**Toxoplasma gondii** in cattle in Brazil: a review

**Toxoplasma gondii** em bovinos no Brasil: uma revisão

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**Abstract**

*Toxoplasma gondii* is an apicomplexan protozoan that is frequently found in both humans and animals worldwide. The aim of this review was to list important aspects of *Toxoplasma gondii* infection in cattle in Brazil. The frequency of occurrence of *T. gondii* antibodies in Brazilian cattle ranges from 1 to 89.1%, depending on the region evaluated, based on data from 1978 to 2018. However, some characteristics of *T. gondii* infection in cattle remain uncertain, such as the role of meat intake in transmitting the parasite to humans. Most information regarding *T. gondii* infection among Brazilian cattle is limited to evaluations of the frequency of occurrence of antibodies. About 70% of the diagnoses of infection in these ruminants in Brazil are made via the indirect fluorescence antibody test (IFAT). Nevertheless, little is known about the population structure of this protozoan in cattle. It is necessary to expand the studies on toxoplasmosis in cattle, in order to better understand *T. gondii* infection in these animals and its implications for Brazilian public health.

**Keywords:** Toxoplasmosis, cattle, Brazil, epidemiology, public health.

**Resumo**

*Toxoplasma gondii* é um protozoário apicomplexa de distribuição mundial prevalente em seres humanos e animais. A presente revisão objetiva elencar aspectos de importância relacionados à infecção por *Toxoplasma gondii* em bovinos no Brasil. A soroprevalência de anticorpos anti-*T. gondii* em bovinos do rebanho brasileiro varia de 1 a 89,1%, a depender da região avaliada, baseando-se em dados disponíveis de 1978 a 2018. Todavia, algumas características da infecção por *T. gondii* na espécie ainda são incertos, como o papel da ingestão da carne bovina na transmissão do parasita ao homem. A maior parte das informações relativas à infecção no rebanho nacional restringem-se a estudos de soroprevalência. Cerca de 70% do diagnóstico da infecção nesses ruminantes no Brasil é realizado por meio da Reação de Imunofluorescência Indireta (RIFI). Contudo, o conhecimento acerca da estrutura populacional do protozoário em bovinos ainda é limitado. Assim, é necessário ampliar os estudos sobre a toxoplasmose em bovinos, tendo em vista uma melhor compreensão da infecção na espécie, bem como de suas implicações para saúde pública brasileira.

**Palavras-chave:** Toxoplasmosese, bovino, Brasil, epidemiologia, saúde pública.
**Introduction**

Toxoplasmosis is one of the most common parasitic infections in both humans and animals. It is a zoonosis caused by *Toxoplasma gondii* (OIE, 2017; Tenter et al., 2000), which is an obligate intracellular coccidian that has the capacity to infect most homeothermic species and form tissue cysts (Dubey & Jones, 2008). It has been estimated that more than a third of the human population is infected with *T. gondii* (OIE, 2017). However, the worldwide frequency of occurrence of antibodies against *T. gondii* in cattle reaches up to 92%, depending on the region studied (Hosein et al., 2016). In Brazil, frequencies of up to 89% have been reported from studies conducted between 1978 and 2018 (Costa & Costa, 1978; Oliveira et al., 2018; Santin et al., 2017).

Some points regarding the epidemiology of *T. gondii* infection in cattle remain uncertain (Dubey & Jones, 2008; Opsteegh et al., 2011a). Because of the resistance to disease development that cattle present and difficulty in isolating this parasite from the tissues of infected cattle, the infective capacity of this protozoan in beef has been questioned (Dubey & Jones, 2008; Tenter et al., 2000). Additionally, there is great concern regarding the representativeness of negative results from tests for direct detection of parasites from tissue samples, given the limited size of these samples, especially considering the high potential for dissemination and low parasitemia levels of *T. gondii* (Esteban-Redondo et al., 1999; Opsteegh et al., 2019).

The role of meat intake in the transmission of *T. gondii* to humans remains unclear (Dubey & Jones, 2008). In fact, the presence of this protozoan or its DNA in bovine tissues has been reported in studies conducted under both natural and experimental conditions (Costa et al., 2011; Dubey, 1992; Hosein et al., 2016; Opsteegh et al., 2011a, 2019). In addition, toxoplasmosis outbreaks caused by consumption of meat from infected cattle have been reported in the literature (Eduardo et al., 2007; Kean, et al., 1969).

