Toxoplasma Gondii Infection and Toxoplasmosis in Different Species: A Review

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Abstract: Toxoplasmosis is a zoonotic parasitic disease caused by *Toxoplasmosis gondii* – a protozoan of important medical and veterinary significance. Its unique sexual cycle (transmission between intermediate and definitive hosts) and asexual cycle (transmission between intermediate hosts via carnivorism) make transmission and infection vary according to complex outer and inner environment. IFA, ELISA and qPCR are the mainstream detection method. In this article, the strengths and weaknesses of diverse commonly used detection approaches were compared based on their specificity and sensitivity. Though no licensed vaccines have been applied to human clinical treatment, a few potential antigens for vaccine development are discussed in this article. Overall, this article aims to summarize the knowledge on the prevalence and effects of infections with *T. gondii* in the most important species, including human being and livestock, and how the Toxoplasmosis is detected and treated.

1 INTRODUCTION

Almost every homoeothermic vertebrates worldwide share a common parasitic zoonosis which caused by *Toxoplasma gondii* - an obligate intracellular protozoan parasite, and it is assumed that *T. gondii* have infected over one-third of human population (Hosseini et al. 2019). Almost every infection happens in intermediate hosts of *T. gondii* will experience three stages: a rapidly dividing invasive tachyzoite stage which causes tissue destruction and pathogen proliferation; a slowly localized dividing stage in CNS or muscle tissue where tachyzoites convert to tissue cysts or bradyzoites; and eventually achieve an environmental stage which characterized by sporozoites (contained within oocysts) shedding (Gangneux et al. 2012). And for the only definite host – felines, since they lack enzyme delta-6-desaturase, which is required during linoleic acid metabolism in intestine, this deprivation results in systemic linoleic acid accumulation which is unique in mammals and makes *T. gondii*'s sexual development possible. Feline's parasited epithelial cells can remove from original tissue and release oocysts into feline's feces, from which oocysts can spread to wide range of environment (Weiss et al. 2004).

Ascribing to high vitality of feline's oocysts, which can survive and remain parasitic in extreme condition for several months, we need to pay attention on the foodborne transmission routes. The contaminated food includes meat (especially chicken, lamb, and pork) or shellfish (such as clams, mussels, and oysters) or unwashed vegetable are highly likely to be pathogenic when expose to this parasite (Dubey wt al. 2011). What's more, for people keeping felines as family pet, accidentally ingesting oocysts after cleaning a feline's litter box is regarded as a highly possible approach to this parasite. For other people who do not contact feline directly, ingesting oocyst via contaminated soil or water can also lead to the infection of *T. gondii*. In addition to food contamination and contact with oocysts via infected feline, congenital transmission is also regarded as a fatal route. That is, because women during or just before pregnancy may not show any symptoms, without effective pregnancy check-up *T. gondii* can remain undetected and possibly result in miscarriage, stillborn and child disability including mental retardation, liver damage etc. (Dubey et al. 2004).

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2 SEVERITY AND SYMPTOM

It is estimated that the global prevalence of toxoplasmosis is around 30%, affecting more than 1 million people annually and is ranked as the third highest burden of foodborne disease, about 17% of the total foodborne disease burden in the European Region (WHO 2017). The main symptoms and severity will be illustrated by diverse species, including family pets, human beings and farm animals.

