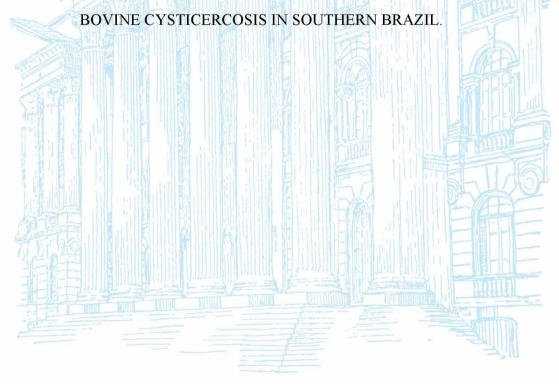
UNIVERSIDADE FEDERAL DO PARANÁ

CAMILA FRANCO



PREVALENCE, GEOSPATIAL DISTRIBUTION AND RISK FACTORS ASSOCIATED TO



CAMILA FRANCO

PREVALENCE, GEOSPATIAL DISTRIBUTION AND RISK FACTORS ASSOCIATED TO BOVINE CYSTICERCOSIS IN SOUTHERN BRAZIL.

Dissertação apresentada ao curso de Pós-Graduação em Ciências Veterinárias, Setor de Ciências Agrárias, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Mestre em Ciências Veterinárias.

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Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em CIÊNCIAS VETERINÁRIAS da Universidade Federal do Paraná foram convocados para realizar a arguição da dissertação de Mestrado de CAMILA FRANCO intitulada: PREVALENCE, GEOSPATIAL DISTRIBUTION AND RISK FACTORS ASSOCIATED TO BOVINE CYSTICERCOSIS IN SOUTHERN BRAZIL., que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

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DEDICATION

À minha família.

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A Deus.

Aos meus pais, por serem a minha força nos dias difíceis e meu suporte nas difículdades. É impossível expressar em palavras o que sinto por vocês!

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EPIGRAPH

"You're going to make it. Trust me."

RESUMO

A cisticercose bovina é uma doença de caráter zoonótico que afeta os seres humanos e os animais. É causada pela Taenia saginata, também conhecida como "tênia da carne". Os humanos são os hospedeiros definitivos deste cestódeo, e os animais intermediários. Os bovinos adquirem o parasita pela ingestão de ovos viáveis no ambiente, presentes em fezes humanas. Já os humanos o adquirem através do consumo de carne crua ou malcozida. As maiores taxas de prevalência da cisticercose bovina são observadas em países em desenvolvimento e nos menos desenvolvidos, especialmente naqueles com condições sanitárias precárias. Embora a pecuária de corte no Brasil seja relevante, o país ainda é considerado em desenvolvimento, de maneira que as questões sanitárias observadas em vários estados são fatores de risco para a doença. As principais medidas preventivas e de controle consistem na detecção dos cisticercos durante a inspeção da carne. Além dos diagnósticos, os dados gerados nos frigoríficos são considerados fontes confiáveis para se obter um conhecimento regionalizado sobre a doença. Assim, o objetivo deste estudo foi avaliar a prevalência, distribuição geoespacial e os fatores de risco associados a cisticercose bovina no Paraná, estado que possui o oitavo maior rebanho bovino do país, bem como apresentar as condições sanitárias desta região. Para a determinação dos parâmetros foram utilizados dois anos (2018-2020) de dados da Agência de Defesa Agropecuária do Paraná e do Instituto Brasileiro de Geografía e Estatística, o que permitiu a análise da distribuição geoespacial de 18.741 bovinos (2%), este valor representa os lotes a que pertenciam todos os animais acometidos. O número de animais diagnosticados com cisticercose bovina inspecionados pelo SIE foi de 1.461, resultado que aponta a prevalência de (0,14%). Na região sul do Estado, Éneas Marques (0,66%), Planalto (0,50%) e Pinhão (0,50%) apresentaram as maiores prevalências. Já na região Centro-Norte, Paiçandu indicou a maior prevalência para cisticercose bovina (1,00%), enquanto Ourizona e Floraí apresentaram (0,75%). A prevalência estabelecida neste estudo pode ser justificada em razão do método utilizado para o diagnóstico da cisticercose, do banco de dados usado para a realização das análises e em virtude da quantidade de animais oriundos de cada cidade. Os principais fatores de risco associados a cisticercose bovina consistiram nas condições sanitárias precárias dos municípios, a elevada densidade populacional e a intensificação da pecuária extensiva. Em conclusão, os dados de animais abatidos em frigoríficos continuam sendo uma ferramenta de grande relevância para a análise da distribuição da cisticercose bovina.

