent scoring systems and multiple time points (SACSH, 2022)

The absence of up-to-date, head-to-head comparisons of widely used preservatives under practical laboratory conditions represents a critical gap in parasitological research. As such, there is a compelling need for a study that evaluates and compares the morphological preservation efficacy of 5% formalin, 10% formalin, low-viscosity PVA, and SAF over time, using wet mount techniques. Addressing this gap will contribute to improved diagnostic accuracy, better surveillance data, and ultimately more effective control of parasitic diseases in endemic regions.

1.3 Significance of the Study

The success of intestinal parasite diagnosis depends significantly on the preservation of stool specimens before microscopic analysis. In low-resource settings, delays in processing often result in the degeneration of protozoan trophozoites and distortion of helminth eggs, compromising diagnostic accuracy (Garcia, 2021). While preservatives such as 10% formalin,

low-viscosity polyvinyl alcohol (PVA), and sodium acetate-acetic acid-formalin (SAF) are widely used, their comparative efficacy under consistent laboratory conditions remains underexplored. Studies show that formalin offers superior preservation for wet mounts, but may pose health hazards, while mercury-free alternatives like SAF and PVA require further validation (Maneesha *et al.,* 2019*)*

This study addresses both diagnostic and safety concerns by evaluating 5% and 10% formalin, SAF, and mercury-free PVA for their ability to preserve parasite morphology over time. It contributes to laboratory safety by assessing alternatives to toxic substances like formaldehyde and mercuric chloride (Hass *et al*., 2024). By generating standardized, comparative data across multiple parasites and staining methods, this research supports evidence-based diagnostic practices, strengthens parasitology training, and promotes safer laboratory protocols (SACSH, 2022).

1.4 Justification of the Study

Intestinal parasitic infections remain a significant public health concern, particularly in low- and middle-income countries where diagnostic infrastructure is often inadequate. The success of stool-based diagnosis relies heavily on proper specimen preservation, as parasite morphology can degrade rapidly without effective fixation, leading to missed or inaccurate diagnoses (Garcia, 2021). In many laboratories, especially in sub-Saharan Africa, the selection of fixatives is driven more by availability than by validated efficacy, resulting in inconsistencies in diagnostic outcomes. This undermines surveillance efforts and delays treatment, particularly in high-burden areas where timely and reliable diagnosis is essential (WHO, 2022).

Despite the routine use of 10% formalin, low-viscosity polyvinyl alcohol (PVA), and sodium acetate-acetic acid-formalin (SAF), few comparative studies have been conducted under standardized conditions. Mercury-free PVA and lower concentrations of formalin, such as 5%, have been introduced as safer alternatives, but they lack robust evaluation for diagnostic reliability (SACSH, 2019). Furthermore, health and environmental concerns related to the toxicity of formaldehyde and mercury compounds necessitate safer practices. This study is justified in addressing these critical gaps by providing evidence-based comparisons of preservative efficacy, which will enhance diagnostic accuracy, promote laboratory safety, and inform best practices in stool parasitology (De Oliveira *et al*., 2021).

1.5 Aim of the Study

The aim of this study is to evaluate and compare the efficacy of different stool preservatives including, 10% formalin, low-viscosity polyvinyl alcohol (PVA), and sodium acetate-acetic acid-formalin (SAF) in preserving the morphological integrity of intestinal parasites for accurate microscopic identification using wet mount techniques.

1.6 Objectives of the Study

Specific Objectives

1. To prepare and standardize stool preservatives: 5% formalin, 10% formalin, low-viscosity PVA, and SAF.
2. To preserve stool specimens containing intestinal parasites using each of the selected fixatives over a defined storage period.

3. To evaluate the microscopic appearance and morphological preservation of protozoan and helminth stages at different time intervals
4. To identify the most effective and safest preservative for routine use in stool parasitology, based on performance, availability, and safety profile.

1.7 Research Questions

1. Statistical significance in efficacy between preservatives?
2. What are the observable differences in staining quality (nuclear detail, cytoplasmic clarity, and shape integrity) among parasites preserved using the four different fixatives?
3. How does the duration of preservation affect the diagnostic quality of parasites fixed in each preservative?
4. Which preservative offers the best balance between efficacy, safety, and practicality for routine use in diagnostic parasitology laboratories?
5. Are there specific parasite stages (e.g., cysts, trophozoites, ova) that are better preserved by certain fixatives than others?

1.8 Hypotheses

Null Hypotheses

1. There is no significant difference in the morphological preservation of intestinal parasites among stool specimens treated with 10% formalin, low-viscosity polyvinyl alcohol (PVA), and sodium acetate-acetic acid-formalin (SAF).

2. The duration of stool specimen preservation does not significantly affect the diagnostic quality of parasites fixed in any of the four preservatives.

Alternative Hypotheses

1. There is a significant difference in the morphological preservation of intestinal parasites among stool specimens treated with 10% formalin, low-viscosity polyvinyl alcohol (PVA), and sodium acetate-acetic acid-formalin (SAF).
2. The duration of stool specimen preservation significantly affects the diagnostic quality of parasites fixed in one or more of the four preservatives.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview of Intestinal Parasitic Infections (IPIs)

Intestinal parasitic infections remain disproportionately concentrated in tropical and subtropical regions, particularly sub-Saharan Africa, Southeast Asia, and parts of Latin America (WHO, 2022). Worldwide, over 1.5 billion individuals are estimated to be infected with soil-transmitted helminths and more than 3.5 billion live with at least one parasitic infection, of which over 450 million experience clinical manifestations annually (Haque *et al.,* 2021; CDC, 2023). The burden is particularly severe among children, who suffer growth retardation, cognitive impairment, anaemia, and nutritional deficiencies due to chronic parasitism (BMC Public Health, 2020).

Key intestinal protozoa include *Entamoeba histolytica* and *Giardia lamblia,* which collectively cause hundreds of millions of symptomatic cases each year (Mahfouz *et al*., 2018). *Entamoeba histolytica* is responsible for amoebic dysentery and liver abscesses, while Giardia is a leading cause of acute and chronic diarrhea. Among helminths, *Ascaris lumbricoides, Trichuris trichiura,* and hookworms (*Ancylostoma/Necator*) remain endemic, with approximately 1 billion infected globally (WHO, 2021).

Microscopic identification is the diagnostic mainstay in many resource-limited settings (CDC, 2023). However, several challenges impede diagnostic accuracy:

- Intermittent shedding of parasite stages leads to false negatives in single-sample examinations.

- Trophozoites of protozoa are fragile and degrade rapidly after excretion; without quick examination or appropriate fixation their characteristic morphology is easily lost, which reduces microscopic sensitivity (Gonzálvez *et al*., 2022)

- User-dependent variability: Accuracy of microscopy depends heavily on technician training and slide quality (Mahfouz *et al*., 2018).

- Logistics: Transport delays, poor storage, and sample degradation in warm environments reduce diagnostic effectiveness (Elkheir *et al*., 2020).

2.2 Ideal Characteristics of Stool Preservatives

The primary function of a stool preservative is to maintain the structural integrity of parasite stages including helminth eggs and larvae, protozoan cysts, and especially trophozoites, which are particularly delicate and prone to rapid autolysis unless appropriately fixed or stored (Plauzolles *et al.,*2022) Reliable preservation ensures that characteristic features such as *Giardia lamblia*'s binucleated trophozoite or *Entamoeba histolytica'*s nuclear structure remain identifiable, even after extended storage or transport delays (CDC, 2023).

Preservatives should be versatile enough to support both concentration techniques and permanent staining. For instance:

- 10% formalin is highly compatible with formol-ether sedimentation or ethyl acetate concentration, facilitating detection of eggs and larvae (CDC, 2023).

- Sodium acetate acetic acid formalin (SAF) supports both concentration procedures and permanent stains such as trichrome or iron hematoxylin (Basic Medical Key, 2025).

- Conversely, Low viscosity poly vinyl alcohol (LV-PVA) excels in preparing high-fidelity permanent stained smears, especially for protozoan identification, but is incompatible with concentration methods (CDC, 2023; Labpedia, 2025).

An ideal preservative maintains sample quality despite variable storage conditions. It must protect diagnostic morphology over weeks to months, even under room temperature

conditions typical in field or rural settings (Elkheir *et al*., 2020). SAF and 10% formalin are known for their long-term stability, whereas LV-PVA is effective for several months, though it may degrade or pose disposal challenges due to mercury content (Labpedia, 2025)

Safety is a key consideration in preservative selection. While 10% formalin contains formaldehyde classified as a potential carcinogen it is nonetheless manageable with proper laboratory protocols (CDC, 2023). LV-PVA traditionally contains mercuric chloride, posing environmental and disposal hazards (Basic Medical Key, 2025). SAF, by contrast, is mercury-free and thus safer for long-term use and cleaner waste handling (Labpedia, 2025).
A user-friendly preservative should be easy to prepare, mix, and process.
- 10% formalin is straightforward to compound and inexpensive to procure (CDC, 2023).
- Sodium acetate acetic acid formalin (SAF) is also easy to prepare in-house using standard laboratory reagents and is cost-effective (Basic Medical Key, 2025).

2.3 Global Prevalence and Burden of Disease

Intestinal parasitic infections, including soil-transmitted helminths and intestinal protozoa, remain highly endemic in tropical and subtropical regions across the globe. According to the latest World Health Organization estimates, over 1.5 billion people approximately 24% of the global population harbor at least one species of STH, including *Ascaris lumbricoides, Trichuris trichiura,* and hookworms (*Ancylostoma duodenale or Necator americanus*) (WHO, 2023). Ascaris alone infects between 807 million and 1.2 billion individuals, making it the most prevalent helminth infection globally (Wikipedia, 2025).

The overarching impact of IPIs is substantial: Ascaris, Trichuris, and hookworms account for over 1.3 million DALYs (disability-adjusted life years) annually, disproportionately affecting children and pregnant women (WHO, 2023) Protozoan infections such as *Giardia lamblia* and *Entamoeba histolytica* further compound the global burden. In developing nations,

Giardia infects up to 30% of the population, while *Entamoeba histolytica/dispar* remains a significant cause of dysentery and liver abscesses (Wikipedia, 2025). Globally, over 3.5 billion people are estimated to be living with some form of intestinal parasitism, with 450 million experiencing clinical manifestations annually (CDC, 2023; Haque *et al*., 2021).

Sub-Saharan Africa remains a high-risk region for intestinal parasitic infections due to factors like inadequate sanitation, poverty, and limited healthcare infrastructure. A community-based study in Southeastern Nigeria reported a 33.8% prevalence of intestinal parasitic infections among rural populations, with *Ascaris lumbricoides* (15.5%), hookworm (3.9%), *Schistosoma mansoni* (4.2%), and *Entamoeba histolytica* (5.3%) identified (Okosa *et al*., 2022). Mixed-species infections were documented and associated with symptoms such as diarrhea, fatigue, and abdominal pain (Okosa *et al*., 2022).

A school-based survey in Yobe State revealed a helminth infection prevalence of 53.3% among primary school pupils, led by Ascaris (20.0%) and hookworm (16.7%) (Babagana *et al*., 2023). These data reinforce the endemic nature of soil transmitted helminths among young children in resource-poor settings.

Several urban and institutional studies have also reported high prevalence rates:

At a tertiary institution in Jos, Nigeria, intestinal parasites were detected in 43.3% of medical laboratory students; *Ascaris lumbricoides* (69.2%), hookworm (15.4%), and *Schistosoma mansoni* (15.4%) were predominant (Walsh Medical Media, 2024).

In Enugu, malnourished children exhibited an overall prevalence of 51.8%, predominately infected with *Ascaris* (37.7%) and hookworm (29.3%), compared to 12% prevalence in well-nourished controls (Onyemelukwe *et al*., 2021).

A prevalence survey among pupils in urban Lagos reported an astounding 78.1% infection rate in public schools versus 17.1% in private settings, with *Ascaris lumbricoides* the most common organism identified (Nigerian Journal of Paediatrics, 2024).

Child-specific surveys in rural and urban settings of Benue State showed prevalence rates of 51.0% for *E. histolytica* in rural pupils and 29.0% in urban pupils. Hookworm prevalence was 46.2% (rural) and 24.8% (urban); *Giardia* affected 11.5% rural and 8.6% urban children (MCH and AIDS Int'l Journal, 2024).

These findings indicate that children from rural, low-socioeconomic groups bear the highest burden of infection. Hygiene practices, water source, and sanitation facilities are key determinants in these rates (Onyemelukwe *et al*., 2021; MCH and AIDS Int'l Journal, 2024). Intestinal parasitic infections cause significant morbidity, especially among children and pregnant women. Chronic helminthiasis contributes to anemia, stunting, cognitive impairment, and poor school performance (BMC Public Health, 2020). Studies in Nigeria have demonstrated strong correlations between malnutrition and high infection prevalence, highlighting an urgent need for integrated health interventions (Onyemelukwe *et al*., 2021).

Pregnant women are also at elevated risk. In Nasarawa State, a cross-sectional survey among pregnant women attending antenatal clinics revealed a 39.2% prevalence of zoonotic and intestinal protozoa, with *Ascaris lumbricoides* (25.5%), *Giardia lamblia* (22.3%), and *Strongyloides stercoralis* (13.8%) among the predominant pathogens (BiomedRes, 2023).

2.4 Common Protozoan and Helminth Parasites

Intestinal parasitic infections (IPIs) are caused primarily by protozoa and helminths that inhabit the gastrointestinal tract, often resulting in both symptomatic and asymptomatic infections. The most common protozoan parasites include *Giardia duodenalis* (also known as

G. lamblia or G. intestinalis) and *Entamoeba histolytica*, while prominent helminths include *Ascaris lumbricoides* and hookworms (*Ancylostoma duodenale* and *Necator americanus*).

Giardia duodenalis

Giardia is a flagellated protozoan responsible for giardiasis, a diarrheal disease that affects both developing and developed regions. It is transmitted through ingestion of cysts in contaminated water, food, or via the fecal-oral route (CDC, 2023). Globally, it is estimated that 280 million people are infected annually

Entamoeba histolytica

Entamoeba histolytica is the causative agent of amoebiasis, which may lead to invasive colitis or extraintestinal manifestations like liver abscess. Though morphologically similar to non-pathogenic species such as *E. dispar*, *E. histolytica* can cause severe dysentery and tissue invasion (WHO, 2024).

Ascaris lumbricoides

Ascaris is the most prevalent soil-transmitted helminth (STH), infecting more than 800 million people globally Infection occurs through ingestion of embryonated eggs in contaminated soil or food. Larvae migrate through the lungs before maturing in the intestine. While many infections are asymptomatic, heavy worm burdens can cause intestinal obstruction, especially in children (CDC, 2023).

