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IDENTIFICATION OF PARASITES FROM POULTRY FAECES BY WET MOUNT METHOD AND CONCENTRATION TECHNIQUES

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ABSTRACT

Background: Poultry faeces may contain many harmful parasites, which may pose significant health risks to the consumer. These parasites may then be transmitted to man by contaminated soil, drinking water supply or by mechanical vectors like flies and rodents. This is a neglected area of public health and deserves more scientific attention. We explored Scientific literature and also assessed our findings. **Methods:** One hundred (100) poultry faeces samples were assessed for viewing parasites by wet mount method. A concentration technique was performed in saturated saline solution, followed by slide preparation and microscopic identification. **Results:** In our experiment, coccidian oocysts, *Entamoeba* oocysts and rarely some *Giardia* oocysts, larvae of *Strongyloides* spp. and trematode eggs were found. **Conclusion:** Keeping in view the public health importance of parasites in poultry faeces, open poultry shops should be cleaned and properly sanitized to mitigate health risks to humans and also the environment.

KEYWORDS: Poultry, faeces, parasites, one health.

INTRODUCTION

Poultry is one of the most important and fastest growing sectors of agriculture in India. Poultry sector plays a crucial role in meeting the protein and nutritional requirements of the population.

India, today is one of the largest manufacturers of eggs and broiler meat. Over the past two decades, the poultry sector in India has undergone a remarkable transformation, evolving from a modest and backward farming practice into a thriving mega-industry with a substantial workforce.

Various domesticated bird species, including chickens, ducks, geese, guinea fowl, and turkeys, fall under the purview of the poultry sector. Among these, chickens (Gallus gallus domesticus) hold particular prominence due to their pivotal role in providing both eggs and meat, contributing significantly to protein-rich diets. Diverse breeds of chickens, such as Rhode Island Red, Leghorn, Plymouth Rock, Wyandotte, and Orpington, are integral components of the poultry industry.

Chicken particularly stands out as an excellent dietary option. It serves as a low-calorie and low-fat source of high-quality protein, making it a favourable choice for health-conscious consumers. Beyond its macronutrient composition, chicken renders a nutritional profile rich in essential nutrients, playing a vital role across various stages of life. Additionally, chicken contains tryptophan, an amino acid known for its role in elevating serotonin levels in the brain, thereby contributing to overall well-being. Dark and white meat chickens contain vitamin B1 and choline which promote brain development in children.

In summary, the poultry industry in India has not only become a key player in meeting the protein needs of the population but has also undergone a transformative journey, emerging as a significant contributor to the country's agricultural and economic landscape.

Chicken faeces, also known as droppings, contain 0.5%- 0.9% Nitrogen, 0.4% - 0.5% Phosphorus and 1.2% -1.7% Potassium. A single chicken produced approximately 8-11 pounds of chicken faeces monthly. Chicken faeces serve as a potent natural fertilizer, enriching the soil with nutrients vital for plant development.

Poultry may also be infested with numerous kinds of parasites. Parasitic diseases in poultry chickens are common due to several reasons such as mismanagement, malnutrition, diseases and predation. Haemoparasites are often transmitted by bloodsucking arthropods like mosquitoes, biting midges or flies [1]. Protozoan parasites can infect the human intestinal tract causing serious diseases. Most prominent intestinal protozoan pathogens found here are *Entamoeba* histolytica, Cystoisospora and *Entamoeba* coli. Others are *Giardia* lamblia, Cryptosporidium, *Strongyloides* stercoralis (Rhabditiform larvae), Blastocystis hominis and Fasciola. We regard these organisms as obligate pathogens because they may cause symptoms in otherwise completely healthy individuals and disappear after clearance by the immune system and/or successful chemotherapy.

Poultry coccidiosis is considered as one of the most significant protozoan diseases affecting poultry worldwide, causing substantial economic losses to the industry. The disease, predominantly caused by intracellular parasites of the genus Eimeria, is endemic in many tropical and subtropical regions, primarily transmitted through the feco-oral route. Its impact is particularly notable among young chickens and those managed intensively.

