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Investigation of a Toxoplasmosis Outbreak in a Rural Family in Iguaraçu, PR, Brazil

Investigação de surto de toxoplasmose em família rural no município de Iguaraçu, PR, Brasil

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ABSTRACT

Introduction: Toxoplasmosis is a disease caused by the parasite *Toxoplasma gondii*, which is transmitted through the consumption of contaminated food or water, ingestion of infected meat, or via transplacental transmission. Humans act as intermediate hosts, while felines serve as the definitive hosts. **Objective:** To investigate an outbreak of toxoplasmosis affecting a family residing in the rural area of Iguaraçu, Paraná; southern Brazil. **Method:** DNA analysis of *T. gondii* was performed on soil, water, and cat feces samples collected at the outbreak site. The samples were sent to the Parasitology Laboratory of the State University of Maringá, PR, and the affected individuals were monitored in an outpatient setting. **Results:** A toxoplasmosis outbreak was confirmed in a family residing in a rural area, leading to severe outcomes, including miscarriage and ocular manifestations of the disease. The environment exhibited several risk factors, such as the presence of cats, chickens, pigs, cows, a ground-level water tank, a well, and untreated sewage discharged directly into a nearby river. The residents consumed water from both the tank and the well. However, environmental analysis using conventional PCR did not detect *T. gondii* DNA in the water, soil, or cat feces samples. **Conclusion:** The infection is likely attributed to an external factor that has not yet been investigated or to the limited sensitivity of the collected samples for detection via PCR. This underscores the difficulties in identifying *T. gondii* oocysts in environmental samples and highlights the necessity of conducting further studies in the region, along with a thorough investigation into the family's food sources.

Keywords: Toxoplasmosis, Congenital; Toxoplasmosis, Ocular; Zoonoses; Epidemiology.

RESUMO

Introdução: Toxoplasmose é uma doença causada pelo parasito *Toxoplasma gondii*, transmitida por alimentos ou água contaminados, carne infectada, ou via transplacentária, com humanos como hospedeiros intermediários e felinos como definitivos. **Objetivo:** Investigar surto de toxoplasmose que atingiu uma família residente da zona rural de Iguaraçu, Paraná, Sul do Brasil. **Método:** Foi realizada a pesquisa do DNA de *T. gondii*, em amostras de solo, água e fezes de gatos, coletadas no local do surto. As amostras foram encaminhadas ao Laboratório de Parasitologia da Universidade Estadual de Maringá, PR, e os envolvidos foram acompanhados via ambulatorial. **Resultados:** Foi confirmado um surto de toxoplasmose em uma família residente em área rural, resultando em consequências graves, como aborto e manifestação ocular da doença. O ambiente apresentava fatores de risco, como a presença de gatos, galinhas, porcos, bois, caixa d'água localizada ao nível do solo, um poço e esgoto sendo despejado diretamente em um rio nas proximidades. Os moradores consumiam água da caixa d'água e do poço. Contudo, a análise ambiental por PCR convencional não detectou DNA de *T. gondii* nas amostras de água, solo e fezes de gatos. **Conclusão:** Acredita-se que a infecção tenha sido causada por um fator externo ao domicílio, ainda não investigado, ou que as amostras coletadas tenham sido insuficientes para detecção pela PCR. Isso reforça o desafio da identificação de oocistos de *T. gondii* em amostras ambientais, destacando a necessidade de estudos mais aprofundados na região e de uma investigação detalhada sobre a procedência dos alimentos consumidos pela família.

Palavras-chave: Toxoplasmose Congênita; Toxoplasmose Ocular; Zoonoses; Epidemiologia.

INTRODUCTION

Toxoplasmosis is a zoonotic disease caused by the protozoan *Toxoplasma gondii* (*T. gondii*), an intracellular parasite that requires multiple hosts to complete its life cycle[1]. Felines are the main hosts, excreting oocysts in their feces, which can infect humans and other animals[2]. *T. gondii* uses multiple routes of transmission, contributing to its widespread prevalence among humans and animals. The main routes include foodborne transmission through the consumption of undercooked meat containing tissue cysts or contaminated products, environmental transmission through the ingestion of sporulated oocysts in soil or water, and congenital transmission from mother to fetus during pregnancy via transplacental transmission[3]. Less common routes comprise organ transplants, blood transfusions and laboratory accidents[1,4].