Palavras-chave: Carne; Cisticercos; Higiene; Inspeção.

ABSTRACT

Bovine cysticercosis is a worldwide zoonotic disease that affects humans and animals, which is caused by Taenia saginata or ''beef tapeworm''. Humans are the definitive host for this cestode, and cattle are the most common intermediate host. Cattle become infected through ingestion of eggs viable present in human feces, while humans acquire the parasite through consumption of raw or undercook meat. The bovine cysticercosis highest prevalence rates are present in developing and least developed countries, especially in regions with poor sanitary conditions. Even though the meat production and the beef livestock in Brazil has increased, the country is still considered in developing, and issues related to the sanitary status in several regions are considered risk factor for the development of several diseases, such as BCC. The main preventive and control measures consist on the detection of cysticerci during the meat inspection, beyond diagnoses slaughterhouse's data are considered a reliable source to obtain the knowledge of the disease in certain regions. Thus, the aim of this study was to evaluate the prevalence, geospatial distribution and risk factors associated to BCC in Paraná state, which have the eight largest cattle herd of the country, and also to present the sanitary status of this region. To determinate the parameters were used two-years of data (2018-2020) from Paraná Agricultural Defense Agency, (State Inspection Service) and the Brazilian Institute of Geography and Statistics. In this study the geospatial distribution of 18.741 cattle (2%), was analyzed. This value represents the batches of overall affected animals, considering the twoyears of analysis. The number of bovine cysticercosis diagnosed in slaughterhouses through overall inspected animals by the SIE was 1.461, establishing a prevalence of (0.14%) for BCC in the state. Regarding the prevalence by municipality, in the Southern region of the state, Eneas Marques (0.66%), Planalto (0.50%) and Pinhão (0.50%) presented the highest prevalence's. In the Central North region of the state, Paiçandu had the highest prevalence for bovine cysticercosis (1.00%), while Ourizona and Floraí presented (0.75%) in the same region. The prevalence established in this study may be justified from the method used to diagnose the BCC, the database used to perform the analyses and the number of animals originated in each municipality. The main risk factors associated to BCC were the poor sanitary conditions of the municipalities, high human population density and the intensification in the extensive livestock sector. Despite this, the data obtained from cattle slaughtered remains as an extremely relevant tool to analyze the distribution of the BCC.

Keywords: Beef; Cattle; Cysticerci; Hygiene; Inspection; Sanitary *status*.

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LIST OF ABREVIATIONS

BCC Bovine cysticercosis

IBGE Brazilian Institute of Geography and Statistics

CWE Carcass Weight Equivalent

FAO Food and Agriculture Organization

SIF Federal Inspection Service

GDP Gross Domestic Product

GPS Global Positioning System

SIM Municipal Inspection Service

NHSS National Household Sample Survey

ADAPAR Paraná Agricultural Defense Agency

PCR Polymerase Chain Reaction

SIF State Inspection Service

USDA United States Department of Agriculture

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1. GENERAL INTRODUCTION

1.1 BACGROUND

Bovine cysticercosis or known as BCC is a parasitic infection of cattle caused by the larval stage of the human intestinal cestode, *Teania saginata*. The life cycle of the parasite is totally dependent of the connection between humans, cattle and the environmental, that still remains prevalent on farms and slaughterhouses around the word (ALEMNEH et al., 2017).