2.1 Toxoplasmosis in Family Pet

Felines are the most commonly known carrier of *T. gondii*, and they are the only natural species that excrete environmentally resistant oocysts. The global pool of *T. gondii* seroprevalence suggests positive samples take up 35% (95% Cl: 32-38%) (Montazeri et al. 2020). Apart from felines, dogs are also susceptible to this ubiquitous parasite, even though the toxoplasmosis is more common in felines than in dogs which promote the potential cross infection to the families that keep felines and dogs at the same time. The high infection rate of *T. gondii* ascribes to low morbidity and mortality in these two species. Meanwhile, the high-efficiency of direct fecal-oral cycle also contribute to ascending seropositivity (Lindsay 2009). Furthermore, once infected with *T. gondii*, animals will carry toxoplasmic cysts lifetime long, and worse, their oocyst-shedding periods are highly unpredictable (Lindsay et al. 2009), which makes the infected domestic felines of greater potential danger. Other kind of pets that occupy smaller niche such as rabbits and birds are also susceptible to *T. gondii* and its effect to human being are often being underestimated. For instance, a cross-sectional study in Egypt - ELISA was used to analyze a total number of 150 rabbits for *T. gondii* IgM and IgG antibodies, the seropositivity is 40 (26.7%) of 150 rabbits raised in Cairo, Qalyubia, and Sharkia Governorates (Hassanen et al. 2017). This index suggests that domestic rabbits has already become a source of *T. gondii* infection endemically. Also, in Japan, serum samples of 337 rabbits were examined and the seropositivity for *T. gondii* was 0.89% (3/337) and 0.29% (1/337) in IgG and IgM ELISA respectively （Salman et al. 2014）. Besides, human had long been keeping birds as family pet to replace little mammals for cities' limitation. To illustrate, recent research that covers three representative administrative region in Gansu province suggests the overall *T. gondii* seroprevalence was 11.21% (77/687) (Wang et al. 2014). Therefore, since pet animals have frequent daily interaction with human beings, both pet owners and public health workers should develop more unique and comprehensive prevention plans against *T. gondii*.

2.2 Toxoplasmosis in Human

Only occasional inflammatory response in intestinal system, or temporary fever and muscle soreness are reported suggest that *T. gondii* is not a deadly parasite to most healthy people (Watanabe et al. 2018). Thus, healthy population generally do not need special treatment for toxoplasmosis infection. However, people with immunodeficiency and women during pregnancy may show some more vital symptoms which require medical support.

For immunocompromised cohort, especially those diagnosed with the acquired immunodeficiency syndrome (AIDS), deficiency of immune system renders early recognition less quickly and effectively. Also, current therapy for acute toxoplasmosis is not applying to clear chronic infection because of the relatively slow dividing of bradyzoite and asynchronous growth, which generally result in long–lived affection (Dunay et al. 2018). After ingesting bradyzoites, except for possible ileitis and other severe lesions of digestive system, *T. gondii* can spread beyond the gut to deeper tissues through lymphatics and blood, including spleen, liver, lungs and gradually reaching the brain which led fatal infection within CNS (Rinkenberger et al. 2021). Even though human being can take advantage of a special mechanism named immune privilege to protect CNS from infection in most circumstances, study has shown that to *T. gondii* and some other pathogens, this privilege is invalid since they can across blood-brain barrier (Barragan et al., 2019 Rinkenberger et al. 2021). This explains the high incidence of focal necrotizing encephalitis due to *T. gondii* among patients with AIDS (Ringenberger et al. 2021). Moreover, not only AIDS patients react this way to *T. gondii*. Some clinical cases also suggest that the clinical findings of immunodeficient patients without AIDS are similar to those of AIDS, such as severe combined immunodeficiency (SCID)

