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Prevalence and risk factors of cysticercosis in cattle tracking

[*Prevalência e fatores de riscos da cisticercose no rastreamento de bovinos*]

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ABSTRACT

The aim of this study was to identify the prevalence and the main risk factors related to the transmission of bovine cysticercosis based on tracking animals sent for slaughter and coming from properties located in the micro-region of Uberlândia, Minas Gerais, Brazil. The properties were previously evaluated for the occurrence of cysticercosis during post-mortem inspection in the 12 months prior to the beginning of the research, and those with animals with bovine cysticercosis found at least once during this period were considered positive. A cross-sectional study was carried out on 87 properties, from which 1024 bovine serum samples were collected. Indirect ELISA performed serological diagnosis and Immunoblot confirmed positive sera. The prevalence found in this study was 5.1% (95% CI = 3.74-6.42). The risk factors identified were cattle origin (RC = 4.9), grazing (RC = 6.4) and sewage destination on the property (RC = 3.6). These environmental factors suggest that sanitation control measures and the restriction of pastures beyond the property boundary can help prevent disease in the study area. A control system based on risk analysis was discussed and proposed as a strategy to control bovine cysticercosis in the *Triângulo Mineiro* region and other regions of the country.

Keywords: bovine cysticercosis, diagnosis, risk factors, public health, screening

RESUMO

Este estudo teve por objetivo identificar a prevalência e os principais fatores de risco relacionados com a transmissão da cisticercose bovina em animais enviados para abate e provenientes de propriedades localizadas na microrregião de Uberlândia, Minas Gerais, Brasil. As propriedades foram previamente avaliadas quanto à ocorrência de cisticercose durante a inspeção post mortem *nos 12 meses antecedentes ao início da pesquisa; aquelas que revelaram animais com cisticercose bovina pelo menos uma vez durante esse período foram consideradas positivas. Foi realizado um estudo de corte transversal em 87 propriedades, onde foram coletadas 1024 amostras de soro bovino. O diagnóstico sorológico foi realizado por triagem pelo teste ELISA indireto, e os soros positivos foram confirmados pelo Immunoblot. A prevalência identificada neste estudo foi de 5,1% (IC 95%= 3,74-6,42). Os fatores de risco identificados foram: a origem dos bovinos (RC=4,9), o pastejo (RC=6,4) e o destino do esgoto da propriedade (RC=3,6). Esses fatores ambientais encontrados sugerem que medidas de controle do saneamento e restrição a pastagens além do limite da propriedade possam auxiliar na prevenção da doença na área de estudo.*

Palavras-chave: cisticercose bovina, imunodiagnóstico, fatores de risco, saúde pública, rastreamento

INTRODUCTION

Bovine cysticercosis is among the communicable diseases considered to be of socioeconomic and public health significance in countries in South America, Africa, and Asia and which have significance for international trade in animals and animal products, according to the World Health Organization (Who, 2018). The presence of bovine cysticercosis (CTC) leads to socioeconomic losses in the beef production chain, especially in developing countries, such as Brazil, and poses an obstacle to the export of Brazilian beef (Rossi *et al*., 2015; Rossi *et al*.,

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2017). CTC prevalence resulted in a financial loss of approximately US$ 5,829,103.99. The presented data showed that even though prevalence seems to be decreasing over the years, it is still necessary to adopt prophylactics and control measures in population (Comin *et al*., 2021).

The anatomopathological inspection currently performed in slaughterhouses is not sensitive enough to detect all positive cases of cysticercosis. The actual prevalence of the disease is therefore underestimated. An alternative to such visual inspection are serological methods, which could assist in monitoring bovine cysticercosis and clarify the risk factors involved in its transmission in different regions (Dorny *et al*., 2010; Silva *et al*., 2015a). Among these main factors identified worldwide are water supply to animals, flooding of pasture, proximity to properties with wastewater effluents, direct deposit of human excrement, leisure activities near grazing areas and the free access of the animals to these areas (Dorny *et al*., 2010; Calvo-Artavia *et al*., 2013; Alves *et al*., 2017; Pinto *et al*., 2019).