As previously mentioned, the life cycle of *T. saginata* includes humans and animals. Humans are the definitive hosts of the parasite and harbor the mature or adult form of the cestode in their intestines (taeniosis). The terminal segments containing eggs are detached from the adult parasite and millions of eggs may be released daily to the environment, resulting in the spread of the parasite in different regions (LARANJO-GONZÁLEZ et al., 2016).

The BCC highest prevalence rates are present in developing and least developed countries, especially in regions with poor sanitary conditions, associated to socioeconomic and cultural aspects of consumption. The disease also has been commonly related to the environmental factors, such as contaminated sources of water or wastewater, the use of untreated or partially treated human waste in agriculture and also by the flooding pastures (BRAAE et al., 2018).

In humans, the parasite migrates to cardiac and striates muscles where it develops into cysticerci, causing the taeniosis. The *T. saginata* infections are generally characterized by mild or absence of clinical symptoms. Complications, such as abdominal discomfort, weight loss, gall bladder perforation, appendicitis and bowel obstruction cases are rarely identified, one of the reasons why the disease is underreported (OKELLO; THOMAS, 2017).

The infection in cattle occur accidentally after the ingestion of the pastures or sources of water containing *T saginata* eggs, while, in humans the taeniosis occurs through consumption of raw or undercooked beef containing viable cysticerci. Natural infections in cattle are normally asymptomatic. However, they cause countless financial losses to the industry due to downgrading, condemnation, extra handling, refrigeration and transport of the infected carcasses (ROSSI et al., 2020).

The post-mortem inspection procedures performed through macroscopic examination of carcass in slaughterhouses are generally used to diagnose the BCC. This method is still widely used due the possibility to identify the *T. saginata* cysts based exclusively on their visual

or morphological appearance in the intact and cut surfaces of the carcass or striated muscles (JANSEN et al., 2017).

The parasite presents a widely distribution. Regarding the countries of America's, Brazil, has one of the highest prevalences of BCC, that still being the most prevalent parasitic disease diagnosed in slaughterhouses during the post-mortem inspection. Several factors can be associated to this prevalence such as, the animal's extensive breeding system, production size and mainly the absence of ideal structure and hygiene conditions (AVELAR et al., 2016).

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2 LITERATURE REVIEW

2.1 BRAZILIAN LIVESTOCK

In 2019, the Gross Domestic Product (GDP) was R\$ 7.3 trillion (\$ 1.32) a nominal growth of 6.8 percent over the previous year. Mostly of this growth was due to the Livestock, which in the same period registered a slight growth, going from 8.3 percent to 8.5 percent of the total GDP, demonstrating the strength of the sector in the Brazilian economy (ABIEC, 2020).

Brazilian livestock has increasingly attracted attention worldwide. The country has the largest commercial cattle heard, with over 214 million heads of cattle. Since 2004, Brazil's has been one of the highest world exporters of beef. Furthermore, most part of these exports are destined to a large number of countries, (around 154 countries), an economic strategy (IBGE, 2020).

In recent years, Brazil's was considered the world's largest exporter of beef, providing about 20 percent of the total beef exports, outpacing India, the second-largest exporter by 527,00 metric tons carcass weight equivalent (CWE). Increased beef demand worldwide has stimulated the production, and also the production gains (USDA, 2019).

However, in the last's decades there have been significant changes in the production systems adopted by livestock beef farmers. These changes include oftentimes exchanges from extensive livestock systems to confinement or semi-confinement, which have already resulted in the expansive number and size of beef herds in different areas. Thus, gains in productivity such as, heavier carcasses slaughtered and animals slaughtered earlier (NETO et al., 2014).