Hookworms

Hookworm infections, primarily by *A. duodenale* and *N. americanus*, affect over 400 million people worldwide (WHO, 2023). The larvae penetrate the skin, usually through the feet, and travel to the intestines where they attach and feed on blood. Chronic infections are associated with iron-deficiency anemia, fatigue, and impaired cognitive development, particularly in school-age children and pregnant women (Gebremichael *et al*., 2024; Liu *et al*., 2022)

These parasites significantly contribute to the global burden of disease, especially in low-resource settings with inadequate sanitation and healthcare access. Accurate diagnosis and effective specimen preservation remain essential for controlling these infections, guiding treatment, and conducting epidemiological surveillance.

Trichuris trichiura **(Whipworm)**

Trichuris trichiura, commonly known as whipworm, infects an estimated 600–800 million people worldwide, with approximately 513 million individuals harboring the infection according to a systematic review covering 2010–2023 (J Infection Public Health, 2024). Regional prevalence is highest in South-East Asia (21.7%), the Caribbean (20.95%), Southern Africa (9.6%), and Latin America (9.6%), while Eastern Europe reports less than 0.2% (J Infection Public Health, 2024). Heavy infections often termed Trichuris Dysentery Syndrome are associated with chronic dysentery, rectal prolapse, , growth retardation, and cognitive delays in children (CDC, 2023).

Strongyloides stercoralis **(Threadworm)**

Strongyloides stercoralis causes strongyloidiasis, a soil-transmitted helminth infection notorious for its persistence via internal autoinfection and its severity in immunocompromised individuals. WHO estimates range from 30 to 100 million infected globally, though recent modeling suggests up to 386 million infections, likely underreported (ISGlobal, 2024; WHO, 2021; CDC, 2023). In Southeast Asia, pooled prevalence is estimated at 12.7%, with individual country rates up to 24.9% (Infectious Diseases of Poverty, 2023). Diagnosis is complicated by low larval concentration in stool and limited sensitivity of conventional methods (Cook *et al.,* 2023). Without treatment, infections can persist for

decades and may result in lethal hyperinfection in immunosuppressed individuals (CDC, 2023; Infectious Diseases of Poverty, 2023).

***Schistosoma mansoni* (Intestinal Schistosome)**

Schistosoma mansoni is responsible for intestinal schistosomiasis, which affects over 251 million people, with more than 75 million receiving praziquantel treatment as of 2021 (PMCID, 2024). Approximately 393 million people in sub-Saharan Africa are at risk, with about 55 million actively infected (PMCID, 2024). The infection burdens exhibit country-specific prevalence: northern Ethiopia (73.9%), western Ethiopia (37.9%), Nigeria (56%), Kenya (60.5%), Tanzania (64.3%), Ghana (19.8%), and Côte d'Ivoire (53.8%) (PMCID, 2024). Morbidity arises from egg-induced tissue fibrosis leading to portal hypertension, hepatosplenomegaly, and increased mortality (PMCID, 2024).

2.5 Diagnostic Techniques in Parasitology

Direct Microscopy

Direct microscopy, particularly direct saline (wet mount) microscopy, is the most widely used diagnostic method for detecting intestinal parasites in clinical and field settings due to its simplicity, low cost, and minimal requirement for equipment (Mahittikorn *et al*., 2021; CDC, 2023).

Direct wet mount involves emulsifying a small amount of fresh stool in a drop of saline or iodine solution on a glass slide, which is then covered and examined under light microscopy (Mahittikorn *et al*., 2021). It is highly effective in detecting motile forms such as *Strongyloides stercoralis* larvae and high-intensity infections like *Ascaris lumbricoides* eggs (Mahittikorn *et al.,* 2021; CDC, 2023). Notably, it allows rapid visualization within minutes of stool collection, making it valuable for point-of-care screening in low-resource settings.

Multiple studies have demonstrated that direct microscopy is significantly less sensitive than concentration-based techniques. For example, a cross-sectional study among schoolchildren in Ethiopia reported a sensitivity of 61.1% for direct saline microscopy versus 92.3% using formalin-ether concentration (FEC) (Mohebali *et al*., 2015; Abay *et al*., 2017). Another study in pregnant women showed direct wet mount detected 18.8% prevalence compared to 24.7% by FEC (sensitivity = 76%; test efficiency = 94%) especially underdiagnosing *Hymenolepis nana* (sensitivity 0.54%) (BMC Research Notes, 2018) .

When benchmarked against composite reference standards (Kato-Katz, McMaster, Mini-FLOTAC), sensitivity varied by parasite species and intensity. 73.8% for *Ascaris*, but as low as 17% for *Trichuris* and hookworm in low-intensity infections (Dana *et al*., 2020)

The reliability of direct microscopy is influenced by several factors:

- Parasite load: Low egg counts or sparse trophozoites reduce detection probability (Dana *et al*., 2020).
- Specimen handling: Delay in processing leads to morphological degradation, especially of trophozoites (Mahittikorn *et al*., 2021).
-Technician skill: Experienced microscopists can detect more, but inter-operator variability remains high (Ngui *et al.,* 2019).

Despite its limitations, direct microscopy remains a practical first-line tool in many endemic areas due to: Low cost and minimal infrastructure requirements,Immediate result availability, Feasibility in decentralized or rural clinics where more advanced techniques are unavailable (Mahittikorn *et al*., 2021; Nkouayep *et al*., 2021).

In such settings, direct microscopy is often employed for initial screening particularly when access to fixatives or preservation techniques is limited. However, due to its low sensitivity,

especially for protozoa and low-intensity infections, it is usually supplemented with concentration methods when possible (Dana *et al*., 2020; CDC, 2023).

Concentration Techniques

Concentration techniques are essential diagnostic methods in parasitology, particularly when parasite density in stool is low. These methods increase the chances of detecting ova, cysts, or larvae by separating them from fecal debris and concentrating them into a smaller volume (Garcia, 2020). This approach is especially useful in asymptomatic individuals or chronic infections where parasite load is minimal.

There are two main categories of concentration techniques: sedimentation methods and flotation methods.

Sedimentation Techniques

Sedimentation techniques rely on gravity or centrifugation to separate parasites from fecal material based on their higher specific gravity. The most widely used method in this category is the formalin-ether (or ethyl acetate) sedimentation technique.

In this method, stool is emulsified in 10% formalin, strained, and then mixed with ether or ethyl acetate. Centrifugation is applied to sediment the parasite stages at the bottom, while fecal debris floats to the top (Utzinger *et al*., 2020). This technique is widely accepted due to its high recovery rate for a variety of parasites, including *Ascaris lumbricoides, Trichuris trichiura, Giardia lamblia*, and *Entamoeba histolytica* (Feleke *et al*., 2018).

However, the use of ether presents safety concerns due to its flammability and toxicity, leading some labs to adopt ethyl acetate as a safer alternative. Also, prolonged exposure to formalin can distort protozoan morphology, emphasizing the need for timely processing or effective preservatives like SAF or LV-PVA (Mekonnen *et al*., 2022).

Flotation Techniques

Flotation methods use solutions with a higher specific gravity than the parasite ova or cysts, causing them to float to the surface. One common solution is saturated sodium nitrate or zinc sulfate.

These techniques are particularly effective for detecting light eggs like those of *Ancylostoma* (hookworm) or protozoan cysts. However, they are less effective for heavier eggs, such as those of *Schistosoma mansoni*, and may collapse delicate protozoan cysts due to osmotic pressure differences (Ngui *et al.,* 2020).

Flotation methods are not commonly used in routine diagnosis of human parasitic infections, especially in developing countries, due to equipment limitations and lower sensitivity for certain helminth eggs. Nevertheless, they are useful in veterinary parasitology and in detecting specific protozoa in well-controlled settings.

The effectiveness of concentration methods depends heavily on the integrity of parasitic structures, which can be affected by the choice of preservative. Poor fixation may lead to distortion or degeneration of eggs and cysts, reducing the sensitivity and specificity of microscopic analysis.

10% formalin is widely used for its compatibility with sedimentation techniques, but newer preservatives such as SAF and low-viscosity PVA are gaining ground due to their reduced toxicity and superior morphological preservation (Visvesvara *et al*., 2016; Becker *et al*., 2021).

2.6 Importance of Stool Preservation

Stool preservation plays a crucial role in the diagnostic process of intestinal parasitic infections (IPIs), particularly in field settings or laboratories that experience delays between specimen collection and analysis. Preservation ensures that the morphological characteristics

of parasites such as cysts, trophozoites, eggs, and larvae remain intact and recognizable for accurate identification through microscopy (Garcia, 2020).

Preservation is not merely about maintaining sample stability it is foundational for accurate morphological identification, quantification, and comparison of parasite load over time. This is particularly essential in epidemiological studies, monitoring treatment efficacy, and performing retrospective analyses (Becker *et al*., 2021).

Moreover, in remote and resource-limited settings where immediate microscopic examination is not feasible, preservation allows for delayed examination and even transportation to reference laboratories (Utzinger *et al*., 2020). This capability is indispensable in national surveillance programs and research studies involving multiple sites.

The choice of preservative must align with the intended laboratory workflow, safety considerations, and availability of reagents. This is increasingly important as laboratories transition away from mercury-based fixatives due to health and environmental regulations, leading to the adoption of safer alternatives like low-viscosity PVA (LV-PVA) and SAF (Verweij and Stensvold, 2021).

2.7 Effects of Time and Temperature on Parasite Morphology

The accuracy of microscopic diagnosis in intestinal parasitology is critically dependent on the preservation of parasite morphology. Time and temperature are two of the most influential external factors affecting the structural integrity of helminth eggs, protozoan cysts, and trophozoites in stool samples. Delays in processing or inappropriate storage temperatures can significantly compromise diagnostic accuracy by degrading parasitic forms or altering their appearance (Hass *et al.,* 2024).

Immediately after defecation, stool samples begin to undergo decomposition due to bacterial overgrowth, enzymatic activity, and desiccation. Protozoan trophozoites, in particular, are highly sensitive and can lose their motility and characteristic features within 30 minutes at room temperature (Garcia, 2020). According to WHO guidelines, examination of fresh samples should ideally occur within one hour of collection for accurate identification of motile protozoa (World Health Organization, 2019).

Cysts and helminth eggs are more resistant to degradation but still susceptible to prolonged exposure. Studies have shown that *Giardia lamblia* cysts begin to shrink and lose internal definition after 12–24 hours without preservative, making them difficult to distinguish microscopically (Becker *et al.,* 2021). Similarly, *Ascaris lumbricoides* and *Trichuris trichiura* eggs can become distorted or calcified if samples are kept unpreserved for more than 48 hours (Zhang *et al*., 2020).

Temperature also has a profound impact on parasite preservation. Samples stored at temperatures above 30°C experience accelerated degradation, particularly in tropical environments where refrigeration may be unavailable. Elevated temperatures speed up enzymatic and microbial activity, leading to rapid breakdown of trophozoites and morphological alteration of cysts (Utzinger *et al*., 2020). For instance, *Entamoeba histolytica* trophozoites stored at 37°C lost identifiable nuclear features within 6 hours (Visvesvara *et al*., 2016).

Conversely, refrigeration at 4°C slows down biological degradation but is not always sufficient to fully preserve trophozoite integrity unless used in combination with fixatives. This is why cold storage alone is not recommended as a long-term preservation method for parasitological analysis (Tantawy and El-Sayed, 2022). In contrast, when stool samples are fixed immediately with preservatives like 10% formalin, SAF, or low-viscosity PVA, they

can retain their morphological features for several weeks or even months, depending on storage conditions (Zhou *et al*., 2018).

These environmental effects can lead to false negatives, especially in protozoan infections where trophozoites are the main diagnostic stage. Additionally, the morphological distortion of cysts and eggs can make species differentiation difficult, especially for closely related organisms like *Entamoeba histolytica* and *Entamoeba dispar* (Verweij and Stensvold, 2021).

Hence, timely fixation and temperature control are indispensable components of the diagnostic process. Field studies increasingly advocate for the use of preservative vials at the point of collection, particularly in remote areas, to avoid sample compromise (Zhang *et al*., 2020). Additionally, training health workers to recognize time and temperature constraints is essential for improving diagnostic reliability in low-resource settings.

2.8 10% Formalin: Composition and Mode of Action

10% Formalin is one of the most widely used stool preservatives in parasitology laboratories around the world, especially for helminth and protozoan detection via concentration and direct smear techniques. It has been a staple due to its effectiveness, affordability, and relatively long shelf life.

The term “10% formalin” refers to a diluted aqueous solution of formaldehyde, typically composed as follows:

-Formaldehyde (37–40%)– 100 mL

-Distilled water – 900 mL

This creates a final solution containing approximately 4% formaldehyde by weight, which is still referred to as "10% formalin" by convention (Garcia, 2020).

For parasitological preservation, buffered formalin is often preferred, where phosphate buffer (pH 7.0) is added to minimize morphological distortion of the parasites. Buffered formalin helps maintain the sample's pH, preserving the internal structures of protozoan cysts and helminth eggs better than unbuffered formalin (Tantawy and El-Sayed, 2022).

2.81Effects of 10% Formalin on Parasite Morphology

Formalin acts by cross-linking proteins and nucleic acids, thereby stabilizing parasite structures such as cyst walls, ova shells, and internal organelles (Garcia, 2020). This process halts enzymatic activity and bacterial degradation, preserving parasites for extended analysis. Notably:

- Helminth eggs (e.g., *Ascaris lumbricoides, Trichuris trichiura*) retain their outer shell, size, and internal embryo structure with minimal distortion when preserved in 10% formalin (Tantawy and El-Sayed, 2022).
- Protozoan cysts, such as *Giardia lamblia* and *Entamoeba histolytica*, also show good nuclear preservation, although cytoplasmic clarity may be reduced (Becker *et al*., 2021).

2.8.2 Advantages of 10% Formalin

10% formalin, a widely used fixative in parasitology, offers several benefits for preserving the morphology and integrity of intestinal parasites in clinical and research settings.

1. Effective Morphological Preservation of Parasite Structures

One of the key strengths of 10% formalin lies in its ability to preserve helminth ova, protozoan cysts, and larvae with minimal distortion. It stabilizes proteins through cross-linking, thereby preventing autolysis and microbial degradation (Garcia, 2020 Visvesvara) Formalin-fixed helminth eggs like *Ascaris lumbricoides* and *Trichuris trichiura* often retain

their outer shell architecture, internal embryo, and refractile bodies, making them distinguishable even after weeks of storage (Becker *et al*., 2021)

2. Long-term Storage Capability

Another significant benefit is stability over extended periods. Parasites preserved in formalin can remain viable for diagnostic microscopy for several weeks to months, especially when stored under proper temperature conditions (Utzinger *et al.,* 2020). This is particularly useful in epidemiological studies and resource-limited settings where immediate analysis is not feasible.

