

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; 10(4): 532-537
www.biochemjournal.com
Received: 25-02-2026
Accepted: 29-03-2026

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Microscopic techniques in the diagnosis of food-borne parasitic zoonoses: A comprehensive review

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i4g.8219>

Abstract

The food-borne parasitic zoonoses pose a high-profile burden on the global population especially in tropical and subtropical regions where the sanitation and surveillance systems are limited. Often the most vulnerable and marginalized communities are greatly affected. Timely diagnosis is highly mandatory to implement effective treatment, disease control strategies, preventive measures and promote wellness of the humans and animal population. This review focuses on the role of microscopic techniques in the diagnosis of major food-borne zoonotic parasitic infections. The modern approaches for detecting parasitic organisms evolved from the initial breakthroughs in microscopic examination. Various approaches like direct wet mount, concentration techniques, quantitative methods and staining techniques are discussed for their applications, merits and demerits. Microscopy being the gold standard technique, requires skills and expertise and is a widely used tool even in resource limited areas. The use of microscopy in combination with other advanced diagnostic methods will provide enhanced accuracy in modern diagnosis of infections.

Keywords: Parasitic zoonosis, microscopy, staining, smartphone microscopy

Introduction

Zoonotic diseases are those that are naturally transmitted from non-human animals to humans. These are caused by bacterial, viral or parasitic agents that spread by direct contact or through contaminated food, water and environment (WHO, 2020) [20]. Parasitic zoonosis even though seems overshadowed, have a high-profile burden on the global population especially in tropical and subtropical regions (Deiana *et al.*, 2024) [6]. Some of the neglected parasites such as *Taenia solium*, *Trichinella*, *Echinococcus* and *Fasciola* are overlooked due to their milder, chronic symptoms, obscure economic losses, infecting the most vulnerable and marginalized communities. According to the World Health Organisation, *Taenia solium* caused 2.8 million disability adjusted life years (DALYs) and at least 2.4 million people from more than seventy countries were infected by *Fasciola* infections (WHO, 2022) [21]. The escalating burden of zoonotic diseases significantly affects both human and animal health, necessitating the robust surveillance and diagnostic frameworks to prevent future outbreaks. These infections lead to weakness, malnutrition, increasing the susceptibility to other diseases, impairs productivity and learning skills in children.

Amidst the increasing risks due to infectious agents, the timely diagnosis of the diseases is highly mandatory to implement effective treatment, disease control strategies, preventive measures and promote wellness of the humans and animal population. This facilitates the easement of the clinical symptoms, prevention of complications and enhances the quality of living. The invention of microscope aided scientists to observe and identify various parasitic organisms such as helminths-roundworms, tapeworms and flukes, protozoa, and arthropods. The modern approaches for detecting parasitic organisms evolved from the initial breakthroughs in microscopic examination and chemical staining techniques (Parija and Poddar, 2025) [14].

Major Food Borne Parasitic Zoonosis are,

Toxoplasmosis, cryptosporidiosis, sarcocystosis, amoebiasis, giardiasis, balantidiasis, fascioliasis, fasciolopsiasis, dicrocoeliasis, paragonimiasis, opisthorchiasis, clonorchiasis, taeniasis (cysticercosis), echinococcosis, trichinellosis, and toxocariasis.

Microscopic Identification

The microscopy mainly focuses on indication of parasitic infections by identification of eggs, cysts and parasites. It is a direct method to determine the presence of parasitic infection (Dabrowska *et al.*, 2024) ^[5].

The conventional microscopy is considered as the gold standard method for the diagnosis of intestinal parasitic diseases. Specimens examined are faeces, blood, duodenal contents, saliva, sputum, endoscopy or biopsy tissue samples. Coproscopy is performed to identify cysts and trophozoites of protozoa as well as eggs, larvae and adult forms of helminths.

Sample collection: Stool samples are collected over a period of 10 days, at two-three days interval, in order to increase the chances of detecting parasites, especially protozoan cysts that are excreted irregularly.