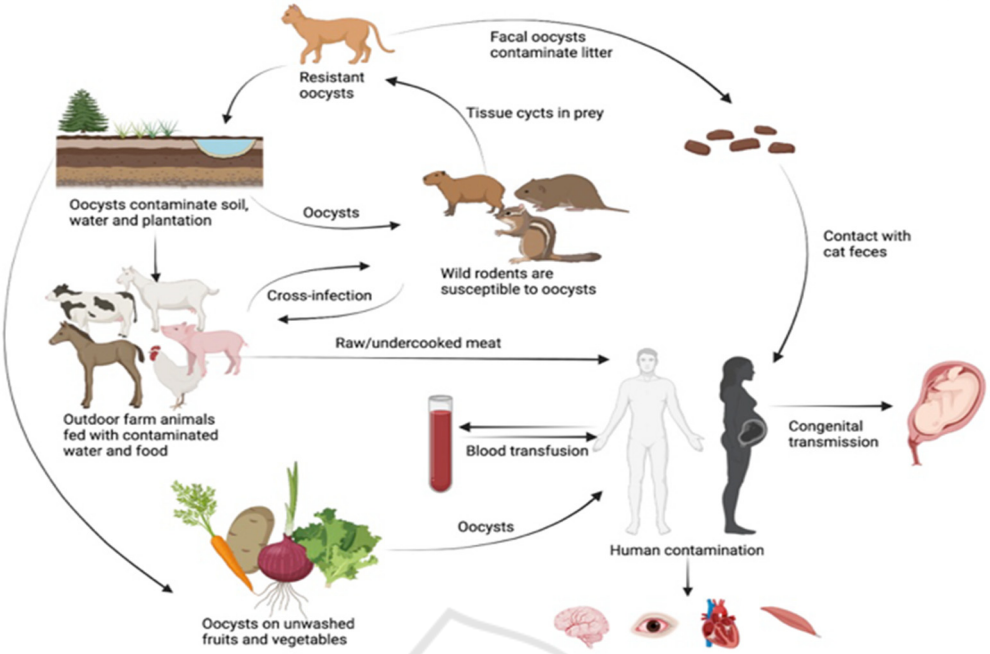

Fig1. Transmission route of T. gondii

Apart from immunocompromised population, infection in pregnant women also shows fetal morbidity and other subclinical neonatal infection which usually developed into ocular and neurological sequelae (Fanigliulo et al. 2017). The most commonly occurred complication of congenital toxoplasmosis are abortion, stillborn during pregnancy and major ocular and neurological sequelae, ranging from slight diminution of vision to retinochoroiditis, intracerebral calcifications and hydrocephalus after parturition (Khan et al. 2018). Besides, the risk of congenital infection and the severity depends on the gestational age when *T. gondii* infection occurs – if the infection occurs in early pregnancy, the chance of transmission is relatively low. However, in later phase, the transmission rate is much higher which is quite common in other laboratory animals. Clinical cases of chorioretinitis shows that as the gestational phase develops, the rate of transmission to fetus can rise from 15 to 65% (Remington et al. 2006).

2.3 Toxoplasmosis in Farm Animals

In addition to felines and human, hazards of toxoplasmosis to farm animals are observable but usually overlooked. In outdoor farms, some highly uncontrollable factors, such as the presence of infected felines and rodents in farms, as well as the pollution of the diet, water and soil, may lead to infection and spread of toxoplasmosis within farm animals. For example, pork is considered to be a crucial food resource to human being except for Muslim area. However, acute toxoplasmosis has long been reported after ingesting contaminated pig meat. The seropositive in Estonia is 5.8% (22/382) and the proportion of seropositive pig in one herd varies between 0 and 43% (Santoro et al., 2017), the collection of 89 indoor-reared sows, 128 indoor finishers and 37 outdoor-reared finisher in Denmark found that 33.7% sows reared indoors, 3.1% indoor-reared finishers and 10.8% outdoor reared finishers were *T. gondii* seropositive (Kofoed et al. 2017). Also, many pig producers are unaware that *T. gondii* infection in pigs are important, and the public impacts and risk of *T. gondii* are uncommon knowledge to producers which makes the toxoplasmosis spread more easily (Wagenberg et al. 2020). Overall, both farm owners and public health workers should complement specific measure to improve the control of *T. gondii* in pigs.

Besides, as an important farm animal, horse meat and milk are significant food resources in some region. The prevalence of horse racing also increase vulnerability to the infection of toxoplasmosis. Even the clinical toxoplasmosis is very limited now, we still cannot rule out the possible damage that horses can rise. The life cycle of *T. gondii* within horses are similar to those of other intermediate hosts species –

the cyst wall dissolved in horses' digestive system, releasing oocyst, and invade intestinal epithelial to differentiate into tachyzoites. Tachyzoites can form special vacuoles within host cells, rapidly replicating tachyzoites multiply cell will lead severe rupture and possible death. This process generally results in lesion characterized with necrosis and granuloma. Besides, this kind of lesion often found in lungs, livers and spleens of horses, gastrointestinal tract, central nerve system and heart are also commonly infected among reported samples (Kimble et al. 2021, Shaapan et al. 2008). Case study suggest that the initial infection may happens in caecum, and the infection of gastric area is limited to slight serositis, no neurological signs was observed and no lesion was detected in heart (Kimble et al. 2021). Even though few clinical horse's toxoplasmosis has been reported previously, serological evidence has shown a worldwide infection of *T. gondii* among horse.

Except for pigs and horses, sheep and goats are also common livestock human share high exposure. Apart from potential danger to infect human, as worldwide economic livestock providing functional wool, infection of *T. gondii* triggers sheep abortion which is a great loss to sheep owners. Toxoplasma oocysts picked up from hay or feed tend be contaminated by fines and cause toxoplasmosis, and cysts shed by an adolescent cat can infect more than a thousand ewes (Shaapan et al. 2008).

