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MOLECULAR SOURCE ATTRIBUTION OF FOODBORNE AND WATERBORNE ZOONOTIC PARASITES: A ONE HEALTH REVIEW OF TRANSMISSION PATHWAYS AND SURVEILLANCE GAPS

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Abstract

Foodborne and waterborne zoonotic parasites remain major One Health threats because their transmission connects humans, livestock, companion animals, wildlife, food systems, water sources, soil, wastewater, and contaminated environments. This review synthesizes current knowledge on molecular source attribution of three key protozoan parasites, Cryptosporidium spp., Giardia duodenalis, and Toxoplasma gondii, which represent major models for multi-host and environmentally mediated transmission. The review highlights how conventional detection methods confirm parasite presence but often fail to identify the origin, reservoir, or direction of transmission. Molecular tools, including PCR, qPCR, sequencing, gp60 subtyping, multi-locus genotyping, PCR-RFLP, whole-genome sequencing, metabarcoding, environmental DNA, and spatial molecular epidemiology, provide higher-resolution evidence for linking human infections with animal, food, water, and environmental sources. Major transmission pathways discussed include meat-borne exposure, fresh produce contamination, raw milk and dairy products, drinking water, recreational and surface water, wastewater reuse, irrigation water, soil persistence, and direct contact with animal reservoirs. The review further examines livestock, companion and stray animals, wildlife, and human activities as drivers of reservoir overlap and environmental contamination. Despite major advances, important surveillance gaps remain, including fragmented human–animal–environment monitoring, limited standardized molecular protocols, weak evidence for transmission direction, under-sampling of food and environmental matrices, poor integration of epidemiological and molecular data, and limited genomic capacity in low-resource settings. This review proposes an integrated molecular One Health surveillance framework based on matched sampling, high-resolution typing, data integration, source attribution, and targeted control actions to improve prevention of foodborne and waterborne zoonotic parasite transmission.

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INTRODUCTION

Foodborne and waterborne zoonotic parasites are important One Health threats because their transmission links humans, animals, food systems, water sources, soil, wastewater, and the wider environment. Parasites such as *Cryptosporidium* spp., *Giardia duodenalis*, and *Toxoplasma gondii* can persist outside the host and infect humans through contaminated drinking water, fresh produce, undercooked meat, irrigation water, or environmental exposure(Slifko et al., 2000) . Environmental transmission is especially important because water, soil, and food can act as major vehicles for parasite spread. Therefore, molecular source attribution is needed to identify the true reservoirs, routes, and transmission pathways responsible for human infection (Innes et al., 2020). Molecular source attribution is increasingly important because many zoonotic parasites share similar transmission routes but differ in host range, infective stage, and epidemiological source. For example, *Giardia duodenalis* requires assemblage-level characterization to distinguish zoonotic assemblages from host-adapted lineages, while *Toxoplasma gondii* diagnosis must be interpreted with parasite genotype and exposure route because infection may arise from meat, water, soil, or environmental oocysts (Ryan & Cacciò, 2013). Global burden studies further show that foodborne parasitic diseases contribute substantially to human morbidity and mortality, supporting the need for integrated surveillance across food, water, animal, and environmental systems (Torgerson et al., 2015).

This review is dedicated to molecular source attribution of zoonotic parasites (*Cryptosporidium spp.*, *Giardia duodenalis* and *Toxoplasma gondii*) of food and water sources. These parasites have been chosen because they are important threats to One Health and are represented by parasites of humans, animals, food, water and environment. The goal is to review the use of molecular tools in identification of parasite source, reservoirs and transmission routes. The intent of this review is to both compare the evidence across the selected parasites and to identify gaps in surveillance and suggest future priorities for One Health studies of the source-tracking of parasites. This is not just about detecting parasites; it's about molecularly confirming transmission.

2. Foodborne and waterborne zoonotic parasites as One Health threats

Foodborne and waterborne zoonotic parasites are key threats to One Health as they pose a health risk that extends beyond human infections. These parasites are transmitted via local human, animal, food and environmental systems. Humans are also usually infected indirectly via contaminated drinking water, irrigation water, fresh produce, meat, soil, wastewater, and food-processing facilities (Slifko et al., 2000). Slifko et al. pointed out that for many protozoan and helminth parasites, the environment plays an important role, and water, soil and food are important vectors of parasites. They also reported that many of the parasite stages are hardy and resistant to environmental stressors and can persist for extended periods in moist conditions, which makes them a constant threat to both public and veterinary health (Slifko et al., 2000).

Food-borne parasitic diseases are significant public health problems. A global burden assessment conducted in PLOS Medicine estimated that foodborne parasitic diseases (excluding enteric protozoa) lead to approximately 23.2 million cases, 45,927 deaths and 6.64 million disability-adjusted life years every year (Rossi, Amadoro, et al., 2024). The same study estimated a high proportion of food-borne transmission with almost 50% of cases attributable to the selected food-borne parasitic diseases and food-borne transmission accounting for a much greater proportion of disease burden. This means that zoonotic parasitic diseases are not minor or insignificant infections but that they play a significant role in global morbidity and mortality, in addition to food-safety risk (Robertson et al., 2026). *Cryptosporidiosis spp.*, *Giardiasis duodenalis* and *Toxoplasmosis gondii* are particularly significant protozoan parasites for food or waterborne transmission. *Cryptosporidium* and *Giardia* are frequently linked to contaminated water, animal faecal pollution and environmentally resistant infective stages(Rossi, Santonicola, et al., 2024).). It is a One Health

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approach that is needed for the control of *cryptosporidiosis*, says Innes et al., since a One Health strategy will need to connect veterinary, public health and environmental research. They also noted the need for integrated genotyping to distinguish *Cryptosporidium* parasites in human and animal health monitoring (Ali et al., 2024). Also, Giardia is a large source-attribution challenge due to its zoonotic potential that is dependent on assemblages and genotypes. Assessment of zoonotic transmission of G. *duodenalis* necessitates molecular characterization because there is significant genetic variation among strains of G. *duodenalis* (Ryan & Cacciò, 2013).

Toxoplasma gondii, yet another illustration of the complexity of parasite transmission under the umbrella of One Health. Humans can be infected when they eat undercooked meat containing infection or when they ingest oocysts that have been shed by felids into soil, water or vegetables or the environment (Hill & Dubey, 2018). *Toxoplasmosis* is not only a clinical disease, but also associated with livestock production, cat population, food hygiene, environmental contamination and water safety. The connection between foodborne and environmental exposure creates challenges when trying to identify the source of exposure without molecular and epidemiological integration (İnci et al., 2023). In summary, food and waterborne zoonoses generally pose a significant One Health problem as they are transmitted via a multi-source pathway, are environmentally stable and may also occur within the normal food and water chain. A focused review on molecular source attribution is thus warranted to shift beyond the mere detection of parasites to the identification of the actual source, route and reservoir of human infections.

2.1 From parasite detection to source attribution

The detection of parasites is the first step in zoonotic parasitology but not enough to understand transmission. There are conventional methods that are available to confirm the presence of a parasite in a human, animal, food, water or environmental sample, which include microscopy, staining, immunoassays, and routine PCR (Farrell et al., 2013). However, such techniques are not always able to identify the source of infection. This is significant in foodborne and waterborne zoonotic parasitology, because the same species of parasite may be present in humans, animals, (domestic, wild, or companion), drinking water, irrigation water, wastewater, soil and in food products. So, the primary scientific question is where the parasite came from and how did it get into humans?(Rossi, Santonicola, et al., 2024). The source attribution process is the effort to trace human infections back to a likely source or vehicle or route of transmission. It typically involves the following steps: detection of a parasite, determination of species, genotyping or subtyping, identification of human, animal, food, water and environmental isolates and inference of the possible transmission pathway. This is particularly critical for those parasites that have more than one host and multiple transmission stages, such as *Cryptosporidium spp.*, *Giardia duodenalis*, and *Toxoplasma gondii*. Studies can be misleading when they are based on the premise that infection is zoonotic when the parasite is found in humans and animals, but molecular data is lacking (Wang et al., 2023).

The detection is not enough, as is the case with *Giardia duodenalis*. There are several genetically distinct assemblages of the parasite. Commonly reported in humans as well as in animals, assemblages A and B are more host-adapted than other assemblages (Shamsi et al., 2025). Because there is a lot of genetic variation within G. *duodenalis*, Ryan and Cacciò said it would be important to do molecular characterization to assess zoonotic transmission. So, animal and human occurrence in the same location does not indicate animal to human transmission unless there is a genetic connection (Ryan & Cacciò, 2013). This is the same for *Cryptosporidium*. In humans, cryptosporidiosis can be caused by human-associated species like C. *hominis* or zoonotic species like C. parvum, frequently associated with animals, such as livestock(Ali et al., 2024). Therefore, species-level identification and subtyping is crucial for determining anthroponotic, zoonotic, and environmentally mediated transmission. Improved source tracking,

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epidemiology and surveillance are possible with an integrated genotyping approach for detection and differentiation of *Cryptosporidium* parasites in veterinary and public-health systems, Innes et al. emphasized. Recent studies of *Cryptosporidium* molecular epidemiology have also documented the extensive use of gp60 subtyping for investigating *C. hominis* and *C. parvum* subtyping in humans, livestock, and other hosts which makes it a tool for source-attribution studies (Oladele et al., 2025).

Overall, source attribution has shifted zoonotic parasitology from prevalence reporting. It enables the study of the population of parasites in humans, animals, food, water, and in the environment, and can help determine possible reservoirs or contamination pathways. Combining molecular evidence with epidemiological information like animal contact, food history, water exposure, sanitation, geography, rainfall and outbreak timing are the strongest evidence(Yang et al., 2021). Hence, the focus of modern One Health parasitology, instead of detection studies should now be on integrated molecular source-tracking studies which will be able to pinpoint the actual source and transmission pathway for foodborne and waterborne zoonotic parasites.

2.2 Molecular evidence

However, molecular methods are needed in zoonotic parasitology, as conventional ones are usually not able to differentiate the species, genotype, subtype, assemblage, lineage or transmission origin of a parasite (Feng & Xiao, 2011). There are various methods to confirm infection by microscopy, staining, antigen detection, and routine clinical diagnosis; however, these methods generally yield limited information as to whether the infection originated from humans, livestock, companion animals, wildlife, contaminated food, contaminated water or a common environmental source(Garcia et al., 2018). This restriction is essential for food borne and water borne zoonotic parasites as the same parasite can occupy several compartments of the environment and several hosts simultaneously.

Molecular evidence is key for Cryptosporidium species as they have different epidemiological significance. *C. hominis* is primarily associated with human transmission, while *C. parvum* is often considered to be zoonotic, with livestock being a common source of transmission (Innes et al., 2020). Thus, the designation of "*Cryptosporidium* positive" alone is insufficient for source attribution. Innes et al. highlighted the need for an integrated genotyping method to screen and distinguish *Cryptosporidium* in veterinary and public-health systems as this will aid in source identification, epidemiology, and surveillance (Innes et al., 2020). Molecular subtyping, particularly of gp60, is commonly employed to investigate the diversity of *C. hominis* and *C. parvum* isolates from humans, animals, and livestock to compare isolates from various hosts and sources (Oladele et al., 2025).