Despite the aforementioned changes related to the livestock systems, most part of beef cattle herds in the country still raised mostly in extensive systems and grazing based on native or cultivated pastures. Besides that, this system has been shown effective from the production "viewpoint", the herds size continues to increase and the pasture area remains stable (MARQUES et al., 2014).

The United States Department of Agriculture (USDA), through the livestock annual informative, report that in the next 10 years, the Brazil beef production will keep increasing. Moreover, it will remain as the largest meat exporter among other countries, followed by Australia and United States. The meat production in Brazil, occurs in several regions, these regions count with an expressive number of herds, animals and meat produced (BRAZIL, 2018).

About 60 percent of Brazil's beef production is concentrated in Center West and North regions, mostly in Mato Grosso state, Mato Grosso do Sul and Pará (USDA, 2019). The expansion of cattle in these areas account around 40 percent of the beef herd in the country. In Southern, and South region of the country, Minas Gerais and Paraná represents the most expressives beef herds. Solely the two states represents almost 15 percent from the total (IBGE, 2021).

Paraná state has the 8th largest cattle herd of the country, mostly compounded by animals targeted to beef livestock. The state has on average 170.374 beef production establishments, with evolved on production and zootechnical indexes. In 2019, data from the

Brazilian Institute of Geography and Statistics (IBGE) estimated more than 9 million heads of cattle in this region, besides that, almost 1.500,000 heads slaughtered (ABIEC, 2020).

The high production in the state occurs due to the exportations and also by the domestic market. The Parana's production increased around 2.48 percent between 2019 to 2020, this value represents 532,617 tons, which represents around to 350,000 tons of beef produced (USDA, 2020).

However, this increasing in animal production and processing mentioned also increases the risks related to the food security. Though the impact of these results demonstrates a positive scenario for the economy, the health issues must be more relevant. Ensure the ideal sanitary conditions through the production process is necessary in order to avoid the transmission of Foodborne Diseases or Zoonoses. Considering these aspects, the meat routine inspection performed in slaughterhouses cooperate in diagnosis and prevention (ALVESEIKE, 2018).

2.2 SANITARY INSPECTION

The sanitary inspection performed in slaughterhouses in several countries remains as an obligatory procedure established in order to reduce the risks provided by unsafe products. According to the current legislation, in Brazil, the Federal Inspection Service (SIF) generally contemplates records or data about large establishments that sell products between states and also those who intend to export (BRASIL, 2017).

Similar process occurs at the State Inspection Service (SIE), that operates at state level, with establishments that sell products to other municipalities, at the same state, and also by the Municipal Inspection Service (SIM), that sell products only in the municipality area, not being allowed to trade with others municipalities or states (BRASIL, 2021).

The inspection service was performed according to the Codex Alimentarius, a program created by the World Health Organization that aims to establish international standards about food and food security. The program refers to the conditions and practices that preserve the health quality of food and avoid food-borne diseases (FAO,2019).

The meat inspection in Brazil complies with the Decree No. 9,013-2017, which regulates the Law No. 7,889-1989, to guarantee that the sale of Products of Animal Origin throughout the Brazilian territory occur accordingly to the international standards. In order to obtain this regulation, it is necessary a registration or inspection seal, issued by the Inspection Service (BRAZIL,2017).

The objectives of meat inspection include to ensure that only apparently healthy and physiologically normal animals are slaughtered for human consumption and that abnormal animals are separated, slaughtered and destined accordingly, also to guarantee that meat from animals is certificated free from disease, wholesome and of no risk to human health (FAO 2019; BRAZIL, 2017),

The Decree establish that quality of the Products of Animal Origin as a set of parameters that allows characterizing the specifications of a product in relation to the intrinsic and extrinsic conditions, hygienic-sanitary and technological factors. In official meat inspection plants in slaughterhouses, the process is carried out by trained professional, that includes a Veterinary and agents designed to the function (BRAZIL, 2017)

Slaughterhouses are determined as establishment where animals are slaughtered for human consumption and or/ in the use of the subproducts for animal feed. The development of the slaughter industry ranges between countries due to the local necessities. The regulation of the slaughter industry aims to improve hygiene and reduce contamination risks, furthering a safe meat (COOK et al., 2017).