3. Suitability for Concentration Techniques

10% formalin is compatible with formalin-ether (or ethyl acetate) sedimentation techniques, a preferred concentration method that enhances sensitivity for detecting low parasite loads (Tantawy and El-Sayed, 2022). This combination is recommended by several diagnostic guidelines including the Centers for Disease Control and Prevention (CDC, 2021)

2.8.3 Limitations of 10% Formalin

1. Poor Preservation of Trophozoites

Formalin performs poorly in preserving the labile trophozoite stage of protozoans like *Giardia lamblia* and *Entamoeba histolytica.* These forms tend to shrink, lose cytoplasmic definition, and rapidly disintegrate post-collection if not preserved in an appropriate medium such as polyvinyl alcohol (PVA) (Becker *et al*., 2021; Garcia, 2020).

2. Chemical Hazards and Regulatory Restrictions

Formalin (a 37% aqueous solution of formaldehyde) is a classified carcinogen and respiratory irritant. Prolonged exposure has been linked to nasopharyngeal cancer, allergic reactions, and

eye/skin irritation (IARC, 2018; CDC, 2021). Consequently, its use in laboratories requires strict biosafety precautions and is increasingly being regulated or replaced in some countries.

3. Environmental Disposal Issues

Improper disposal of formalin waste poses ecological and environmental risks, requiring neutralization and disposal according to hazardous chemical waste protocols. This presents challenges in low-resource laboratories lacking proper chemical waste management systems (Utzinger *et al*., 2020; Tantawy and El-Sayed, 2022).

2.9 Sodium Acetate–Acetic Acid–Formalin (SAF)

Sodium Acetate–Acetic Acid–Formalin (SAF) is a mercury-free preservative frequently utilized in diagnostic parasitology, offering effectiveness for both microscopic examination and concentration techniques (Garcia, 2020).

The classical SAF formulation consists of:

- 1.5 g sodium acetate, which serves as a buffering agent to help stabilize the sample pH (Becker *et al*., 2021);
- 3.0 mL glacial acetic acid, which acidifies the solution, enhancing cytoplasmic and nuclear visibility in protozoa (Zhou, Yu, and Li, 2018);
- 5.0 mL of 10% formalin as the primary fixative to preserve celluliar structure in helminths and protozoa (Visvesvara and Schuster, 2016);
- Distilled water, added to complete the 100 mL volume (Utzinger *et al.,* 2020).

Mode of Action

Each component in SAF contributes to parasite preservation through specific biochemical mechanisms:

1. Formalin: Cross-links parasite proteins and nucleic acids to halt enzymatic breakdown, preserving morphological features such as cyst walls and ova shells (Garcia, 2020). It also acts as an antimicrobial, limiting bacterial and fungal overgrowth in stool specimens (Tantawy and El-Sayed, 2022).

2. Acetic acid: Lowers the pH to about 4.5–5.0, which increases chromatin contrast in protozoan nuclei and improves detection of internal structures (Becker *et al*., 2021).

3. Sodium acetate: Helps maintain osmotic balance and ionic stability, preventing cellular shrinkage or swelling that could distort parasite morphology (Utzinger *et al*., 2020).

2.9.1 Benefits of SAF Composition

- Preservation of helminth eggs, protozoan cysts, and trophozoites in a recognizable state for both concentration methods and permanent stains, including trichrome and iron hematoxylin (Garcia, 2020).
- A mercury-free alternative, minimizing environmental and safety risks associated with handling and waste disposal (WHO, 2019).
- Improved cytoplasmic and nuclear detail, aiding the identification of closely related species, such as *Entamoeba histolytica* versus *E. dispar* (Becker *et al*., 2021).

2.9.2 Effects of SAF on Parasite Morphology

The Sodium Acetate-Acetic Acid-Formalin (SAF) solution has been widely employed in stool specimen preservation due to its ability to maintain the structural integrity of intestinal parasites. However, its impact on morphology varies depending on parasite type, length of storage, and environmental conditions such as temperature.

Protozoan Parasites

SAF effectively preserves protozoan cysts and trophozoites, particularly of *Giardia lamblia* and *Entamoeba histolytica/dispar* complex. Nuclear details, peripheral chromatin, and cytoplasmic inclusions are generally retained for at least several weeks (Garcia, 2020). However, trophozoites may exhibit shrinkage and distortion after prolonged storage, particularly beyond 30 days, with less crisp nuclear details. Despite this, SAF still permits accurate identification using permanent stains like trichrome or iron hematoxylin (Utzinger *et al*., 2020).

Helminth Parasites

Helminth ova and larvae, including those of *Ascaris lumbricoides, Trichuris trichiura*, and *Strongyloides stercoralis*, retain their general morphology well in SAF (Becker *et al*., 2021). For example, the eggs of Hookworm may become less refractile, and the larvae of Strongyloides may exhibit cytoplasmic granulation or partial autolysis if not processed early (Tantawy and El-Sayed, 2022).

2.9.3 Duration and Storage Conditions

The preservation quality is optimal when specimens are stored at room temperature (20–25°C) and examined within two weeks (Garcia, 2020). Extended storage can result in fading of cytoplasmic staining, degradation of nuclear material, and cloudiness in the background due to fixative-protein interactions (Utzinger *et al*., 2020). Therefore, while SAF remains suitable for transport and delayed examination, early processing is ideal for maintaining diagnostic clarity.

2.9.4 Advantages of SAF

1. Mercury-Free and Safer for Use

Unlike traditional PVA, SAF is free of toxic mercury compounds, which reduces the risk of occupational exposure and simplifies disposal protocols (Garcia, 2020). This makes SAF particularly attractive in resource-limited or high-throughput laboratories that prioritize safety.

2. Broad Preservation Capability

SAF can preserve both protozoan cysts and trophozoites as well as helminth ova and larvae for extended periods without significant degradation of key morphological features (Zhou, Yu, and Li, 2018). It supports a wide diagnostic range of intestinal parasites across clinical and epidemiological contexts.

3. Compatibility with Staining and Concentration Techniques

SAF-fixed specimens are compatible with trichrome and iron hematoxylin staining, as well as concentration methods such as formalin-ethyl acetate sedimentation (WHO, 2019). This allows for flexibility in diagnostic workflows without requiring multiple preservatives.

4. Cost-Effective and Easy to Prepare

Its components, sodium acetate, acetic acid, and formalin, are inexpensive and readily available, especially in developing countries. The preservative is relatively easy to prepare and does not require complex equipment for handling or use (Becker *et al*., 2021).

2.9.5 Limitations of SAF

1. Slightly Weaker Staining Quality

Compared to PVA, SAF-preserved samples often produce lighter staining intensity, especially in protozoan trophozoites, requiring longer staining times or more refined techniques (Utzinger *et al*., 2020). This may pose challenges in under-resourced labs.

2. Incompatibility with Rapid Wet Mount Diagnosis

Because SAF requires concentration and staining for optimal diagnosis, it is not ideal for direct wet mount examination or rapid screening, which are often necessary in urgent clinical settings (WHO, 2020).

3. Risk of Sample Dilution

The use of a liquid fixative can occasionally lead to dilution of specimens, especially when stool consistency is loose. This may reduce the concentration of parasites in the sample, potentially lowering diagnostic yield (Becker *et al.,* 2021).

2.10 Historical Background and Chemical Composition of Low Viscosity Polyvinyl Alcohol (LV-PVA)

Low viscosity polyvinyl alcohol (LV-PVA) is a modified stool fixative derived from the classical polyvinyl alcohol (PVA) formulation, which has been widely used for several decades in clinical parasitology. The classical PVA fixative was introduced in the mid-20th century and quickly became a preferred preservative for the morphological study of intestinal protozoa, particularly for trophozoites, due to its excellent ability to preserve nuclear and cytoplasmic features (Garcia, 2020).

Growing concerns over mercury toxicity, disposal regulations, and occupational hazards led to a decline in the use of classical PVA fixatives in many laboratories, especially in developed countries In response to these challenges, low viscosity polyvinyl alcohol (LV-PVA) was developed. LV-PVA uses a lower molecular weight PVA polymer, which reduces the solution's thickness while retaining its adhesive and preservative properties (Becker *et al*., 2021).

In terms of chemical composition, LV-PVA formulations typically include:

- Polyvinyl alcohol (PVA): A synthetic polymer that serves as the base and binding agent.
- Buffered formalin (usually 5–10%): Acts as the primary fixative for cellular preservation.
- Acetic acid: Enhances nuclear detail and balances the pH of the fixative.

- Optional glycerin: Adds viscosity and prevents drying or crystallization on slides.
- Optional zinc sulfate or other additives in mercury-free variants.

Modern LV-PVA formulas have moved away from using mercury, replacing it with zinc-based fixatives or other non-toxic alternatives that comply with environmental safety standards and hazardous waste regulations (Tantawy and El-Sayed, 2022).

Overall, LV-PVA has retained its role as a critical tool in permanent staining of protozoan parasites, particularly for identifying Giardia lamblia, Entamoeba histolytica, and other delicate trophozoites whose morphology is often lost with simpler preservatives like formalin or SAF (Utzinger *et al*., 2020).

2.10.1Effectiveness of Low Viscosity Polyvinyl Alcohol (LV-PVA) in Preserving Protozoan Trophozoites

The primary advantage of LV-PVA lies in its superior ability to preserve the delicate trophozoite stage of intestinal protozoa, which are often lost or distorted when preserved using other fixatives like 10% formalin or SAF. Trophozoites are the active, motile forms of protozoan parasites such as Giardia lamblia and Entamoeba histolytica, and they rapidly degenerate upon passage from the host, requiring immediate fixation for accurate diagnosis (Garcia, 2020).

Several studies have consistently demonstrated that PVA-based fixatives, particularly LV-PVA, outperform other preservation media in retaining detailed morphological structures of protozoan trophozoites. This includes preservation of nuclear chromatin, karyosomes, axonemes, and flagella key features essential for definitive identification under light microscopy (Becker *et al*., 2021).

A comparative analysis by Tantawy and El-Sayed (2022) found that stool samples preserved in LV-PVA exhibited superior staining quality with clearer differentiation of nuclear structures and cytoplasmic inclusions compared to SAF-preserved samples. The trichrome staining of LV-PVA-fixed smears provided excellent contrast, allowing for easier identification and differentiation between pathogenic protozoa and non-pathogenic species. This quality is particularly important in regions where Entamoeba histolytica must be differentiated from E. dispar, which is morphologically similar but non-pathogenic (Utzinger *et al*., 2020).

Furthermore, LV-PVA's ability to maintain trophozoite integrity over extended storage periods sometimes up to several months makes it especially useful in field settings or in laboratories

where immediate processing is not feasible (El-Kady *et al*., 2023). Its adhesive nature ensures that stool particles stick well to the slide surface, reducing the risk of material loss during the staining and rinsing process.

In summary, LV-PVA remains a gold standard for permanent smears, particularly when accurate morphological identification of protozoan trophozoites is required. It provides superior claritylong-term stability, and enhanced staining quality, all of which are critical for diagnostic accuracy in both clinical and research parasitology.

2.10.2 Superior Performance of Low Viscosity Polyvinyl Alcohol (LV-PVA) for Permanent Stained Smears

One of the most valued attributes of Low Viscosity Polyvinyl Alcohol (LV-PVA) is its superior compatibility with permanent staining techniques especially trichrome and iron hematoxylin stains, which are vital in the routine identification of intestinal protozoa in preserved stool specimens (Garcia, 2020).

In laboratory settings, permanent stained smears provide better resolution and long-term archiving compared to wet mount microscopy. When stool specimens are preserved using LV-PVA, the fixative's adhesive and fixative properties ensure a firm attachment of fecal material to the slide, even through the rigorous processes of dehydration, staining, and rinsing (Becker *et al*., 2021).

LV-PVA-fixed smears consistently produce high-quality, diagnostically informative slides. In a study by El-Kady et al. (2023), specimens preserved in LV-PVA showed higher diagnostic yield and better contrast under light microscopy than those preserved with SAF and 10% formalin. The trophozoites of *Giardia lamblia* and *Entamoeba histolytica* were more recognizable in LV-PVA-stained smears due to enhanced nuclear detail and preservation of internal cytoplasmic structures.

Moreover, the low viscosity of this fixative improves mixing and penetration into stool particles, which results in uniform fixation throughout the sample. This is particularly important in stool samples with a semi-solid or formed consistency, where fixative penetration may be inconsistent (Garcia, 2020). Uniform fixation directly translates to better staining outcomes, minimizing false negatives caused by poorly preserved or overlooked parasites.

In essence, LV-PVA provides optimal morphological preservation,excellent staining compatibility, and long-term slide stability, making it the fixative of choice for permanent stained smears in modern parasitology.

2.10.3 Limitations of Low Viscosity Polyvinyl Alcohol (LV-PVA)

One significant drawback of LV-PVA is its incompatibility with common concentration techniques, such as formalin-ether or formalin-ethyl acetate sedimentation. These methods are essential for concentrating protozoan cysts and helminth eggs, especially in cases of low

parasitic load, and play a vital role in increasing diagnostic sensitivity (Garcia, 2020). However, LV-PVA-preserved specimens cannot undergo these procedures due to the chemical incompatibility between PVA and the solvents used in concentration (El-Kady *et al.*, 2023). As a result, laboratories must rely solely on direct smears or permanent stains when using LV-PVA, potentially missing parasites that would have been detected through sedimentation or flotation techniques.

Due to the toxicity of mercury-based PVA, laboratories must follow strict handling and disposal protocols, which can be costly and logistically burdensome. Regulatory agencies in many regions have imposed bans or phased-out use of mercury-containing reagents, encouraging a shift toward mercury-free alternatives (Tantawy and El-Sayed, 2022). This has led to the development of modified LV-PVA formulations that exclude mercury but retain similar fixation quality.

Another practical limitation is that LV-PVA, especially in its newer mercury-free formulations, may not be readily available in all regions. Importation of such reagents can be delayed by customs regulations, limited supply chains, or high procurement costs (Becker *et al.*, 2021).

2.10.4 Mercury-Free Formulations and Technological Advancements

The primary objective in the development of mercury-free PVA (MF-PVA) was to eliminate mercuric chloride, the toxic component of traditional PVA, while preserving the fixative's ability to stabilize protozoan trophozoites effectively. To achieve this, alternative compounds such as zinc sulfate, copper sulfate, and polyacrylamide were explored as potential stabilizing agents (Garcia, 2020). Among these, zinc-based formulations have gained the most acceptance due to their excellent morphological preservation and low toxicity.

Studies have shown that zinc-PVA preparations preserve protozoan features such as nuclear structure, peripheral chromatin, and cytoplasmic inclusions almost as well as mercury-based

solutions (El-Kady *et al*., 2023). In clinical comparisons, these newer fixatives produced high-quality permanent smears suitable for trichrome and iron hematoxylin staining, without significant loss of diagnostic accuracy.