Clinically, poultry coccidiosis manifests through symptoms such as bloody diarrhea, ruffled feathers, dehydration, and paleness of the comb. Post-mortem examinations often reveal thickening of the intestine, haemorrhage, and necrotic enteritis at specific sites, varying with the species of Eimeria involved. Diagnosis of coccidiosis relies on clinical observation, coprological analysis, and post-mortem examinations, though biochemical and molecular methods have become increasingly common in recent

years. Despite available treatment options such as anticoccidial and sulfa drugs, the emergence of drug resistance poses a significant challenge to effective management.

The economic impact of coccidiosis is substantial, stemming from both production losses and the costs associated with treatment and prevention. Thus, it remains imperative for the poultry industry to prioritize the implementation of stringent management practices, including robust hygiene and biosecurity measures, to effectively control and prevent the spread of this debilitating disease.

Entamoeba histolytica colonizes the colon and, unlike the former pathogens, may invade the colon wall and disseminate to other organs, mainly the liver, thereby causing life-threatening amebiasis. It is the causative agent of amoebiasis, a condition characterized by gastrointestinal symptoms such as diarrhea, abdominal pain, and sometimes severe complications including liver abscesses. In cases where poultry are infected, their faces can serve as a potential source of contamination for the environment. If E. histolytica is suspected in poultry, appropriate measures should be taken to prevent its spread. This may include improving hygiene practices, ensuring clean water sources, and implementing measures to control vectors such as flies that can contribute to the spread of the parasite. *Giardiasis* is caused by faecal contaminations of drinking water, food, or direct contact with faeces, waterborne transmission being regarded as a major source [2]. *Giardia* duodenalis (syn. *Giardia* lamblia or *Giardia* intestinalis) is the species commonly associated with infections in humans and animals. While poultry are not the primary hosts for *Giardia*, they can carry the parasite and shed it in their faeces, potentially contaminating the environment. Pathogenesis and virulence depend on the genotype of the *Giardia* strain and the immune and nutritional status of the host. *Giardia* exists in two morphologic forms: the multi-flagellated trophozoite (four pairs of flagella) and the cyst. The trophozoite is di-nucleated, pear-shaped, multi-flagellated, 9 to 15 µm long, 5 to 15 µm wide, and 2 to 4 µm thick, with an adhesive disk on the ventral surface. While poultry are not considered the primary reservoir for *Giardia* transmission to humans, there is still a potential risk of zoonotic transmission.

Poultry birds are sometimes not covered by the prophylactic program against parasitic diseases. We conducted this cross-sectional study to determine the prevalence of gastro-intestinal helminths, protozoans and hemiparasites in poultry chickens.

The common gut parasites that infect poultry include helminths, cestodes, nematodes, trematode and protozoans with mixed infections being widespread. The poultry chicken becomes infected by picking up worm eggs from litter, soil or droppings. Once in the gut, the eggs hatch and mature [3]. The eggs of the worms are excreted in the birds' droppings and the cycle repeats. These parasites may spread to humans when they consume contaminated or uncooked meat and may cause severe diseases. Parasitosis should not be neglected, as they have an adverse effect on the health of the animals as well as humans around them and can be detrimental [4].

Many concentration methods are present to detect parasites from stool but some of them are very expensive and non-specific hence, there is a need of simple and effective method for concentrating stool samples and viewing parasites. Hence, we tried to find the different parasites causing human infection from poultry faeces. This comprehensive research study aims to provide an in-depth understanding of

the public health implications of important parasitic poultry diseases and how these diseases can be managed to reduce human health impact. As the focus is on smallholder systems, including scavenging extensive systems and small-scale semi-intensified or intensified systems, most of the literature is from Kolkata, West Bengal, where smallholder systems predominate, despite the rapid growth of intensive systems. The isolation of different parasites from poultry birds involves various techniques and methods to identify and study these organisms. Parasites that commonly affect poultry include helminths (worms), protozoa, mites, lice, and ticks. Faecal samples are commonly examined for the presence of helminth eggs (nematodes, cestodes) and protozoan cysts. Techniques such as floatation, sedimentation, and faecal cultures may be used. The concentration method of ova and parasite examination was the commonest method of isolation of Coccidia and other protozoan parasites identified in the current study. In conclusion, Coccidial infection is high in food obtained from poultry birds. Therefore, to tackle the disease, there is a need for a concerted effort in the identification, diagnosis and general prevention and control of the disease.