Beef is the type of meat with the highest average consumption (g/day) per capita among Brazilians, according to the Brazilian Institute for Geography and Statistics (IBGE, 2011). The importance of this food in the epidemiological chain of toxoplasmosis in humans, especially in endemic areas, cannot be ignored (Carmo et al., 2017). Therefore, given the severity of this disease among pregnant women and immunosuppressed individuals, transmission of protozoan diseases through meat consumption should be considered to be of great importance for public health (Dubey & Jones, 2008; Opsteegh et al., 2011a). Moreover, the characteristics of *T. gondii* infection in cattle need to be known.

Although Brazil has one of the largest commercial cattle herds in the world, studies on toxoplasmosis have mostly been restricted to serological surveys. In the light of the scarcity of information, the aim of the present review was to list some important aspects of *T. gondii* infection in cattle in Brazil.

**Basic biology**

*Toxoplasma gondii*, an apicomplexan protozoan, is the only species of the genus *Toxoplasma* and belongs to the subfamily Toxoplasmatinae, along with *Besnoitia* spp., *Neospora* spp. and *Hammondia* spp. (Dubey, 2010). Domestic and wild felines are the only definitive hosts of *T. gondii*, while all homeothermic animals are potential intermediate hosts (Tenter et al., 2000).

**History of Toxoplasma gondii infection in cattle**

According to Dubey (1986), the first report of natural *T. gondii* infection in cattle was in 1953, in Ohio, United States. However, in 1986, Dubey raised questions regarding whether the cases reported were actually related to *T. gondii*, since he had not found any structure similar to that coccidian or to any other protozoan when he re-evaluated the same tissues (Dubey, 1986). These parasites were further investigated through experimental research.
Dubey (1986) described isolation of *T. gondii* from the brain, liver and lungs of cattle that had been parenterally and orally inoculated with oocysts and tissue cysts in Germany, in 1966. Furthermore, under natural conditions, in 1992, *T. gondii* was found in a homogenate from a cow's small intestine (Dubey, 1992).

In Brazil, it is believed that the first documented attempt to detect *T. gondii* in cattle dates back to 1969, in the city of São Paulo (Jamra et al., 1969). At that time, there was an unsuccessful attempt to isolate the parasite from the brains and livers of 61 cows that supposedly were naturally infected (Dubey, 1986; Jamra et al., 1969). In 1977, Costa et al. (1977) isolated this coccidian from experimentally orally infected calves, and the organ most frequently parasitized was the lymph nodes. However, Passos (1984) investigated the presence of this protozoan in the diaphragms of slaughtered cattle, but failed to detect it through mouse bioassays.

Early in the present century, despite difficulties in isolating *T. gondii* from the tissues of naturally infected cattle, Santos et al. (2010) reported the presence of this coccidian in brain samples from beef cattle in the state of Bahia, Brazil. It was also recovered from fetuses in 50 gestating cows that were examined during a study of naturally acquired *Toxoplasma* infection in the state of São Paulo (Costa et al., 2011). With advances in studies on this topic, Macedo et al. (2012a) isolated and genotyped *T. gondii* from cattle in the state of Santa Catarina, and were able to demonstrate the occurrence of type II.

**Population structure of *T. gondii* in Brazil**

*Toxoplasma gondii* isolates from humans and animals in North America and Europe have been classified into three genetic lineages: Type I, Type II and Type III (Howe & Sibley, 1995). This protozoan was considered by these authors to be primarily clonal, with low genetic diversity. In Brazil, Pena et al. (2008) analyzed a dataset composed of 125 isolates that had been obtained from cats, hens and dogs in four states (Pará, São Paulo, Paraná and Rio Grande do Sul). They identified 48 genotypes, among which 26 had a single isolate and 22 had multiple isolates in different hosts and in different geographic areas. Four of the 22 multiple isolates were characterized as common clonal lineages in Brazil and were designated as types BrI, BrII, BrIII and BrIV. Type BrI was considered to be the most virulent isolate because of its virulence in mice, followed by BrII and BrIV with intermediate virulence. Lastly, type BrIII was considered to be a non-virulent isolate. According to these authors, the results suggested the existence of an epidemic *T. gondii* population structure in Brazil, in which a variety of recombination had resulted from genetic exchanges and a few clonal lineages had expanded successfully throughout the country. On the other hand, in North America and Europe, only three clonal lineages predominated, with rare genetic exchanges between them (Pena et al., 2008; Howe & Sibley, 1995).