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Congenital toxoplasmosis (CT) occurs when a pregnant woman is infected with the parasite for the first time and transmits the parasite to her fetus[5]. The risk of vertical transmission increases as pregnancy progresses, but the severity of fetal manifestations tends to be greater when maternal infection occurs early in pregnancy, between 10% before 12 weeks and 71% around 36 weeks[6]. Although many infected newborns are asymptomatic at birth, CT can result in serious consequences, mainly, chorioretinitis, the most common manifestation, in the long term[7-8].

The global prevalence of *T. gondii* infection is high, affecting approximately one-third of the world's population[5]. In Brazil, the rates are remarkable, reaching up to 50% in children and 80% in women of reproductive age[9]. The three classic clinical forms of toxoplasmosis include ocular toxoplasmosis (OT), CT and cerebral toxoplasmosis[4,10]. The most severe forms of toxoplasmosis are found in immunocompromised patients and newborns with congenital infection. These can cause miscarriages, stillbirths, and serious sequelae such as retinochoroiditis, cerebral calcifications, hydrocephalus, developmental delay and epilepsy[7,10].

It is estimated that TC affects around 190,100 newborns a year worldwide, with a global incidence rate of 1.5 cases per 1,000 live births[8]. In Brazil, the prevalence varies from 0.1 to 3.4 cases per 1,000 live births, with significant impairments such as neurological damage (35% of cases), eye alterations (80%), and hearing loss (40%)[11].

Although the central nervous system is the primary target, *T. gondii* has a tropism for various organs, including the eyes, where it can cause irreversible damage[12]. This ocular affinity is evidenced in OT, a serious manifestation of the infection, which can lead to severe complications, including visual loss and blindness. OT encompasses a variety of ocular disorders, such as retinochoroiditis, optic neuritis, and uveitis, with retinochoroiditis of the posterior pole being the most frequent form. This condition is characterized by yellowish-white retinal lesions and reduced visual acuity[10].

Uveitis, characterized by inflammation of the uveal tract (iris, ciliary body and choroid), can cause significant visual loss, and blindness in more severe cases[13]. OT is one of the most frequent infectious causes of posterior uveitis. This condition not only impairs vision but also has a high rate of recurrence or relapses, increasing the risk of severe visual impairment or complete blindness[14].

The consequences of toxoplasmosis have a global impact, but in Brazil, the high prevalence of atypical strains and the occurrence of outbreaks highlight the importance of the disease in the national context[6,15]. Health surveillance requires mandatory notification and investigation of these events, with records dating back to the first documented outbreak, in 1967, in Bragança Paulista, SP. Since then, more than 35 outbreaks have been reported in the country[16]. The main sources of transmission include contaminated water, raw or poorly sanitized food, undercooked meat, and unpasteurized goat's milk. The largest global outbreak was recorded, in 2018, in Santa Maria, RS, with more than 900 cases, surpassing the 2001-2002 outbreak in Santa Isabel do Ivaí, PR, totaling 426 cases[17-18].

Publication of data related to outbreaks is essential to identifying patterns and preventing future similar episodes. Therefore, this study aimed to investigate environmental samples from an outbreak of toxoplasmosis in a rural family in the municipality of Iguaraçu, PR, to identify possible sources of contamination.

METHODS

Region of Investigation

Following the confirmation of an outbreak of toxoplasmosis in a family, a rural property was investigated. The property is located in the municipality of Iguaraçu, from the 15th Health Region of Paraná, about 30 km from Maringá. Iguaraçu is located in the north of the state of Paraná (PR; 23° 11′ 36″ South, 51° 50′ 33″ West), in Southern Brazil. It has an estimated population of 4,404 inhabitants (Figure 1), where the family under investigation lived.