Giardia duodenalis is also highly reliant on the molecular evidence for zoonotic potential, which is heavily dependent on the assemblage identity. There are several genetic assemblages in *G. duodenalis*. Assemblages A and B have been found in humans and some animals while assemblies C to H are more host adapted and, in most cases, do not infect humans (Ryan & Cacciò, 2013). However, molecular characterization is needed to assess zoonotic transmission of *G. duodenalis* as there is significant genetic variation within *G. duodenalis* species (Ryan & Cacciò, 2013). Likewise, Feng and Xiao wrote in Clinical Microbiology Reviews that molecular diagnostic methods have been important for the taxonomy, zoonotic potential and transmission of *Giardia* in humans and animals. They also found that host adaptation at genotype and subtype level might decrease the risk of zoonotic transmission in certain settings. Therefore, when the animal isolates were from host-specific assemblages, molecular data is useful to prevent over-estimation of animal-to-human transmission (Feng & Xiao, 2011). However, molecular proof is required for *T. gondii*, as infection can happen through many routes, all of which are overlapping, including tissue cysts in undercooked meat, oocysts in soil or water that are from the felid feces and contaminated fresh produce. Serology is used to help detect exposure but it is generally not

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effective to identify the specific route of exposure (Robert-Gangneux & Dardé, 2012). Robert-Gangneux and Dardé considered the epidemiology and diagnostics of this disease in Clinical Microbiology Reviews and stressed the need for understanding the diagnostic and epidemiological results according to the parasite genotype. Therefore, molecular typing is important to link the human infection with food animals, environmental oocyst contamination or geographically circulating strains (Robert-Gangneux & Dardé, 2012).

Molecular evidence provides a shift from detection to source attribution and transmission pathway analysis in zoonotic parasitology. It provides researchers the ability to compare parasites found in humans, animals, food, water, wastewater, soil, and wildlife by genetic markers. Molecular information is best used when epidemiological data, including the history of animals, food intake, water exposure, sanitation, geography, rainfall and the timing of the outbreak, is also considered. Foodborne and waterborne zoonotic parasite studies may be descriptive in the absence of molecular evidence, and may not detect the actual reservoirs and routes by which humans are infected as explained **in figure 1**.

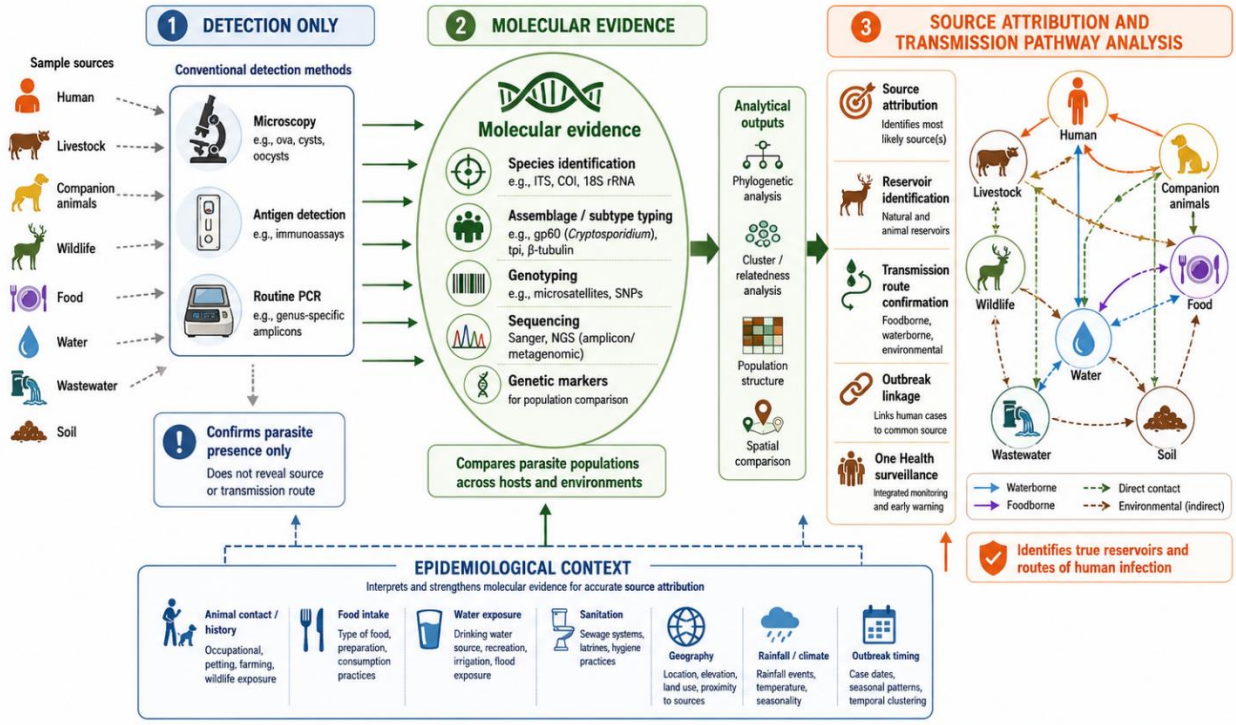


Figure 1: This figure shows how zoonotic parasite research progresses from simple detection to molecular source attribution by integrating genetic markers from humans, animals, food, water, wastewater, soil, and wildlife. It also shows that combining molecular evidence with epidemiological data helps identify the true reservoirs and transmission pathways of foodborne and waterborne infections.

3. Key foodborne and waterborne zoonotic parasites selected for source attribution

3.1 *Cryptosporidium* spp

Cryptosporidium spp. are parasites that infect humans and many animals causing the diarrheal disease *cryptosporidiosis*, and are intracellular organisms. Over 40 species have been identified but *Cryptosporidium spp.* most commonly linked to human infections are *Cryptosporidium parvum* and *Cryptosporidium hominis*(Helmy & Hafez, 2022). Of these, the zoonotic potential of *C. parvum* and its ability to infect livestock and humans make it a special concern. Oocysts are environmentally resistant structures produced by the parasite and

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excreted in the feces, and can persist in water and moist substrates for long periods of time, allowing for the spread of the infection between hosts (Enbom et al., 2023). *Cryptosporidium* is a widely recognised one of the most significant food borne and water borne zoonotic parasites. However, in developing countries, exposure risks are higher due to the presence of inadequate sanitation conditions, and to the fact that many developing countries have poor water treatment practices, and close human-animal contact (Putignani & Menichella, 2010). Young children, immunocompromised persons, and the elderly are particularly susceptible to life-threatening infections. Livestock, and especially calves, have been identified as important reservoirs of zoonotic C. *parvum*, and they have a significant contribution to environmental contamination and infection in humans (Caffarena et al., 2020).

The main route of transmission is fecal-oral route. Water borne transmission is generally believed to be the primary route of infection with oocysts being introduced into water sources through agricultural run-off, livestock wastes, sewage and wildlife waste. *Cryptosporidium* oocysts are very resistant to conventional chlorination, and they can survive in drinking water systems and recreational waters causing outbreaks(Helmy & Hafez, 2022). Many epidemiological studies have associated outbreaks of *cryptosporidiosis* with contaminated municipal water and swimming pools. Foodborne transmission also has a high prevalence, especially with fresh produce, fruits, and vegetables that are contaminated during production, processing, and handling, as well as unpasteurized dairy products. Furthermore, direct exposure to infected animals and contaminated farm settings are potential zoonotic routes of exposure, particularly for farmers, veterinarians, and animal handlers (Abebe et al., 2020). Molecular typing and source attribution studies have contributed to greater knowledge about transmission dynamics by providing information about human infections and their animal and environmental origins. These studies provide justification for the use of a One Health approach to incorporate surveillance of humans, animals, food products and environmental reservoirs. These are vital to detect sources of infection and limit disease spread (Zinsstag et al., 2023).

On a broader scale, *Cryptosporidium spp*. are important zoonotic parasites of global public health importance. Widespread transmission via many different routes, especially contaminated water and food, underscores the need for good source identification and coordinated control efforts to help minimize the global impact of *cryptosporidiosis*.

3.2 Giardia duodenalis

Giardia duodenalis (syn. *Giardia lamblia* and *G. intestinalis* are two of the most prevalent food borne and water borne protozoan parasites found in humans and animals in all parts of the world. It causes giardiasis, a gastrointestinal illness that results in diarrhea, cramps, nausea, malabsorption and weight loss (Zinsstag et al., 2023). The parasite occurs in a wide variety of hosts including man, domestic animals, companion animals and wildlife, thus indicating a high zoonotic potential. Of the genetic assemblages identified, assemblages A and B are associated with human infections and are believed to be significant for zoonotic transmission (Thompson, 2013).

The life cycle of G. duodenalis is direct and consists of two stages: trophozoite and environmental stage (cyst). Infective cysts are shed in the feces and may persist for long periods in moist soil, water and water bodies. Cysts are excreted into the small intestine following ingestion by a susceptible host where they excyst and trophozoites colonize the intestinal mucosa and multiply via binary fission (Adam, 2021). The cysts' survival in the environment is a significant factor in the parasite's ubiquity and the risk of transmission via infected water and food (Rossi, Santonicola, et al., 2024).

A number of important transmission pathways for G. *duodenalis* have been identified through source attribution studies. The main mode of transmission is waterborne and is usually linked to drinking water that has been contaminated with human or animal faeces, either by direct contact or by inadequate treatment of it (Horton et al., 2019). The outbreaks have been associated with municipal water supplies, recreational waters, and

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surface waters receiving agricultural runoff and/or sewage discharge. Cysts have been found to be environmentally stable and can be present over wide geographic areas, making water an important means of contamination and spread(Sinclair et al., 2009).

Gastrointestinal transmission also plays an important role in the transmission of *giardiosis* to man. Fresh fruits, vegetables, and ready to eat food can be contaminated when they are irrigated with contaminated water, if sanitation is not good in the production process, or if there is inadequate food handling. Giardia-infected food handlers can also cause the spread of the infection by contaminating food products directly. Multiple epidemiological studies have pointed to contaminated produce and water processing during food preparation as significant sources of contamination. (Osafo et al., 2022).

Another important route is direct zoonotic transmission. Animals, especially cattle, sheep and goats, and pets like dogs and cats can be infected with *Giardia* and be a source of contamination of the environment. The zoonotic nature of the parasite has been confirmed by molecular epidemiological studies which have shown a genetic similarity between human and animal assemblages(Hatam-Nahavandi et al., 2025). However, the proportion of animal reservoirs will vary geographically and will be determined by circulating assemblages and local environmental conditions. To improve source attribution, modern genotyping approaches have enabled identification of specific transmission links between human, animal, food and environmental sources (Hazards et al., 2019).

In conclusion, G. *duodenalis* is an important worldwide zoonotic parasite of public health importance. This is due to its route of transmission via contaminated water, food products and animal reservoirs, which shows the need for integrated One Health surveillance and source attribution measures to prevent and control giardiasis effectively.

3.3 Toxoplasma gondii

Toxoplasma gondii is a zoonotic protozoan parasite, an obligate intracellular parasite and is one of the most significant zoonotic pathogens transmitted via food or water which is found throughout the world. It can infect nearly all warm-blooded animals; domestic and wild felids are definitive hosts and are responsible for shedding the oocysts which have a long environmental life (Farhab et al., 2025). In humans, infection can result in a disease called toxoplasmosis, usually without symptoms, but sometimes leading to severe illness in the immunocompromised and to congenital abnormalities in pregnant women (Saadatnia & Golkar, 2012). Studies of the sources of infection show that *T. gondii* can be spread in several different ways. The most significant is the consumption of raw or undercooked meat with tissue cysts, especially pork, beef, and small ruminant meats (Symeonidou et al., 2023). It is estimated that a significant proportion of human infections may be foodborne worldwide. Fresh fruits and vegetables can also be contaminated with oocysts via cat feces, contaminated soil or irrigation water (Hazards et al., 2018).