Analyzing the animal movement network to map localities and properties considered as sources of contamination for bovine cysticercosis, Acevedo Nieto *et al*. (2012) and Aragão *et al*. (2017) have warned of the difficulties found in applying bovine cysticercosis control measures, arising from the difficulty in identifying the source of infection, especially since slaughtered cattle usually come from several properties.

However, tracing this parasite also enables identification of foci of bovine cysticercosis, positive animals on properties, the origin of these animals, the management system practiced, and possible specific risk factors involved in transmission of the disease in the respective property. This type of analysis may help to identify endemic regions for the disease and to develop more effective control programs, thus ensuring safe and quality meat products for the consumer (Meliker and Sloan, 2011; Oliveira *et al*., 2020).

According to Rossi *et al*. (2016) and the European Food Safety Authority (Technical…, 2013), it is necessary to develop a risk model based on animal origin to improve detection and control of cysticercosis in endemic areas. EFSA states that epidemiological indicators should be defined by determining the prevalence or concentration of the hazard at a given stage of the production chain. These indicators can be used to carry out risk analyses to support these decisions and to guide adaptations to meat inspection methods, strengthening one of the main methods of controlling bovine cysticercosis (Technical…, 2013; Nastasijević *et al*., 2020).

Thus, this study aimed to identify prevalence and evaluate the main risk factors related to the transmission of bovine cysticercosis in animals sent for slaughter from properties located in the micro-region of Uberlândia-MG, based on cysticercosis cases detected at a slaughterhouse in the same municipality.

MATERIAL AND METHODS

The research was carried out in the *Triângulo Mineiro*, Minas Gerais, Brazil, micro-region of Uberlândia, comprising the municipalities of: Araguari, Indianópolis, Prata, Monte Alegre de Minas and Tupaciguara. The population of these five municipalities is estimated at 200,358 inhabitants, with 172,808 in urban areas and 27,550 in rural areas (IBGE, 2021).

The precipitation index in this region is around 1460mm and the ambient temperature varies between 19 °C and 27 °C. The predominant climate is tropical. The region is in the *Cerrado* biome and the use and occupation of the land in this micro-region are characterized by cultivation of monocultures and pasture used in livestock farming (Alves and Rosa, 2008).

Based on a survey of prevalence data and tracking of cases of bovine cysticercosis recorded in a slaughterhouse located in the municipality of Uberlândia-MG and supervised by the Brazilian System of Animal Product Inspection (SISBI-POA), we selected for the survey the five municipalities mentioned above, which send animals for slaughter at said establishment and where cases of cysticercosis were found during post-mortem inspection.

A cross-sectional study was carried out, covering 87 rural properties located in the micro-region of Uberlândia, based on the records of a slaughterhouse located in the municipality of

Uberlândia-MG. The parameters adopted for calculating this sample were estimated disease prevalence of 4.7% (Duarte *et al*., 2016) and a significance level of 5% (EpiInfo version 7.2.5, CDC 2021).

The properties located in said area of coverage and which sent animals for slaughter at this slaughterhouse were considered primary sampling units. These properties were georeferenced (GPS Etrex Garmim) to map the distribution of cases in the region and had been previously evaluated for cysticercosis during post-mortem inspection in the 12 months prior to the beginning of the research (May/2015 to May/2016). Properties at which animals with bovine cysticercosis were found at least once during said period were considered positive and the others were considered negative.

The minimum number of animals tested (secondary sampling units) at each rural property band was calculated based on the value of aggregate sensitivity and specificity (Dohoo *et al*., 2009). For the purpose of the calculations, the values of 90.8% and 96.7%, respectively, were adopted for the sensitivity and specificity of the test protocol used (Silva *et al*., 2015a) and 4.7% (Duarte *et al*., 2016) for the estimated prevalence. Thus, the number of animals examined was calculated using Herdacc version 3.0 (University of Guelph, Guelph) and the size of the sample chosen was that which enabled aggregate sensitivity and specificity values equal to or greater than 90%. Thus, in properties with up to 50 cattle, 8 animals were sampled; between 51 and 100, 12 and above 101, 20. The choice of the animals at each property was defined with simple random sampling, and only cattle over 3 months old were sampled, except for pregnant females.