Figure 1. Location of the municipality of Iguaraçu, in the state of Paraná, Brazil. Source: Google.

Outbreak investigation

A patient with a history of four previous pregnancies was seen at the gestational toxoplasmosis outpatient clinic at the University Hospital of Maringá (HUM), with a suspicion of incomplete abortion at a gestational age of six weeks, presenting IgG and IgM serology tests for toxoplasmosis that were reactive and IgG with low avidity, confirming an acute gestational infection. She denied symptoms similar to toxoplasmosis and mentioned that she lived in the rural area of the municipality. During the consultation, she presented her husband's toxoplasmosis tests, also showing an acute infection, and reported that he had infarcted lymph nodes in his neck and eye complaints. This prompted an investigation into a probable outbreak of toxoplasmosis.

The two children and their husband were summoned to the University Hospital for tests to diagnose toxoplasmosis. Around ten days after the tests were taken, we received the results from the Central Laboratory of Paraná (LACEN-PR), which showed that all those being investigated were in the acute phase of the disease with low IgG avidity, thus confirming an outbreak of toxoplasmosis (Table 1). This study was approved by the Human Research Ethics Committee (CAAE: 09286918.5.0000.0104).

Table 1. Serological tests for toxoplasmosis in all family members involved in the outbreak.

Member	Date of collection	IgA (UI/mL)	IgG anti-*T. gondii* (UI/mL)	IgM anti-*T. gondii* (UI/mL)	Avidity	Laboratory	Method
Pregnant women	02/06/2021	-	200,0	17,29	17,7%	LACEN	CMIA
Pregnant women	18/06/2021	6,19	1246,4	17,86	14,0%	LACEN	CMIA
Husband	03/05/2021	-	51,40	45,49	18,9%	Santa Terezinha-Iguaraçu	CMIA
Child 1	12/07/2021	-	1543,3	4.47	14,5%	LACEN	CMIA
Child 2	12/07/2021	-	1575,0	15.75	21,9%	LACEN	CMIA

LACEN: Paraná State Central Laboratory; CMIA: Microparticle Immunoassay by Chemiluminescence.

We consider the following reference values as method Microparticle Immunoassay by Chemiluminescence (CMIA):

IgG Reactive: >=3.0 IU/mL; Non-Reactive: <1.6 IU/ML; Inconclusive: >=1.60 and <3.00 IU/mL

IgM: Non-reactive: <0.50; Inconclusive: >=0.50 and <0.60; Reactive: >=0.60.

Avidity: Low < 50%; Intermediate 50.0 - 59.9%; High > = 60.0 %.

Ophthalmologic evaluation

All family members underwent fundoscopy and retinography of both eyes in partnership with the Oftalmar clinic in Maringá, PR. The ophthalmologists assessed the eyes using the TOPCON TRC-NW300 device after dilating the pupil according to the clinic's protocol.

Epidemiological Surveillance

To begin the investigation, the first step was to fill in the epidemiological forms in the SINAN (Information System for Notifiable Diseases) and notify the health authorities in the municipality of Iguaraçu. Therefore, it was hypothesized that the infection was probably due to contact with cats, chickens, and other animals in the peri-domicile area and ingesting unfiltered water. Water and soil were collected from the peri-domicile area and cat feces for *T. gondii* DNA analysis.

Collecting samples at home

Two days after discovering the outbreak of toxoplasmosis, the group went to the home in the rural area of Iguaraçu to collect materials that were likely to be the source of infection. They collected 50 liters of water that the family ingested and stored from different points (well, water tank, tap), and deposited it in previously washed gallons. The water from the water tank was collected from the bottom of the tank.

For soil collection, samples were taken from five equidistant points in the peri-domicile and stored in unused plastic containers. Cat feces near the home were collected in sterile jars and sealed with a lid. These samples were collected in July 2021 and taken to the Parasitology Laboratory at the State University of Maringá for investigation.