Coprological examination

The most popular parasitological examination approach for diagnosing chicken coccidiosis is the coprological examination. It is a qualitative technique for detecting the oocyst by precisely conducting the procedure [5]. The first step is to collect 3-5 gm of faeces from the upper surface of the litter immediately after birds have dropped, followed by dissolving the faeces in 20-30 ml of floatation solution (NaCl), then shifting the faecal suspension through a tea strainer into a beaker. The sample is subsequently centrifuged for 3-5 minutes at 1500 rpm. Finally, it is examined under a microscope under the low power objective lens [5]

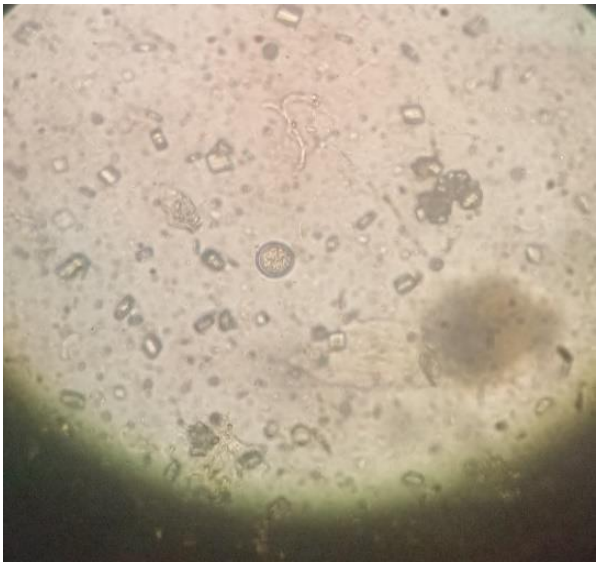

Figure 1 *(Entamoeba histolytica.)*

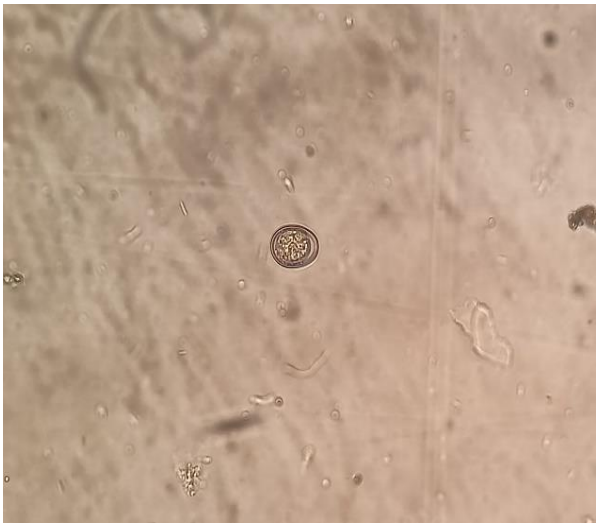

Figure 2 *(Coccidian oocyst.)*

Harada-Mori Method to distinguish between *Strongyloides* and hookworms

The Harada-Mori test-tube filter paper method increases the chances of recovering *Strongyloides* from intestinal samples [6]. This method utilizes the natural water tropism of larvae to concentrate them. A recent, unpreserved and unrefrigerated fecal sample is smeared on a strip of blotting paper. The strip is placed in a 40-mL screw-capped tube containing a few milliliters of water which continuously soaks the paper. The tube is incubated at 24- 28°C for up to 10 days. *Strongyloides* rhabditiform larvae migrate to the water and transform into filariform larvae. The water sediment is screened daily under a low magnification for living larvae, which should be differentiated from those of hookworm. This technique is simple, efficient, convenient, very easy to perform and may be requested from any clinical microbiology laboratory.

Figure 3 *(Strongyloides stercoralis larvae technique.)*

Figure 4 *(Harada Mori filter paper culture.)*

The aim of our study was to determine the presence of parasites in faeces of poultry by wet mount method and concentration technique.