3 DIAGNOSIS AND TREATMENT OF TOXOPLASMOSIS

3.1 Diagnosis

Since *T. gondii* first be discovered in 1908 annamed a year after, its medical and veterinary importance became widely known in 1939 and 1957 for two famous clinical and veterinary cases (Cowen et al. 1939). Researchers after that had strived to figure out the most effective approach to detect and diagnose this protozoan parasite. Direct observation of the parasites in stained tissue sections or other biopsy material is primitive diagnosis method which is one of the most favorable detection approaches before modern molecular biotechnology was invented. But direct microscopy is used less frequently nowadays because of the difficulty to obtain specimens. That is, parasite can only be extract and isolate from body fluids (e.g. cerebrospinal fluid), which is complicated and requires considerable labor and time.

Cellular level detection of Toxoplasma-specific antibodies is the primary diagnostic method in laboratories today, for commercially available kits can tremendously reduce the workload of the scientists (Marques et al. 2020). The immunofluorescence assay (IFA), enzyme-linked immunosorbent assay (ELISA) tests for IgG and IgM antibodies are the tests most commonly used today and had utilized in a large amount of different samples (Barros et al. 2017). The IgG serves as the indicator of the immune status, while IgM indicates precise time when infection happens which is particular important for pregnant women. ELISA's edge lies on its hyper-sensitivity, while IFA shows greater specificity (Garcia et al. 2006). 300 serum samples from sheep slaughtered in main abattoir in in Cairo, Egypt were measured by a comparative serological examination, the result shows ELISA has high sensitivity (90.1%) and IFA, which suggest the lowest sensitivity (80.4%). Conversely, IFA was proved to have the highest specificity (91.4%), and ELISA (85.9%) (Shappan et al. 2008). And the modified agglutination test (MAT) is uniquely designed to adapt a large number of different hosts. For example, When toxoplasmosis abortion storm occurred in a flock of purebred Suffolk ewes on a farm in Texas, MAT was functioned to exam the sheep infection (Edwards et al. 2013). Besides, MAT is also used to detect peafowls with *T. gondii* in Yunnan Province (Tian et al. 2012) and the seroprevalence of domestic donkeys (Equus asinus) in Durango, Mexico (Alvarado et al. 2015). As well as the seroprevalence of *T. gondii* in Harbor Seals (Phaco vitulina) in Southern Puget Sound, Washington (Lambourn et al. 2001).

In addition, Polymerase Chain Reaction (PCR) and real-time PCR (qPCR) is another widely used method to detect *T. gondii* especially for sampling in food market, because *T. gondii* oocysts persist and remain infective in water and soil for a long time which result in food contamination extensively (Marques et al. 2020). However, it is noteworthy that there are imperative but complex preparations before carrying out PCR or qPCR for identification and quantification of *T. gondii* DNA in fruit and vegetable, that is, the concentration of oocysts after washing samples which applies high resolution water filtration and immunomagnetic separation (Marchioro er al. 2016). qPCR is a reformative version of traditional PCR, the major advantages of qPCR is its ability to quantify the infection load of a clinical specimen, and significantly reduce the chance for being false positive since traditional direct PCR

have a different accuracy in detecting *T. gondii* within different sample sizes regardless of sample source (Rani et al. 2020). Besides, for wild animal and other meat products, PCR analyses shows great sensitivity and specificity too, manifested by an excellent discriminating ability for each of the examined tissues (Santoro et al. 2019). Heart and diaphragm sample from wild rabbits in central Portugal are tested by PCR which separately amplified the 5′ and 3′ ends of the surface antigen 2 (SAG2) which has been extensively used for genotyping *T. gondii* isolates (Sabaj et al. 2010).

Table 1: Advantages and limitations of diagnosis methods.