Microscopic examination is usually performed using the 10x objective. When parasite structures are found, the objective should be changed to 40x and 100x for closer examination.

Wet Mount Method

The World Health Organization (WHO) recommends microscopic testing by wet mount method. Direct wet mount is commonly used for the diagnosis of intestinal parasitic infections.

Advantages

- Fast and simple procedure and provides a quick answer when positive
- Estimates the parasitic burden
- Used with unpreserved specimens to detect the characteristic motility of the trophozoites of protozoan species
- **Saline wet mount:** A routine and affordable test. A small volume of faeces is emulsified with a drop of physiological saline. It helps to demonstrate the motility of the trophozoites. It is used for detection of fasciolopsiasis.
- **Iodine wet mount:** A drop of saline is mixed with a small drop of iodine solution followed by addition of stool sample the size of a match head. It aids in differentiation and identification by characteristic morphological features and internal structures. Disadvantages is that the preparation dries within few minutes which can be resolved by combining it with glycerol.
- **Buffered methylene blue wet mount:** A large drop of buffered methylene blue is placed and mixed with small volume of faeces. This stains the internal structures of trophozoites and cysts. It is used when protozoan trophozoites is suspected.
- **Lactophenol cotton blue (LPCB) stain:** LPCB can be used in wet mounts preparation to identify intestinal parasites. The stain is highly effective in visualising trophozoites, cysts, and helminthic ova, making them easily identifiable. This method was not effective in detection of *Cryptosporidium* (Parija and Poddar, 2025) ^[14].

However, the sensitivity and negative predictive value of wet mount method was found to be lower when compared to

formol-ether concentration (FEC) techniques during the stool samples (Demeke *et al.*, 2021) ^[7].

The gold standard for diagnosis of human liver fluke infections is the demonstration of eggs in faeces, bile, or duodenal fluid by microscopic methods (Sadaow *et al.*, 2019) ^[18].

Direct microscopic methods with Lugol's iodine solution has been reported with the highest recovery rate for detection of *B. coli* oocysts (Pinilla *et al.*, 2021) ^[16].

Concentration Techniques

When there is a low level of parasitic load in the specimen, the sample is subjected to concentration techniques such as sedimentation and flotation to concentrate helminth eggs, larvae, protozoan cysts and oocysts. But protozoan trophozoites cannot be recovered by these methods, making the direct wet mount method obligatory.

Sedimentation

The specimen is diluted with solution of lower specific gravity compared to parasitic organisms, thus allowing the sedimentation of organisms concentrating at the bottom. Sedimentation techniques are easier to perform and carry lower risk of error. It is used for detection of fascioliasis.

Formalin-ethyl acetate (FEA) method

Principle: Formalin fixes the egg, larvae, oocysts and spores preserving the morphology and making them no longer infectious. Faecal debris are extracted with ethyl acetate.

Procedure: A small amount of specimen is mixed with 10 ml saline and strained through two layers of gauze into a centrifuge tube. Then centrifuged at 2000 rpm for 10 minutes. Sediment is collected followed by addition of 10 ml formalin (10%). The debris are extracted by adding ethyl acetate and centrifuged for 10 minutes at 1500 rpm. Four layers are obtained (top layer of ethyl acetate, plug of debris, layer of formalin and sediment). Carefully top three layers are discarded. The parasites are sedimented at the base of the tube. The sediment is then examined under 10x and 40x objectives, with or without iodine. This technique is useful for intestinal sarcocystosis, fascioliasis, fasciolopsiasis, dicrocoeliasis and opisthorchiasis (Ali *et al.*, 2025) ^[2].

Shortcomings: Fire and explosion hazard, and it is qualitative rather than quantitative.