Sample preparation

Soil

To test for *T. gondii* DNA in environmental samples (soil and water), we used the "Protocols for the investigation of *Toxoplasma gondii* in environmental and food samples of the Ministry of Health of 2020"[19]. The processes were separated into two parts for soil; the first aimed to separate the denser substances in the soil sample from the lighter (less dense) part of the sample, The second part, called "purification", used sucrose solution to resuspend the supernatant, at which point the oocyst could remain in the meniscus part of the solution; followed by washing the sample with distilled water to remove excess sugar. The final result was stored at 4°C until DNA extraction.

Water

For DNA testing in water samples, the "Protocols for the investigation of *Toxoplasma gondii*in environmental and food samples of the Ministry of Health of 2020"[19-20] were also followed. The water was first filtered through a cellulose ester membrane (47 mm diameter, 0.3-micrometer pore size; Millipore) using a negative pressure 5 charge pump (10-1 mmHg). The membrane was then manually scraped[21]. The material was concentrated by centrifugation; the supernatant was discarded and the pellet was resuspended in 500 uL of distilled water[20].

Felid feces

Stool samples were analyzed using two methods: the Willis method and the Faust method. They were taken at two different times: up to one day after collection and five days after collection[21].

Extraction of *T. gondii* DNA from soil, water and feces samples

After sample preparation, the final volume was frozen in liquid nitrogen (-196 °C) for five minutes, followed by thawing in a dry bath at 65 °C for five minutes. This cycle was repeated five times. The purpose of this process was to break the membrane of the oocyst to expose its genetic material for DNA analysis. Extraction was carried out using the commercial Quick PCR Purification Kit (Pure Link TM), following the manufacturer's recommendations.

Polymerase chain reaction (PCR)

The samples were analyzed by PCR using primers B1 (B22-B23) to amplify approximately 115 base pairs (BP)[22]. In each reaction, a negative control (mixture without DNA) and a positive control (DNA extracted from the Me-49 strain of *T. gondii*) were processed. The amplified products were observed in 4.5% polyacrylamide gels, revealed by silver, and digitally recorded.

RESULTS

Through the prenatal consultation and the patient's anti-*T. gondii* serology, it was possible to identify the first outbreak of toxoplasmosis in a rural area of Iguaraçu. Table 1 shows the results of this family's tests:

Analyzing this family's test results, it was possible to observe that this was an intra-household outbreak, with cases of miscarriage and low avidity in all members, confirming the acute infection. During the ophthalmological assessment, retinography was performed exclusively on the husband and child 1. In both cases, a spherical opacity was observed at the center of the retina, suggesting the onset of ocular damage, accompanied by diffuse opacity near the retinal vessels (Figure 2).

Observing the environmental conditions and after questioning the family about their consumption habits; the following data were obtained: the water consumed by the family came from a well, stored in a water tank located outside the house, characterized by poor conditions, without proper treatment or filtration. The sanitation system in the area was also inadequate, with the sewage from the residence and other properties in the rural area being directed to a nearby river. There were no vegetable gardens on the property, but the house was surrounded by animals such as cattle, pigs, chickens, and domestic cats, which roamed freely nearby. The family reported not consuming beef and pork raised on the property, preferring food purchased in the city, although visits to the city were infrequent since their residence was in a rural area. No wild felines were observed in the region, and there were no reports of food consumption from local restaurants.

In the environmental samples analyzed, no *T. gondii* DNA was detected in any of the soil, water, or cat feces samples using conventional PCR or through microscopic observation at 40x magnification. Since the environmental samples tested negative in this study, we believe the infection may have been caused by an uninvestigated or external factor, such as vegetables contaminated with oocysts or undercooked meat containing *T. gondii* tissue cysts.