Method	Advantages	Limitations	Reference
Microscopy	Simple and direct	Slow Considerable labor Hard to obtain specimen	28
IFA	High sensitivity and specificity	Labor, time and cost consuming	30, 31
ELISA	Low cost and high sensitivity and specificity	Sensitivity and specificity are highly dependent on the antigen used	30, 31
MAT	Medium sensitivity and specificity Low cost	Need well-trained laboratory technicians	2, 32
PCR and qPCR	High sensitivity and specificity qPCR reduce PCR's false positive specimen	Require concentration of specimen Possibility of false-possitive	29, 36, 37, 38, 39
LACA	Very high specificity in chicken	Not applicable for all species yet	51

3.2 Treatment

Felines are only definite host of *T. gondii* and also very prevailing family pets, it is of greater potential danger when their owners and other who have access to them are immunocompromised population or prepare or during pregnancy. Options for diagnosis in felines include fecal examination for oocysts and serologic testing. It can be difficult to accomplish eventual diagnosis of toxoplasmosis (Barrs et al. 2006), so if any clinical improvement is not observed within three days, the diagnosis of toxoplasmosis may be questioned. Besides, general treatment usually involves antibiotic treatment, clindamycin is most commonly used clinically, either alone or in combination with corticosteroids when severe inflammation happens in eyes, or even worse, the central nervous system is involved. Treatment should ideally be started immediately after diagnosis is made and continued for several days after signs have disappeared. Treatment for human toxoplasmosis especially within immunocompromised population and congenital transmission route did not achieve significant breakthrough, while some regular treatment has become increasingly mature. Infants with congenital toxoplasmosis, for example, after maternal seroconversion during the first two trimesters, spiramycin (9 million IU/d) was prescribed until birth. In other condition, such as the third trimester or when the maternal transmission risk is high, Doctors can prescribe pyrimethamine and sulfonamide systemically immediately after the diagnoses are made, and the prescription generally last around one year (Kieffer et al. 2008). However, these drugs are not ideal choices since clinical cases shows they have various serious side effects: some hematological abnormalities, bone marrow suppression etc. and when the parasite encysts in the tissues, these drugs can hardly eliminate them and also poorly tolerated (Antczak et al. 2016). In addition, the follow-ups were generally limited to two years the first two years is generally thought to be a principal end point for diagnosis of a first retinochoroiditis lesion was decided during this period. More importantly, the risk for a lesion to develop in the absence of a previous retinochoroiditis is weaker after the age of 2 years (Antczak et al. 2016, Kieffer et al. 2008). Also, *T. gondii* infection is seen as a main pathogen that leads to the death of immunocompromised people, especially AIDS

patients. Because their susceptibility to cancer (e.g., lymphoma, leukemia, and myeloma etc.) and the subsequent regular antitumor treatment make them have better opportunity to reactivate latent *T. gondii* infection. Recent study has shown that sulfonamides, in conjunction with Pyrimethamine (PYR) are mainstream in toxoplasmosis treatment nowadays, but AIDS patients are unable to tolerate this treatment. Also, there have been several failed reports on the long-term treatment of toxoplasmosis among AIDS patients (Luft et al. 1992). That is, even great progress has made to understand the pathogenesis of *T. gondii* and more effective medicine has been developed. Medicines we currently prefer use to against toxoplasmosis still show some side effects, thus prolonged courses are required, and both effect and side effect may be fluctuated by differences among the virulence of *T. gondii* strains found around the world (Alday et al. 2017).

4 DISCUSSION

Apart from regular detection methods, there are significant number of updated discoveries in detection method of *T. gondii* serving as an extension of traditional means. For example, MAT was found to have greater sensitivity and specificity compared with PCR and qPCR. For instance, in the study detecting the overall *T. gondii* prevalence from Phillip Island, Australia, the 95% confidence interval of qPCR and MAT are 72.6–85.0 and 84.6–95.8, respectively (Adriaanse et al. 2020). Moreover, some targeted novel detection method has now been studied to detect *T. gondii* in certain species. One apt illustration involves luciferase-linked antibody capture assay (LACA), LACA is detected to have an unexpected great sensitivity (90.5%) and specificity (95.4%) when testing chicken with *T. gondii* and some other pathogens, suggesting a better performance in special species compared with its conventional counterparts (Duong et al. 2020). Closer scrutiny to those evolutionary detection methods reveals that they are generally multidisciplinary approaches, and it is this very character that makes them more productive than their conventional counterparts. For example, MAT in conjunction with Bayesian latent class (BLC) analysis, which is a computationally model in Statistic, forms a new method to determine sensitivity and specificity we researchers faced with an absence of sufficient reference samples (Adriaanse et al. 2020). LACA takes advantage of a novel luciferase-linked capture antibody platform by using recombinant nanoluciferase conjugated GRA8 antigen is a perfect example of utilizing molecular science (Rezaei et al. 2019). Thus, the prosperity of interdisciplinary detection of *T. gondii* should be developed in the future studies.