Another important mode of transmission is waterborne. Oocysts shed by infected cats can persist for long periods in the environment, potentially contaminating surface water, ground water and drinking water. Oocysts are able to spread in the environment via rainfall runoff, potentially leading to exposure to humans and animals (Fayer, 2018). Molecular epidemiological and source attribution methods have enhanced knowledge of the relative importance of food, water and environment sources of human infection. There is a need, therefore, for an One Health approach to the integration of animal, human, food and environmental surveillance in order to identify the transmission pathway and to formulate an effective control strategy. The prevention of toxoplasmosis continues to rely heavily on minimizing exposure to contaminated meat, water and fresh produce (Ravel et al., 2017).

3.4 Comparative source-attribution importance

In the study of food borne and water borne zoonotic parasites, source attribution has a fundamental role in understanding the epidemiology. It is the process of assigning the relative proportion of the contribution of different

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pathways and sources to human infections. These can be through contaminated food, drinking water, animal reservoirs, environmental contact, or contact with animals. Since the frequency of the most significant routes of transmission is the critical element in determining the success of disease prevention and public health decision making, source attribution is essential(Pires et al., 2014).

Comparative source attribution is especially of interest for parasites like *Cryptosporidium spp.*, *Giardia duodenalis* and *Toxoplasma gondii*, which have multiple transmission routes. Few studies have been conducted to evaluate the association of waterborne transmission with *Cryptosporidium* and *Giardia*, while *T. gondii* is often associated with contaminated fresh produce and meat. A relative comparison of the importance of these pathways allows researchers to understand what is the main pathway of infection and focus their efforts on the appropriate intervention as explained in figure 2 (Rossi, Amadoro, et al., 2024).

There are several methodological approaches for source attribution studies, such as outbreak investigations, case-control studies, microbial subtyping, comparative exposure assessment and expert elicitation. Both methods offer different perspectives on dynamics of transmission and source contribution (Pires et al., 2018).). Choices of the method are dependent on the pathogen being studied, availability of data, and the public health question being asked. The comparative assessment of transmission pathways also facilitates the One Health approach, providing a set of information from humans, animals, food systems and environmental reservoirs. These integrations enhance surveillance programmes, bolster risk assessment processes, and play a role in food safety policies based on evidence (Al-Khalaifah et al., 2025). Therefore, source attribution is an important tool for decreasing the burden of foodborne parasitic diseases and enhancing public health around the world (Li et al., 2019).

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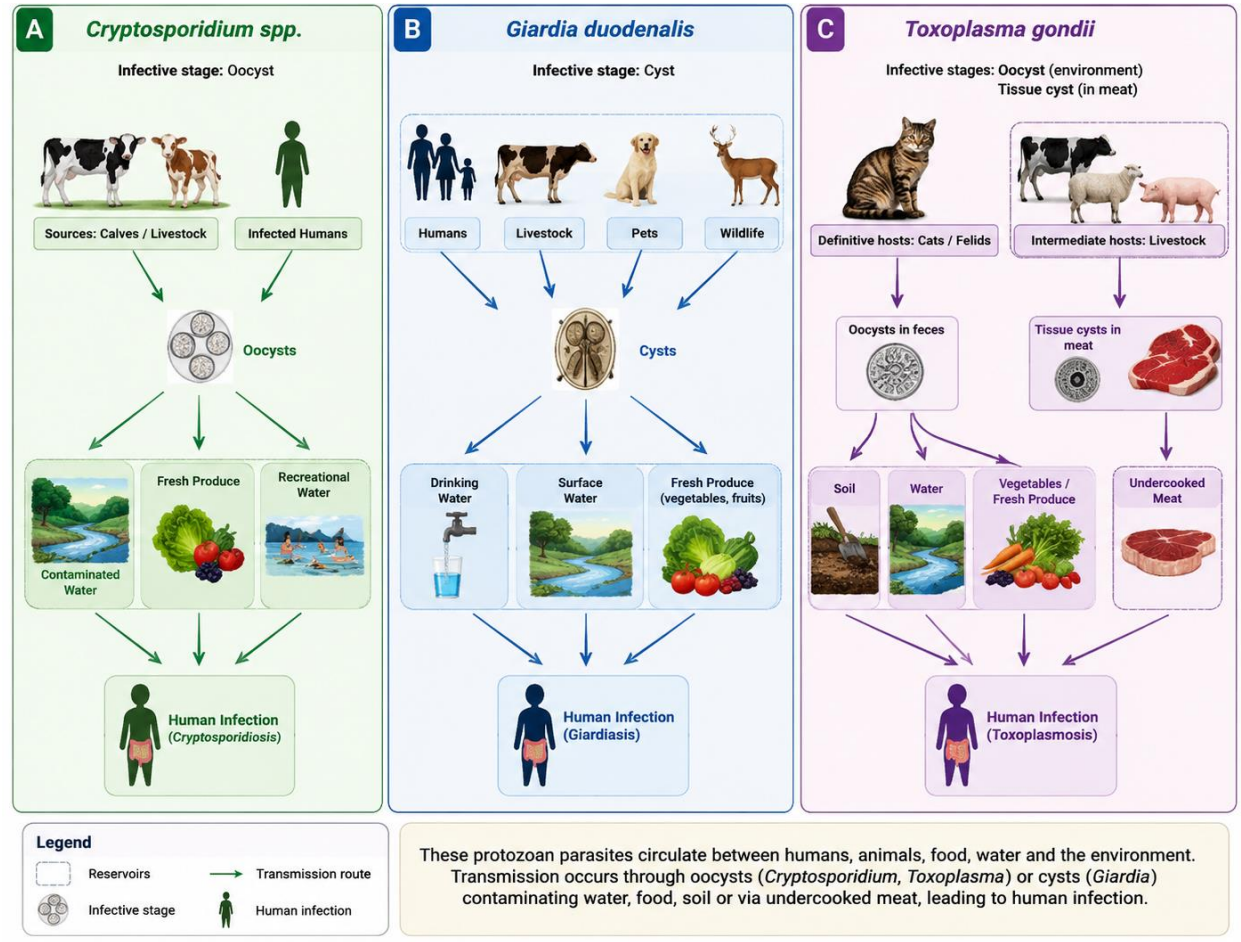


Figure 2: The figure compares the foodborne and waterborne transmission routes of Cryptosporidium, Giardia duodenalis, and Toxoplasma gondii across human, animal, food, water, and environmental interfaces.

4. Foodborne transmission pathways of zoonotic parasites

4.1 Meat-borne transmission

Meat borne transmission is one of the most important routes for zoonotic parasites to be transferred to people. Parasites that are present as infective stages in raw, poorly cooked or undercooked meat can cause infection. Meat borne transmission is linked to several zoonotic parasites such as *Toxoplasma gondii, Taenia saginata, Taenia solium, Trichinella spp. and Sarcocystis spp* (Gabriël et al., 2022). Cattle, pigs, sheep, goats and poultry can be intermediate hosts, carrying parasitic cysts or larvae in their tissues. Human infection occurs when humans eat contaminated meat that has not been cooked enough to kill the infective stages. Parasitic infections in meat are still an important public health problem in the world today and they are also a significant cause of food-borne diseases(Cappelier, 2022). The epidemiology of meatborne parasitic diseases is influenced by animal husbandry practices, slaughterhouse hygiene, meat inspection procedures, and consumer dietary habits (Pozio, 2020). *Toxoplasma gondii* is one of the most significant parasites of meat origin worldwide and undercooked meat is a significant source of human infection. *Trichinella spp*. infection is also often linked to the ingestion of infected pork or wild game products and the ingestion of viable *cysticerci* in beef or pork produces *taeniasis*. The rising population of animal-derived foods and

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globalised food trade have also provided further opportunities to spread these parasites between geographical areas (Steinfeld et al., 2013).

Relative importance of meat borne transmission has been shown to differ between the various parasite species and geographical areas by studying the source. Molecular epidemiology, outbreak investigation and quantitative microbial risk assessment (QMRA) are widely applied to determine the source of meat infections in humans (Hazards et al., 2019). These approaches allow public health authorities to decide on interventions and help to optimize food safety policies. Good farming practices, public awareness of safe food handling, and routine veterinary inspections are all effective methods of prevention, as is cooking meat properly. These steps are vital in minimizing transmission of zoonotic parasites via the food chain and safeguarding public health.

4.2 Fresh fruits Produce Contamination

There has been a growing appreciation that fresh fruits and vegetables serve as vehicles for the spread of zoonotic parasites that are transmitted by food. There has been an increasing appreciation that fresh fruits and vegetables are vehicles for the transmission of foodborne zoonotic parasites. However, unlike foods which are cooked or thermally processed, fresh produce has the potential to be consumed raw and can harbor infective parasitic stages that can survive until the time of consumption (Berger et al., 2010). Several zoonotic parasites, including *Cryptosporidium spp*., *Giardia duodenalis*, and *Toxoplasma gondii* have been found in fresh vegetables, leafy greens, herbs and fruits, making the potential for fresh produce contamination a public health concern. Contamination of produce has been linked to many foodborne outbreaks across the globe, and it is a significant source of human infections(Rossi, Santonicola, et al., 2024)

There are several different points along the farm-to-fork continuum at which contamination can happen. The more significant ways in which people may be exposed to E. coli include using water contaminated with the bacteria for irrigation, using untreated animal manure, coming into contact with soil that has been contaminated, runoff from livestock farms, and contact with infected wildlife or domestic animals. Parasitic pathogens can also be introduced and spread onto produce during harvesting, processing, transport and marketing operations through human handling (Uyttendaele et al., 2015).

Leafy vegetables and other fresh produce with rough surfaces like lettuce, spinach, parsley, coriander and other leafy plants are highly vulnerable because the transmission stages of the parasites could attach well to the surface of the plants (Alshareef et al., 2019).). In some instances, it may occur that the parasite can survive after usual washing operations, which may lead to an infection of the consumers. This increased consumption of fresh and minimally processed produce has thus raised concerns about parasitic diseases associated with produce (Aiyedun et al., 2021). Safe irrigation water, good irrigation water management practices, using safe manure, Good Agricultural Practices (GAPs), good worker hygiene and safe washing and handling practices, throughout the food production chain are effective precautions. These measures are necessary to minimize the problem of foodborne parasitic infection from fresh produce and food safety (Uyttendaele et al., 2015).

4.3 Milk, dairy, and food-processing environments

Milk and dairy products are significant sources of transmission of zoonotic parasites of food origin, especially if consumed raw or undercooked (Sugrue et al., 2019). *Toxoplasma gondii, Cryptosporidium spp.,* and *Giardia duodenalis* are among the parasites that have been linked to milk borne transmission. Infected animals (goats, sheep, cattle, buffaloes) can excrete the parasitic stage in milk making it a potential consumer hazard. Additionally, with the surge in raw milk and traditional dairy product consumption, there are growing concerns about the contribution of dairy products to the transmission of parasites (Bhattacharya et al., 2024). Milk can be contaminated during any of the stages of milk production and processing. Inadequate milking

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hygiene and handling practices, dirty milking equipment, infected animals, and poor sanitation conditions can all lead to the introduction of parasitic pathogens into raw milk and dairy products. Food processing areas also can be contaminated by a worker who is infected or from contaminated water or from handling animals that are infected. This contamination can allow parasites to continue and proliferate in food production systems(Girma et al., 2014).

Pasteurizing milk and dairy products is effective at destroying most of the stages of parasitic disease transmission, however many areas around the globe continue to be at risk from consuming unpasteurized milk and dairy products (de Klerk & Robinson, 2022) . Raw milk has been implicated as a risk vector for transmission of *T. gondii* DNA, and *Cryptosporidium* oocysts have been found in several raw milk samples, underscoring its potential public health importance. In dairy production and food processing, hygiene measures, routine veterinary check and monitoring, pasteurization, and implementing Hazard Analysis and Critical Control Point (HACCP) systems are key measures to help minimize the risks of contamination (Ahmedsham et al., 2018).