Based on a previously tested epidemiological questionnaire applied to the sampled properties, data were collected for later analysis of possible risk factors associated with being a positive property. The questionnaire was designed to verify the absence or presence of management practices and sanitary conditions at the properties which could be possible risk factors for the transmission of bovine cysticercosis.

Blood samples were collected from 1024 animals by puncture of the jugular vein. Subsequently, the samples were centrifuged at 2,250rpm (905g) and the sera stored individually in microtubes at -20 °C.

The serological diagnosis of bovine cysticercosis was performed in triplicate using the indirect ELISA (Enzyme-Linked Immunosorbent Assay) (Silva *et al*., 2015b). Suspected cases (positive ELISA result) were subject to Immunoblot for confirmation (Silva *et al*., 2015a).

The information obtained from the questionnaires was recorded in the Epi Info program version 7.2.5 (2021). Possible risk factors were categorized and coded. The lowest risk category was considered as a reference for comparison with the others, giving a risk of 1.00. The analysis of the risk factors was performed in two steps: simple binary logistic regression and multivariate binary logistic regression. Variables with a value of $p < 0.20$ in the simple binary logistic regression analysis underwent multivariate regression analysis, considering $p < 0.05$. For this purpose, multiple logistic regression (Hosmer and Lemeshow, 2000) was used to define a model that would better identify the risk factors at the properties involved in the study. These analyses were performed using the SPSS program version 20.0.

This study was approved by the Animal Research Ethics Committee (Case 29/2015) and the Human Subject Research Ethics Committee (Case 1,233,967/2015), both of which are linked to the Universidade Federal de Viçosa, following the procedures established in the Plataforma Brasil (project registered on 06/25/2015), guided by the guidelines and norms of National Health Council Resolution 466/2012.

RESULTS

In 37.9% of the properties sampled in the region studied, one or more cattle with cysticercosis in the serological tests were found, with the most positive properties concentrated in the municipalities of Monte Alegre de Minas (10), Tupaciguara (10) and Prata (9) (Fig. 1). Animal prevalence was 5.1% (95% CI = 3.74-6.42) (Table 1). The frequency of seropositive cattle per property ranged from 12.5% to 41.7%.