To date, the population structure of *T. gondii* from infected cattle continue to be only poorly characterized in Brazil. Dubey et al. (2012) summarized the genotyping results from 363 samples obtained in Brazil, but none of them were isolated from cattle. In the south of this country, Macedo et al. (2012a) isolated two *T. gondii* strains: one from a cow (in brain cysts that were observed in bioassayed mice) and the other from a fetus (tachyzoites observed in the peritoneal liquor of bioassayed mice). Macedo et al. (2012a) analyzed 13 markers by means of PCR-restriction fragment length polymorphism (PCR-RFLP) and genotyped both strains (TgBoBr1 and TgBoBr2). They identified the Type II lineage, which is the most common type in North America and Europe but is very rare in Brazil.

**Studies on *T. gondii* infection in cattle and its importance for public health**

After inoculation of $2.0 \times 10^5$ sporulated *T. gondii* oocysts of strain P, Oliveira et al. (2001) found that the mean hematological values generally remained within the normal limits for *Bos taurus*, *Bos indicus* and *Bubalus bubalis*. However, *B. taurus* appeared to be more sensitive to the parasite than *B. indicus* and *B. bubalis*, and showed the highest mean values
for parasitemia and more evident temperature peaks. Besides the common clinical signs presented by all the ruminants (hyperthermia, tachycardia, tachypnea, anorexia, prostration, nasal discharge and tearing), *B. taurus* was the most severely debilitated and also presented photophobia, conjunctivitis and diarrhea. On the other hand, *B. indicus* was more resistant and showed lower mean values for leukocytes and lymphocytes than *B. taurus* and *B. bubalis*. Despite this, according to these authors, there was transient leukocytosis in all species between 14-21 days after inoculation (DAI) and 35-63 DAI, which may have represented proliferation of the parasite in new sites of the organism (Oliveira et al., 2001).

Esteban-Redondo et al. (1999) investigated calves that had been orally infected with $10^3$ and $10^5$ sporulated *T. gondii* oocysts, of isolate M3. They reported that only at the concentration of $10^5$ oocysts did the calves develop parasitemia, which started on the 2nd DAI. All the infected calves presented tachypnea and hyperthermia during the first week after infection, but the values returned to normal between the 8th and 9th DAI. The febrile response of the calves started belatedly and ended earlier than was seen among sheep that were subjected to the same experimental protocol. No histopathological changes were detected in the brain, heart and skeletal muscle from calves orally infected with $10^3$ and $10^5$ *T. gondii* oocysts (Esteban-Redondo et al., 1999).

Therefore, *T. gondii* infection in cattle does not seem to be an important cause of either clinical disease or reproductive disorders such as abortion among these animals (Dubey, 2010; Dubey & Jones, 2008). Since toxoplasmosis is the fourth most common foodborne infection in the world (Fao, 2012) and the meat of these ruminants is a possible source of infection to humans, studies on *T. gondii* infection in cattle are therefore mainly founded on interest relating to public health (Opsteegh et al., 2011a).

Some aspects of the epidemiology of toxoplasmosis in cattle remain uncertain. On the one hand, the resistance to infection among cattle and difficulty in isolating *T. gondii* from these infected animals raise questions about whether this parasite is really present in cattle meat and what its real infective capacity among humans is, along with questions about the sensitivity of the diagnostic methods applied for detecting this protozoan in cattle (Burrells et al., 2018; Tenter et al., 2000). Although natural infections usually appear to be asymptomatic, presence of this coccidian in the viscera and tissues of these ruminants at the age of slaughter has been reported, under both experimental and natural conditions (Burrells et al., 2018; Costa et al., 2011; Dubey, 1992; Hosein et al., 2016; Macedo et al., 2012a; Opsteegh et al., 2019). Moreover, although quantification of the risk of transmission of this coccidian to humans through consumption of cattle beef is complex, this risk is real (Belliuco et al., 2016; Opsteegh et al., 2011a). From a homogenate prepared from the small intestine of an infected cow, Dubey (1992) reported the presence of a *T. gondii* isolate identified as CTI, which was considered highly pathogenic to mice.

Failure to isolate this protozoan in cattle may also be explained by difficulty in detecting *T. gondii* in tissues consequent to the limited size of the samples examined in these studies (Opsteegh et al., 2019). Especially in livestock animals, the low levels of parasitemia and wide dissemination of this coccidian, which has the capacity to infect any nucleated host cell, mean that it is possible that this parasite may be present in non-evaluated tissues. Therefore, a negative result from a tissue fragment does not necessarily imply absence of *T. gondii* from the whole tissue (Esteban-Redondo et al., 1999; Santos et al., 2010). In this regard, Opsteegh et al. (2019) found that cattle that tested positive in a bioassay (on the liver) tested negative in magnetic capture quantitative PCR (MC-PCR, on the diaphragm), and vice-versa. These authors also pointed out that the low *T. gondii* DNA concentrations revealed by qPCR on mouse brains and bovine diaphragms suggested that the numbers of parasites in the bovine liver and diaphragm are low (Opsteegh et al., 2019).