MATERIALS AND METHODS

Type of study: - Laboratory based observational study.

Time of study: - 16 January 2024 to 15 April 2023 (3 months).

Place of study: - Department of Microbiology, Bidhan Nagar campus, All India Institute of Hygiene and Public Health, Kolkata.

Sample size: - One hundred (100) poultry faeces samples.

Location of sample collection: - Kolkata, West Bengal.

Methodology: -

1. Slide preparation (wet mount) and microscopic identification.
2. Concentration in saturated saline solution, followed by slide preparation and microscopic identification.

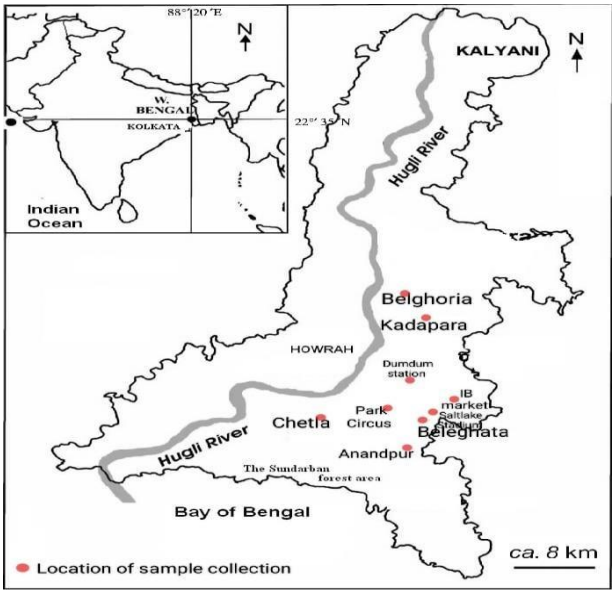

Figure 5 *(Location of faeces sample collection from poultry shops present in different parts of Kolkata.)*

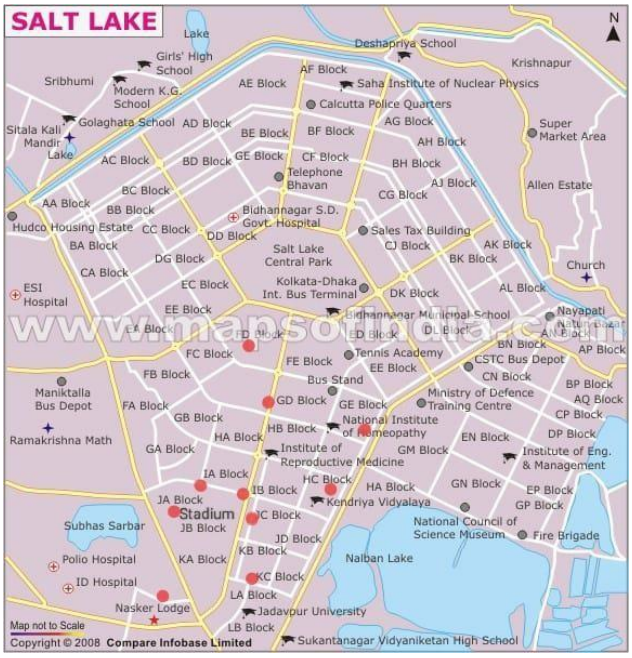

Figure 6 *(Location of poultry faeces sample collection from different markets present in Salt Lake City (marked in red circle).)*

SL. NO.	REAGENTS REQUIRED	Purpose
1	70% alcohol	Cleaning slides
2	Normal saline	Preparing wet mount with normal saline
3	Lugol's iodine (stock and working)	Preparing Iodine mount
4	Sodium chloride	Concentration technique

5	Distilled water	Concentration technique (for dissolving NaCl)
6	0.1% Methylene Blue	Concentration technique and ZN stain
7	4% sulphuric acid	ZN stain
8	Decolourizer	ZN stain
9	Carbol Fuchsin	ZN stain

- **Formula of Lugol's Iodine (Stock solution)**

- 5 g Iodine (I2)
- 10 g Potassium iodide (KI)
- 100 ml distilled H2O

(At first Potassium iodide was added to the distilled water and then Iodine was added)

- **Working solution of Lugol's iodine**

- 1ml of stock sol of Lugol's Iodine was added to 9ml of distilled water.