Flotation

Specimen is mixed with solutions of higher specific gravity such as zinc sulphate solution, saturated sugar solution (Sheather's solution), mercuric iodide solution and sodium chloride solution than the parasites, resulting in flotation of parasites at top of the solution. The filtrate is taken into a test tube up to the brim, and a coverslip is placed on the bulging surface of the suspension of the test tube. The test tube is left undisturbed for 30 minutes, followed by removal of coverslip and placed on slide for microscopic examination. Protozoan cysts are successfully detected by flotation techniques. This method should not be prolonged for longer duration as "light" eggs and cysts will eventually absorb water and reach the bottom mixing with debris. Flotation is used for detection of balantidiasis, fascioliasis and cryptosporidiosis (Pinilla *et al.*, 2021) ^[16].

Advantages

- Separates most parasites from faecal debris
- Flotation gives a cleaner wet-mount preparation than sedimentation

Disadvantages

- Heavy eggs like eggs of *Fasciola*, *Clonorchis* spp., or *Opisthorchis* spp., will sink and be lost with the sediment

McMaster flotation technique

It is used to count the number of parasitic elements per gram of faeces such as eggs per gram (EPG), oocysts per gram (OPG), cysts per gram (CPG) and larvae per gram (LPG), by use of a McMaster slide. The counting chamber is filled with 2 g of faecal suspension and diluted in flotation solution. It is used for intestinal sarcocystosis, balantidiasis, fascioliasis (Ali *et al.*, 2025; Pinilla *et al.*, 2021) ^[2, 16].

FLOTAC

Principle: The FLOTAC apparatus is a cylindrical-shaped device made of polycarbonate amorphous thermoplastic. It comprises three physical components, the base, the translation disc and the reading disc. There are two flotation chambers, for optimal examination of large faecal sample suspensions in each flotation chamber (5 ml). The two versions of FLOTAC apparatus are FLOTAC-100, that permits a maximum magnification of x100 and FLOTAC-400, with maximum magnification of x400 giving a fourfold higher magnification than FLOTAC-100 best suited for protozoan parasites. Whereas FLOTAC 100 is suggested for helminthic eggs and larvae (Cringoli, 2012) ^[4]. Fascioliasis and dicrocoeliasis can be detected by this technique.

Procedure: The faeces is homogenized and centrifuged. Supernatant is discarded, flotation solution is added and filled in both flotation chambers followed by centrifugation at 1000 rpm for 5 mins. Later, apical part of chambers are examined under microscope.

Mini-FLOTAC

It is a simplification of FLOTAC. A very accurate multivalent, qualitative, and quantitative diagnosis of parasitic eggs, larvae, oocysts, and cysts in faecal sample. It comprises of two physical components, the base and the reading disc. There are two 1-ml flotation chambers, are designed to examine faecal suspensions in each chamber and which permits a maximum magnification of x400 (Cringoli, 2012) ^[4]. It is used for detecting intestinal protozoa like *Giardia lamblia* or *Entamoeba histolytica*, fascioliasis and dicrocoeliasis.

Advantages

- Higher sensitivity and accuracy than McMaster and Kato-Katz method
- Performed on both fresh and preserved faecal material
- Requires only 12-15 minutes of preparation time before microscopic analysis

Kato-Katz Method

It is a commonly used and quantitative method recommended by WHO for detection of soil-transmitted helminths. Faeces (10-15g) is taken on a petri dish/paper, and a wire screen is placed and pressed. A cardboard is placed on a clean slide, and the hole of the cardboard is filled with faeces passed out through the wire screen, and the cardboard is discarded. Then, a malachite green-soaked cellophane is placed on the smear and examined within 30 to 60 minutes. This method is widely used in human hospitals and diagnostic centers (Lightowers *et al.*, 2023) ^[10]. It is used for detection of intestinal sarcocystosis, fascioliasis, fasciolopsiasis, dicrocoeliasis, opisthorchiasis and clonorchiasis.