In Brazil, depending on the region studied, high frequency of occurrence of *T. gondii* antibodies is reported in cattle herds (Dubey et al., 2012), thus indicating that this protozoan has widespread circulation and that infected animals are commonly present.
Toxoplasma gondii in cattle

Even with low frequency of *T. gondii* infection in these ruminants, Opsteegh et al. (2011b) showed that beef consumption is one of the main sources of infection among humans in the Netherlands. Moreover, case-control studies have indicated that meat intake is a risk factor for acquisition of toxoplasmosis (Cook et al., 2000; Jones et al., 2009).

Although the role of beef consumption in the transmission of toxoplasmosis to humans is unclear (Dubey & Jones, 2008), outbreaks related to meat consumption in Brazil and worldwide are well documented (Batz et al., 2012; Eduardo et al., 2007; Kean et al., 1969). Since beef is widely consumed by Brazilians (IBGE, 2011), toxoplasmosis must be considered to be an issue of public health importance, especially in endemic areas (Carmo et al., 2017). Consumption of raw or undercooked beef is culturally widespread in many countries, with strong presence also in Brazil, and this favors infection of the human population by *T. gondii* (Belluco et al., 2016; Tenter et al., 2000).

Biological cycle of the parasite in cattle

Infection of cattle by *T. gondii* is most plausibly established through ingestion of sporulated oocysts that are dispersed in pastures and other food and water sources (Robert-Gangneux, 2014). After ingestion of this infective form, the oocyst wall is digested, with consequent release of sporozoite forms. The sporozoites invade intestinal epithelial cells and become tachyzoites. In parasitic vacuoles, they multiply rapidly through endodyogeny. In the blood and lymphatic circulation, tachyzoites can also reach extraintestinal tissues, including the placenta. They are able to invade any nucleated cell, and can also infect developing fetuses (Rougier et al., 2017; Tenter et al., 2000).

After repeated replication, the tachyzoites leave the host cell, and destroy it. As free organisms, they can then spread and continue the lytic cycle: they invade, replicate in and exit from the parasitized structure, or initiate differentiation into bradyzoites. In differentiation, the parasitophorous vacuole membrane gives rise to the tissue cyst wall, which houses the new forms of the coccidian (Dubey, 2010). This process occurs as a reaction to the development of the bovine immune response. There is no information regarding how long it takes, but a mean period of seven to ten days post-infection has generally been described in relation to mammals (Rougier et al., 2017).

Tissue cysts are the final stage of *T. gondii* in intermediate hosts, and they may remain in the infected organism for an indeterminate period (Rougier et al., 2017; Tenter et al., 2000). In cattle, Dubey & Thulliez (1993) isolated viable *T. gondii* 1191 days after infection; therefore, in animals of slaughter age. Through mechanisms of resistance that are not yet completely understood, these ruminants may be able to eliminate the parasite or to reduce it to undetectable levels within a short period of time (Dubey, 1986; Dubey & Jones, 2008). From this perspective, Yildiz et al. (2017) evaluated the behavior of bovine and ovine neutrophils against *T. gondii*. They observed that extracellular trap formation by neutrophils (NETs) from cattle had a lethal effect on tachyzoites, whereas those from sheep only immobilized the parasites. Thus, these authors suggested that these structures contributed to the bovine resistance mechanism. Nonetheless, when present, the cysts retain their infective capacity, so that if they are ingested by a new host, including humans and cats, a new asexual phase is initiated.

Geographic distribution and frequency of occurrence

*T. gondii* has the capacity to infect all homeothermic animals and is found worldwide. It has been described in more than 350 host species, including both birds and mammals (Robert-Gangneux, 2014). The establishment of *T. gondii* infection can be influenced by several elements, encompassing environmental factors and host resistance. In theory, areas of dry climate are less favorable for parasite sporulation, which develops more efficiently in tropical climates. In Brazil, a predominantly tropical country, but with
heterogeneous climatic conditions across its vast territory, this coccidian is widely distributed in both humans and animals (Dubey et al., 2012).

Dissemination of the parasite among intermediate hosts, via carnivorism, confers a certain degree of independence from edaphoclimatic factors for the distribution of *T. gondii*. Host feeding behavior has also been correlated with the risk of infection among humans and animals (Robert-Gangneux, 2014). For cattle, which tend to feed on the higher portions of the pasture grass, the possibility of oocyst intake is lower than for sheep, for example, which graze on grasses that are close to the ground (Magalhães et al., 2016).