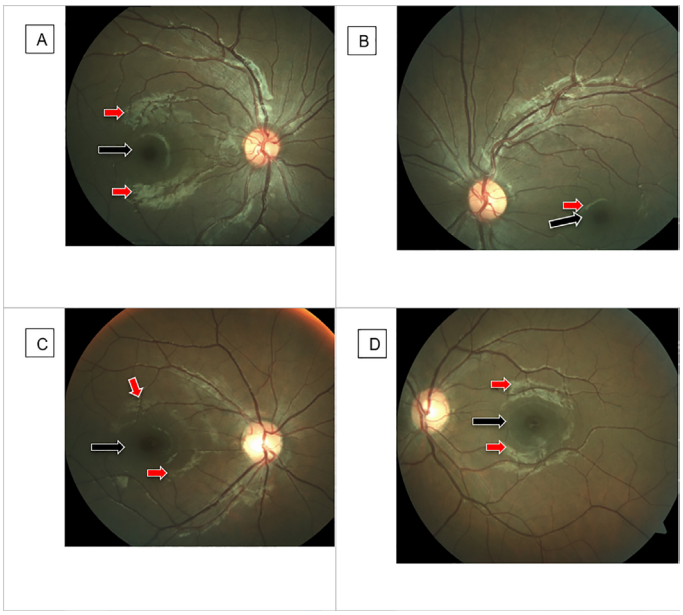

Figure 2. Retinography images of the right and left eyes of the child 1 and husband. Subtitle: A-B: Retinography of child 1: right and left eye. C-D: Retinography husband: right and left eye. Black arrow: beginning of ocular lesion caused by toxoplasmosis. Red arrow: inflammatory process. Retinography reveals rounded choroidal scars in the posterior pole of both eyes and a choroidal neovascular membrane of the two patients analyzed.

Although the family denies eating poorly washed vegetables or undercooked animal meat.

DISCUSSION

This study is the first to investigate an outbreak in a family living in a rural area in the municipality of Iguaraçu, southern Brazil. This has reported the occurrence of an abortion and ocular toxoplasmosis in the same family; a rare combination not previously described in the literature. The miscarriage probably resulted from congenital toxoplasmosis.

Infections occurring during the early stages of pregnancy can significantly impact fetal development, often leading to severe anomalies or spontaneous abortion. Congenital toxoplasmosis (CT) presents a wide range of clinical manifestations, varying from asymptomatic cases to severe multisystem disorders. The classic CT triad includes ventricular dilatation, intracranial calcifications and chorioretinitis[23].

The prevalence of acute gestational toxoplasmosis in regions similar to the one studied is 1.21 cases per 1,000 live births[24]. The high seropositivity for IgG antibodies against *Toxoplasma gondii* among pregnant women in rural areas confirms findings from studies that associate exposure to the parasite with poor environmental conditions, including insufficient access to basic sanitation and treated water[25-26]. This corroborates findings in other countries such as Nigeria, Africa[27] and Warsaw, Poland[28], where the highest seroprevalence occurred among pregnant women living in rural areas.

Multivariate analysis reinforces this relationship, identifying residence in rural areas as a significant risk factor and associating increased seropositivity with advancing age[26]. These data highlight the urgent need for more agile diagnoses, improvements in sanitation infrastructure, and educational actions on food safety in these regions. In addition, inefficient screening of seronegative pregnant women contributes to late diagnosis, leading to serious outcomes such as miscarriages[29].

In addition to GT and CT, there is also ocular toxoplasmosis (OT). It could be from congenital or acquired infections, presenting clinical manifestations that often make it difficult to clearly distinguish between the two forms[10]. However, specific clinical patterns can help identify the source of the infection. Congenital OT is commonly bilateral, and it is characterized by retinochoroidal scarring, involvement of the macula and posterior pole, as well as a high degree of visual loss, even in cases of less extensive inflammation[14,30]. These patients have high recurrence rates over the years. On the other hand, acquired OT usually affects only one eye, with less involvement of the macula. It is marked by significant inflammation, such as retinal vasculitis, retinochoroiditis and vitritis. Initial symptoms often include floaters and reversible visual changes[14,30].