4.4 Food-chain source-attribution gaps

Although great progress has been made in source attribution tools for zoonoses, there are key gaps in knowledge regarding transmission of foodborne zoonoses in the food chain (Hazards et al., 2018). The life cycles of parasites, numerous modes of transmission, and the presence of various animal, environmental and food reservoirs often make it difficult to determine the source of the parasite. Many parasitic infections lack adequate surveillance data and the number of cases may be under-reported, making it difficult to determine the source for human infections. Thus, in many regions, the relative importance of particular food commodities and environmental pathways are unknown (Beshearse et al., 2021).). There is a significant lack of harmonisation of parasite detection and molecular characterisation at various points in the food chain. Comparisons between studies are hard to make due to differences in sampling methods, lab methods, and how these data are reported, which can result in different estimates of source attribution (Ryan et al., 2017). In addition, in many developing countries, no monitoring programmes are in place for foodborne parasites and there is significant data gaps.

Another challenge is the lack of integration of human, animal, food and environmental surveillance data. The One Health approach has been promoted widely, but has been underutilized in many places. This means that the water borne, fresh food borne, livestock, wild animal and food processing environment borne routes of transmission are not properly evaluated (Ogunseitan, 2022). New molecular methods, like whole genome sequencing and high throughput genotyping, can help to increase the accuracy of source attribution, but are not widely used in parasitic diseases. Harmonised surveillance systems, better diagnostic tools, better data sharing and better collaboration between public health, veterinary, food safety and environmental sectors will be essential to address these gaps. Such activities are crucial to obtain reliable estimates of source attribution and to form effective measures to lower the burden of food-borne zoonotic parasitic diseases on a global scale (Struelens et al., 2024).

Table 1: Major foodborne transmission pathways of zoonotic parasites

Foodborne pathway	Representative parasites	Main source	Human exposure route	Reference
Raw or undercooked meat	*Toxoplasma gondii*, *Trichinella* spp., *Taenia* spp.	Livestock and wild game	Ingestion of tissue cysts, larvae, or cysticerci in insufficiently cooked meat	(Belluco et al., 2018)

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Fresh produce	*Cryptosporidium* spp., *Giardia duodenalis*, *T. gondii*	Contaminated irrigation water, manure, soil, and animal feces	Consumption of raw vegetables, fruits, or herbs carrying cysts or oocysts	(Belluco et al., 2018)
Raw milk and dairy products	*T. gondii*, *Cryptosporidium* spp., *G. duodenalis*	Infected animals or fecal contamination during milking	Consumption of unpasteurized milk or dairy products	(Marín-García et al., 2022)
Fish and shellfish	Anisakids, fish-borne trematodes, cestodes, *T. gondii*	Aquatic animals and contaminated water	Consumption of raw or undercooked aquatic foods	(Chai et al., 2005)
Food-handler contamination	*Giardia duodenalis*, *Cryptosporidium* spp.	Infected food handlers	Fecal–oral transfer to ready-to-eat foods	(Ziarati et al., 2022)

5. Waterborne and environmental transmission pathways

5.1 Drinking-water transmission

It is known that drinking water is one of the most significant routes of spread of zoonotic protozoan parasites. Surface and groundwater can become contaminated with human sewage, livestock waste, and wildlife feces, as well as agricultural runoff (Rossi, Santonicola, et al., 2024). *Cryptosporidium spp., Giardia duodenalis,* and *Toxoplasma gondii* are waterborne parasites of particular public health concern, as the environmentally resistant transmission stages are able to persist in aquatic environments for prolonged periods of time and are also capable of infecting humans. However, drinking-water-associated outbreaks still occur across the world, highlighting the importance of this transmission pathway in both developed and developing countries (Moratal et al., 2020).

Cryptosporidium and Giardia are common causes of outbreaks of waterborne disease because they are resistant to standard water treatment methods, can survive in the environment for long periods and have a low infectious dose. Oocysts and cysts can be released in the feces and find their way into drinking-water sources, and survive for weeks to months (Karanis et al., 2007). These parasites are of particular concern as drinking-water pathogens, as they are found in a large number of aquatic environments and are highly resistant to the disinfectants commonly used to control these diseases, including chlorine. Thus, poorly treated drinking water is still a significant source of human infection (Omarova et al., 2018).

Cryptosporidiosis and *giardiasis* have been associated with many outbreaks caused by contaminated municipal drinking water supplies. One of the most notable is the *Cryptosporidiosis* outbreak in Milwaukee, USA, which infected over 400,000 people with the parasite in contaminated drinking water (Graczyk et al., 2011). In all the reviews of waterborne protozoan disease outbreaks that have been done worldwide, *Cryptosporidium* and *Giardia* are always the most common causes of the outbreaks. From 2017 to 2022, *Cryptosporidium* was reported responsible for about 77% of the waterborne protozoan outbreaks, and Giardia for approximately 17% of the outbreaks (Bourli et al., 2023).

Toxoplasma gondii is another waterborne zoonotic parasite, but it is not as commonly reported. Shedding by the definitive host, cats, produces oocysts which are resistant to the environment and can contaminate reservoirs, wells and other potable water sources(Dabritz & Conrad, 2010). Several outbreaks of *toxoplasmosis* due to oocysts have been reported from various areas, indicating that oocysts are capable of surviving in aquatic environments to cause infection in humans. As the incidence of waterborne *toxoplasmosis* has

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grown, interest has focused on the monitoring of *T. gondii* contamination in drinking-water systems has increased (Yan et al., 2016).

In terms of source-attribution, drinking water can be viewed as a key link between environmental contamination, animal reservoirs and human exposure. Advances in molecular epidemiology and outbreak investigations have facilitated the identification of the source of contamination and have led to more precise identification of infection as a result of livestock, wildlife or a sewage discharge or environmental reservoir (Fong & Lipp, 2005). As such, strengthening water treatment systems, conserving watershed protection and adopting integrated One Health surveillance are key measures to decrease the burden of waterborne zoonotic parasitic diseases (Singh et al., 2024).

5.2 Surface water, recreational water, and wastewater

Surface water systems such as reservoirs, ponds, streams, water bodies, lakes and rivers have been noted to be important in zoonotic parasite transmission. Frequent sources of contamination are livestock operations, agriculture run-off, wildlife feces and untreated or partially treated sewage. Consequently, they are critical environmental reservoirs of parasites like Cryptosporidium spp., *Giardia duodenalis* and *Toxoplasma gondii* (Putignani & Menichella, 2010). Many communities depend on surface water as their source of drinking water and recreational activities, therefore contamination of these systems has a significant public health impact. A consistent finding in all studies that have been conducted is that surface waters all over the world are contaminated with protozoan parasites, making them an important factor in waterborne disease transmission (Putignani & Menichella, 2010).

Another pathway of exposure is through recreational waters (swimming pools, water parks, lakes and rivers). Human infection can be due to accidental exposure to contaminated water while swimming or participating in other water-related activities (Stec et al., 2022). An interesting note about *Cryptosporidium* is that it is a critical pathogen in a recreational water outbreak since its oocysts are particularly resistant to chlorine disinfection and can survive for an extended period of time in treated swimming pools (Hlavsa et al., 2021). Effective monitoring and water quality management are important since many outbreaks of *cryptosporidiosis* and *giardiasis* have been associated with recreational water exposure. The contamination of surface waters for recreation purposes can occur from sewage discharges, stormwater runoff, wildlife and infected bathers (Hlavsa et al., 2021). Wastewater is one of the major sources of environmental dissemination of zoonotic parasites. Municipal wastewater will bring in faecal material containing infected humans and animals, so that large numbers of parasite cysts and oocysts enter the treatment system. An extensive review showed that up to 60,000 Cryptosporidium oocysts/L and 100,000 Giardia cysts/L have been detected in untreated wastewater (Zahedi et al., 2021). Wastewater treatment will significantly reduce the number of parasites, however, it may not be effective in removing them entirely, and parasite contamination in receiving waters may result from wastewater treatment plants. Thus, effluents treated may lead to the establishment of parasites in water bodies and could affect the quality of drinking and recreational waters downstream (Castro-Hermida et al., 2008).

There is information in the literature that suggests that *Giardia* and *Cryptosporidium* can still be detected in effluents from typical wastewater treatment plant effluents worldwide. The result points out the problems in existing wastewater treatment systems and the need for the use of advanced treatment technologies and periodic monitoring (Zahedi et al., 2021). In terms of source attribution, knowledge of the movement of parasites in surface water, recreational water and wastewater systems is crucial in determining the source of contamination and effective control measures. A One Health approach to environmental monitoring alongside human and animal surveillance could greatly benefit the prevention and control of waterborne parasitic pathogens(Singh et al., 2024).

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5.3 Irrigation Water and Wastewater Reuse

Irrigation water is one of the main sources for the spread of food-borne zoonotic parasites, especially if surface water, untreated wastewater or reclaimed wastewater is utilized in agricultural operations (Adegoke et al., 2018).). In many parts of the world, treated wastewater is used for irrigation in agriculture and is being reused owing to the growing scarcity of water and increasing demand for water in agriculture. The reuse of wastewater as a water source is an alternative to conventional water, but can also result in the entry of parasitic pathogens into the food chain if wastewater treatment is inadequate. The environmentally resistant stage of the parasites are of particular concern since *Cryptosporidium spp., Giardia duodenalis* and *Toxoplasma gondii* can survive in water and contaminate food crops for human consumption (Nasser, 2016).

Fresh produce can become contaminated with parasite cysts or oocysts if irrigation water with parasite cysts or oocysts touches the produce. Infective stages in raw food products will remain infective after consumption because there is no cooking process to destroy them other than leafy greens, herbs, and other foods eaten raw (Osafo et al., 2022). The presence of parasitic cysts and oocysts has been shown to remain viable on plants and can be infectious until eaten. Therefore, water quality in irrigation is recognized as a crucial element in investigations of foodborne parasitic diseases as a means to food safety (Amahmid et al., 2022).

There are concerns that the reuse of wastewater may expose agricultural workers, their families, local residents and consumers to higher risks of exposure (Adegoke et al., 2018). The epidemiological studies suggest that the use of partially treated or untreated wastewater can contribute to the spread of intestinal parasitic infection when people come into contact with it, drink it, or swallow contaminated produce, and/or when it is present in the environment. Parasites can also survive in soils for extended periods, both as cysts and oocysts, which further facilitates their transmission and environmental spread(Adegoke et al., 2018).

Therefore, wastewater reuse in agriculture has gained momentum, and effective technologies, regular monitoring programs and internationally accepted safety protocols are needed. Implementing advanced wastewater treatment methods, combined with Good Agricultural Practices (GAPs) and risk-based water quality management can significantly lower the risk of zoonotic parasites being transmitted from wastewater used for irrigation to food crops (Ratnadass & Sarter, 2025). In terms of source-attribution, knowledge of the role of irrigation water and wastewater reuse in the transmission of parasites is also critical to provide evidence to inform a One Health approach to control measures (Hazards et al., 2018).