In general, extensive, semi-intensive and intensive rearing systems present similar risks of infection for beef cattle (Fajardo et al., 2013). In extensive management, especially near to forests, coexistence of wild felids within cattle herds is common. These animals can enter the pastures and share the same water source as used by the cattle, thus increasing the cattle’s exposure to the parasite through dissemination of oocysts in the environment. On Fernando de Noronha, an Atlantic island in the state of Pernambuco, Brazil, Magalhães et al. (2016) showed that the exposure of cattle to *T. gondii* was related to extensive rearing. On this island, *T. gondii* antibodies were detected in 66% of the feral cats (Costa et al., 2012). In semi-intensive and intensive dynamics, close contact between cattle herds and domestic cats leads to infection of these herbivores. Among cattle, Albuquerque et al. (2011), in the state of Rio de Janeiro, and Fajardo et al. (2013), in the state of Minas Gerais, both in Brazil, reported that the more intensive the cattle rearing system became, the higher the frequency of occurrence of anti-*T. gondii* antibodies was. In this context, food storage is indicated as the main risk factor for ruminant infection. Storage of grains and other products increases the presence of rodents, which attracts cats. Once infected, the cats would disseminate oocysts contaminating the environment through their feces (Fajardo et al., 2013). Also, on Brazilian farms, use of cats for controlling rodents is still a common practice (Albuquerque et al., 2011).

*T. gondii* infection in cattle has been reported from around the world, with large variations in the frequency of occurrence within and between countries (Burrells et al., 2018; Dubey et al., 2012). The frequency of occurrence of *T. gondii* antibodies has been reported to range from 1 to 92% worldwide, with significant variation depending on the region studied. Natural infection among cattle has been reported in Central, South and North America (Arias et al., 1994; Dubey, 1992; Dubey et al., 2012), Africa (Tonouhewa et al., 2017), Europe (Lopes et al., 2013) and Asia (Sarvi et al., 2015). Similarly, to the global picture, the frequency of occurrence of *T. gondii* antibodies in Brazilian cattle herds is geographically variable, with rates that have been found to range from 1% to 89% in studies conducted between 1978 and 2018 (Table 1). It should be emphasized, however, that any comparison and interpretation of epidemiological data needs to be done with caution, because of the limited number of studies per Brazilian state and the use of different diagnostic techniques with different cutoff points in heterogeneous sample groups (Hosein et al., 2016).

Table 1. Serologic studies of the presence of *T. gondii* antibodies in cattle from Brazil conducted between 1978 to 2018.

| Geographical area/State | Number of Tested Animals | % Positive Animals | Diagnostic Method | Cut-off | Population (age months/sex/breed or aptitude)* | Reference |
|------------------------|--------------------------|--------------------|-------------------|--------|-----------------------------------------------|-----------|
| Northern Brazil        |                          |                    |                   |        |                                               |           |
| Amazonas               | 25                       | 12                 | IH                | 128    |                                               | Ferraroni et al. (1980) |
| Pará                   | 765                      | 87.4               | ELISA             | 200**  | 36/-/Nelore                                    | Silva et al. (2015) |
| Pará                   | 500                      | 40.6               | IFAT              | 64     | /M and F/Beef                                  | Carmo et al. (2017) |
| Pará                   | 500                      | 52.0               | IFAT              | 64     | 36/F/-                                        | Silva et al. (2017) |
| Pará                   | 1749                     | 34.4               | ELISA             | 200**  | 0-24/M and F/-                                | Oliveira et al. (2018) |
| Pará                   | 1749                     | 44.1               | IFAT              | 64     | 0-24/M and F/-                                | Oliveira et al. (2018) |
**Toxoplasma gondii** in cattle

Table 1. Continued...