Several diseases can be associated to the lack of standards to perform the meat inspection in slaughterhouses. Training for meat handlers and inspectors have been known as an extremely important tool to identify multiple injures present in meat inspection routine. As mentioned, the diagnose of bovine cysticercosis it is mostly recognized during the visual inspection in the country (REZENDE et al., 2018).

The identification of risks associated to public health generally must be observed by the Veterinary and also by the trained agents in order to ensure the safety of the final product. The ante-mortem inspection can be described as the visual examination of animals collectively and individually to observe characteristics that may compromise the safety of processing (BRASIL, 2017).

The ante-mortem inspection is an activity performed obligatory by the Veterinary (have this requirement in this stage, due the fact that the animals are still alive), the inspection agents may assist if it is necessary but never preform this activity without support. This inspection includes the visual analyze and the data, origin, sex, age and health of animals (BRASIL, 2017).

In contrast, the post-mortem inspection consists of examinate the organs and tissues by palpation, visual macroscopic and cuts in several organs. The BCC is most commonly prevalent in striated and cardiac muscles. Therefore, in these muscles are performed cuts in the parenchyma to evaluate the presence of cysticerci, viable or calcified (BRASIL, 2017).

In Brazil, the meat inspection routine to identify BCC include to 'cut' the surface for the macroscopic visualization of the: masseter and pterygoids muscles; tongue; cardiac muscle; liver; esophagus and diaphragm according to the number of cysts identified the organ or the entire carcass should be commended (BRASIL, 2017).



Figure 1. Cattle tissue: Viable cysticerci, larval stage of *Taenia saginata*. Identified during the meat inspection in slaughterhouse. (CHINO; Inspection Department of Products of Animal origin (DIPOA), 2006).

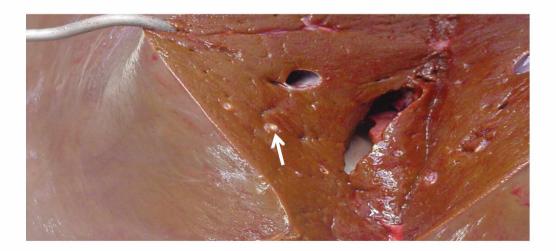


Figure 2. Cattle liver: Calcified cysticerci, larval stage of *Taenia saginata*. Identified during the meat inspection in slaughterhouse. (CHINO; Inspection Department of Products of Animal origin (DIPOA), 2006).

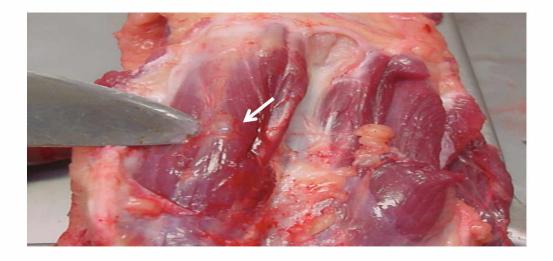


Figure 3. Cattle tissue: Viable cysticerci, larval stage of *Taenia saginata*. Identified during the meat inspection in slaughterhouse. (CHINO; Inspection Department of Products of Animal origin (DIPOA), 2006).

2.3 CHARACTERIZATION of Taenia saginata

The *Taenia saginata*, is among the oldest known human helminthiases and the most commonly and widely spread of overall taenias species that affects humans and animals. Written descriptions of the disease and history of the name taenia reach into antiquity. This ''tapeworm'' how it is known, has global distribution and for this reason can be founded in all continents, meantime, is endemic only in Eastern Europe, Southeast Asia, Africa and Latin America (KONYAEV et al., 2017).