- **Saturated saline solution**

- Working saturated saline solution was prepared by adding enormous amount of salt to 200ml of distilled water. The solution was boiled till it dissolved. Again, salt was added and the solution was boiled for few minutes. The solution was allowed to cool down before using for our experiment.

- **Modified (cold) ZN staining was done whenever we found coccidian oocysts in wet mount.**

1. A clean glass slide was taken and made grease free with 70% ethanol.
2. A smear was made at the centre of the slide.
3. The smear was heat fixed and allowed to dry.
4. Then few drops of Carbol Fuchsin (strong) was added and kept for 10-15 minutes without heating, and then washed with tap water.
5. Then 4% H2SO4 was added for 4 min or till the colour disappears
6. The slide was washed with running tap water.
7. Then the smear was counterstained with 0.1% aqueous methylene blue for 1 minute.
8. In the end, the smear was washed, dried and observed under 100X objective

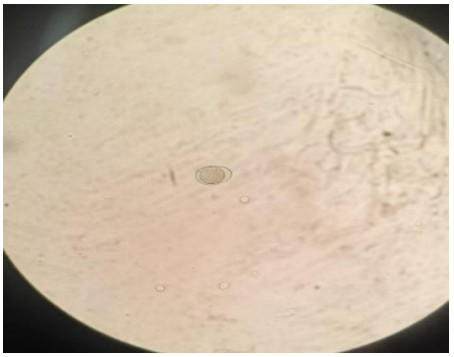

Figure 7 *(Coccidian, Cystosiospora oocyst.)*

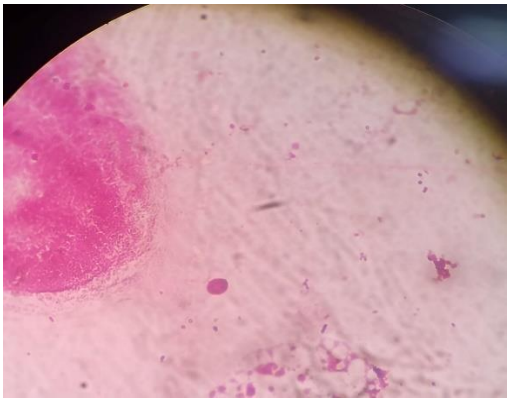

Figure 8 *(oocyst of Cystoisospora in modified ZN stain.)*

Steps: -

1. Sample collection- Poultry faecal samples were collected using sterile forceps into sterile universal containers from different poultry shops present in different regions of Kolkata and Saltlake as depicted in figures 1 and 2.
2. Sample transportation- After collection, faeces sample were immediately transported to the microbiology laboratory within 30 minutes to prevent drying up and for various laboratory testing.
3. Emulsification- Solid or dry samples were emulsified using normal saline for further processing.
4. Vortexing – The sample were mixed in normal saline and vortexed for 5 minutes.
5. Slide preparation - In a clean glass slide, 1 drop of Lugol's iodine and 1 drop of normal saline was added. To that 0.02g (one loopful) of faeces was added and a smear was prepared and coverslip was put.
6. Saturated saline preparation – A saturated solution is a solution that contains the maximum amount of solute that is capable of dissolving. Concentration technique was used with saturated saline.
7. Concentration technique- The above saturated saline solution were taken in small sterile test tubes and to that 0.02g faeces was added and mixed properly. 5µl of 0.1% Methylene blue was added for colour contrast and kept at 4 degree C for 1 hr. It was kept at 4ºC so that specific gravity increases and sedimentation is better seen.
8. Centrifugation- After 1 hr the tubes were centrifuged at 2000 rpm for 5 minutes. Then 40 µl was pipetted out from the supernatant as well as deposit and put in a glass slide and mounted with cover slip.
9. Observation- The slides were observed under light microscope at 10X and 40X objectives.