Advantages

- Higher sensitivity than direct microscopy
- More time consuming and relies on collection of multiple stool samples to maximize sensitivity

Staining Methods

Staining facilitates differentiation of parasites from background in faecal smears. Common stains used include trichrome stain and iron haematoxylin for protozoan cysts and trophozoites. In contrast, blood films are typically stained with Giemsa or Wright's stain (Parija and Poddar, 2025) ^[14].

Trichrome stain is used for detection of *E. histolytica* and *Giardia lamblia*. Ziehl-Neelsen stain can be used to detect *Cryptosporidium* spp. and for differentiation of *Taenia saginata* and *T. solium* eggs. For coccidian parasites such as cryptosporidiosis and sarcocystosis, Kinyon modified Ziehl-Neelson method and Auramine phenol method are performed (Leitch and He, 2012) ^[9]. Thin smears are prepared with formalin ethyl acetate and air dried.

Kinyoun's Acid-Fast Stain

The smear is fixed with absolute methanol (1 minute) and stained with Kinyoun's carbol fuchsin for 5 minutes. Then, rinsed briefly (3 to 5 seconds) using 50% ethanol and immediately rinsed with tap water. Decolourized with 1% sulfuric acid (2 minutes) followed by thorough rinsing with water and counterstained with methylene blue for 1 minute. Slide is rinsed and air dried. Stained slides are focused under x100 and x400 with confirmation at x1000 magnification using light microscope (Abou El-Naga and Gaafar, 2014) ^[11].

Auramine-Phenol Stain

The smear is stained with auramine-phenol for 10 minutes and rinsed with water, decolourized with 3% acid alcohol (0.5% HCl in 70% ethanol) for 2 minutes. Again rinsed in tap water and counterstained with 0.1% aqueous potassium permanganate for 2 minutes. Then, rinsing and air dry done. The stained slides are focused at x100 and x400, followed by confirmation at magnification of x1000 using a fluorescent microscope (Abou El-Naga and Gaafar, 2014) ^[11].

S. No	Kinyoun's Acid-Fast Stain	Auramine-Phenol Stain
1	Requires less time (10 minutes) and screening needs around 5 minutes	Time required is around 15-17 minutes and screening can be done immediately within a minute
2	Smears are difficult to interpret, requiring confirmation for every field at x1000 oil magnification	Extremely easy as fluorescent organisms could be detected at x100, with confirmation at a magnification of x400
3	Misdiagnosis due to artifacts is common	Misdiagnosis is uncommon
4	Does not require fluorescent microscope	Requires fluorescent microscope
5	Less costly	Stain is costlier than Kinyoun's Acid Fast Stain

- In sarcocystosis, oocysts/sporocysts do not consistently take up acid-fast stain. Hence Ziehl-Neelsen method should not be recommended (Poulsen and Stensvold, 2014)^[17].
- For toxoplasmosis, the oocysts in samples are enriched by filtration or centrifugation. Giemsa and Haematoxylin and Eosin (HE) staining is simple and cost-effective. Periodic acid schiff (PAS) can stain amylopectin granules in bradyzoites.
- For amoebiasis, the gold standard for diagnosis is detection of parasite in the faeces and rectal scrapes, to identify cysts and rarely trophozoites. This identification is carried out by direct microscopy with Faust-Ferreira concentration method (specifically used for amoebiasis using saturated zinc sulphate solution). Trophozoites can only be seen in a loose 'hot stool' (a stool that is examined, ideally within 20 minutes) (Cooney *et al.*, 2025^[3]; Moran *et al.*, 2023)^[11].
- Microscopic examination of giardiasis involves Ritchie technique, a formalin-ether sedimentation method which involves mixing of stool with formalin, filtering, adding ether, and centrifuging. This separates the debris and ether from the concentrated sediment, where parasites like *Giardia* cysts and trophozoites are found more effectively (Vicente *et al.*, 2024)^[19].