The cestode affect humans in the 'adult form 'of *T. saginata* tapeworm, the infection occurs through ingestion of raw meat or undercooked meat. Humans are considered the definitive hosts for this specie, while cattle are accidental hosts that became infected by ingesting pasture contaminated (EICHENBERGER et al., 2020).

Human taeniasis is generally asymptomatic, although abdominal discomfort and weight loss have been reported, and carriers may suffer some distress from observing proglottids in their feces. Rarely reported sequelae to intestinal taeniasis include gall bladder perforation, appendicitis and bowel obstruction (OKELLO et al., 2017).

Despite the relative ease with which this diagnostic method can be undertaken in resource-limited settings, a major disadvantage of this is the sensitivity of microscopy, due to the intermittent nature of egg shedding. Traditional diagnosis includes Microscopic,

immunological and molecular methods have been used to detect and differentiate between *Taenia* spp., while a combination of two or more methods appears to provide higher sensitivity (SYMEONIDOU et al., 2018)

2.3.1 MORPHOLOGY

Briefly describing the parasites of the genus *Taenia*: the *T. saginata* is a long-flattened ribbon-like tapeworm, opaque white or yellowish and exceptionally long segmented parasites, measuring 1 to 12 m in their adult stage. The head or scolex is the attachment organ, and has four suckers and a rostellum with rudimentary hooklets. The hooks are organized in two rows of 22 to 32 and range in size from 110 to 180 μm, and the scolex is the size of a pin-head (CDC, 2020).

Taenia saginata cysticerci can be seen macroscopically between two- and four-weeks post-infection as nodules in striated and cardiac muscles, measuring 2-5 mm in diameter, including the surrounding inflammatory tissue reaction. The cysticercus, sometimes referred to as cysticercus bovis is an oval bladder (7-10 mm by 4-6 mm), fluid filled and containing the invaginated scolex of the tapeworm (WHO, 2020).



Figure 4. Morphology of the *Taenia saginata*: scolex 0.6-2.0mm (modified from American Society of Parasitology/OIE).



Figure 5. Morphology of the *Taenia saginata*: mature proglottid 2.1-4.5mm (modified from American Society of Parasitology/OIE).



Figure 6. Morphology of the *Taenia saginata*: Gravid proglottid 0.2-2.2cm/Eggs 16-45μn (modified from American Society of Parasitology/OIE).

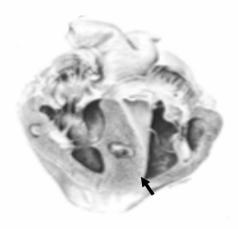


Figure 7. Cysticerci: cattle heart (modified from American Society of Parasitology/OIE).

2.4 LIFE CYCLE

After the ingestion, the tapeworm becomes sexually mature after three months, producing gravid proglottids, which are mobile and either migrate from the host's anus spontaneously, usually in chains of six to nine segments, or are shed daily in the feces. Proglottids may migrate between the legs onto clothes, bedding or the ground, releasing eggs in the process. Occasionally, a large part of the strobila may be discharged and the expulsion ceases for a short period (FAO, 2020).

The eggs may remain viable for several weeks or months in sewage, water or on pasture. When eggs are ingested by cattle, embryos hatch and activate under the influence of gastric and intestinal juices, and penetrate the intestinal mucosa to reach the general circulation. The oncospheres develop in skeletal and cardiac muscles and less frequently in fat and visceral organs but begin to degenerate within a few months after infection, and by nine months a substantial proportion of them are dead and calcified (WHO, 2020).