Figure 9 *(Slide preparation in normal saline (NS) and Lugol's Iodine (LI))*

Figure 10 *(Poultry shops with poor sanitation.)*

RESULTS

Many parasites of human and zoonotic importance were found in the poultry fecal samples.

The results of our study revealed that the direct microscopic method is more suitable in medical diagnostic laboratories due to its time saving and simplicity of testing.

Out of 100 samples, 37 were found to be positive for parasites.

Out of 100 samples, 5 were such that they contained more than one type of parasites.

pH of all faecal samples ranged from 6.5 to 7

Sl. no.	Regions of samples collection: -	Total no.	Percentage in %
1	Salt Lake City	86	86 %
2	East Kolkata	7	7 %
3	South Kolkata	6	6 %
4	North Kolkata	1	1 %

Table 1 *(Total number of samples collection from different regions.)*

Sl. no.	Sample type	Total no.	No. of positive sample	% of positive sample
1	Semi – solid	62	25	40.32
2	liquid	22	8	36.36
3	Dry	16	4	25.00

Table 2 *(Number and percentage of positive samples in different consistency type of samples.)*

Serial no.	Names of parasites identified in descending order of commonness	Total number	Percentage
1	Coccidia	18	18
2	*E. histolytica*	15	15
3	*E. coli*	4	4
4	*Strongyloides stercoralis*	1	1
5	*Giardia* spp.	1	1
6	*Fasciola hepatica*	1	1
7	*Blastocystis hominis*	1	1

Table 3 *(Number and percentage of parasites identified in descending order of commonness.)*

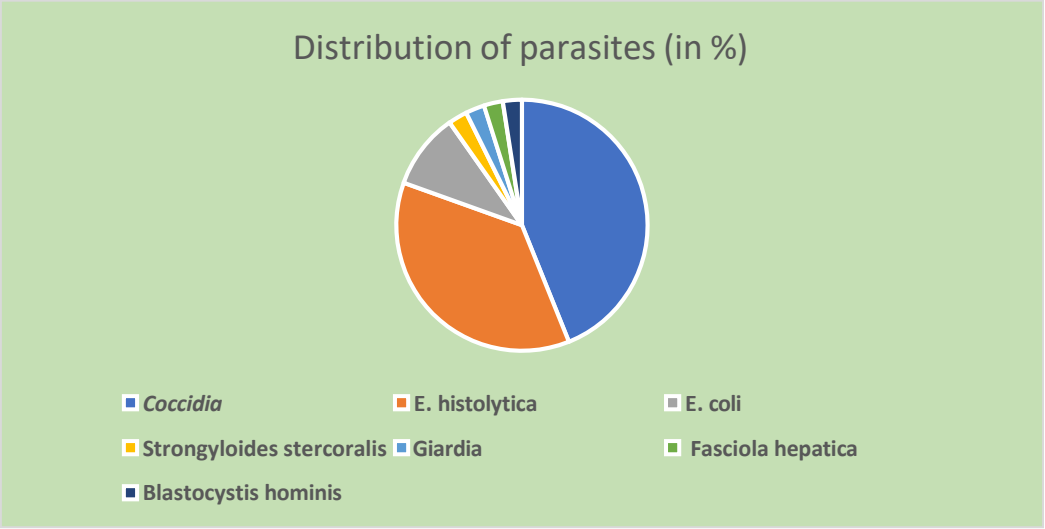

Figure 11 *(Pie chart for distribution of parasites found in poultry faeces (in %))*

DISCUSSION

Once the parasites are identified in poultry stool, appropriate treatment and control measures can be implemented. These may include the use of anthelmintic drugs, acaricides, or other specific treatments depending on the type of parasite. Implementing preventive measures such as proper hygiene, sanitation, and vaccination can help reduce the risk of parasitic infections in poultry flocks. Regular

monitoring and a proactive approach to parasite control contribute to the overall health and productivity of poultry birds. In our experience, coccidian oocysts, *Entamoeba* oocysts and rarely some *Giardia* oocysts and larvae of *Strongyloides* spp. larvae and trematode eggs were found commonly in poultry faeces.