The clinical findings observed in the ophthalmologic evaluation corroborate the characteristics described in the literature about the manifestations of acquired ocular toxoplasmosis. The active ocular lesions, described as white foci with indistinct borders and often associated with atrophic or pigmented scars, are consistent with the signs of active retinochoroiditis[14,31]. In the case reported, the retinography performed on the husband and son 1 revealed spherical opacities in the center of the retina, possibly indicative of initial damage, as well as diffuse opacities near the vessels, suggesting inflammation in progress.

The incidence of toxoplasmosis is higher in rural populations due to poor sanitation conditions and greater exposure to sources of infection, such as untreated water[25]. Toxoplasmosis is a zoonosis that affects several hosts, including humans, and has high genetic variability[2,8]. In South America, atypical strains of the parasite predominate, which are more diverse and generally more virulent[15]. This genetic characteristic contributes to more severe clinical forms. The geographical distribution and variability in virulence of the strains highlight the relevance of molecular epidemiology in understanding the patterns and outcomes of toxoplasmosis[5,15].

In this study, although it was not possible to precisely identify the source of the infection; several risk factors were identified, such as the storage of water in outdoor bins; the presence of slaughtered animals and felids in the peridomicile, as well as poor basic sanitation and hygiene conditions. These characteristics, common in rural areas, favor exposure to *T. gondii*, especially due to the proximity between humans and animals, such as oxen, pigs, chickens and domestic cats[1,2,32-33]. Cats, in particular, as definitive hosts of the parasite, play a central role in the transmission cycle by excreting oocysts in areas close to water sources or directly into the soil, increasing the risks of environmental contamination[26,32].

Similar cases have been described in previous outbreaks, such as the one, in 2001, in Santa Isabel do Ivaí, PR, which was attributed to contamination of the water supply system by oocysts shed by cats in the treatment plant. This episode resulted in 426 confirmed cases of toxoplasmosis[34]. Similarly, another major outbreak occurred, in 2018, in Santa Maria, RS, with more than 900 reported cases. These outbreaks highlight the vulnerability of water supply systems and the possibility of widespread contamination of entire communities, especially in rural areas[19]. In addition, foodborne outbreaks, usually related to inadequate handling and hygiene practices, can also affect specific groups at family gatherings, events or work environments[34-35].

On the property investigated, the absence of vegetable gardens does not eliminate the risk of infection from vegetables, since food purchased at local markets or fairs can act as sources of contamination. Inadequate hygiene practices for these foods are well-documented risk factors for the transmission of *T. gondii*[36]. Although the family lived near livestock raised for slaughter, they reported not consuming locally

raised beef or pork, instead opting for food occasionally purchased in town. This preference suggests that exposure could be linked to other sources, such as contaminated water or externally acquired food.

The detection of oocysts in environmental samples, such as soil, water, and food, remains challenging due to factors like morphological similarities with other coccidia and the presence of inhibitors in the samples. Most toxoplasmosis outbreaks are concluded based on epidemiological aspects, as isolating the parasite is rare[37].

Although conventional PCR is widely used to detect *T. gondii* DNA in biological samples, its application to environmental samples faces technical limitations. Laboratory studies using artificially contaminated water have shown better results in oocyst detection[38]. More advanced molecular methods, such as quantitative PCR, demonstrate higher sensitivity, but they are costly, making them unsuitable for routine use in public health.

These findings emphasize the need for effective control strategies, including improvements in basic sanitation infrastructure, proper food handling practices, and interventions aimed at raising awareness among rural populations about the risks of exposure to *T. gondii*.

CONCLUSION

This study highlights the risk factors associated with toxoplasmosis, particularly, in rural areas, where environmental conditions such as the presence of animals, inadequate water storage, and lack of filtration increase transmission risks. The outbreak in a rural family resulted in severe health consequences, including miscarriage and ocular toxoplasmosis, demonstrating the severity of prolonged exposure and late diagnosis. Although PCR tests did not detect the parasite's DNA in environmental samples; the case underscores the challenges of identifying sources of infection and the need for more sensitive detection methods. The outbreak also highlights the vulnerability of rural populations, emphasizing the need for improved sanitation, food safety practices, and tailored preventive strategies to reduce the risk of zoonotic diseases.

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