5.4 Soil and environmental persistence

Many zoonotic parasites are present in soil and are significant environmental reservoirs and play a key role in transmission to humans and animals. The passage of the parasite stages oocysts, cysts, and eggs in the feces of infected hosts may lead to their release into the environment and to the accumulation in soil which can survive for a considerable period (Ahmed, 2023). Livestock manure, wildlife feces, domestic animal wastes, wastewater application and agriculture cause soil contamination. Therefore, soil is an important link in source-attribution studies (Nieder et al., 2018). *Toxoplasma gondii, Cryptosporidium spp., and Giardia duodenalis* are protozoan parasites that are extremely resistant to the environment. *T. gondii's* oocysts have a tough, extremely resistant wall that allows them to survive in a variety of environmental conditions. Sporulated oocysts have been found to survive for up to 18 months in moist soil, which can lead to long-term environmental contamination and exposure via contaminated food, water, and direct contact with contaminated soil. In the same way, *Cryptosporidium oocysts* and *Giardia cysts* are also widely distributed, due to their ability to survive for several months in cool and damp environments (Yan et al., 2016).

Parasites are sensitive to environmental conditions and will survive and persist within them. Transmission stages can survive at different

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temperatures, humidities, soil compositions, in sun, and in relation to rainfall. Generally, parasite viability is promoted by moist conditions and moderate temperatures, while extreme heat, desiccation and intense ultraviolet radiation decrease parasite viability. So, environmental distribution and abundance of parasites may be affected by seasonal variations (Kim et al., 2016). Flooding and heavy rainfall could also help to spread parasites from contaminated soils to surface water, irrigation systems and agricultural land, allowing them to be picked up by humans (Short et al., 2017).

Elimination is the biggest problem with source attribution; parasites can be infectious long after initial contamination. Because they can survive in soil and other environmental matrices, finding the source of infection is difficult (Mazur-Panasiuk et al., 2019). The recent molecular and environmental surveillance approaches have enabled better identification of parasites in soil, water, and food matrices, thereby bringing better understanding of the possible routes and environmental reservoirs of transmission(Shaha et al., 2026). Therefore, in order to correctly identify sources of infection and formulate effective control interventions, it is important to integrate the monitoring of the environment with the monitoring of humans and animals in a One Health approach (Singh et al., 2024).

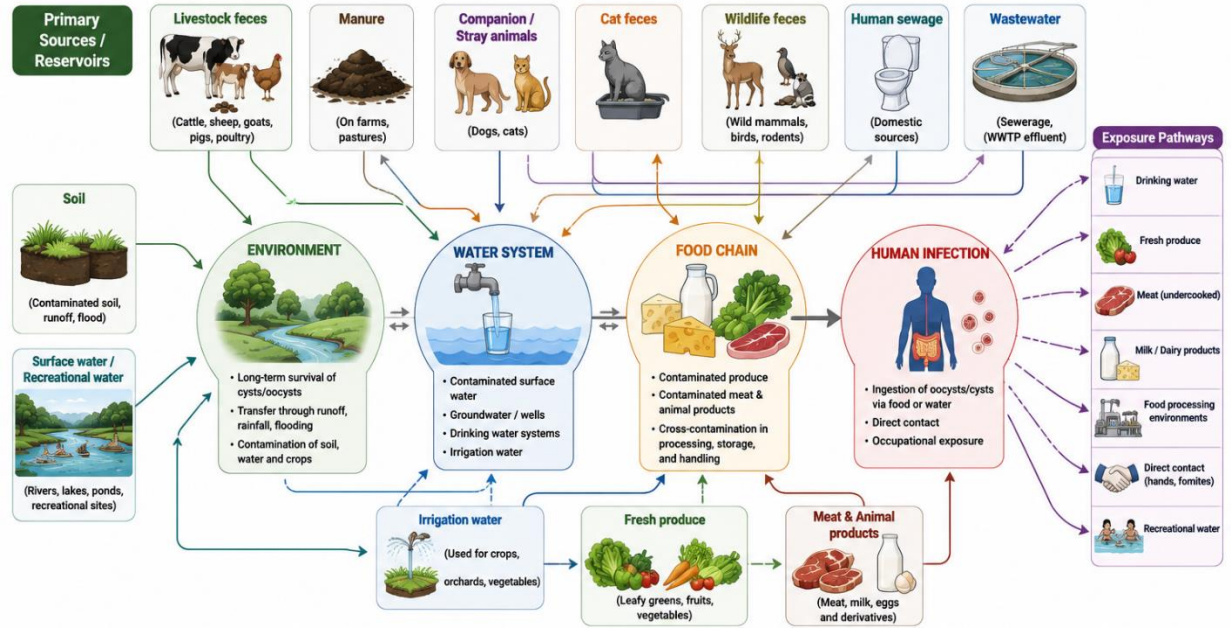


Figure 3: The figure presents a One Health transmission network linking animal and human sources with environmental, water, and food-chain compartments. It shows how parasite stages move through manure, sewage, wastewater, soil, irrigation water, fresh produce, meat, dairy products, and recreational or drinking water before reaching humans. The interconnected arrows emphasize that molecular surveillance across all compartments is required to identify reservoirs, transmission routes, and effective control points.

6. Animal reservoirs and One Health interfaces

6.1 Livestock reservoirs

Livestock are an important reservoir and a direct link in the One Health continuum between animal health and environmental contamination, food safety and human health due to zoonotic parasites. *Cryptosporidium spp.*, *Giardia duodenalis*, *Toxoplasma gondii*, *Taenia spp.*, and *Trichinella spp* are all zoonotic parasites that can be found in domestic animals, such as cattle, sheep, goats, pigs, and buffaloes(Nabarro & Wannous, 2014). These animals transmit and maintain parasites by shedding infective stages into the environment, the water, soils, pastures and food (Nabarro & Wannous, 2014). This means that the livestock-associated route is still a significant part of the

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global burden of food-borne and water-borne parasitic diseases (Singh et al., 2024).

Cryptosporidium parvum is one of the most important zoonotic species among the livestock associated parasites. Molecular epidemiological investigations have shown that cattle, especially young calves, are the main source of zoonotic *C. parvum* infections in humans (Ibrahim et al., 2016). Millions of oocysts may be shed per day from infected calves, leaving the farm environment and water systems around them very contaminated. A worldwide evaluation projected that livestock waste contributes to a tremendous amount of *Cryptosporidium oocysts* being released each year, the bulk of which are from cattle. Direct interactions with infected animals and indirect exposure through contaminated drinking water are known to be important routes of transmission(Ibrahim et al., 2016). Sheep and goats, in addition, are sources of zoonotic parasites. These animals can be zoonotically infected with *Cryptosporidium* and *Giardia* species and are able to contaminate grazing areas and watersheds by shedding the organisms in their feces (Putignani & Menichella, 2010). The public health importance of the role of sheep and goat in giardiasis is still uncertain; however, there is evidence that these animals can serve as environmental reservoir species and participate in waterborne outbreak situations when conditions are favorable. They are of great significance in pastoral and rural production systems, where humans are in close contact with livestock on a regular basis (Alders et al., 2021).

Livestock also have a key role in foodborne transmission pathways. Worldwide, the ingestion of undercooked meat infected with tissue cysts of *Toxoplasma gondii* or with the larval form of other parasites continues to be a significant route of human infection. Pigs, sheep and goats are known reservoirs of *T. gondii* and food-producing animals are indirectly involved by contaminating the farm environment (Cenci-Goga et al., 2011). The contamination of agricultural soils, irrigation systems and surface waters by livestock waste further increase the likelihood of parasite transmission, providing several chances for exposure along the food chain (Cenci-Goga et al., 2011).

Livestock reservoirs are important points in the reservoir chain and the source of attribution from a One Health perspective. A range of farm control measures, such as good farm biosecurity practice, manure management, keeping a watch on livestock infection rates, safe water management and regular monitoring of livestock infections, can significantly minimise environmental contamination and, in turn, human exposure to the disease. Therefore, a necessary step for transmission dynamics understanding and designing evidence-based approaches on zoonotic parasitic diseases is the integration of animal, environmental, and human health data (Singh et al., 2024).

6.2 Companion animals and stray animals

Because of their intimate and frequent association with humans, companion animals (most commonly dogs and cats) are an important source in the epidemiology of zoonotic parasitic diseases. These animals are a critical interface between human, animal and environmental health, within the One Health framework. There are several zoonotic parasites that can be found in dogs and cats such as *Giardia duodenalis, Cryptosporidium spp, Toxoplasma gondii, Toxocara spp* and *Echinococcus spp*(Esch & Petersen, 2013). With the increasing trend of pets and zoonotic disease transmission globally, there is a need to gain a deeper understanding of the role that companion animals play in the dynamics of zoonotic diseases(Overgaauw et al., 2020).

The parasitic stage can be shed by dogs or cats in their faeces, causing contamination of the environment (e.g. households, public areas, parks, gardens and water sources). The zoonotic potential of *Giardia duodenalis* and *Cryptosporidium* species has been shown in molecular studies in companion animals; however, the zoonotic potential differs by species and genotype (Barrera et al., 2026).). Some zoonotic species and assemblages have been observed in pets, with some strains being host-adapted, so there is a potential for transmission between animals and humans. Pets and their owners are closely associated,

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providing opportunities for direct and indirect exposure especially for children, elderly and immunocompromised people (Stull et al., 2013)

Cats are a significant host in regard to their role as definitive hosts of *Toxoplasma gondii* among the companion animals. Cats infected with the parasite can shed millions of oocysts, which are environmentally resistant, in their feces, which can spread to soil, water and food production areas. The viability of these oocysts can be extended for many months in relatively favorable environmental conditions and they can play a significant role in environmental transmission. Direct transmission from pet cats to human beings is suspected to be less common than food-borne transmission, however, cats are an important ecological role in maintaining the life cycle and environmental spread of the parasite Free-roaming and stray animals are even more of a public health threat due to limited veterinary care, parasite control and routine health monitoring. They can be introduced to infected organisms through contaminated outdoor surroundings and animals such as wild animals and infected prey, and spread parasites to a great distance (Gerhold & Jessup, 2013). They often defecate on streets, in public parks, water bodies and on farmland, thus providing avenues for human exposure. Recent reviews have pointed out how the proliferation of stray animals can be a major cause of environmental contamination by zoonotic parasites and can be of importance in urban and peri-urban environments(Sanhueza Teneo et al., 2025).

Companion and stray animals are important, but are often under investigated reservoirs of zoonotic parasites, from a source-attribution perspective. Effective control strategies include improved surveillance, responsible pet ownership, regular deworming programs, vaccination, population management of stray animals and public education (Torgerson et al., 2020). A One Health approach to integrating veterinary surveillance, environmental surveillance and public health surveillance can improve understanding of parasite transmission and minimise zoonotic parasite transmission from companion and stray animals to people (Torgerson et al., 2020).

6.3 Wildlife reservoirs

Wildlife reservoirs are integral in maintaining, amplifying and transmitting many zoonotic parasites in nature (Karesh et al., 2012). Within the One Health approach, wildlife species are a critical nexus between the environmental health system, domestic animal health and human health systems. Parasites may be found in many wild mammals, birds, and other vertebrates which can infect humans and livestock without clinical signs being apparent. This can lead to long term reservoirs of wildlife maintaining transmission of parasites, and to spillover events when environmental or human activities increase contact among wildlife, domestic animals, and humans (Hassell et al., 2017).

Many zoonotic parasites have found wildlife hosts. *Cryptosporidium spp., Giardia duodenalis, Toxoplasma gondii, Echinococcus spp., Trichinella spp.,* and *Babesia* species are known hosts of wild ungulates, rodents, carnivores and primates (VanWormer et al., 2014). It has been shown that wildlife will carry the same or similar genotypes of parasites found in humans and domestic animals, indicating their epidemiological role. Wildlife reservoirs can be a source of contamination in many ecosystems, as they shed cysts, oocysts and eggs into soil and water sources, promoting indirect transmission (Feng & Xiao, 2011) .