Effective management of gut parasites in poultry needs proper sanitation, implementing biosecurity measures, and also routine deworming activities. It is crucial for poultry farmers to work in tandem and liaison with veterinarians in order to develop a comprehensive parasite control strategy tailored to the specific needs of their flock. Regular monitoring and prompt treatment are essential for maintaining the health and productivity of poultry birds. So, keeping in view of public health security, more studies should be done in this regard. Poultry can harbour various parasites that are harmful to both the birds and humans. Identifying and isolating these parasites allow for effective disease control measures to be implemented, preventing outbreaks and minimizing economic losses in the poultry industry.

Some poultry parasites are zoonotic, meaning they can be transmitted from animals to humans. By isolating these parasites, public health authorities can take appropriate measures to prevent human infections, such as implementing proper hygiene practices and treatment protocols.

Knowledge of the types and prevalence of parasites in poultry populations helps veterinarians develop targeted treatment and prevention strategies. This includes selecting appropriate dewormers and vaccines, as well as implementing biosecurity measures to minimize parasite transmission.

Over time, parasites can develop resistance to commonly used dewormers and other control measures. Isolating parasites allows researchers to monitor for resistance development and adapt control strategies accordingly, such as rotating dewormers or developing alternative treatment methods. Parasites present in poultry faeces can contaminate the environment and potentially contaminate poultry products if proper hygiene practices are not followed. By isolating parasites, measures can be taken to reduce the risk of contamination and ensure the safety of poultry products for human consumption. These parasites present in poultry faeces, can also contaminate the soil and water and thus enter the human food chain, thus posing grave health risks and is thus s public health problem. In a study from Uttar Pradesh and Uttarakhand, out of 58 poultry farms that were tested for gastrointestinal parasites, 81.03 % were positive for Eimeria spp., 15.52 % for Ascaridia galli, 3.45 % for Hetarakis gallinarum, 1.72 % for Syngamus trachea, 5.17 % for Capillaria spp, 1.72 % for Raillietina spp., 1.72 % for Trichostrongylus tenuis, 1.72 % for Choanotaenia infundibulum and 1.72 % for *Strongyloides* avium [7]. The last percentage roughly matches our study. More such studies are needed in this context, with more advanced tools also, to assess the true burden of protozoa and STH (Soil transmitted helminths) that can be carried via poultry faeces. Also, we here devised a new method of concentration technique, by keeping it at 4ºC for one hour.

Future prospects

The One Health concept acknowledges that the health of humans is intricately linked to the health of animals and the environment they share. It emphasizes the importance of understanding and addressing the underlying factors contributing to the emergence and spread of diseases, such as zoonotic diseases (those transmitted between animals and humans).

By adopting a One Health approach, stakeholders can work together to develop comprehensive strategies for disease surveillance, prevention, and control. This approach also promotes sustainable practices that benefit both human and animal populations, as well as the environment.

Once the parasites are identified, appropriate treatment measures can be implemented. These may include the use of anthelmintic drugs, acaricides, or other specific treatments depending on the type of parasite [8]. Implementing preventive measures such as proper hygiene, sanitation, and vaccination can help reduce the risk of parasitic infections in poultry flocks [9]. Regular monitoring and a proactive approach to parasite control contribute to the overall health and productivity of poultry birds.

Effective management of gut parasites in poultry involves proper sanitation, biosecurity measures, and routine deworming programs [10]. It's crucial for poultry farmers to work with veterinarians to develop a comprehensive parasite control strategy tailored to the specific needs of their flock. Regular monitoring and prompt treatment are essential for maintaining the health and productivity of poultry birds. Parasites should be looked for in poultry faeces to ensure safety of poultry products and also to minimize risk of Soil transmitted helminth infections.

CONCLUSION

In conclusion, detection of parasites from poultry faeces is important for disease control, effective veterinary management, monitoring parasite resistance, public health protection, and also maintaining food safety standards. It enables targeted interventions to minimize the impact of parasites on both poultry and human health, as well as ensuring the sustainability and profitability of the poultry industry. These are significant from a one health viewpoint.

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