| Geographical area/State | Number of Tested Animals | % Positive Animals | Diagnostic Method | Cut-off | Population (age months/sex/breed or aptitude)* | Reference |
|-------------------------|--------------------------|--------------------|-------------------|--------|-----------------------------------------------|-----------|
| Central-Western Brazil  |                          |                    |                   |        |                                               |           |
| Mato Grosso             | 516                      | 73.0               | ELISA             | 200**  | 36/-Nelore                                    | Silva et al. (2015) |
| Mato Grosso             | 2000                     | 71.0               | IFAT              | 64     | -/F/Dairy                                     | Santos et al. (2009) |
| Mato Grosso do Sul      | 466                      | 4.3                | IH                | 64     | -/Nelore and Crossbreed                      | Santos et al. (2010) |
| Mato Grosso do Sul      | 388                      | 5.1                | DAT               | 25     |                                               | Marques et al. (2009) |
| Goiás                   | 119                      | 89.1               | ELISA             | 200**  | >24/F/Curraleiro and others                  | Santin et al. (2017) |
| Goiás                   | 32                       | 10.9               | IFAT              | 64     |                                               | Passos (1984) |
| Southern Brazil         |                          |                    |                   |        |                                               |           |
| Minas Gerais            | 740                      | 10.2               | IFAT              | 64     |                                               | Passos (1984) |
| Minas Gerais            | 350                      | 12.0               | IFAT              | 64     |                                               | Costa & Costa (1978) |
| Minas Gerais            | 1195                     | 2.6                | IFAT              | 64     | -/M and F/Gir                                | Fajardo et al. (2013) |
| São Paulo               | 204                      | 32.3               | IFAT              | 64     |                                               | Costa et al. (1978) |
| São Paulo               | 50                       | 18.0               | IFAT              | 64     | -/F/-                                        | Costa et al. (2011) |
| São Paulo               | 200                      | 11.0               | ELISA             | 100**  |                                               | Meireles et al. (2003) |
| Rio de Janeiro          | 589                      | 14.7               | IFAT              | 64     | >36/F/-                                      | Albuquerque et al. (2011) |
| Rio de Janeiro          | 459                      | 1.9                | IFAT              | 64     | -/M and F/-                                  | Luciano et al. (2011) |
| Rio de Janeiro          | 77                       | 49.4               | ELISA             | 500**  |                                               | Frazão-Teixeira & Oliveira (2011) |
| Southern Brazil         |                          |                    |                   |        |                                               |           |
| Paraná                  | 503                      | 48.5               | IFAT              | 64     | -/M and F/Dairy                              | Marana et al. (1995) |
| Paraná                  | 400                      | 25.8               | IFAT              | 64     | -/M and F/Holstein, Crossbreed               | Garcia et al. (1999) |
| Paraná                  | 348                      | 41.4               | IFAT              | 64     | -/M and F/-                                  | Daguer et al. (2004) |
| Paraná                  | 385                      | 26.0               | IFAT              | 64     | -/F/Dairy                                     | Ogawa et al. (2005) |
| Paraná                  | 250                      | 30.8               | IFAT              | 64     | -/-Beef                                       | Moura et al. (2010) |
| Santa Catarina           | 120                      | 29.1               | IFAT              | 50     | -/F/Holstein, Jersey                         | Macedo et al. (2012b) |
| Rio Grande do Sul       | 112                      | 5.4                | IH                | 64     |                                               | Chaplin et al. (1984) |
| Rio Grande do Sul       | 134                      | 6.7                | IH                | 64     |                                               | Braccini et al. (1992) |
| Rio Grande do Sul       | 532                      | 3.4                | IH                | 64     |                                               | Silva et al. (1983) |
| Rio Grande do Sul       | 121                      | 17.4               | IFAT              | 64     |                                               | Santos et al. (2013) |

*When described in the study; **serum dilution; DAT = direct agglutination test; ELISA = enzyme-linked immunosorbent assay; F = female; IFAT = indirect fluorescence antibody test; IH = indirect hemagglutination; LAT = latex agglutination test; M = male.
Although Brazil has one of the largest commercial cattle herds in the world, data on frequencies of occurrence are only available from some states (Table 1). Among the studies carried out in this country, considering simple averages according to each region, the frequency of occurrence of antibodies against *T. gondii* in the herds in the north region (45.5%) is highest, followed by the center-west (42.2%), south (23.4%), southeast (17%) and northeast (13.4%). According to IBGE (2018), the three Brazilian states of the center-west region (Goiás, Mato Grosso and Mato Grosso do Sul) are where the largest proportion of slaughter of cattle in Brazil takes place, with participation of 36.9%. Two other states in the northern region, Pará and Rondônia, are also among the seven main states for cattle slaughter. Thus, the importance of *T. gondii* infection in cattle for Brazilian public health should not be neglected.

Among these Brazilian studies separately, low frequency of *T. gondii* infection in cattle (1%) was reported by Gondim et al. (1999), in the Recôncavo Baiano and Caatinga areas of the state of Bahia, using a latex agglutination test (LAT ≥ 64). On the other hand, the highest frequency (89.1%) was observed by Santin et al. (2017), in the state of Goiás, using ELISA (≥ 200), in an evaluation on cattle in two municipalities.