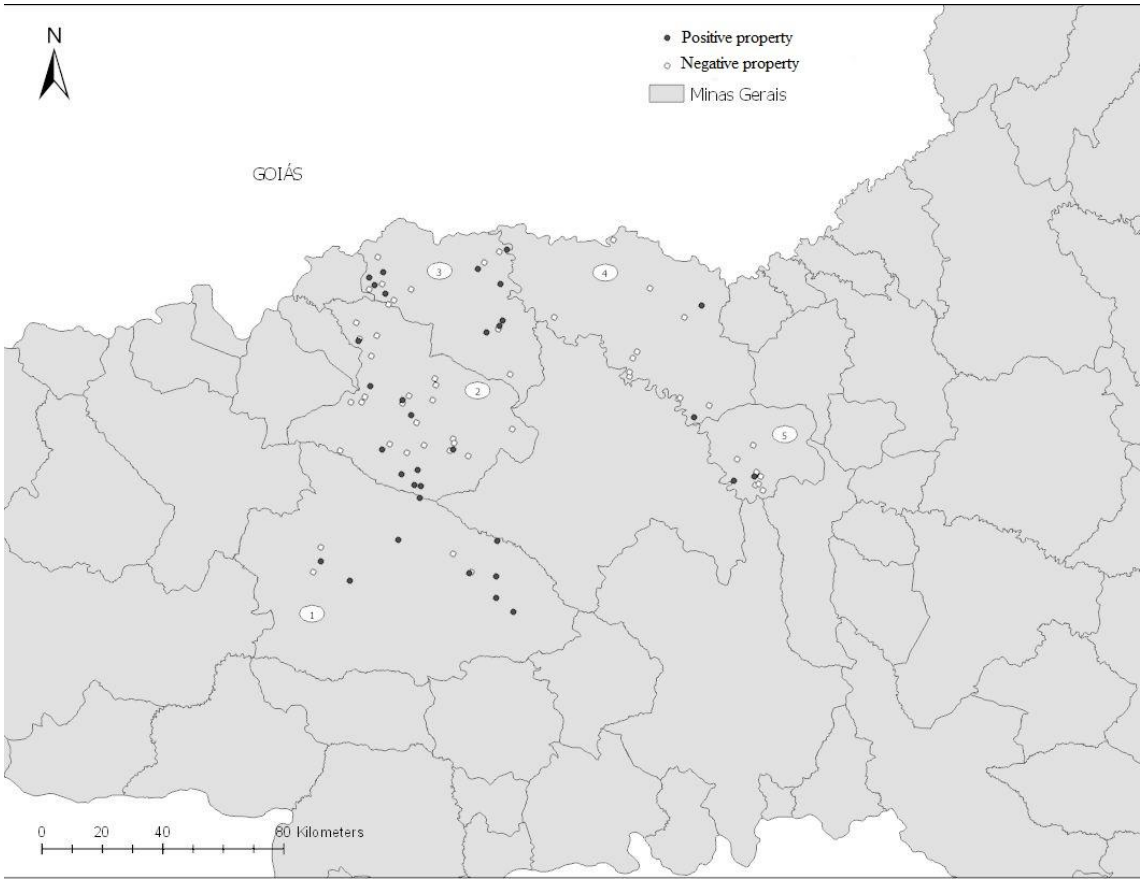

Figure 1**.** Geographical distribution of positive and negative properties for bovine cysticercosis in the municipalities Prata (1), Monte Alegre de Minas (2), Tupaciguara (3), Araguari (4) e Indianópolis (5).

Table 1. Prevalence by properties and sampled animals for bovine cysticercosis in the microregion of Uberlândia, Minas Gerais, Brazil.

	Properties		Animals	
	N	Prevalence (%)	N	Prevalence (%)
Positive	33	37.9	52	5.1
Negative	54	62.1	972	94.9
Total	87	100	1024	100

The variables related to the risk of bovine cysticercosis transmission which were selected ($p < 0.2$) for the multivariate binary logistic regression analysis were: size of the property, number of years of schooling of the manager or owner of the property, origin of cattle, feeding on pasture, use of anthelmintic, water source offered to animals, sewage destination, veterinary medical care, property type (urban / rural) and municipality.

In the final logistic regression model, the risk factors identified were type of access to pasture, the origin of cattle and destination of the human sewage from the property, with the respective risk impacts defined in Table 2.

Table 2. Risk factors associated with the prevalence of bovine cysticercosis in five municipalities in the microregion of Uberlândia, Minas Gerais, Brazil.

Risk factor	OR	p – valor	CI 95%
The origin of cattle			
Born on the property	Reference		
Purchase	16.0	0.000	5.2 – 49.5
Pasture			
Property	Reference		
Other property	6.4	0.002	2.0 – 20.2
Destination of the human sewage			
Septic tanks	Reference		
River/stream	3.6	0.000	2.3 – 5.6

OR= Odds Ratio; CI= Confidence Interval

DISCUSSION

This study confirms the occurrence of bovine cysticercosis in the Uberlândia micro-region, as occurred in another study conducted in the Patrocínio microregion (Duarte *et al*., 2016), both belonging to the *Triângulo Mineiro* region, where is concentrated the largest cattle herd in the state of Minas Gerais, around 4 million head (17%) (Bovinocultura…, 2017). These numbers show the importance of detecting bovine cysticercosis and its possible economic repercussions in this region for the cattle in the State and in the Brazilian scenario, where the disease is endemic (Pinto *et al*., 2019).