Ziehl-Neelsen method

A thin layer of faeces is spread on the centre of the glass slide using a cotton swab. Let air-dry, fix with methanol for 5 min and add carbol fuchsin for 4 minutes, and rinse until the water runs clear. Then, wash and decolourize with the hydrochloric acid-ethanol and rinse until the water is clear. The slide is flooded with methylene blue for 1 minute, washed with tap water and dried. The slide is to be observed microscopically with immersion oil at 40x and 100x.

Application: Cryptosporidiosis and Balantidiasis

Giemsa staining

It is the gold standard staining technique for both thin and thick smears to examine blood parasites. Giemsa stain is a differential stain with combination of reagents such as Azure, Methylene blue, and Eosin dye.

Procedure

Thin film staining

- On a clean slide, a thin film of blood smear is made and let to air dry
- The smear is dipped (2-3 dips) into pure methanol for fixation, let air dry for 30 seconds
- Flood the slide with 5% Giemsa stain solution for 20-30 minutes
- Flush with tap water and leave to dry

Thick film staining

- Add a thick smear of blood and air dry for 1 hour on a staining rack
- Dip the thick blood smear into diluted Giemsa stain
- Wash the smear by dipping in buffered distilled water for 3-5 minutes
- Let air dry

Periodic Acid Schiff (PAS) staining

Procedure: Deparaffinize the sections and hydrate to water. Oxidize in 0.5% Periodic acid solution for 5 minutes. Rinse with distilled water. Place in Schiff's reagent for 15 minutes (Sections become light pink at this step). Wash in tap water (5 min) until the sections turn dark pink. Counterstain in Mayer's haematoxylin (1 minute). Wash in tap water for 5 minutes then rinse in distilled water. Dehydrate, place coverslip and mount using Xylene based mounting media. PAS effectively stains bradyzoites within tissue cysts in toxoplasmosis. This stain helps to differentiate tissue cysts from other tissue components.

Haematoxylin and Eosin (HE) staining

Principle: Eosin dye is acidic, negatively charged and stains the basic structures of a cell (acidophils) as red or pink colour and haematoxylin is basic, positively charged thus staining the acidic structures, tissues and cell structures (basophilic) as purplish blue. Haematoxylin is not basic by itself and requires to be conjugated with a mordant (aluminium salt) before it is used so as to strengthen its positive charge for efficient binding to the tissue components.

Procedure

1. Clean the sections with distilled water
2. Then stain nuclei with the alum haematoxylin (Mayer's) to fix the tissue for about 5 minutes
3. Rinse with running tap water
4. Using the differentiator, 0.3% acid alcohol and note the endpoint (background becomes colourless)
5. Rinse the stain in running tap water
6. Rinse the stain in Scott's tap water substitute which shortens the time for the correct end-point
7. Rinse with running tap water
8. Flood the smear with eosin for 2 minutes
9. Dehydrate the smear, clear, and mount using a clean coverslip

Nuclei are stained blue, whereas the cytoplasm and extracellular matrix are stained pink.

Stoll's ova counting method (Fascioliasis)

3g of faeces is mixed with 42mL of 0.1N NaOH solution. The suspension is sieved, and 0.15ml of the filtrate is transferred to a clean glass slide, then covered with a cover slip. Then observed under microscope. The average number

of each sample is calculated, and EPG is estimated simply by multiplying the average number of eggs with 100. The method is very simple and useful for calculating EPG in helminth infections caused by trematodes (Ali *et al.*, 2025) [2].

Modified Stoll's ova dilution technique

3g faeces is mixed with 42 ml of water to make 45 ml suspension, which is sieved and 0.15ml suspension is spread on slides, covered with cover slips and examined where number of cyst/trophozoites is counted. The sample is considered positive when atleast one cyst (round shape) or ciliated trophozoite is identified in the microscopic field. This technique detected balantidiasis (Paul *et al.*, 2019) [15].