Among the Brazilian states where at least three surveys were carried out (Bahia, Minas Gerais, Pará, Paraná, Pernambuco, Rio de Janeiro, Rio Grande do Sul and São Paulo) (Table 1), the highest average frequency of occurrence of *T. gondii* antibodies in cattle was in Pará (51.8%, in the northern region) (Carmo et al., 2017; Silva et al., 2015, 2017), followed by Paraná (34.5%, in the southern region) (Daguer et al., 2004; Garcia et al., 1999; Marana et al., 1995; Moura et al., 2010; Ogawa et al., 2005). These results corroborate the copious capacity for dissemination and adaptability of this parasite, both in tropical and subtropical areas, from the north to the south of the country, respectively. Thus, bovine exposure to this coccidian has been described in all five regions of Brazil (north, northeast, center-west, southeast and south), in all biomes (Amazon, Caatinga, Cerrado, Atlantic Rainforest, Pampa and Pantanal) and in at least 15 of the 27 Brazilian states. Figure 1 illustrates the locations of the cattle sampled in the different Brazilian studies on the presence of *T. gondii* antibodies.
Transmission and sources of *T. gondii* infection

In general, transmission of *T. gondii* can occur either horizontally or vertically among cattle (Dubey, 2010). Like in other herbivores, the main form of transmission of *T. gondii* among cattle is via ingestion of sporulated oocysts, through consumption of contaminated pasture grass, silage, feed and water (Robert-Gangneux, 2014). Dispersion of oocysts in the environment may occur by means of the wind, rain, surface water, food harvesting or invertebrate organisms (Tenter et al., 2000). Access to animal food containing tissue cysts, although less plausible and more unlikely, should not be ruled out as a source of infection (Fajardo et al., 2013).
Congenital toxoplasmosis in cattle has been known since 1980, when Stalheim et al. (1980) detected *T. gondii* in the placenta of two cows that had been inoculated experimentally, and in the gastric contents of two fetuses. Furthermore, it was reported that these fetuses were aborted 24 days after inoculation of the tachyzoites. However, Canada et al. (2002) pointed out that Stalheim's study was conducted before the discovery of *Neospora caninum*, and that "structures similar to *T. gondii*" were obtained via cellular passages, but not via bioassay. Canada et al. (2002) used intraperitoneal inoculation in mice to document isolation of *T. gondii* from the brain tissues of a fetus naturally infected that had been aborted in the 5th month of gestation and from those of a newborn calf. Wiengcharoen et al. (2011) also reported the presence of this protozoan in the placenta and in two fetuses that were aborted at 156 and 161 days of gestation, respectively, six and 11 days after subcutaneous inoculation of $3 \times 10^8$ *T. gondii* tachyzoites (strain RH). However, it should be noted that subcutaneous inoculation is not a natural infection route in this species.

In Brazil, in the state of São Paulo, Costa et al. (2011) evaluated two situations: natural congenital infection and experimental inoculation of oocysts in pregnant cows. *T. gondii* was detected in the brain and retina of three calves that had been naturally exposed to the parasite. The dams of these three calves were positive for anti-*T. gondii* antibodies. However, presence of this protozoan could not be confirmed using IFAT, histopathological examination or bioassay in fetuses whose mothers were experimentally infected in the first, second or last trimester of pregnancy. However, the infection in the mother was confirmed using IFAT. In Santa Catarina, Macedo et al. (2012a) demonstrated a transplacental transmission rate of 23.3% (14/60) in dairy cows at a local abattoir, with identification of *T. gondii* in the blood, brain and fetal lung via bioassay in mice. In ten of these 14 fetuses, the parasite was not isolated from the cow's blood.

Although toxoplasmosis is not considered to be an important cause of miscarriage or stillbirth among cattle (Dubey, 2010), transplacental transmission may occur under both experimental and natural conditions (Canada et al., 2002; Costa et al., 2011; Macedo et al., 2012a; Wiengcharoen et al., 2011). It is also believed that the pathogenicity and frequency of occurrence of the congenital infection are influenced by the virulence of the strain (Costa et al., 2011; Wiengcharoen et al., 2011).

Viability of sexual transmission of *T. gondii* among cattle was reported in Brazil by Scarpelli et al. (2009). It was detected using IFAT and isolated via bioassay in mice from the semen of several cattle that had been experimentally inoculated with tachyzoites and oocysts, between the 7th and the 84th DAI. Also, tissue parasitism was confirmed in seminal vesicles by means of the polymerase chain reaction (PCR), amplifying a 194 bp segment of the *T. gondii* B1 gene. The possibility that positive fetuses might be generated from the semen of positive bulls was not discussed by these authors (Scarpelli et al., 2009). According to Dubey (2010), the significance of these findings within the natural epidemiology of bovine toxoplasmosis remains uncertain.