According to Jahed Khaniki *et al*. (2010) and Rezende *et al*. (2018) even when rates of bovine cysticercosis are very low, the economic impact can still be significant since it causes significant economic losses to the meat industry in relation to slaughter. Such losses are due to intensified control in the herds at the properties affected by the *T. saginata* parasite and the use of procedures adopted in the routine inspection of meat, such as total condemnation of affected carcasses or their economic declassification, through routine treatments for inactivating the cysticerci by cold, heat or salting (Multicriteria…, 2014; Brasil, 2020).

Seroepidemiological studies of bovine cysticercosis in the state of Minas Gerais have found a prevalence of around 4% to 5% (Garro *et al*., 2015; Duarte *et al*., 2016; Pinto *et al*., 2019), similar to that found in this study (5.1%). However, when these numbers are compared to those obtained from slaughterhouses, lower prevalence is observed, around 1 to 2%, which can be explained by the difference in diagnostic methodologies adopted, immunodiagnosis in the first case and anatomopathological examination in the second, the latter with lower sensitivity (Eichenberger *et al*., 2011; Comin *et. al*., 2021).

Thus, use of these immunodiagnostic methods together with the anatomopathological examination carried out at slaughterhouses is important not only as a new proposal for diagnosing the disease, but as an epidemiological tool, providing more realistic prevalence data on areas with risk of transmission and enabling the health of slaughtered herds from these cysticercosis endemic areas to be monitored (Guimarães-Peixoto *et al*., 2015). To support a risk-based control system, an appropriate monitoring system is essential and should include slaughterhouse, laboratorial, provenance and health authority records (Guidelines…, 2014). The worldwide recommendation is that proposals to control cysticercosis should include the whole production chain, from the property to the consumer's table, which requires better knowledge of the origin of the animals involved in the control programs to define a strategy that is more streamlined for each region (Pawlowski *et al*., 2005; Duarte *et al*., 2016; Nastasijević *et al*., 2020).

In the study in question, at properties where the animals had access to external pasture, this grazing management was identified as a risk factor for disease transmission in the study region (Tab. 1). In a study of cattle outbreaks at two dairy farms in New Zealand, this management practice also occurred in one of the investigated properties, which showed high prevalence (43%) (Mcfadden *et al*., 2011).

In 20.7% of the properties sampled the infrastructure was inefficient, such as lack of fences, handling pens and containment. Consequently, at these properties the animals had access to other pasture, such as on neighboring properties or the roads that bordered them, and thus also had access to water sources of unknown origin. Such free access by the animals to pasture, water from flooded areas and pasture contaminated with *T. saginata* eggs, derived from directly human feces or through sewage sediment, are known to be significant environmental risk factors in the transmission of bovine cysticercosis in the herd (Calvo-Artavia *et al*., 2013, Duarte *et al*., 2016; Maia *et al*., 2017; Pinto *et. al*., 2019).

Regarding the origin of cattle at the properties sampled in this study, the purchase of animals from other properties or at auction was identified as a risk factor for the prevalence of the disease. A similar study, conducted in the state of Paraíba, also identified this variable as a risk factor for the prevalence of cysticercosis in the bovine herd (Maia *et al*., 2017).

Of the properties sampled in this study, 43.7% reported acquiring their animals at other properties in the *Triângulo Mineiro*, the North of Minas Gerais and Southern regions of the state of Goiás; as well as at auctions that sell animals from other regions in the state of Minas Gerais with high rates of cysticercosis as well as from other states, such as São Paulo, for example. Studies of the prevalence of bovine cysticercosis have shown that the state of São Paulo has rates between 3 and 9.5% for this parasite (Rossi *et al*., 2015).

Another risk factor identified in the study area was the sewage destination from the residences of the sampled properties. When the sewage destination was open or destined to a river/stream, the animals at these properties were more likely to contract the disease compared to those at properties that had septic tanks. The cattle breeding system, coupled with low socioeconomic and cultural level of the population and precarious health conditions, contribute to contamination of pastures, and are directly related with the occurrence of cysticercosis in cattle (Scandrett *et al*., 2009; Duarte *et al*., 2016). This risk factor is reinforced by Murrel (2005), who reports the main routes of transmission of CTC from humans to cattle: outdoor defecation near premises or pasture; use of untreated sewage, sludge, or human effluent to irrigate or fertilize crops and grazing; presence of human carriers involved in cattle rearing; and indiscriminate deposition of feces in camps, along highways and along railways.