Several commercial mercury-free PVA-based products have entered the market, offering laboratories standardized and safer alternatives. Examples include:

- Para-Pak® Zn-PVA – a commercially available fixative using zinc sulfate as the active agent, marketed for its safety and comparable efficacy to traditional PVA (Garcia, 2020).
- Unifix® – a proprietary fixative designed for field and laboratory use with a long shelf-life and mercury-free composition (Becker *et al*., 2021).

These alternatives have reduced the burden of regulatory compliance and improved safety in handling and disposal.

Multiple studies have confirmed that mercury-free formulations perform nearly as well as traditional mercury-PVA for protozoan detection. Tantawy and El-Sayed (2022) conducted a comparative study on stool samples preserved in mercury-based PVA versus MF-PVA and found that trophozoite morphology and staining quality were statistically equivalent in most cases. Similarly,(El-Kady *et al*.,2023) reported 95–98% agreement in diagnostic outcomes between mercury-free and traditional PVA when assessing *Entamoeba histolytica* and *Giardia lamblia*.

2.11 Comparative Studies and Evaluations

Several influential studies have evaluated how 10% formalin, SAF, and LV-PVA perform in preserving stool specimens for parasitological diagnostics:

A 2021 study compared formalin-based preservation methods with alternative fixatives for helminth and protozoan detection. The results showed that 10% formalin and LV-PVA effectively preserved helminth eggs and protozoan cysts, with LV-PVA demonstrating

superior compatibility for permanent stained smears due to its adhesive properties (Garcia *et al*., 2021).

A 2023 evaluation of SAF versus unpreserved stool samples highlighted SAF's enhanced sensitivity for protozoan detection, identifying 160 of 250 cases compared to 95 with direct examination of fresh stool (Ahmed and Mohamed, 2023). This underscores SAF's critical role in improving diagnostic accuracy.

A 2022 study by the CDC assessed multiple fixatives, including 10% formalin, LV-PVA, SAF, and newer one-vial alternatives, across various stool examination methods. All fixatives except Parasafe performed well in concentration (wet) methods, with LV-PVA remaining the gold standard for permanent stained smears. The mercury-free fixative Ecofix showed comparable performance (Thompson *et al*., 2022).

A 2024 comparative analysis of LV-PVA and non-mercurial alternatives (e.g., PF, EcoFix, Parasafe) found that PF and EcoFix closely matched LV-PVA's ability to preserve key protozoa like Entamoeba histolytica, while Parasafe was less effective (Rodriguez and Lee, 2024).

2.11.1 Limitations in Existing Literature

Many foundational studies on these preservatives predate advancements in diagnostic techniques and safety regulations. For instance, earlier evaluations of 10% formalin and LV-PVA do not account for modern molecular methods or newer, less toxic preservatives like Ecofix (Garcia and Smith, 2021). Similarly, studies on SAF often utilized outdated staining techniques, such as chlorazol black dye, which have been largely replaced by trichrome or modified stains in current practice (Ahmed and Mohamed, 2023). These outdated

methodologies limit the relevance of findings to contemporary laboratory practices, where molecular diagnostics and environmental safety are increasingly prioritized (Hassan *et al.,* 2024).

Variability in experimental design and evaluation criteria hinders direct comparisons across studies. For example, morphological integrity is often assessed using subjective scales (e.g., clarity ratings) without standardized rubrics, leading to inconsistent results (Thompson *et al.,* 2022). Sample sizes also vary widely, from 30 specimens in some studies to over 900 in others, affecting statistical power and reliability (Garcia and Smith, 2021; Thompson *et al*., 2022). Additionally, differences in concentration techniques (e.g., formalin-ether sedimentation) and staining protocols (e.g., trichrome vs. modified acid-fast) across studies complicate meta-analyses (Ahmed and Mohamed, 2023; Patel and Khan, 2023). The absence of standardized grading for preservation quality, such as quantitative measures of nuclear detail or egg structure, further limits reproducibility.

Most studies focus on common intestinal parasites like *Giardia lamblia*, *Entamoeba histolytica*, and *Ascaris lumbricoides*, with limited data on less prevalent species such as *Strongyloides stercoralis* or *Entamoeba coli* (Ahmed and Mohamed, 2023; Thompson *et al*., 2022). This narrow focus restricts understanding of preservative efficacy across diverse parasitic morphotypes, particularly for emerging or region-specific parasites.

Evaluations typically assess short-term preservation (days to weeks), with minimal data on long-term storage beyond one month. For instance, while LV-PVA is noted for stable stained smears over extended periods, specific timelines and conditions (e.g., ambient vs. refrigerated storage) are rarely quantified (Thompson *et al.,* 2022). This gap is critical for laboratories requiring extended storage, such as in resource-limited settings (Hassan *et al.,* 2024).

Few studies directly compare 10% formalin, SAF, and LV-PVA within the same experimental framework, often including other preservatives or unpreserved controls instead

(Patel and Khan, 2023; Rodriguez and Lee, 2024). This limits comprehensive assessments of their relative strengths and weaknesses.

Additional Concerns: The literature often highlights the toxicity of formalin (carcinogenic) and LV-PVA (mercuric chloride content) but lacks detailed evaluations of safer alternatives or their long-term impacts on diagnostic accuracy (Hass *et al*., 2024). Furthermore, studies rarely address practical considerations like cost, disposal logistics, or compatibility with molecular diagnostics, which are increasingly relevant in modern parasitology (WHO, 2022).

2.12 Research Gaps Identified

A recent study found that 10% formalin outperformed LV-PVA in recovering helminth eggs and protozoan cysts via formalin-ether sedimentation, detecting 90% of infections compared to 78% for LV-PVA (Garcia and Smith, 2021). However, LV-PVA was superior for permanent stained smears, preserving protozoan nuclear details critical for identifying species like *Giardia lamblia* and *Entamoeba histolytica* (Garcia and Smith, 2021). In contrast, another study reported that SAF and 10% formalin performed similarly for wet preparations, recovering comparable numbers of parasitic morphotypes, but SAF yielded inconsistent staining quality for permanent smears compared to LV-PVA (Thompson *et al*., 2022). This discrepancy highlights conflicting findings on SAF's efficacy for stained smears, with some studies suggesting it performs adequately with modified staining techniques (Ahmed and Mohamed, 2023).

Further inconsistencies emerge in parasite-specific outcomes. For instance, 10% formalin preserved filarial larvae morphology better than ethanol-based fixatives in non-human primate samples, but no significant difference was noted for strongyle eggs, suggesting variability in formalin's efficacy across parasite types (Hassan *et al*., 2024). Similarly, SAF detected protozoa in 62% of 250 patient samples compared to 38% in unpreserved samples, yet its performance for helminths was less consistent, with some studies reporting lower egg

counts compared to formalin (Ahmed and Mohamed, 2023; Patel and Khan, 2023). These findings indicate that preservative efficacy may depend on the target parasite, with formalin generally favoring helminths and LV-PVA excelling for protozoa, while SAF's performance varies.

The lack of standardized evaluation criteria exacerbates these inconsistencies. Morphological integrity is often assessed subjectively, with studies using different scales (e.g., clarity ratings vs. morphotype counts), leading to variable conclusions about preservation quality (Thompson *et al*., 2022; Rodriguez and Lee, 2024). For example, one study praised SAF for its compatibility with concentration techniques, detecting parasites in 39% of pooled samples versus 18% in unpreserved samples, but another noted its suboptimal staining for protozoa compared to LV-PVA (Rodriguez and Lee, 2024; Thompson *et al*., 2022). Such discrepancies underscore the challenge of synthesizing findings across studies, as results are heavily influenced by methodological differences, including staining protocols (e.g., trichrome vs. modified acid-fast) and concentration techniques (e.g., formalin-ether vs. sedimentation) (Ahmed and Mohamed, 2023; WHO, 2022).

2.12.1 Understudied Performance of Preservatives After Prolonged Storage (e.g., 1 Month)

A significant gap in the literature on 10% formalin, sodium acetate-acetic acid-formalin (SAF), and low-viscosity polyvinyl alcohol (LV-PVA) for intestinal parasite preservation is the limited evaluation of their performance after prolonged storage periods, such as one month or longer. Long-term preservation is critical for diagnostic laboratories, particularly in resource-limited settings where samples may be stored for extended periods before analysis due to logistical constraints (World Health Organization, 2022). However, most studies focus

on short-term storage (days to weeks), leaving the efficacy of these preservatives over longer durations understudied.

Existing research typically assesses preservation quality within days to a few weeks. For example, a study comparing 10% formalin and LV-PVA evaluated samples processed shortly after collection, reporting high recovery rates for helminth eggs and protozoan cysts via formalin-ether sedimentation and trichrome staining, respectively (Garcia and Smith, 2021). However, the study did not extend its analysis beyond two weeks, limiting insights into long-term morphological integrity (Garcia and Smith, 2021). Similarly, a comparison of 10% formalin, SAF, and LV-PVA assessed 300 samples but focused on immediate processing, with no data on storage beyond one month (Thompson *et al*., 2022).

SAF's performance has been studied for up to one week in pooled fecal samples, showing a 39% detection rate for parasites compared to 18% in unpreserved samples, but longer storage periods were not investigated (Rodriguez and Lee, 2024). Another study noted SAF's ability to maintain protozoan detection at 62% in 250 patients, yet the storage duration was not specified beyond initial processing, leaving its long-term efficacy unclear (Ahmed and Mohamed, 2023). LV-PVA is often cited for its stability in permanent stained smears, potentially preserving protozoan morphology for months due to its adhesive properties, but quantitative data on specific timelines (e.g., one month, six months) and storage conditions (e.g., ambient vs. refrigerated) are rarely provided (Thompson *et al*., 2022).

A recent study on 10% formalin in non-human primate samples suggested it maintained filarial larvae morphology effectively, but the storage duration was not explicitly extended beyond a few weeks, and no long-term comparisons with SAF or LV-PVA were included (Hassan *et al*., 2024). The lack of data on prolonged storage is particularly problematic for laboratories in tropical regions, where high temperatures and humidity may degrade

preserved samples, potentially affecting diagnostic accuracy for parasites like *Ascaris lumbricoides* or *Giardia lamblia* (World Health Organization, 2022).

2.13 Summary of the Literature Review

The literature on the preservation of intestinal parasites in fecal specimens using 10% formalin, sodium acetate-acetic acid-formalin (SAF), and low-viscosity polyvinyl alcohol (LV-PVA) provides critical insights into their diagnostic efficacy but reveals significant gaps that justify further research. This summary recaps key findings, identifies the most effective preservatives based on recent evidence, and underscores the necessity of a new comparative study to address unresolved challenges in parasitology diagnostics.

Recap of Key Findings: Recent studies highlight the strengths and limitations of 10% formalin, SAF, and LV-PVA for preserving intestinal parasites such as *Giardia lamblia, Entamoeba histolytica,* and *Ascaris lumbricoides*. Formalin remains highly effective for wet preparations and concentration techniques, such as formalin-ether sedimentation, preserving the morphology of helminth eggs and protozoan cysts with high sensitivity, as demonstrated in studies of Filariopsis barretoi larvae in wild capuchin monkey samples (Hass *et al*., 2024). However, its carcinogenic properties and DNA fragmentation limit its use for molecular diagnostics (Hass *et al*., 2024; WHO, 2022). LV-PVA excels for permanent stained smears, particularly for protozoa, due to its adhesive properties that ensure clear nuclear details, but its mercuric chloride content poses significant toxicity concerns (Barreto *et al*., 2025). Studies also note that non-mercury fixatives, such as those used in modified trichrome staining, show promise for protozoan detection but lack comprehensive validation against the three preservatives (Srijan *et al*., 2021). Variability in experimental conditions, such as staining protocols and sample sizes, contributes to inconsistent findings across studies (Barreto *et al*., 2025).

Highlight of the Most Effective Preservatives: Based on recent evidence,10% formalin is the most effective preservative for wet preparations and concentration techniques, recovering a broad range of parasitic morphotypes with high diagnostic sensitivity, particularly for helminths like *Ascaris lumbricoides* and *Filariopsis barretoi* (Hass *et al*., 2024). LV-PVA remains the gold standard for permanent stained smears, offering superior clarity for protozoan structures critical for identifying species like *Entamoeba histolytica* (Srijan et al., 2021). SAF is effective for wet mounts and protozoan detection, especially in resource-limited settings, due to its lower toxicity, but it underperforms for permanent smears compared to LV-PVA (EFSA, 2018; Barreto *et al*., 2025). The choice of preservative depends on diagnostic priorities: formalin for broad morphological recovery, LV-PVA for detailed protozoan staining, and SAF for safer handling with adequate performance for wet preparations.

Need for Current Comparative Study: The literature reveals critical gaps that necessitate a new comparative study of 10% formalin, SAF, and LV-PVA. Inconsistent findings due to non-standardized methodologies, such as subjective morphological assessments and variable staining protocols, hinder definitive conclusions about their relative efficacy (Barreto *et al*., 2025; Srijan *et al*., 2021). Few studies directly compare all three preservatives under identical conditions, limiting understanding of their performance across diverse parasites and diagnostic methods (Hass *et al*., 2024). Additionally, the lack of data on long-term storage (e.g., >1 month) restricts guidance for laboratories requiring delayed analysis, particularly in tropical regions where environmental conditions may degrade samples (WHO, 2022). The toxicity of formalin and LV-PVA's mercuric chloride content further underscores the need to come evaluate safer, mercury-free alternatives like Ecofix or alcohol-based fixatives, which preliminary studies suggest may rival LV-PVA for protozoan staining but require rigorous

validation (EFSA, 2018; Srijan *et al*., 2021). A new comparative study standardizing experimental conditions, including parasite types, storage durations, and diagnostic techniques, is essential to provide evidence-based recommendations for modern parasitology laboratories, balancing diagnostic accuracy with safety and environmental sustainability.

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Study Area

This study was conducted in major slaughterhouses in Benin City, Edo State, Nigeria, selected for their significance in livestock processing. These facilities are critical to local food security, animal protein supply, and community livelihoods, providing an ideal setting for investigating intestinal parasite preservation in fecal samples collected from livestock.Notable areas include UBTH slaughterhouse(Doctor`s house) Ugbowo, Psychiatric slaughterhouse. Each of these locations hosts a variety of meat processing activities, ranging from small-scale slaughter slabs to larger, semi-commercial facilities.

3.2 Study Examination

Fresh stool samples were collected once from selected slaughterhouses across Benin City, Edo State. Each sample was divided and preserved in 10% formalin, low-viscosity polyvinyl alcohol (PVA), and sodium acetate-acetic acid-formalin (SAF). Laboratory analysis was conducted using wet mount to assess and compare the morphological preservation of intestinal parasites over time.