**Diagnosis**

Direct diagnosis of *T. gondii* in cattle can be performed using PCR, histological analysis and bioassays in cats and mice (OIE, 2017). The main obstacles to detecting the parasite in infected hosts are due to: 1) widespread dissemination of this protozoan in the body, with the capacity to infect any nucleated host cell; 2) the limited size of the samples evaluated; and 3) host resistance, which relates to the possibility of elimination of the parasite or gradual reduction of its levels over time (Dubey & Jones, 2008; Opsteegh et al., 2011a; Tenter et al., 2000). Thus, direct investigation may generate repeated negative results, even though the protozoan is present (Esteban-Redondo et al., 1999).

Currently, the only method available for distinguishing the viability of *T. gondii* is the bioassay method (OIE, 2017), which can be performed on mice or cats. Briefly, for bioassays
in mice, the bovine organs and tissues that are to be tested are digested *in vitro* and inoculated into these rodents, with monitoring of seroconversion and development of clinical signs. Bioassays in cats are considered to be the gold standard method because of the significant susceptibility of these animals, as definitive hosts, to infection by this protozoan. The technique consists of feeding the cats with the bovine organs and tissues that are to be tested and analyzing the excretion of oocysts, three to 14 days after ingestion of the samples (Belluco et al., 2016). However, despite the high sensitivity, these trials are time-consuming, laborious, questionable from the point of view of animal welfare and poorly enforceable in large-scale screenings (OIE, 2017; Opsteegh et al., 2011a).

In this context, indirect diagnosis is often used to determine the exposure of ruminants to *T. gondii* (Fajardo et al., 2013; Hosein et al., 2016). However, knowledge about the sensitivity and specificity of the indirect methods for diagnosing the presence of *T. gondii* in cattle is limited (Dubey & Jones, 2008). So far, no serological test that could be classified as a gold standard for cattle has been developed (Dubey & Jones, 2008).

Among the serological techniques available, it is known that the Sabin-Feldman test (dye test), which has been indicated for humans, produces unreliable results among cattle (Dubey & Jones, 2008). False positives may occur due to the non-specific IgM reaction of natural occurrences in bovine serum (Esteban-Redondo & Innes, 1997). In contrast, the modified agglutination test (MAT), indirect fluorescence antibody test (IFAT) and enzyme-linked immunosorbent assay (ELISA) have been recommended for confirmation of bovine exposure to *T. gondii* and are suitable for epidemiological surveillance studies (OIE, 2017). With the aim of seeking greater efficiency regarding detection of specific antibodies against *T. gondii*, different types of ELISA have been applied as bovine serological tests. Indirect ELISA, using whole antigen extracts, is commonly used (Liu et al., 2015; Gondim et al., 2017). However, given specificity limitations, this is being replaced by techniques using recombinant antigens, derived from immunodominant antigenic fractions (Gondim et al., 2017; Sudan et al., 2019). Sudan et al. (2019) described detection of antibodies against *T. gondii* in cattle via recombinant SAG2-ELISA and considered that the protein SAG2 was a promising candidate for making serodiagnoses of toxoplasmosis in livestock. Nevertheless, use of serological methods to obtain an estimate of the presence of *T. gondii* in cattle may be unsuitable, given that Opsteegh et al. (2019) found that there was a lack of concordance between direct detection and the presence of antibodies in cattle.

In Brazil, studies on the frequency of occurrence of *T. gondii* among cattle have mostly been based on IFAT (Dubey et al., 2012). Out of 35 studies that have been conducted in Brazil, 24 (68.5%) used IFAT to make the diagnosis, among which 22 (91.6%) established 64 as the cutoff (Table 1). It has been suggested that the low antibody titers usually presented by these ruminants (Opsteegh et al., 2011a) are due to presence of the chronic phase of *T. gondii* infection, in association with resistance mechanisms against the parasite (Carmo et al., 2017).

**Conclusion**

From the present review, it is evident that *T. gondii* infection in cattle is prevalent in the different regions of Brazil. Moreover, studies in Brazil have mostly been restricted to serological surveys. Among these ruminants, little is known about the possible mechanisms of resistance to *T. gondii*, or the persistence of this coccidian in tissues. Since beef is widely consumed in Brazil, and because isolation of this protozoan in bovine tissues has been described, the importance of this infection in cattle cannot be neglected. It is necessary to go into greater depth in evaluating the importance of this bovine infection in the context of public health, especially in terms of the viability and permanence of the tissue cysts in infected animals. Lastly, it is necessary to gain greater knowledge of the population structure of *T. gondii* in cattle in Brazil, in order to improve the understanding of the infection in cattle and its implications for public health.
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