Of these properties, 18.4% of sanitary sewage was inadequate, an important feature that contributed to cysticercosis transmission in the study area, contaminating pasture and water for the animals. On the other hand, at rural properties in the municipality of Divinésia-MG, it was found that 74% of the rural properties destined sewage to cesspools. This high frequency observed in the correct destination of sewage favored the prevention and control of cysticercosis, preventing the animals from having access to human feces (Felippe *et al*., 2014).

Tracking cattle between the slaughterhouse and the rural property can provide epidemiological information on animals suspected positive for *T. saginata* during slaughter at the slaughterhouse and may also help in investigating relevant production risk factors based on positive carcasses (CAC, 2007; Rossi *et al*., 2017). However, detailed epidemiological studies regarding the occurrence of bovine cysticercosis are scarce, most of the citations are limited, circumscribed or timely (Blagojevic *et al*., 2017).

In the research in question, bovine cysticercosis screening enabled the identification of positive animals in the herd at the sampled properties, as well as the origin of the animals diagnosed positive. Moreover, poor management systems were observed at 54.5% of the positive properties and inadequate sanitary infrastructure at properties with positive diagnosis for this zoonosis, thus exposing consumers to the risk of contracting the teniasis. These results showed which herds were most affected by the disease, which enables intensive control of the disease in the animals, in relation to the positive properties, in line with the new concepts of cysticercosis control established by Codex Alimentarius (Guidelines…, 2014) and by the European Union (Technical…, 2013).

According to the Codex Alimentarius Commission - CAC (Guidelines…, 2014), the

tracking of animals between the slaughterhouse and farms, in particular the registration of the place of production, must be implemented so that the information on carcasses positive for *T. saginata* can be used to apply control measures at properties, when deemed appropriate by the competent authority. In this scenario it could also include notification of "suspect" animal groups to be sent to the slaughterhouse for the application of intensified post-mortem inspection procedures.

The main elements of the new European meat safety system have been identified as risk categorization at the level of animal, farm, and slaughterhouse (EFSA, 2013). The categorization proposed by this system simulates the targeting of different inspection service procedures, that is, on a scale varying from only visual for low-risk herds/batches to more rigorous for those high risk ones. To this purpose, the European system proposes the use of three control indicators similar to that of this study to be considered in the epidemiological investigation of bovine cysticercosis; the first indicator is to audit conditions on the property, the second is based on the prevalence of positive animals detected by serology and the third concentrates on the confirmation of *T. saginata* cysticerci through the use of PCR (Technical…, 2013; Nastasijević *et al*., 2020).

These risks should be based on official inspection service data, serological results, information on known risk factors and on case history of properties (EFSA, 2013). This scenario was developed in the study in question, determining some of these risk categories, which may help the Brazilian government to implement a similar system, reducing costs and assisting in the programming of more effective policies to control bovine cysticercosis.

However, further studies are needed in other regions of Brazil, both in the investigation of more accurate sanitary records from the official inspection service (slaughterhouses) and at field level (cattle raising), to identify positive properties for the disease, to investigate the risk factors involved, as well as the improvement and validation of high sensitivity and specificity serological tests, which should be considered for the implementation of this risk-based inspection system. According to Blagojevic *et al*. (2017) this system should be implemented in association with a consistent and up-to-date zoonosis surveillance system as new risks may emerge and low priority risks such as *T. saginata* occurrence may increase over time or in different regions.

CONCLUSION

Based on tracking cases of bovine cysticercosis in the region studied, it was verified that the disease is endemic and that properties have undesirable characteristics that consequently may lead to contamination of pasture areas and water sources accessible to the animals. Thus, control strategies for the prevention of this parasitosis must be based on measures applicable to the human and bovine population and to the environment. Risk categorization for the disease should be implemented in association with a zoonosis surveillance system, leading to cost reduction and aiding in the development of more streamlined and effective control programs for this parasite, especially in the *Triângulo Mineiro* region.

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