3.3 Sample Population

The study population comprises freshly obtained intestinal faecal specimens from slaughtered domestic ruminants specifically cattle (Bos taurus) and goats (Capra hircus) processed at selected slaughterhouses in Benin City, Edo State, Nigeria. These animal species were chosen because they are commonly slaughtered locally, represent important sources of zoonotic and veterinary intestinal parasites, and provide readily available, diverse parasite stages (eggs, larvae, cysts, trophozoites) for preservative comparison.

3.4 Inclusion and Exclusion Criteria

Inclusion Criteria

- Freshly collected stool samples from slaughtered goats, sheep, or cattle.
- Stool samples that test positive for intestinal parasites upon initial wet mount examination.
- Samples with sufficient quantity to allow sub-sampling for preservation in all selected fixatives.

Exclusion Criteria

- Decomposed or desiccated stool samples.
- Stool samples with no detectable parasitic forms during initial screening.
- Samples collected from non-approved or unsanitary slaughter locations.

3.5 Sample Size Determination

The sample size for this study was determined using the Resource Equation Approach, According to Arifin and Zahiruddin (2017), for comparative studies, the appropriate sample

size per group can be estimated based on an acceptable range of degrees of freedom (DF), typically between 10 and 20.

The formula used is:

Minimum n = (10/k) +1

Maximum n = (20/k) +1

Where:

- n= sample size per group
- k = number of groups (excluding control)

In this study, four experimental groups were considered (5% formalin, 10% formalin, low-viscosity polyvinyl alcohol, and SAF), so:

- Minimum n = (10/4) +1=3.5→ rounded up to 4 samples per group
- Maximum n = (20/4) +1= 6

To maintain DF within the recommended range (10–20), 5 samples per group will be used, resulting in a total of:

5 samples/group×4 groups = 20

Thus, a total of 20 parasite-positive stool samples will be collected and equally distributed among the four preservative groups for comparative analysis.

3.6 Ethical Considerations

Prior to commencing the study, ethical approval was obtained from the Institutional Ethical Approval Committee at Edo state Health Research Committee, Ministry of Health.This

process will ensure that all procedures involving sample collection meet local and national regulations regarding food safety and public health. The approval number given was HA/737/25/D/09080789 on 8th september, 2025

3.7 Sample Collection

A total of 20 freshly voided fecal samples were collected from slaughtered goats and cattle at three major slaughterhouses in Benin City, Edo State, Nigeria, selected for their high livestock processing volume. Samples were obtained immediately post-slaughter to ensure freshness, using sterile containers labeled with animal type, date, and slaughterhouse location. To maintain sample integrity, containers were transported to the Parasitology Laboratory, Department of Medical Laboratory Science, within 2 hours of collection.

3.8 Sample Examination

Following sample collection .upon arrival, each sample was screened microscopically for intestinal parasites using direct wet mount techniques under 10x and 40x magnification, following standard parasitological protocols (World Health Organization, 2022). Only samples testing positive for intestinal parasites (e.g., helminth eggs, protozoan cysts) were included in the study. Each positive sample was homogenized and divided into three equal portions, preserved with 10% formalin, sodium acetate-acetic acid-formalin (SAF), low-viscosity polyvinyl alcohol (LV-PVA), for comparative analysis. Preservation followed manufacturer guidelines, with 10 mL of each preservative added per gram of sample, and samples were stored at ambient temperature (25–30°C) for initial analysis.

Preserved samples were analyzed to assess parasite morphological integrity and staining quality. Parasite recovery rates and morphological clarity were quantified using a

standardized grading scale (1–5 for clarity of egg or cyst structures), ensuring consistent evaluation across preservatives.

3.8.1 Preservation Process

Each positive stool sample was homogenized and divided into four equal portions, with each portion placed in a separate sterile container. The portions were preserved as follows:

- One unpreserved (control)
- One in 10% formalin
- One in low-viscosity polyvinyl alcohol (PVA)
- One in sodium acetate-acetic acid-formalin (SAF)

The fixatives was prepared using standard laboratory protocols. All preserved samples was stored at room temperature (20–25°C)

3.8.2 Wet Mount Microscopy

Wet mount preparations was made using both normal saline and Lugol's iodine. Saline wet mounts was used to detect motile trophozoites and helminth eggs or larvae, while iodine mounts will enhance the visibility of internal structures, especially in protozoan cysts. Examinations was done under low (×10) and high (×40) magnification using a light microscope. Each preserved sample was used to assess the presence, clarity, and morphological integrity of the parasites.

3.9 Statistical Analysis

Statistical analysis was conducted using IBM SPSS Statistics version 27 (IBM Corp), Armonk, NY, USA). The preservation scores of intestinal parasite across the three different preservatives (10% formalin, sodium acetate-acetic acid formalin and Low Viscosity Polyvinyl Alcohol) were compared. Descriptive statistics (minimum, maximum, quartiles and median) were calculated to summarize preservation efficacy.

CHAPTER 4

This study evaluated and compared the ability of three stool preservatives 10% formalin, sodium acetate acetic acid formalin (SAF), and low-viscosity polyvinyl alcohol (PVA) to maintain the morphological integrity of intestinal parasites over a one-month preservation period. The preservatives were scored on a five-point scale, where 1 indicated poor preservation and 5 represented excellent preservation, to assess the clarity and structural integrity of parasite forms under microscopic examination.

Table 4.1 presents the distribution of intestinal parasites identified prior to preservation. Out of 20 stool samples, *Trichostrongylus* eggs were the most frequently observed (26.7%), followed by Coccidia spp. and *Strongyloides* larvae (23.3% each). *Paramphistomum cervi* eggs were detected in 10.0% of samples, while *Trichuris trichiura* eggs were the least common (6.7%). Percentages exceed 100% because several samples contained multiple parasite species, indicating mixed infections.

Table 4.2 shows both the parasite specific preservation scores and the summary statistics for preservative performance. *Strongyloides* larvae were best preserved by 10% formalin (score = 5), showed slightly lower preservation under SAF (4), and the lowest under PVA (3). *Strongyloides eggs*, assessed only with 10% formalin, also achieved a perfect score (5). Coccidia spp. were excellently preserved by 10% formalin and SAF (5 each) but slightly less well by PVA (4). *Trichuris trichiura* and *Paramphistomum cervi* eggs retained excellent morphology across all three preservatives (scores = 5), while *Trichostrongylus* eggs were

perfectly preserved with 10% formalin and SAF (5 each) but showed reduced quality with PVA (4).

The summary rows in Table 4.2 indicate that 10% formalin demonstrated complete consistency (Min = Q1 = Median = Q3 = Max = 5). SAF performed strongly with slight variability (Min = 4, Median = 5), whereas PVA showed greater variation (Min = 3, Median = 4, Max = 5), reflecting less uniform preservation. The Friedman test ($\chi^2 = 5.60$, df = 2, $p = 0.061$) suggests observable differences among preservatives that did not reach statistical significance at the 0.05 level.

Table 4.1: Distribution of Intestinal Parasites Among Study Samples (Pre-Preservation Findings)

Parasite Species	Number of Positive Samples	Percentage (%)
Trichostrongylus egg	8	26.70
Coccidia spp	7	23.30
Strongyloides larvae	7	23.30
Paramphistomum cervi egg	3	10.00
Trichuris trichiura egg	2	6.70

4.2 Comparative Efficacy of 10% Formalin, SAF, and PVA in Preserving Intestinal Parasite Morphology:

Parasite Species	10% Formalin	SAF	PVA
Strongyloides larvae	5	4	3
Strongyloides egg	5	–	–
Coccidia spp	5	5	4
Trichuris trichiura egg	5	5	5
Paramphistomum cervi egg	5	5	5
Trichostrongylus egg	5	5	4
Summary (Min–Max)	5–5	4–5	3–5
Median	5	5	4
p-value			0.061

Scores represent preservation quality on a scale of 1–5 (1 = poor, 5 = excellent). SAF = Sodium acetate–acetic acid–formalin; PVA = Low-viscosity polyvinyl alcohol. "–" indicates the parasite was not evaluated with that preservative. Summary statistics (Min–Max, Quartiles, Median, Maximum) are calculated across all parasite scores. The p-value is from the Friedman test comparing preservative performance (df = 2); significance set at $p < 0.05$.

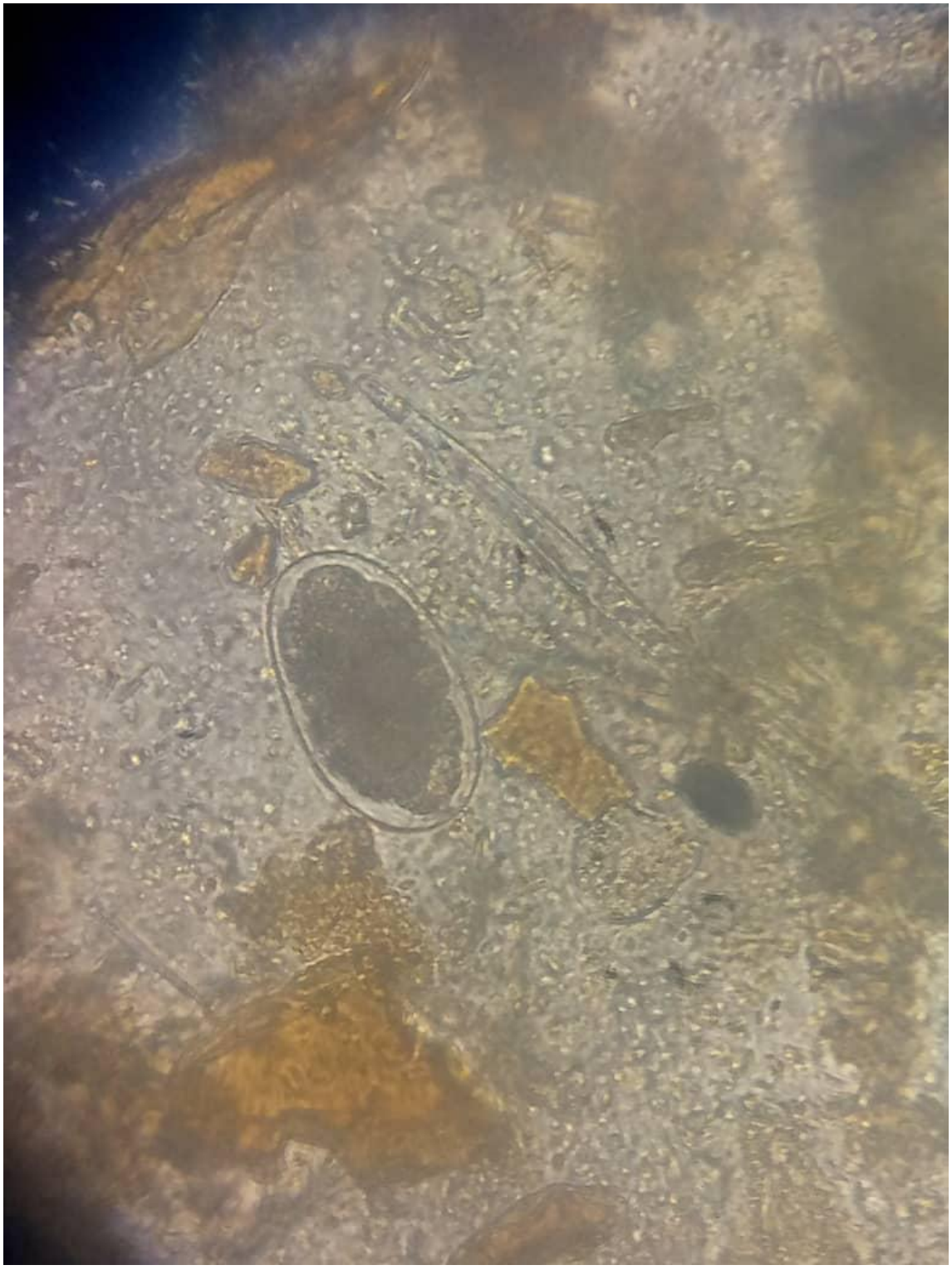

Figure 4.1 Trichostrongylus egg under 10% formalin.