Giardia duodenalis is a good example of a protozoan parasite that is transmitted from wildlife. Beavers, muskox, deer and other rodents have been demonstrated to contain zoonotic *Giardia* assemblages (Tsui et al., 2018). Secondary reservoirs can develop when parasites are spread from humans and livestock (domestic animals) to wildlife, which allows for the parasites to be circulated via this new source of contamination. Likewise, the involvement of wildlife in the maintenance of a Cryptosporidium species and genotype that can infect humans has been reported, as has the involvement of domestic animals and environmental sources (Appelbee et al., 2005).

Toxoplasma gondii also has an important role in the ecology of wildlife. Many wild herbivores, omnivores and carnivores act as intermediate hosts and the shedding of oocysts by wild felids

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leads to environmental contamination(Wilson et al., 2024). Predation, scavenging, and interactions in the food web can aid in the spread of parasites among wildlife, and may indirectly affect the risk of infection in livestock and humans. Wildlife reservoirs also play a special role in the maintenance of parasites in remote ecosystems where the direct human participation is minimal (Tompkins et al., 2011).

Environmental alterations caused by humans have greatly affected wildlife-parasite relationships. Habitat fragmentation, wildlife translocation, climate change, agriculture and deforestation all increase interactions between wildlife and livestock and humans (Chakraborty et al., 2019). These activities provide the chance for wildlife to become hosts of parasites to humans and humans to become hosts to parasites to wildlife. More surveillance and molecular epidemiological studies have identified the importance of studying wildlife reservoirs to know where a zoonotic disease is coming from and what the risk is. Therefore, integrated monitoring of wildlife, domestic animals and environmental reservoirs in a One Health approach is crucial for effective control (Banik & Basu, 2025).

6.4 Human Activity as a Driver of Reservoir Overlap

The relationships between wildlife, livestock, companion animals and humans are tightly linked and have been altered by human activities, which can lead to zoonotic parasites emerging and spreading. (White & Razgour, 2020).). In a One Health context, reservoir overlap occurs when several host species occupy the same habitat, use the same resources, or share the same environmental portals that would provide a greater opportunity for exchange of pathogens. The close encounters of people and animal reservoirs, driven by population growth, intensification of agriculture, urbanization, deforestation, land-use change, and environmental degradation are growing and increasing the risk of zoonoses spreading from animal reservoirs to humans. One of the most significant factors that led to reservoir overlap is the agricultural expansion which is a human modification.(Esposito et al., 2023) Natural habitats are frequently replaced by pasture, and agriculture by livestock, which can increase the likelihood of livestock coming into contact with wildlife populations (Mereu et al., 2016). This greater contact allows for the spread of *Cryptosporidium spp., Giardia duodenalis* and *Toxoplasma gondii* parasites from wild to domestic hosts. Pathogens can pass to humans via livestock, which can act as bridge hosts and carry them from wildlife to humans via food, water, or environmental contamination. As a result, agricultural areas are often suitable breeding grounds for zoonotic parasite vectors(Esch & Petersen, 2013).

The effects of urbanization and growth of human populations add to reservoir overlap by disrupting human, companion animal, stray animal and wildlife relationships. Free-roaming dogs, cats, rodents and other species of synanthropic wildlife are common in urban areas, and may harbor zoonotic parasites (Hassell et al., 2017). Stray cats are the source of contamination of the environment by *Toxoplasma gondii* oocysts and rodents as well as other urban wildlife may sustain transmission cycles for several parasitic pathogens. These interactions can be exacerbated by poor waste management, inadequate sanitation and environmental pollution that can lead to increased exposure to humans (Ziraba et al., 2016).

Waterborne and foodborne transmission pathways are also affected by human-induced changes in the environment. De-forestation, mining operations, irrigation projects and wastewater discharge can disrupt the transmission of parasites in ecosystems, causing contamination of water bodies, agricultural areas and food production areas. Reservoir overlap also occurs due to effects of climate change on distribution of hosts, persistence of environmental conditions and survival of parasites. Temperature and precipitation changes could increase the distribution area of both hosts and parasites, resulting in new opportunities for transmission and disease emergence as explained in figure 4 (Short et al., 2017).

Human activities make the source attribution of infections difficult from a source-attribution

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perspective, as transmission pathways are becoming more complex and multi-faceted. The inter-sectoral transmission of zoonotic diseases, where humans, wildlife, livestock and companion animals overlap, is complex and cannot be addressed with a single-sector perspective (Mughini-Gras et al., 2019). Thus, integrated One Health surveillance, sustainable land-use planning, and enhanced biosecurity and environmental management practices are crucial to minimise reservoir overlap and the potential for zoonotic parasites to be transmitted. Understanding the role of human activities in shaping host–parasite interactions is crucial for developing effective prevention and control programs (Plowright et al., 2024).

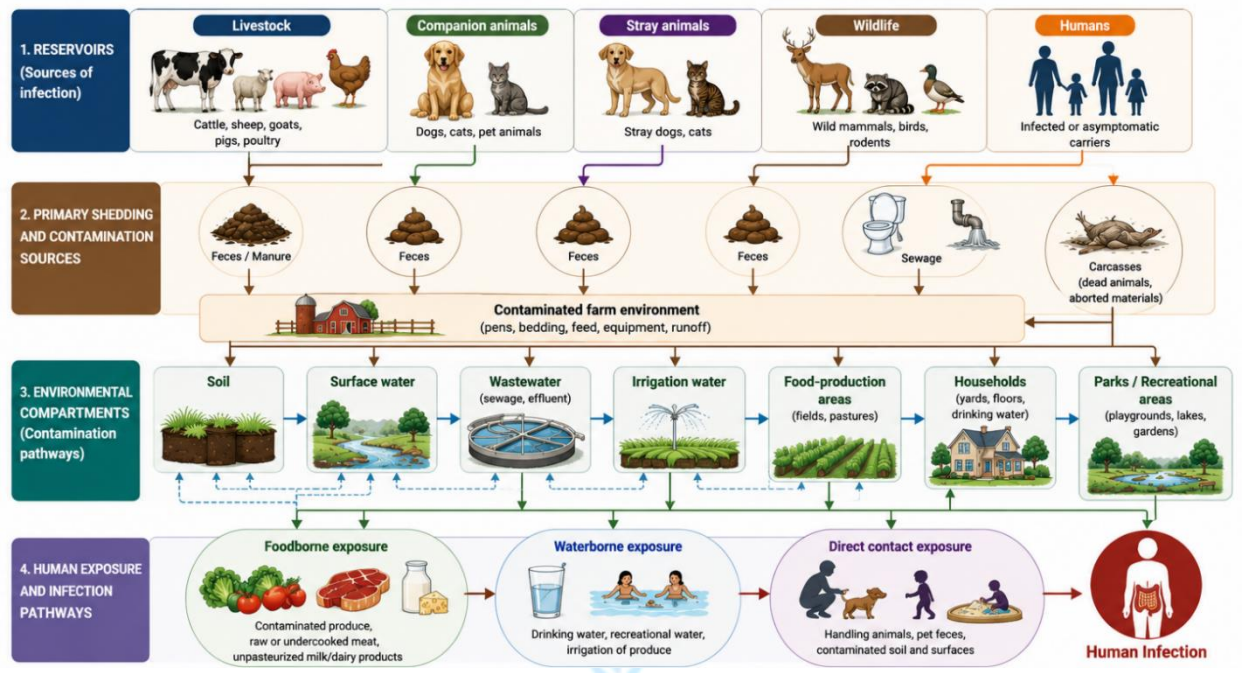


Figure 4: The figure illustrates how livestock, companion animals, stray animals, wildlife, and humans release zoonotic parasite stages through feces, manure, sewage, carcasses, and contaminated farm environments. These stages enter soil, surface water, wastewater, irrigation systems, food-production areas, households, and recreational sites. The resulting contamination produces interconnected foodborne, waterborne, and direct-contact pathways that ultimately lead to human infection.

7. Molecular tools and source-attribution approaches

7.1 Molecular Identification: Species, Assemblages, and Lineages

For most of the 18th century, phyla and genera were the only taxonomic ranks recognized. For much of the 18th century only phyla and genera were recognized. The molecular identification has revolutionized the study of zoonotic parasites by providing accurate tools to identify the species and to establish the source of the parasite (Antunes & Antunes, 2026). Traditional microscopic techniques cannot differentiate morphologically similar species and genotypes of parasites. Molecular techniques such as polymerase chain reaction (PCR), quantitative polymerase chain reaction (qPCR), and DNA sequencing can be used for the exact identification of parasites at the species, assemblage, lineage and subtype levels (Oliveira et al., 2011). These methods have greatly contributed to the knowledge of the transmission pathways and host specificity and are an integral part of current epidemiological investigations. In the case of *Cryptosporidium*, molecular identification has shown that there is a great amount of genetic variation among the species

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that infect humans and animals. There are over 40 species described but *Cryptosporidium parvum* and *Cryptosporidium hominis* are responsible for most human infections (Feng et al., 2018). Two genes have been widely used for distinguishing species and transmission subtypes: the small subunit ribosomal RNA (SSU rRNA) gene and the gp60 gene. These markers have shown that in many instances C. *parvum* is associated with transmission from livestock to humans (zoonotic transmission) while C. *hominis* is mainly associated with human-to-human transmission. These differences are important when conducting source attribution studies and outbreak investigations(Guy et al., 2021).

In the same way, G. *duodenalis* has been molecularly characterized with 8 major genetic assemblages (A – H). Other assemblages are host-specific whereas Assemblages A and B are the predominant cause of human infections and have great zoonotic potential. Molecular studies of genes (e.g., glutamate dehydrogenase (gdh); triose phosphate isomerase (tpi); β-giardin (bg)) have allowed transmission trackers to follow the spread from humans to livestock, companion animals, and wildlife (Ankarklev et al., 2015). Shared assemblages between hosts is a good indicator for zoonotic transmission and environmental spread (Carlson et al., 2022).

Molecular identification has also helped to gain knowledge of population structure and diversity of lineage of *Toxoplasma gondii*. Several major clonal groups (Type I, Type II and Type III) and many atypical strains, which are found worldwide, have been identified by genetic studies (Su et al., 2012). The different types of these lineages vary in virulence, host range, geographic distribution and transmission. Thus, molecular typing has now emerged as a valuable tool to trace human infections to animal reservoirs and environmental sources. Other zoonotic parasites such as *Echinococcus*, *Trichinella* and *Taenia* species have been analysed using similar methods, leading to the identification of different strains and the elucidation of transmission cycles (Maher & Shaapan, 2025).

Species-, assemblage- and lineage-level identification is the basis from which species transmission networks and reservoir contributions are understood from a source-attribution perspective. Molecular identification can facilitate the determination of genotype match in the human and livestock hosts as well as in the other hosts (companion animals, wildlife, food, and environmental samples) and environmental samples, which improves the accuracy of the epidemiological investigations and helps to develop specific control measures under One Health approach (Xiao & Ryan, 2007).