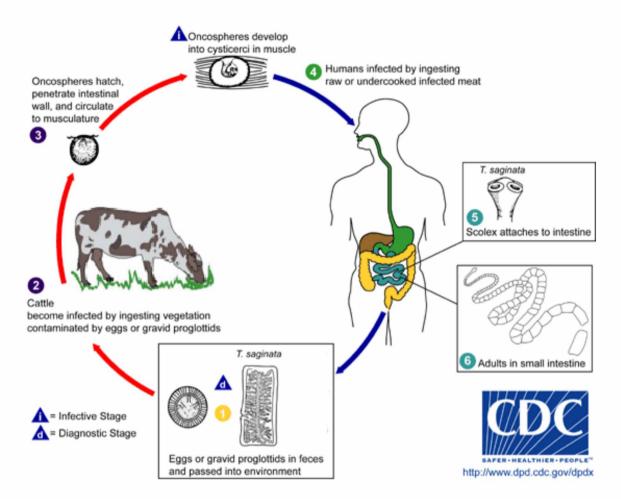


Figure 8. Taenia saginata life cycle.

2.5 PREVALENCE of BCC

Several risk factors can be associated with the appearance of bovine cysticercosis in several countries, such as: the supply of contaminated fodder to animals, the use of agricultural land for leisure activities without sanitary control, regions with a high prevalence of tourism practices, access to sources of contaminated water, little or no basic sanitation (MAIA et al., 2017; ROSSI et al., 2015).

The less developed countries have higher rates of prevalence of bovine cysticercosis. Dermauw (2018) through a retrospective study demonstrated that in southeastern Africa the most developing or least developed countries present or have already presented cases of human taeniasis and bovine cysticercosis. Slaughterhouse data used to identify the proportion rates of bovine cysticercosis in southern Africa showed prevalence rates of 0.70%, similar to the values found in several Brazilian states (QEKWANA et al., 2016).

Another study, conducted by Hendrickx (2019) in different regions of Africa with an analysis period similar to the previous one (1990-2017) observed the prevalence and geographic

distribution of human taeniasis and bovine cysticercosis in 27 countries. Taeniasis had a lower prevalence rate, 7 of the 27 regions analyzed positive. While bovine cysticercosis was reported in 14 of the 27 countries analyzed (HENDRICKX, 2019)

With a taeniasis prevention program, Russia remains as another country considered endemic for *T. saginata* and bovine cysticercosis. According to official country reports, during the study period (1991-2017) more than 17,000 cases of taeniasis were recorded. There has been a decline in the rate of onset of the disease in both cattle and humans in recent years, not only due to the prevention of taeniasis and cysticercosis, but the decrease in local meat production and increased imports (BÓBIC et al., 2018).

The distribution of the parasite in Central and Western Asia is also notable. Reports from countries such as Belgium and Switzerland relate the high incidence to inadequate basic sanitation and the access of animals to water contaminated by effluents. Most of the data on bovine cysticercosis and taeniasis come from countries like Iran, considered the lowest consumer of beef among those analyzed (3.4 kg / year peer captures), suggesting that the real situation in other countries is even more relevant, but less reported (TORGERSON et al., 2019).

Latin American countries such as Colombia have an average of 725 cases of taeniasis and cysticercosis reported per year. The measure in which there is a positive impact on the social conditions of the population, the number of cases tends to reduce. In the study conducted by Afonso et al (2018), between the years 1990 and 2010 access to better sanitation increased by 15% in the region, as well as other factors associated with surveillance and food safety education would explain the reduction of the parasite according to years (AFONSO et al., 2018).

The wide distribution of the parasite is related to the various factors mentioned, but the consumption of raw meat that has not been inspected rigorously is the main risk factor for maintaining the disease in humans and cattle. The main preventive and control measure are the detection of cysticerci during meat inspection (LARANJO-GONZÁLEZ et al., 2018).

2.6 BRAZIL HYGIENE CONDITIONS AND DISEASES

Brazil has around 15 million people without access to safely managed water in urban areas, in rural areas 25 million only have a basic level of service, and 2.3 million access unimproved sources of drinkable water, personal and house hygiene. In terms of sanitation, over 100 million people live without access to safely managed sanitation, of which 21.6 million

people used an unimproved sanitation facility, and 2.3 million practice open defecation, risk factor to spread several diseases, including BCC (IBGE, 2020).