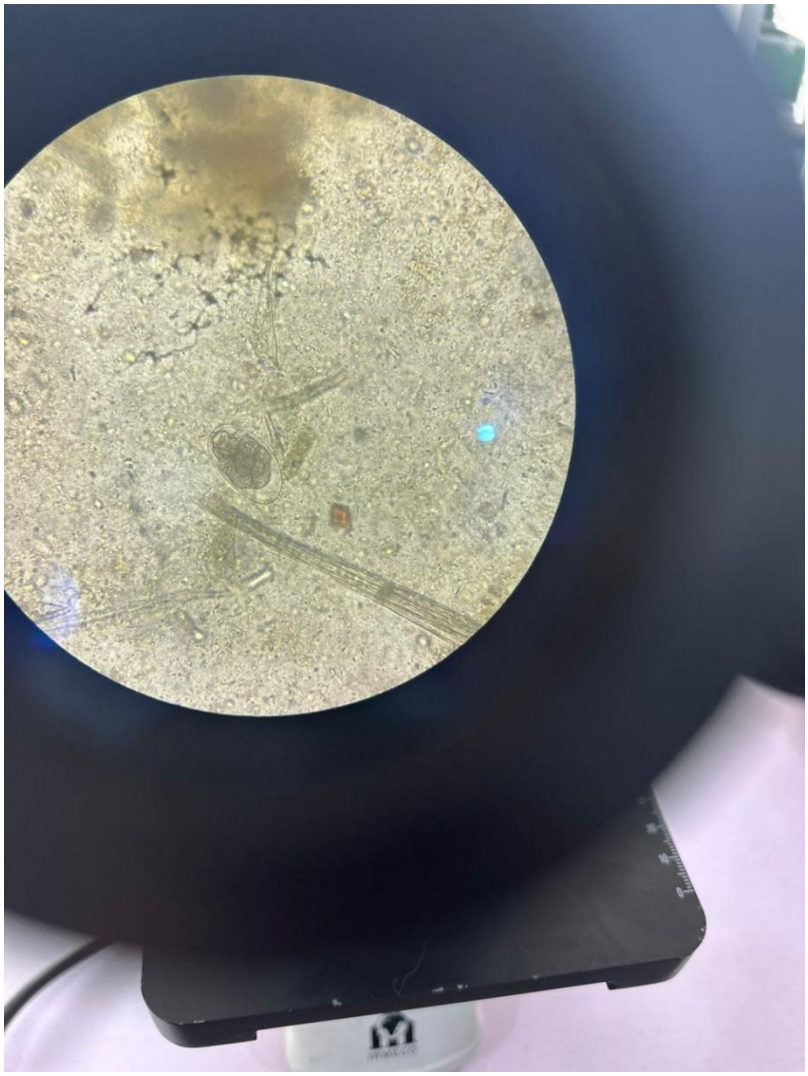

Figure 4.2 Trichostrongylus egg under low viscosity polyvinyl alcohol

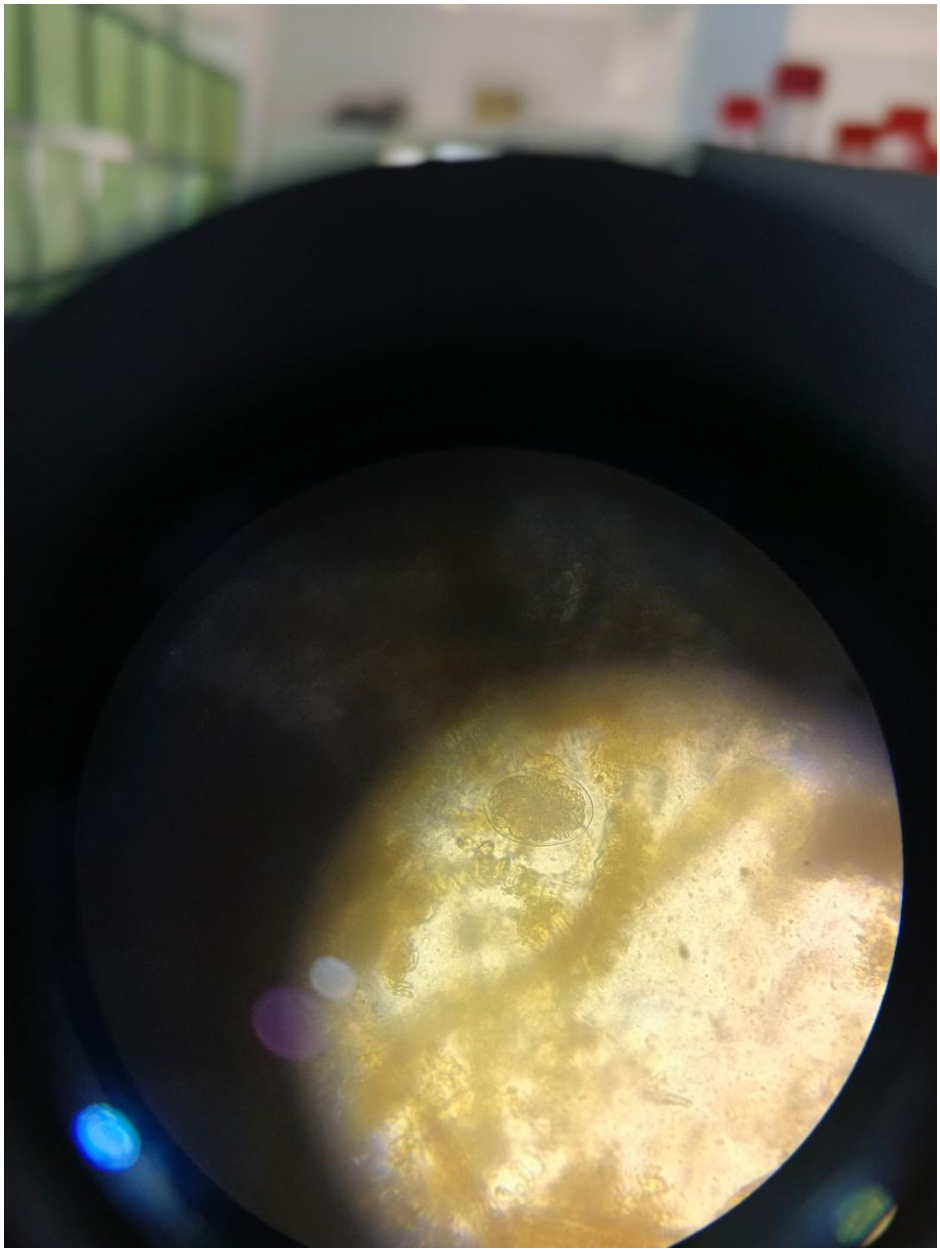

Figure 4.3 Trichostrongylus egg under sodium acetate acetic acid formalin.

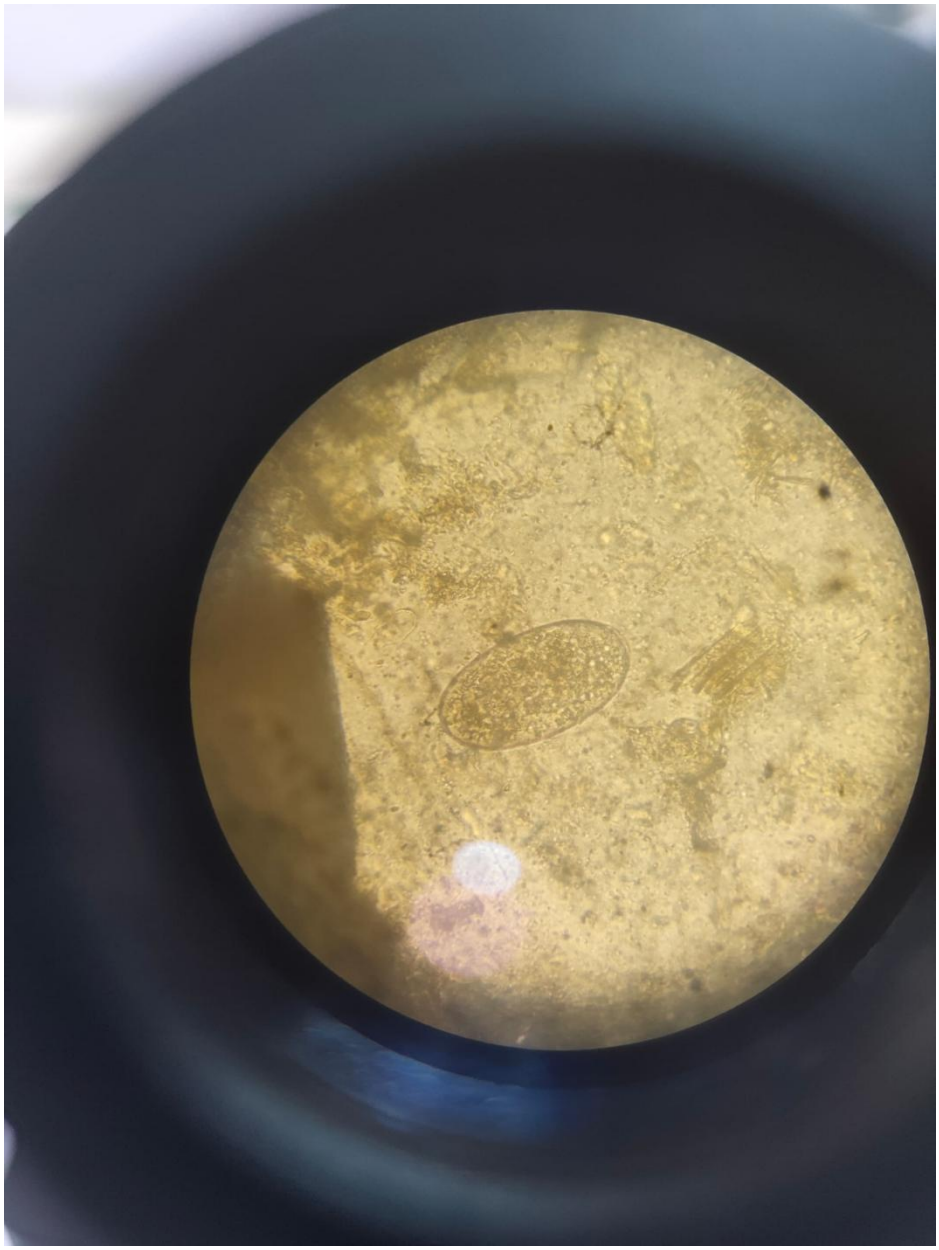

Figure 4.4 Paramphistomum cervi under sodium acetate acetic acid formalin.