Table 2. Molecular identification markers and epidemiological significance of major zoonotic parasites

Parasite	Molecular marker(s)	Molecular classification	Epidemiological significance	Key reference
Cryptosporidium spp.	SSU rRNA, **gp60**	Species and subtype	Differentiates zoonotic (*C. parvum*) from anthroponotic (*C. hominis*) transmission; supports outbreak investigation and source attribution	(Li et al., 2021)
Giardia duodenalis	**gdh, tpi, bg**	Assemblages (A–H)	Identifies zoonotic assemblages (A, B), host specificity, and transmission among humans, livestock, pets, and wildlife	(Carlson et al., 2015)

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Toxoplasma gondii	PCR-RFLP, Microsatellite typing, MLST	Lineages (Types I, II, III and atypical strains)	Determines strain diversity, virulence, geographic distribution, and links human infections to animal and environmental reservoirs	(Robert-Gangneux & Dardé, 2012)
Echinococcus spp.	cox1, nad1, ITS	Species and genotype	Differentiates transmission cycles, host adaptation, and geographic distribution	(Hui et al., 2013)
Trichinella spp.	ITS1, ITS2, mtDNA (cox1)	Species and genotype	Identifies wildlife and domestic transmission cycles and geographical origin	(Wells et al., 2018)
Taenia spp.	cox1, nad1, ITS	Species and haplotype	Distinguishes *T. solium*, *T. saginata*, and *T. asiatica*; supports food-chain surveillance and outbreak tracing	(Zolfaghari Emameh et al., 2018)

7.2 Genotyping and subtyping for transmission linkage

To gain insight into the transmission dynamics of zoonotic parasites and to create a link between human and animal cases, zoonotic food, and zoonotic environmental contamination, genotyping and subtyping are essential molecular tools to be used(Feng & Xiao, 2011). The species identification identifies the parasite species being studied but genotyping and subtyping give more precise information and can help to trace transmission routes and determine possible sources of infection (Grad & Lipsitch, 2014). Due to the fact that many zoonotic parasites are shared between hosts and environmental reservoirs, these approaches have become more and more relevant in source-attribution studies. Molecular characterization can be used to identify if the infection of humans and animals is from the same transmission network or if it is independent (Mossong et al., 2016).

For *Cryptosporidium spp.*, genotyping and subtyping are commonly performed using the gp60 gene, which exhibits considerable genetic variability. This marker enables differentiation of transmission-associated subtype families and has been widely used during outbreak investigations (Feng et al., 2018). Specific gp60 subtypes have been identified as being shared between humans, calves, and environmental samples, indicating zoonotic and waterborne transmission. The molecular subtyping has since enhanced knowledge of the relative importance of livestock, contaminated water, and human-to-human transmission in the epidemiology of cryptosporidiosis in humans (Oladele et al.).

Lebbad et al. (2011) have investigated transmission linkage in *Giardia duodenalis* using multilocus genotyping based on the genes β-giardin (bg), glutamate dehydrogenase (gdh), and triose phosphate isomerase (tpi). These markers enable the differentiation of assemblages and sub-assemblages and enable the comparison of isolates from humans, livestock, companion animals, wildlife and environmental samples. Identical assemblages in several hosts are indicative of possible zoonotic transmission, and are useful for the identification of significant reservoirs in disease transmission. Multilocus studies have markedly enhanced the accuracy of transmission-linkage studies over single gene analyses (Lebbad et al., 2011). _Toxoplasma gondii has been differentiated into major clonal lineages and atypical strains by multilocus genotyping techniques (Wardeh et al., 2020). These methods are used to determine possible links between humans, animals, wildlife, and environmental pollution. Other species such as *Echinococcus*, *Taenia* and *Trichinella* species are treated similarly, and genetic markers give useful information on the transmission cycles, host adaptation and

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geographical distribution. This type of data is becoming increasingly utilized in outbreak investigations and risk assessments(Power et al., 2017).

7.3 Advanced tools: WGS, metabarcoding, eDNA, and spatial molecular epidemiology

The capacity to source-attribute zoonotic parasites has been greatly improved over the last few years by the advent of molecular technologies. While traditional genotyping methods focus on a small number of genetic loci, advanced molecular tools yield vast amounts of genomic and ecological data that can be used to gain a higher resolution of parasite movement, transmission, and the movement of host organisms (Bass et al., 2015).). In the context of a One Health approach, these technologies, in particular Whole-Genome Sequencing (WGS), DNA metabarcoding, environmental DNA (eDNA) and spatial molecular epidemiology, have been developed as very useful tools to investigate parasite diversity, transmission networks and contribution from reservoir. These tools enhance the capacity to establish connections between human infections, animal reservoirs, food sources and environmental reservoirs.

Whole-Genome Sequencing (WGS) offers the most genetic resolution currently available for molecular epidemiological investigations. WGS differs from traditional PCR-based typing methods because it can detect fine scale genetic polymorphisms among isolates, as it is able to sequence the entire genome of the parasite (Kao et al., 2014). This helps with outbreak investigations, identification of clusters of transmission, evolutionary analysis and reconstruction of the route of transmission. WGS has been used more widely in parasites like *Cryptosporidium spp.*, *Giardia duodenalis*, and *Toxoplasma gondii*, to show the ability to differentiate between closely related strains and to more accurately identify potential sources of infection than traditional genotyping methods. Whole genome typing of human, animal, food and environmental isolates aids direct epidemiological connections and better source attribution (Pistone et al., 2026).

The method called DNA metabarcoding is a combination of high-throughput sequencing and DNA barcoding for the simultaneous identification of multiple species from the same sample. This technique has revolutionized the monitoring of parasites, allowing for the quick characterization of the complex parasite communities found in faecal, soil, water and environmental samples (Yuan et al., 2025). Metabarcoding offers superior taxonomic resolution, sensitivity and detection of mixed infections compared to conventional microscopy. In the field of parasitology, metabarcoding has become more and more useful to evaluate the diversity of parasites in animals (livestock, wild, environmental) in this context. These attributes enhance knowledge on parasite ecology and aid in source-attribution investigations, which include the identification of possible reservoirs and transmission routes (Matoute et al., 2024).

Another new tool with significant promise for parasite surveillance is environmental DNA (eDNA). eDNA is the nucleic acids that organisms shed into environmental matrices like water, soil, and sediments (Bass et al., 2015). Researchers can identify parasite presence by sampling environment and examining the DNA of these environmental samples. Elastin DNA assays are especially beneficial for the surveillance of environmental parasites transmitted by fish and the identification of water quality pollutant hotspots within the aquatic environment. Recent studies have indicated that eDNA surveillance is also a promising tool to enhance detection of zoonotic parasites and vectors and to decrease labor and sampling expenses. The technology is particularly useful for monitoring wildlife reservoirs that are difficult to sample and large environmental systems (Sengupta et al., 2019).

Spatial molecular epidemiology combines molecular information with the spatial data science tools and environmental information, such as geographic information systems (GIS), to explore the distribution and spread of infectious diseases (Chowell & Rothenberg, 2018). Using genetic data with spatial data, transmission hotspots can be identified, the movement of parasites across landscapes can be tracked, and

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environmental factors can be assessed as a factor in parasite distribution (Parratt et al., 2016). This method has gained in significance when studying the impacts of livestock production, wildlife habitats, water systems, climate variability and human activity on the spread of parasites. Spatial molecular epidemiology can offer important evidence for targeted surveillance, risk assessment and source-attribution research and support One Health disease control strategies(Cable et al., 2017).

These next-generation technologies have many benefits, but specialized laboratory facilities, bioinformatics skills and extensive reference databases are necessary. However, as technology advances and the cost of sequencing continues to drop, they are likely to be used even more in the field of parasitology. By combining these four approaches—WGS, metabarcoding, eDNA, and spatial molecular epidemiology—more opportunities arise to enhance source-attribution, surveillance systems, and the control of foodborne and waterborne zoonotic parasites.

7.4 Barriers to molecular source attribution

Although significant progress has been made in the molecular epidemiology of food and waterborne zoonotic parasites, there remain several obstacles to molecular source attributions being effective. A source attribute seeks to identify the source and route of infection by establishing a link between human cases and animal reservoirs, food and environmental sources (Hazards et al., 2019). The cumbersome life cycles of parasites, the fact that they can be transmitted by many different methods and the large genetic variation are often complicating factors in these efforts. Thus, the relative importance of various reservoirs is still a great problem in parasitology and public health (Cable et al., 2017).

Standardization of molecular markers and typing methods in laboratories and research studies is one of the main obstacles. When using different genes, different species are identified and different genes are used for genotyping, and different analytical methods are used for the identification and the different parts of the gene are used as targets for the sequencing, which is possible with different investigators (Van Belkum et al., 2001). This variability can make it challenging to compare between studies and can lead to different estimates of source attributions. Multilocus typing methods (MLT) often produce inconsistent results for parasites like *Giardia duodenalis, Cryptosporidium spp.*, and others as a result of genetic recombination, mixed infections, and differences in marker resolution (Cody et al., 2019). Without universally accepted typing schemes, comparability and reproducibility of molecular investigations are, therefore, limited.

Another big problem is that there are not very many good reference databases available. Source attribution relies on extensive and complete genotypic sampling of parasites from people, animals, pets, wildlife, food and environment. Many regions, however, do not have well established molecular surveillance programs, especially in low and middle income countries (Søe et al., 2018). This means that the genetic diversity of the parasites found in the available databases may not accurately reflect the genetic diversity found in natural populations and, consequently, uncertainty exists when assigning the source of infection. Molecular data is also limited in its interpretation because of geographic gaps in sampling, and in its usefulness for source attribution analysis.

There are also hurdles that are a matter of technical limitations. In food, water, soil and environmental samples, parasites are frequently found at low levels and may be difficult to extract and amplify DNA. Inhibitors in the environmental samples can decrease the sensitivity of the PCR-based detection and may lead to false negative results. Molecular analysis is made more difficult by mixed infections with several species or genotypes of parasites, that can further mask transmission patterns. These are the kinds of questions which are important when studying contamination pathways in the environment(Søe et al., 2018).

Technological advances like Whole-Genome Sequencing (WGS), metabarcoding and the use of environmental DNA (eDNA) surveillance are also limited by the costs and logistics involved. A number of these methods involve specialized

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laboratory equipment, high throughput sequencing platforms, computational resources and bioinformatics skills that may not be widely available in many research laboratories (Maljkovic Berry et al., 2020).). The current large amount of data produced by these technologies brings with it several difficulties concerning data storage, analysis, interpretation and standardization. As a result, they are not often used routinely in source-attributing studies, despite their very powerful potential (Davis-Turak et al., 2017).

Lastly, transmission pathways can not be definitively determined based on molecular evidence alone. To achieve accurate source identification, integration of molecular information with epidemiological, environmental, veterinary and ecological information is needed within a One Health paradigm. Lack of collaboration between sectors, weak surveillance systems and limited data sharing may limit full analyses and effectiveness of source-attribution efforts (Djordjevic et al., 2024). Standardized methodologies, better global surveillance efforts, more comprehensive reference databases, investments in molecular facilities and better interdisciplinary efforts to gain a more complete understanding and control of zoonotic parasitic diseases will be necessary.

8. Surveillance gaps and unresolved transmission questions

8.1 Fragmented Surveillance Across One Health Sectors

Surveillance is essential to the understanding of the transmission of foodborne and waterborne zoonotic parasites. But the surveillance system is extremely disjointed between the human health and veterinary, food safety and environmental sectors (Kabiraz et al., 2026). In many countries these sectors are not coordinated to a high level, and cause incomplete datasets and a lack of capacity to identify transmission pathways. Zoonotic parasites are frequently found in humans, livestock and wildlife, companion animals, food and environmental reservoirs, and outbreaks are therefore under-detected by isolated surveillance systems(Tsai et al., 2010). Therefore, broken surveillance is identified as one of the biggest challenges for source attribution and disease control (Tsai et al., 2010). While human health surveillance is usually geared towards clinical cases and outbreak investigations, veterinary surveillance tends to be oriented towards animal health and production-related issues (Kabiraz et al., 2026). Water quality or environmental contamination can be monitored independently from environmental monitoring programs and not in conjunction with human and animal health databases. This means that the epidemiological connections between infected hosts, environmental reservoirs, contamination sources are often not identified. The absence of co-ordinated surveillance is especially problematic for parasites with several hosts and many modes of environmental transmission, like *Cryptosporidium spp.*, *Giardia duodenalis*, and Toxoplasma gondii (Kabiraz et al., 2026).