Fluke Finder Kit

This kit consists of two sieves with mesh sized around 125 and 30nm and each 2 inches wide. Procedure is mixing 2g of faeces with water and liquid soap, pouring the mixture into the Fluke finder unit, and rinsing it thoroughly. The kit left to settle for 2 min, and the supernatant is discarded. The leftover material is examined under a dissecting microscope with a 10x magnification by adding three drops of methylene blue. Fluke finder is the most accurate method for *Fasciola* egg detection, with a sensitivity of 80% and a specificity of 90% compared to sedimentation and 100% in artificially spiked stool samples (Ali *et al.*, 2025) [2].

Detection of paragonimiasis

- **Sputum:** Morning sputum samples are ideal for direct wet smear examination to detect the parasite ova. The rusty brown or blood-stained sputum contains numerous ova and Charcot-Leyden crystals.
- **Pleural fluid:** The centrifuged deposit of pleural fluid are examined for *Paragonimus* ova in cases of pleural effusion.
- **Stool:** Stool examination for ova is recommended in children who swallow sputum and those patients' whose sputum samples are apparently negative for ova. Ideally, two to three stool samples collected at consecutive days should be examined by formalin-ether sedimentation.

Blood: Blood is tested for the presence of blood protozoa and rarely in case of intestinal parasites. Identification of peripheral eosinophilia is one of the initial steps in the diagnosis of parasitic infection.

- Thin blood smear-used to determine parasite morphology
- Thick blood smear-used when parasitic density is low

The sample is collected from capillary blood. Both thin and thick smears are prepared after 30 mins of collection. The smears are usually stained using Giemsa stain or H&E stain. Microscopic examination is performed under 100x objective. It can be time-consuming, intrusive and unreliable. The lack of specificity in the early signs makes the diagnosis even more challenging. However, microscopic examination of blood smear is reliable in acute infection as there is high level of parasitaemia in the blood.

Advantages and disadvantages of microscopic examination

Advantages

- Relatively low cost

- Wide accessibility

Disadvantages

- Time consuming and labour intensive
- Highly dependent on the skilled and expertise
- Fails when number of parasites is very low
- Lower sensitivity and specificity compared to molecular methods
- Cannot differentiate between some pathogenic and non-pathogenic species, like *Entamoeba histolytica* and *E. dispar*
- Stool tests cannot diagnose extraintestinal infection

Hence microscopy is to be implemented in combination with other diagnostic techniques in order to attain more reliable results (Ndao, 2009) [13].

Smartphone-based microscopic systems

These advanced version of microscope allows the user to magnify and visualize the microorganisms on the phone screen immediately and also capture images and videos with the smartphone (Nakarmi *et al.*, 2023) [12]. The microscope consists of a single-ball lens of 0.8 mm in diameter inserted into a plastic jacket that is fixed to a smartphone. The smaller the ball lens, the larger will be the magnification. This method was used for cases of Giardiasis and Cryptosporidiosis (Kobori *et al.*, 2016) [8].

Advantages

- Portable, cost effective, and more accessible than traditional brightfield microscopes
- Smartphones offer capabilities such as telediagnosis, store-and-forward imaging, live-streaming and the software development for complex image analyses

Disadvantages

- Requires trained technicians and unavailable in resource-limited settings
- Ball lens device has reduced resolution at the periphery of the field of view, due to the spherical nature of the focal surface (Kobori *et al.*, 2016) [8].

Conclusion

The socioeconomic burden due to parasitic infections are severe in regions with most prevalence, with lack of robust healthcare resources and surveillance programmes. This significantly deteriorates the public health, lifestyle, work productivity, and nation economic stability. To mitigate these rising risks across nations, it is mandatory for the timely diagnosis for the precise care of individual wellness and effective control of infection. The traditional microscopic examination being the gold standard, when combined with other advanced diagnostic methods will enhance accuracy in diagnosis and greatly improve the economic growth and standard of livelihood worldwide.

Acknowledgements

Funding: The authors declare that no funds were received during the preparation of this manuscript. The authors have no relevant financial or non-financial interests to disclose.

Conflict of Interests: None

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