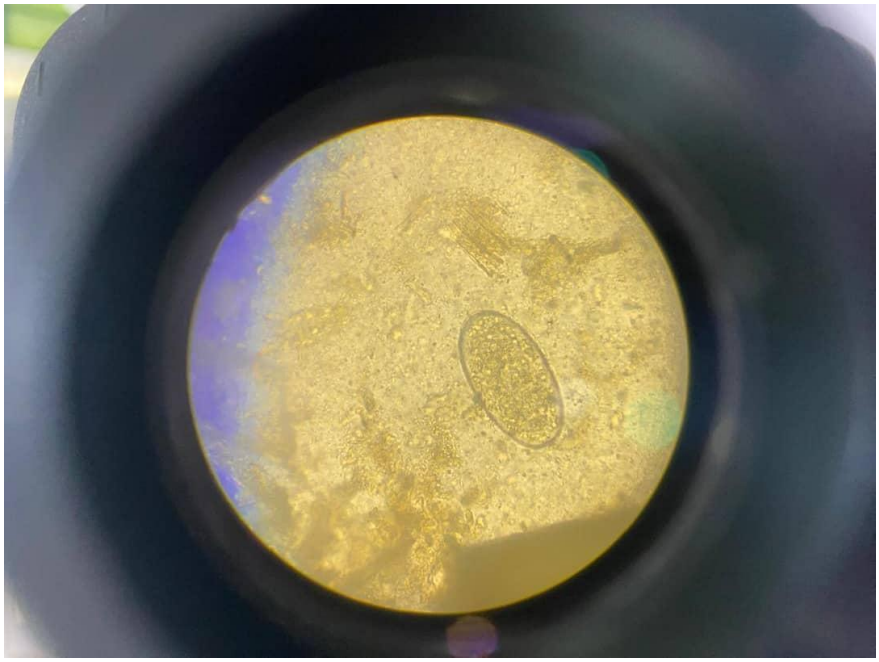

Figure 4.5 Paramphistomum cervi under 10% formalin

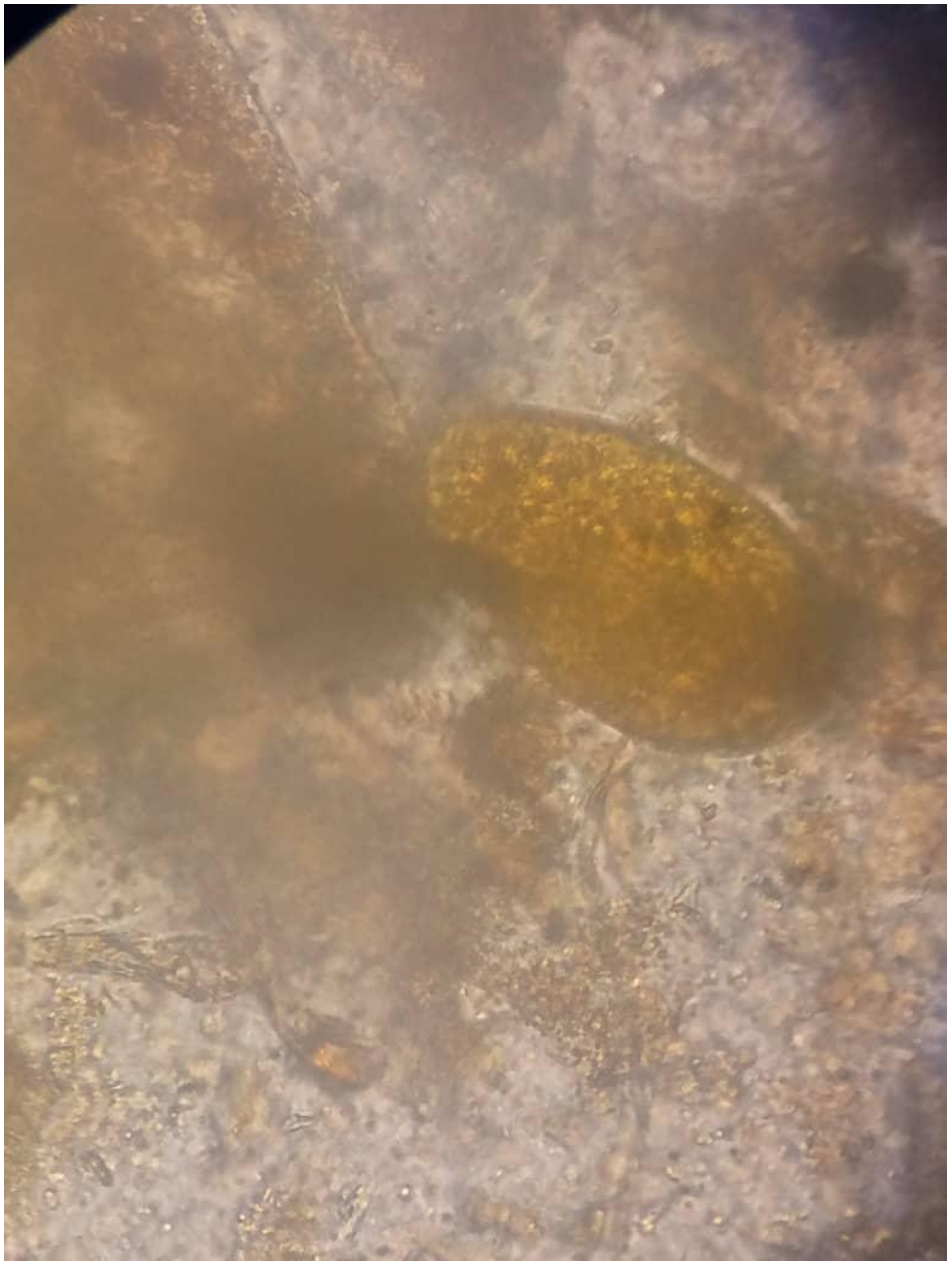

Figure 4.6 Paramphistomum cervi under low viscosity polyvinyl alcohol

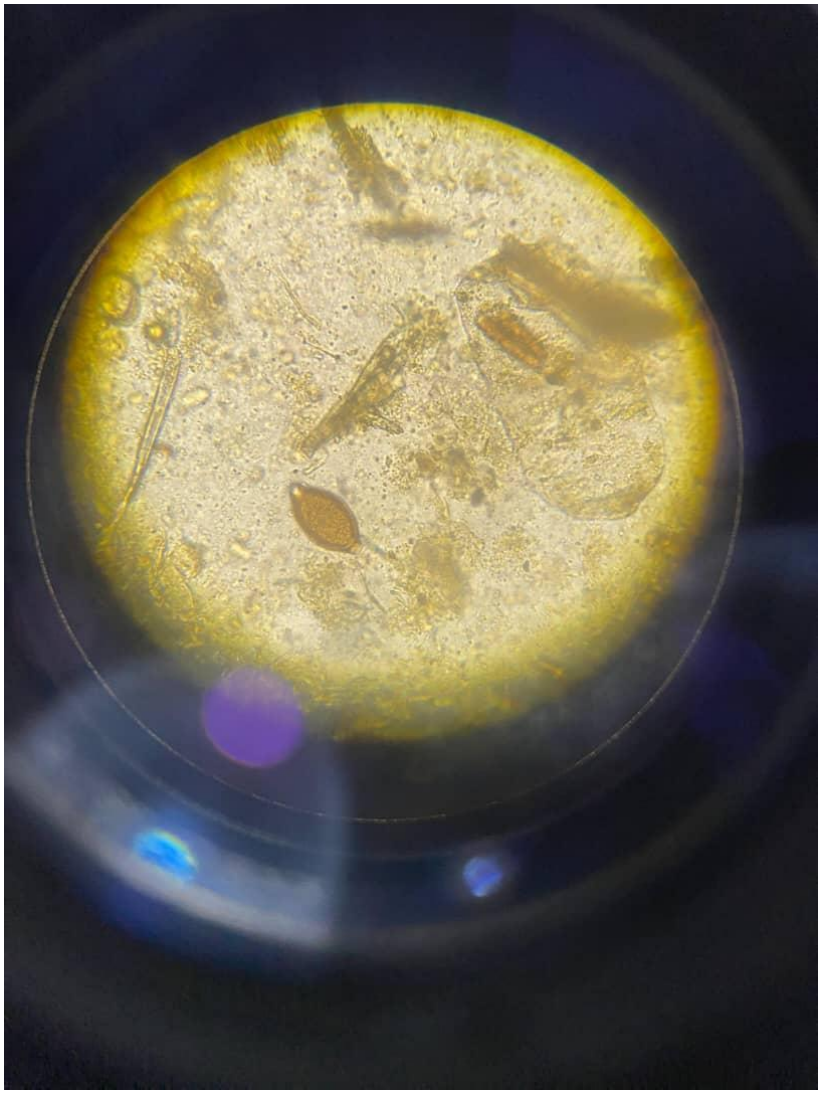

Figure 4.7 Trichuris trichuria under low viscosity polyvinyl alcohol

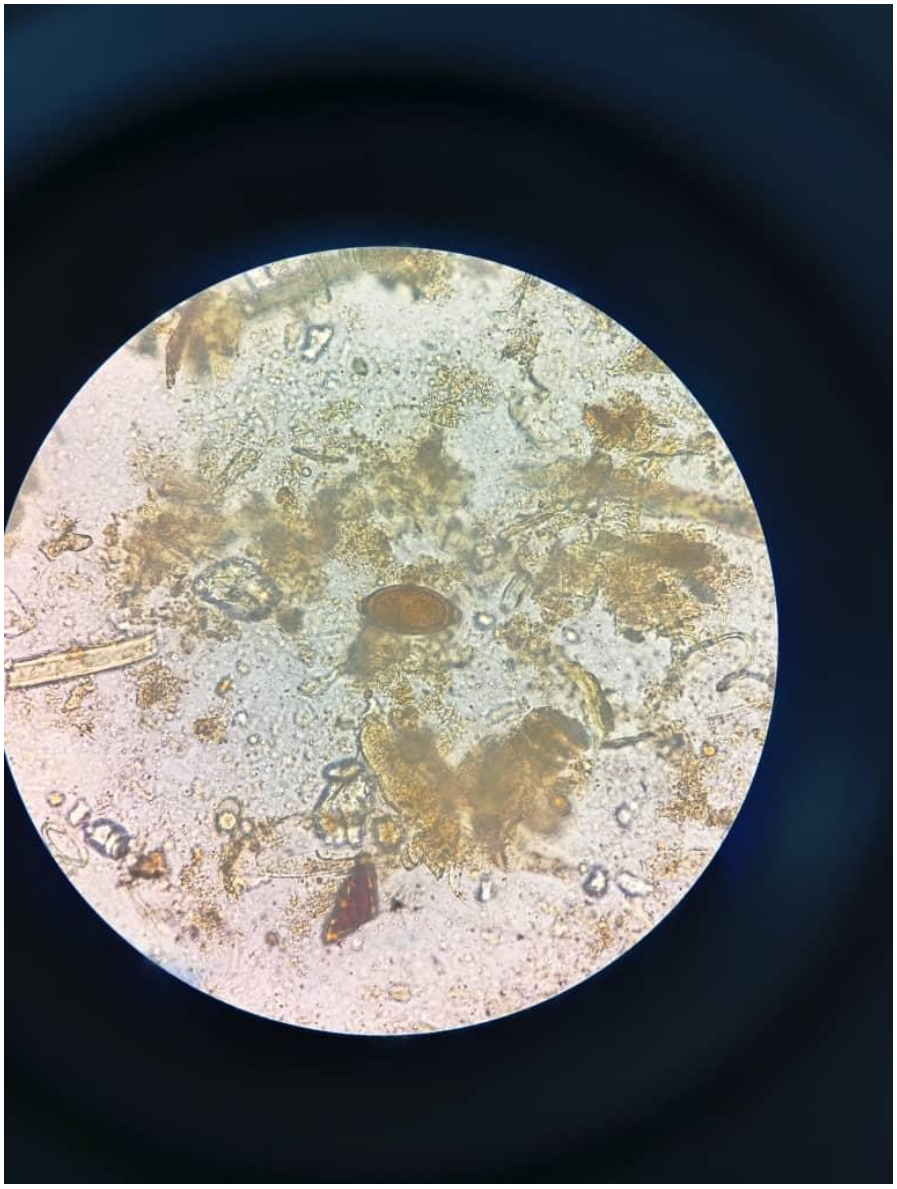

Figure 4.8. Trichuris trichuria under 10% formalin

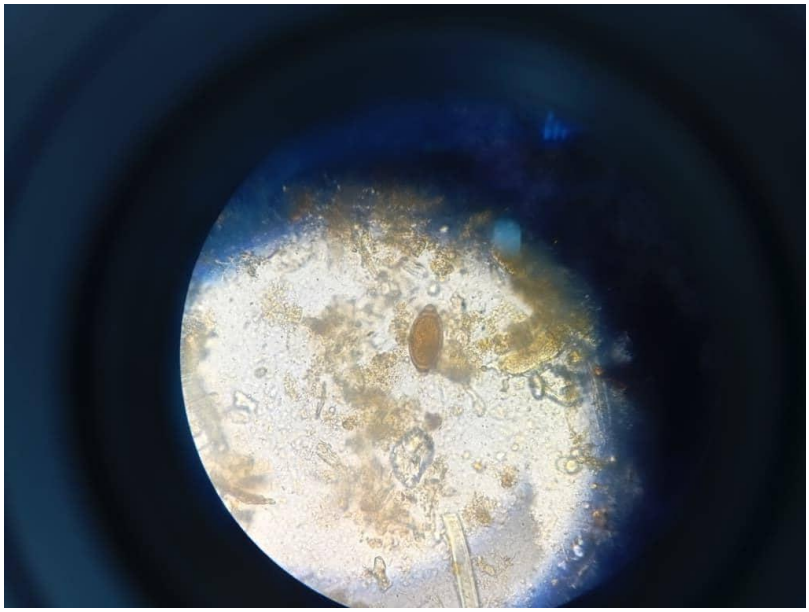

Figure 4.9 Trichuris trichuria under sodium acetate acetic acid formalin

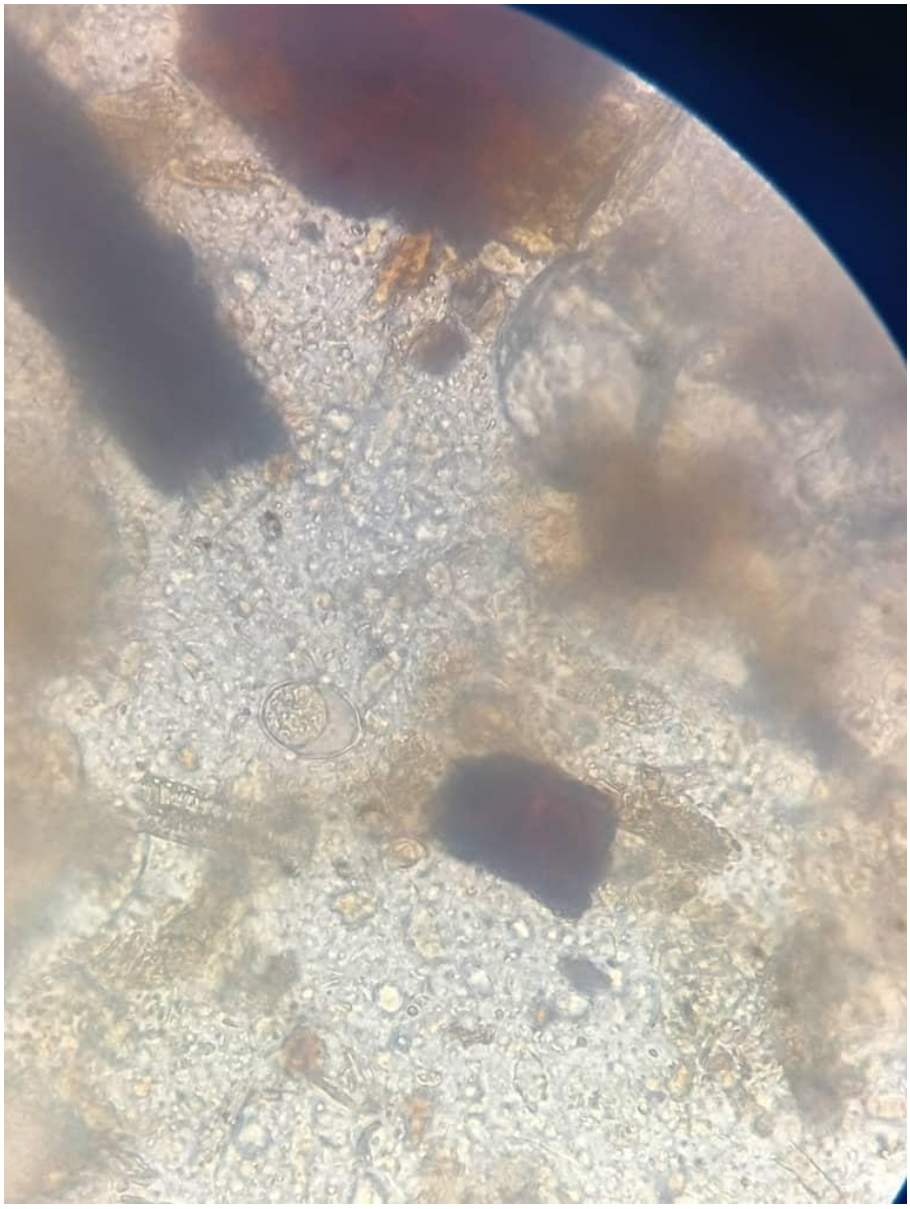

Figure 4.10. Coccidia spp under 10% formalin

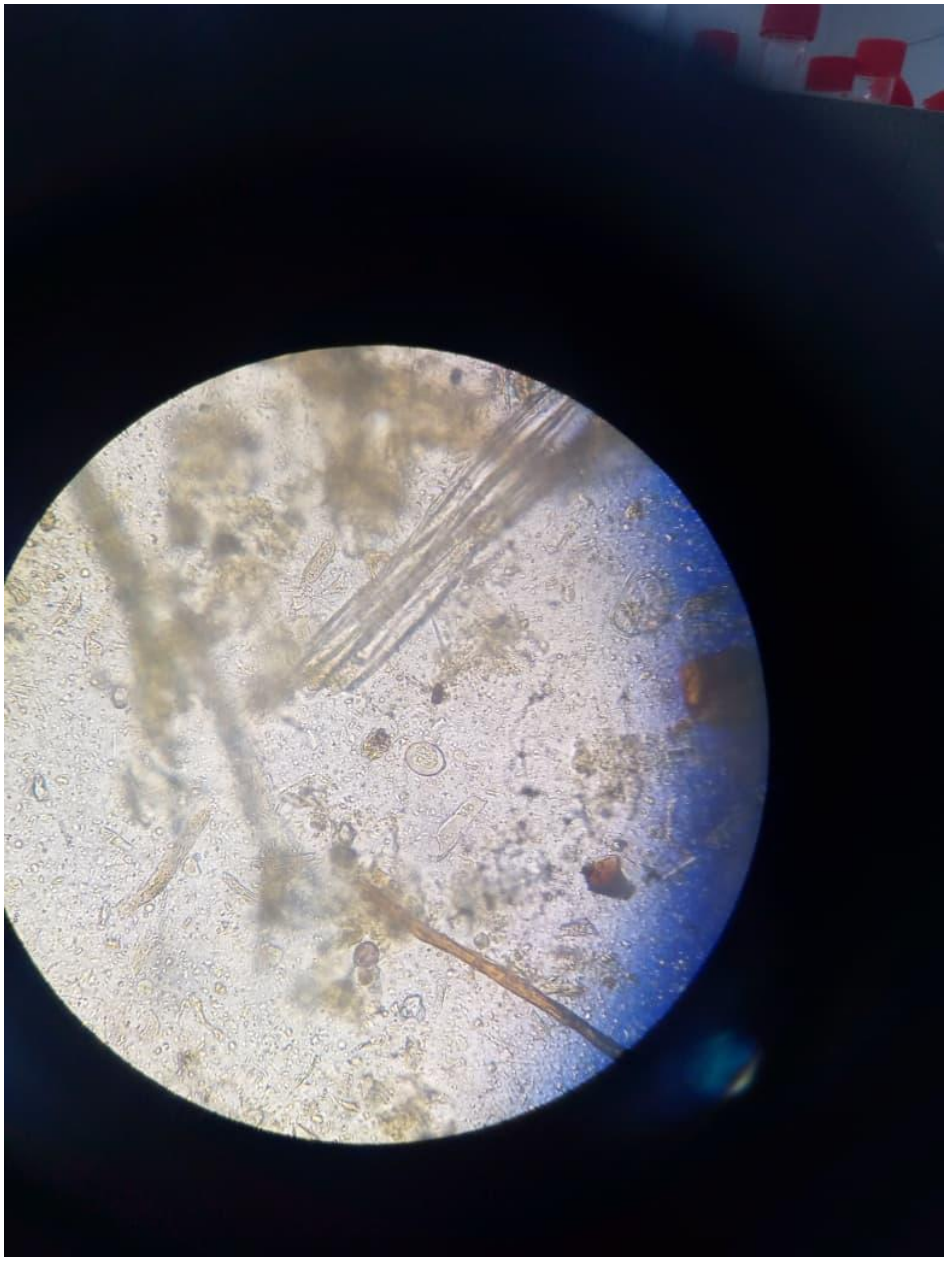

Figure 4.11. Coccidia spp under Sodium acetate acetic acid formalin

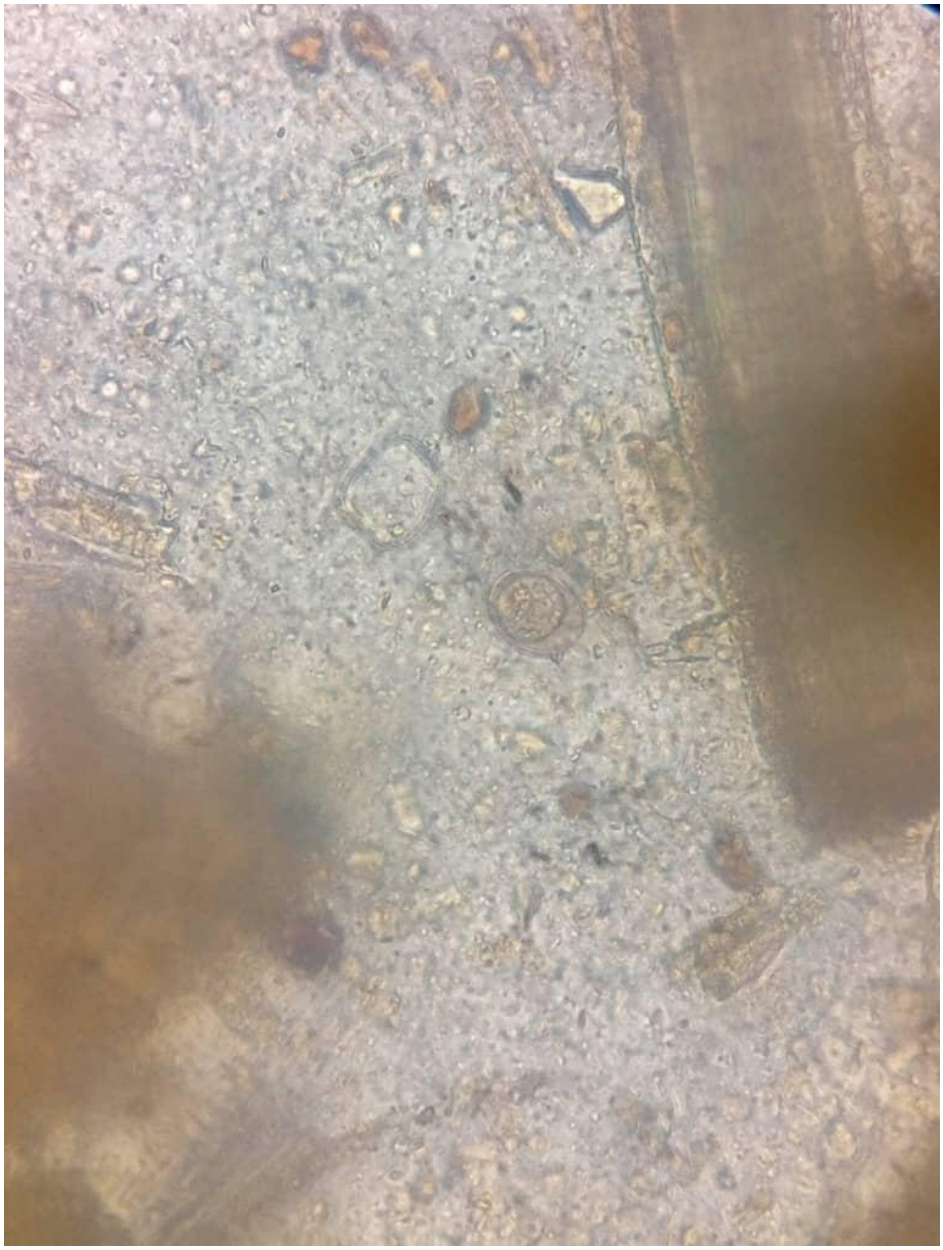

Figure 4.12. Coccidia spp egg under low viscosity polyvinyl alcohol

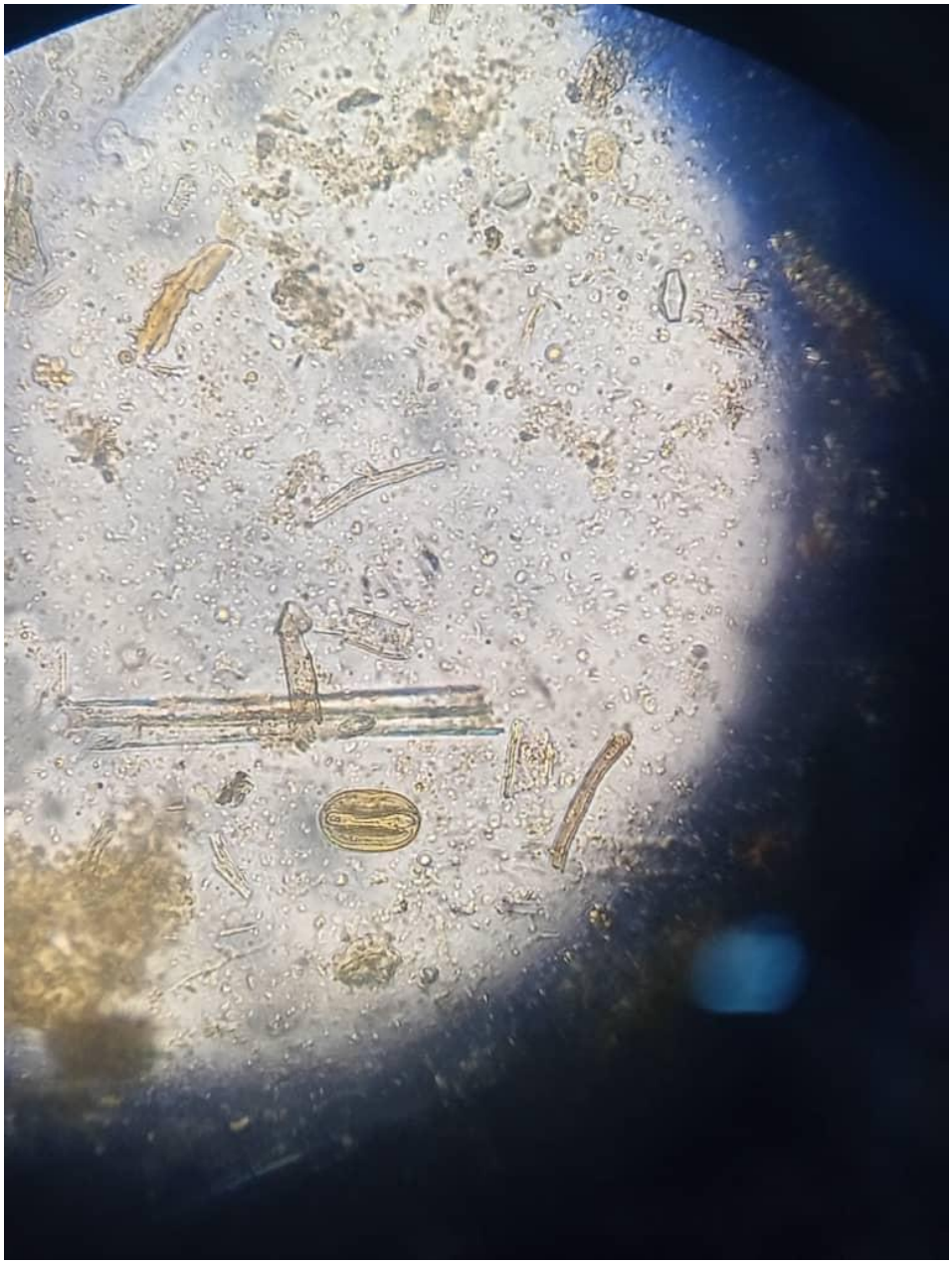

Figure 4.13. Strongyloides pappilossus egg, seen under 10% formalin alone

Figure 4.14. Larvae strongyloides pappilossus under 10% formalin

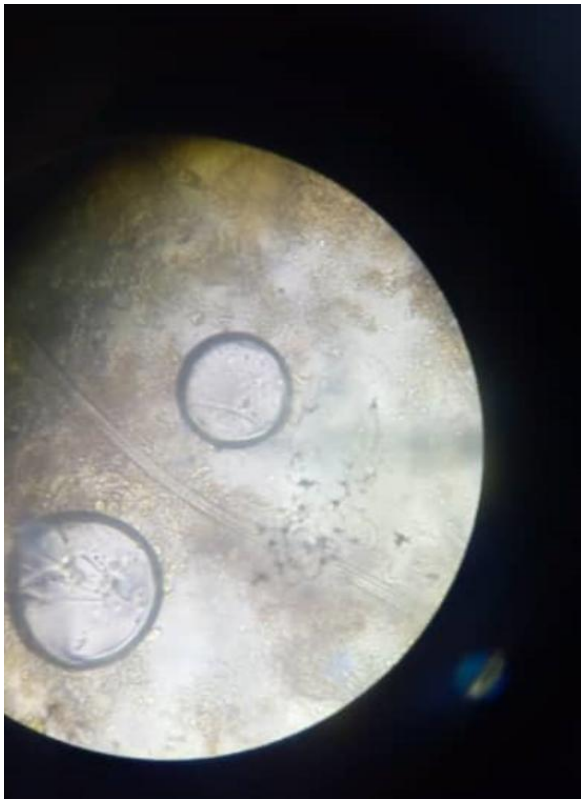

Figure 4.15 Larvae of strongyloides pappilossus under sodium acetate acetic acid formalin

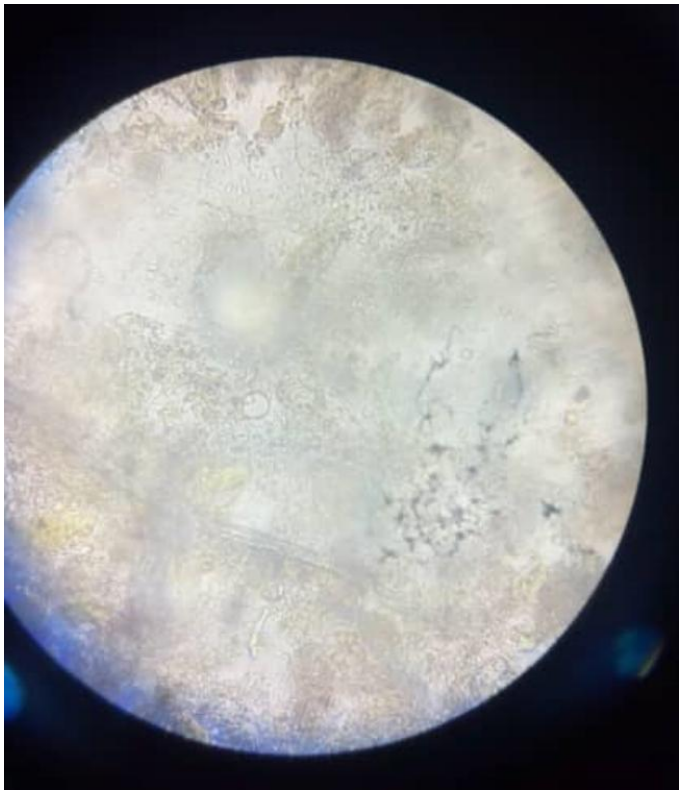

Figure 4.16 Larvae of strongyloides pappilossus in low viscosity polyvinyl alcohol

CHAPTER FIVE

5.1 Discussion and Conclusion

This study assessed the efficacy of 10% formalin, sodium acetate acetic acid formalin (SAF), and low viscosity polyvinyl alcohol (LV-PVA) in preserving the morphology of intestinal parasites for accurate microscopic identification. All three fixatives maintained the recognizable morphology of helminth eggs and protozoan cysts for at least one month, confirming their suitability for diagnostic use under routine laboratory conditions. Although the Friedman test (p = 0.061) indicated no statistically significant differences among the preservatives, qualitative evaluation revealed subtle distinctions in performance.

Across parasite species, 10% formalin consistently achieved the highest preservation scores, reflecting excellent retention of morphological detail. SAF performed almost as well, showing only minor variability while preserving helminths and protozoa with clarity sufficient for accurate diagnosis. LV-PVA displayed slightly reduced consistency, particularly for helminth eggs, but provided valuable staining compatibility for protozoan trophozoites in permanent smears.

These findings agree with earlier studies reporting formalin's effectiveness for helminth ova and protozoan cysts but its poor preservation of fragile trophozoites (Garcia, 2020; CDC, 2023; Visvesvara *et al.,* 2016). SAF's strong performance corroborates its reputation as a versatile, mercury-free preservative suitable for both wet mounts and permanent staining (Becker *et al.,* 2021; WHO, 2019). LV-PVA's reduced helminth preservation but superior staining compatibility supports reports by Garcia *et al.* (1983), Mank *et al.* (1995), and Fedorko *et al.* (2000), who documented similar strengths and limitations.