Another key issue is the level of surveillance each country and region has. Advanced laboratory facilities and molecular surveillance systems are common in high income countries while many LMIC countries do not have routine surveillance of zoonotic parasites(Dunga et al., 2025). This gap results in large regional differences in epidemiological information and makes it more difficult to compare regional transmission patterns. Moreover, disease burden estimates are incomplete due to underreporting, and source-attribution studies are uncertain because of the incompleteness of burden estimates, varied disease definitions, and uncertainty about how the disease can be attributed to a specific source.

It has been suggested that a One Health surveillance system would be a solution to these challenges. Integrated surveillance systems that integrate human, animal, food and environmental data can help to detect outbreaks, support source attribution, and inform risk assessment **as explained in figure 5** (Singh et al., 2024). New technologies, such as molecular epidemiology, data-sharing platforms and geographic information systems (GIS) further facilitate inter-sectoral surveillance integration. However, institutional constraints, variations in data gathering techniques, under-resourcing and inadequate inter-sectoral coordination still

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remains as challenges. Addressing these issues is crucial to improve the understanding and control of zoonotic parasitic diseases worldwide (Kaminsky & Mäser, 2025).



Figure 5: The figure presents a stepwise molecular One Health surveillance model, beginning with matched sampling from human, animal, food, water, soil, and wastewater compartments. Molecular detection and high-resolution typing are integrated with epidemiological, spatial, environmental, food-chain, and outbreak data to identify reservoirs, transmission routes, hotspots, and high-risk groups. These outputs guide targeted water, food-chain, farm, wastewater, animal-surveillance, and public-health interventions through a continuous feedback system.

8.2 Weak Evidence for Transmission Direction

One of the key areas of research in zoonotic parasites is working out the exact flow of the infection from humans to animals, or from animals to humans, to food or to environmental vectors. Depending on the type of parasite, the determination of human-to-animal or animal-to-human transmission, as well as transmission among humans, is difficult, despite the use of molecular tools for the identification and characterization of parasites (Desquesnes et al., 2022). Many studies have found the same genotype of parasites in different hosts but the identification of common genotypes is not sufficient to prove origin or direction of transmission. The transmission route remain uncertain, and transmission uncertainty remains as a result of uncertainties in source attribution studies (Nkhoma et al., 2020).

In *Giardia duodenalis* and *Cryptosporidium spp.*, molecular techniques often show the presence of similar assemblages or subtypes in humans, animals, pets and wildlife. The results of these studies indicate possible transmission routes; however, they do not necessarily pinpoint the source of infection (Feng & Xiao, 2011). The same molecular pattern may result from shared environmental exposure, indirect contamination, and multiple transmission events, which could make interpretation difficult. Moreover, cross-

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sectional studies are limited in their ability to provide information on infection dynamics as they typically do not account for temporal changes, and they are generally not well equipped to provide confidence with regards to establishing the direction of transmission, due to the lack of adequate longitudinal studies or environmental sampling, or complete molecular and epidemiological data integration(Gandini et al., 2021) Whole genome sequencing, spatial molecular epidemiology, and other more sophisticated methods provide better resolution, but are not widely used in many applications (Hill et al., 2021). Integrated One Health surveillance systems that incorporate genomic and ecological, temporal and epidemiological data to better elucidate transmission dynamics and enhance the accuracy of source-attribution analyses for zoonotic parasites are needed for future research.

8.3 Understudied Matrices and High-Risk Settings

Although there has been significant advances in knowledge on the transmission of zoonotic parasites, there are several knowledge gaps on some of the environmental matrices and high risk settings. Many surveillance and source-attribution studies have been conducted on humans, livestock and drinking water systems, while other possible reservoirs like soil, wastewater, fresh produce, wildlife habitats and food processing environments are relatively under-studied (Fletcher et al., 2012). This imbalance will hinder the knowledge of complex transmission routes, and can lead to underestimation of significant sources of human infections. As a result, infectious diseases related to environmental exposure, frequently remain poorly characterised and hard to assign to a specific reservoir(Rohani et al., 2009).

However, viable cysts, oocysts, and eggs of zoonotic parasites can persist in environmental matrices (soil, sediments, wastewater, irrigation water) for long periods of time. They are not always monitored routinely due to methodological problems, low numbers of pathogens, and the lack of standardized sampling procedures (Hoorfar, 2011). Therefore, the role of environmental contamination in the spread of *Cryptosporidium spp.*, *Giardia duodenalis*, and *Toxoplasma gondii* is still not well understood. The reuse of wastewater in agriculture and contamination of fresh produce are of particular concern but have not been extensively studied in many parts of the world(Putignani & Menichella, 2010).

There are also a number of high-risk settings that are poorly researched. These include refugee camps, informal settlements, day care centers, livestock markets, slaughter houses, intensive farming systems and areas with poor sanitation facilities. These can lead to close contact between human beings and animals and contaminated environmental sources, which can promote the spread of parasites(Smit et al., 2006).). However, epidemiological and molecular research in these contexts is less common, especially in low- and middle-income countries where parasitic infections are likely to be more prevalent. Targeted surveillance and research in under-studied matrices and high-risk environments are therefore needed to enhance source attribution, risk assessment, and effective One Health control strategies (Saibu et al., 2026).

8.4 Standardization and data-sharing gaps

One of the most difficult issues in zoonotic parasite surveillance and source attribution is the fact that study design, sampling, laboratories and reporting systems are not standardized. Comparisons among different studies are complicated because different research groups may use different molecular markers, genotyping methods, and analytical methods, as well as different diagnostic techniques (Sini et al., 2023). This variability can result in inaccuracies in determining prevalence, sources of parasite transmission, and the significance of the reservoir. Consequently, data sharing across regions and sectors is still challenging, so the effectiveness of global monitoring is hindered and there is less trust in data source attributions (Perestrelo et al., 2023).

One area where standardization problems are especially prominent is molecular epidemiology. There are differences in sensitivity and discriminatory power from one study to another

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that may result in different genetic locations being used to identify, genotype, or subtype a species (Kosoy et al., 2018). Moreover, variations in DNA extraction, PCR conditions, sequencing technologies and pipelines in bioinformatics software can affect the results, making it difficult to directly compare datasets. There are no universally accepted protocols for detection and characterization of parasites, and this is, thus, a great obstacle to source attribution and tracking the transmission of parasites(D'Amore et al., 2016).

Limited data sharing further hinders surveillance and research. The health, vet, environment and food sectors often have individual databases with limited interoperability with each other. The sharing of information between organizations and countries is often inefficient due to legal restrictions, privacy concerns, institutional policies, and differences in data formats (Ifedinezi et al., 2024). This means that useful epidemiological and molecular information may not be available on the hands of the researchers and health authorities, which would lower the probability to find the transmission routes across sectors and emerging risks (Coque et al., 2023).

To resolve these challenges, the implementation of harmonized surveillance protocols, the adoption of a standardized molecular approach, sharing of reference databases and integration of One Health data platforms are widely recommended. There are opportunities due to advances in genomic technologies and international cooperation to enhance data comparability and enable real-time exchange of data. Increased standardization and data sharing will increase the accuracy of the source attribution, outbreak investigation, and evidence-based decision making in the prevention and control of foodborne and waterborne zoonotic parasites (Struelens et al., 2024).

9. Future One Health source-attribution framework and conclusion

9.1 Molecular One Health framework

The transmission of zoonoses is becoming increasingly complex, necessitating an integrated approach in surveillance of the human, animal, environmental and food chains with the deployment of advanced molecular-epidemiological tools. Traditional surveillance systems are frequently used separately and are not able to detect the transmission pathways and to correctly identify the sources of infections. A One Health step-wise molecular approach to help with source attribution by step-by-step integration of data from various fields has therefore been suggested. This method helps to identify parasite reservoirs, the mechanisms of parasite transmission, and the environmental interfaces, which ultimately helps in evidence-based disease prevention and control measures (Singh et al., 2024).

The first, is coordinated sampling of human, livestock, companion animal, wildlife, food, water and environmental reservoirs. A comprehensive surveillance is necessary as zoonotic parasites often are found in more than one environmental matrix and host. Rep. sampling of all relevant sectors will increase the chances of finding potential sources of infection and help to make source-attribution analyses more accurate. There is a need to adopt standardized sampling procedure to ensure the comparability of the data between regions and surveillance programs (Sharan et al., 2023). The second step involves molecular characterisation (species identification, genotyping and subtyping). Parasite isolates from various hosts and environmental sources can be compared using techniques like PCR and multilocus genotyping, as well as whole genome sequencing. Identical or highly similar genotypes in multiple areas can give clues to links in transmission and reservoir origins. The use of molecular results in conjunction with epidemiology and environment further bolsters the source-attribution analysis(Ahmed et al., 2006).

Data integration and risk modelling are the third step. Using the tools of geographic information systems (GIS), spatial epidemiology and quantitative risk assessment modelling, information gained from molecular analysis, environmental monitoring, food chain investigations and epidemiological studies should be brought together. This can help identify "transmission hot spots", high-risk populations and critical control points in the food and water

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supply chain. These analyses can inform specific interventions and enhance outbreak preparedness (Liao et al., 2026).

The last step highlights the need for ongoing information exchange and cooperation between public health officials, veterinarians, environmental health and water bodies, food safety organizations and food research institutions. Scientific results need to be translated into practical disease-control decisions, which requires good interaction, and a harmonized surveillance system. The proposed framework incorporates the molecular epidemiology approach to One Health surveillance, bringing together a holistic approach for enhanced source attribution, better risk assessment, and global burden reduction of foodborne and waterborne zoonotic parasitic diseases (Struelens et al., 2024).

9.2 Future research

Although much has been learned regarding foodborne and waterborne zoonotic parasites, there remain a number of gaps in knowledge that hinder efforts in effective source attribution and disease control. This study calls for further research into the development of integrated One Health tools and approaches to better understand transmission dynamics through integration of human, animal, environmental, and food-chain data. The available evidence suggests that for many parasites, infections occur as a result of interactions between several reservoirs and pathways, but to date relative contributions of these reservoirs are not known.

Standardization of molecular approaches, surveillance, and data-sharing strategies should also be a priority of future research. The harmonization of methodologies would enhance the comparability of the data from the different studies and bolster the global surveillance efforts. In addition, a combination of molecular epidemiology with quantitative risk assessment, spatial analysis and mathematical modeling may help to better understand the dynamics of parasite transmission. The interdisciplinary approaches are crucial for deriving interventions based on evidence and enhancing public health outcomes.

9. Conclusion

Foodborne and waterborne zoonotic parasites remain major One Health challenges because their transmission involves humans, livestock, companion animals, wildlife, food chains, water systems, soil, wastewater, and contaminated environments. This review highlights that conventional detection methods are useful for confirming parasite presence but are insufficient for identifying the true source, reservoir, or route of infection. Molecular tools such as PCR, qPCR, sequencing, gp60 subtyping, multilocus genotyping, WGS, metabarcoding, eDNA, and spatial molecular epidemiology provide stronger evidence for source attribution and transmission-pathway analysis. For *Cryptosporidium* spp., *Giardia duodenalis*, and *Toxoplasma gondii*, integrated molecular evidence is essential to distinguish zoonotic, anthroponotic, foodborne, waterborne, and environmental transmission. However, fragmented surveillance, limited standardization, poor environmental sampling, and weak data integration remain major gaps. Future control should rely on coordinated One Health surveillance, matched sampling, high-resolution molecular typing, and targeted interventions to reduce zoonotic parasite transmission.

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