Although the life circle and pathogenetic mechanisms of *T. gondii* has already been revealed, we do not have any vaccine for human been licensed by now (Rezaei et al. 2019). However, experiment in model animals suggests great progress in vaccine development. Some essential antigens have been discovered: dense granule antigens (GRAs), rhoptry antigens (ROPs), surface antigens (SAGs) and microneme antigens (MIC). Among them, with active involvement in parasites virulence, survival and replication processes, serving as major proteins of the excretory secretory antigens, GRAs are considered as a predominant vaccine candidate and in recent studies, common laboratory animals such as ewe, mouse, pig and sheep were used for a wide variety of experiments to evaluate humoral responses (Rezaei et al. 2019). Researchers deem GRA7 the most competitive candidate for vaccine experiment since TgGRA7 has been found in almost every infectious stages of parasite, Tests to evaluate immune response of different GRAs in little mammals such as sheep indicates GRA7 present greater IFN γ level within whole experiment process (Hiszczyńska-Sawicka et al. 2011). ROPs takes part in the cell invasion and the formation of parasitophorous vacuole (PV) both process are essential for survival of *T. gondii* in host cells (Boothroyd et al. 2008), recent study in mice indicates multi-epitope ROP8 DNA vaccine can induce strong humoral and cellular responses and extends the survival time (Foroutan et al. 2020). Furthermore, it is noteworthy that a nonselective beta-adrenoceptor antagonist – propranolol as adjuvant in association with tachyzoite SAG-1 as an antigen can leads to stronger immune reactions to Th1 and cellular immunity (Abasi et al. 2019). Since MIC plays a crucial role in entering host cells and parasites gliding motion, an increasing number of articles about MICs suggests their growing importance as vaccine candidates that can induce intense immune responses against toxoplasmosis.

As the improving consumer demand for “animal-friendly” or “organic” animal products, farm animals have an increasing opportunity to be infected by *T. gondii*’s oocysts because of the better chance of outdoor activities. More importantly, grazing animals raised by nomadic people on pasture are directly threatened by infected wild felines and rodents, at the same time the uncovered feed and the contaminated soil and water are dangerous infection source too. One apt illustration involves horses and sheep: the

overall prevalence of *T. gondii* in the horses was 5.15% (5/97) for Jilin Province, China, 5.55% (3/54) for Liaoning Province, China, and 7.50% (6/80) for Xinjiang Uygur Autonomous Region, China (Ren 2019). And the regions in Qinghai Province, specific IgG against *T. gondii* are detected in 21.33% (95% confidence) (Liu 2015); a cross-sectional study carried out in 319 random sheep in Northwestern Rio Grande do Sul State, Brazil shows 70.2% (224/319) *T. gondii* detection (Consalter et al. 2019). Although many farm owners are aware of potential risk and consequences of *T. gondii* infection within pigs, the more profound and extensive risks regarding of public health are not yet comprehensive knowledge to all farm owners, even in developed countries, such as Dutch. Furthermore, farm owners vary in motivation and capability to control and address *T. gondii* infections. We should warn farm owners that they should not expect some tangible symptoms on livestock when toxoplasmosis infection happens, because *T. gondii* infection do not have sensible performance in most circumstances. Moreover, we should suggest farm owners to be more conscientious about stray cats and rodents living in the farms and call for their consciousness about farm health condition as well as breeding method.

5 CONCLUSIONS

T. gondii as a world-widely distributed parasite disease can severely infect pregnant women and people with immune deficiency. Since no apparent symptom in healthy individuals and other intermediate hosts, which makes *T. gondii* hard to be detected. So far, PCR and qPCR are utilized more commonly in large-scale detection, while ELISA is wildly accepted in clinical detection. Other targeted detecting methods such as LACA for the specific species are urgently needed. When individual get infected by *T. gondii*, the common treatment is antibiotic treatment to control the proliferative tachyzoite cycle and no specific medicines for all stages of *T. gondii* development. Additionally, the cases of farm animals infected by *T. gondii*, such as pigs, horses, and sheep, are gradually increasing and causing economic loss. Therefore, the spread of *T. gondii* in farm should be paid attention. Since there are growing number of vulnerable groups and worldwide distribution infection cases, the infection of *T. gondii* is worth heeding.

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