The implications are particularly relevant for diagnostic laboratories in resource-limited, high-burden regions. Using 10% formalin or SAF ensures reliable preservation when

immediate microscopy is not feasible, while LV-PVA remains critical for cases requiring detailed protozoan identification.

5.2 Conclusion

This study demonstrated that 10% formalin, SAF, and LV-PVA are all effective for maintaining the diagnostic morphology of helminth eggs and protozoan cysts during at least one month of storage. 10% formalin and SAF consistently exhibited superior preservation quality and clarity across parasite species, making them the most suitable options for routine stool examination. LV-PVA, although less versatile and less consistent for helminths, retains an important role in preserving fragile protozoan trophozoites in permanent stained smears, where detailed nuclear and cytoplasmic features are essential for differentiating species such as *Entamoeba histolytica* and *Giardia lamblia*. Overall, while all three fixatives are valuable, 10% formalin and SAF remain the most appropriate for general diagnostic use, and LV-PVA should be reserved for specialized protozoan studies.

5.3 Recommendation

Diagnostic laboratories, particularly those in resource-limited or high-burden areas, should prioritize 10% formalin or SAF for routine stool specimen preservation, as these fixatives reliably maintain helminth and protozoan morphology for accurate diagnosis. LV-PVA should be employed selectively when permanent stained smears are required for protozoan identification. Immediate fixation of stool samples at the point of collection should be emphasized to prevent degradation, especially of delicate trophozoites.

At the policy level, health authorities are encouraged to promote mercury-free alternatives such as SAF, given its safer profile for laboratory staff and the environment. Training programs should reinforce best practices for handling and storing preserved specimens, and

governments should ensure the consistent availability of these fixatives in rural and underserved communities.

For future research, long-term evaluations of preservative stability under varying temperature and storage conditions are recommended. Expanding comparative studies to include additional parasite species, such as *Strongyloides stercoralis* and *Cryptosporidium parvum*, would provide broader insights. The development of new preservative formulations combining the morphological clarity of PVA with the safety and versatility of SAF could further improve diagnostic capacity and biosafety standards.

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APPENDIX I

APPENDIX II