The largest deficits are concentrated in the North and Northeast of the country. Lack of access is especially acute in the lower income segments, in indigenous villages, in the urban peripheries and slums, or ''favelas'', where approximately 13 million Brazilians live. One in three people worldwide still do not have access to drinkable water, two in five do not have adequate basic facilities to wash their hands with soap and water, and more than 673 million people do not have toilets or latrines (BRASIL, 2020).

The recent disclosure of results from the Continuous National Household Sample Survey (NHSS), referring to basic sanitation conditions in 2019, demonstrates current unresolved needs. Some indicators support this statement. The general water distribution network, which served 85.8% of households in 2016, has remained practically unchanged at 85.5% in 2019. In addition, regional distribution of the general water network is uneven, varying from 58.8% in the North region of the country to 92.3% in the Southeast (IBGE, 2020).

Analysis of the water sources and infrastructure used to supply Brazilian municipalities, shows that 31% of the population live in places of low water security, that is, they face rationing, collapse or warning in periods of drought; and 41% live in regions where production systems require expansion. Only 27% of the population live in municipalities where the supply was considered satisfactory. Water distribution across income brackets is very unequal, as 40% of the unserved population are in the 1 minimum wage or less income bracket (ANA, 2020).

These regional inequalities are more intensely noticed when looking at the proportion of households with access to the general sewage system: in 2019, the North and Northeast regions of the country had the lowest coverage, with 27.4% and 47.2%, respectively, whilst coverage in the Southeast region reached an estimated 88.9%; and the South and Midwest regions had the same coverage of 68.7% (IBGE, 2020).

Countrywide, 19.1% of households are connected to septic tanks but they are not connected to the general network; substantial regional variations are seen, with 42.9% of households in the North, 30.7% in the Northeast, and 5.5% in the Southeast using this modality of connection, thus corroborating the evident heterogeneity in the access to this essential service. (PAIVA, 2018).

Approximately 9 million households (12.6%) had a ditch, rudimentary cesspit, river, lake or sea, in addition to other forms of waste disposal. In the North region, 29.6% of households (1.6 million) were in this condition, exceeding the estimated 27.4% of households connected to the general network (IBGE, 2020).

The relevance of sanitation infrastructure in the health-disease process was highlighted in a study on hospitalizations due waterborne diseases. It was estimated that, in 2015, these diseases corresponded to 2.35% of all hospitalizations in Brazil, totaling 0.7% of total Unified Health System (SUS) spending on hospitalizations in that period (PAIVA, 2018).

Overall, in 2015, diarrhea was estimated to be one of the main causes of death in all age groups (1.31 million), and one of the main causes of Disability-adjusted life years - DALYs (71.59 million DALYs) due to its disproportionate impact on children under 5 years old. In general, rotavirus is the leading cause of death from this disease, followed by *Shigella* spp and *Salmonella* spp. (TROEGER et al., 2017).

This zoonosis is directly related to risk factors such as disordered urbanization, precarious conditions of basic and hygienic sanitation, close contact with pigs, and poor health surveillance present in the regions where the human infection is endemic, as well as cultural behavior and internal migration of people from rural areas to urban centers (KONGU et al., 2017).

The World Health Organization reported that inadequate water, sanitation and hygiene conditions were responsible for 829,000 deaths from diarrhea in the world in 2016. Some waterborne diseases, such as gastroenteritis, are on the Brazilian list of primary care-sensitive conditions. Hospitalizations for primary care-sensitive conditions represent potentially preventable conditions, which can reduce the risk of unnecessary hospitalizations and are a powerful indicator of primary care access and quality (WHO, 2020).

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3. RATIONALE AND OBJECTIVES

3.1 RATIONALE

The knowledge of the prevalence rates of bovine cysticercosis in the state of Paraná, based on the notifications made by the SIE, provide the possibility to plan and control actions not only at the slaughterhouses level, but also in the knowledge of the population, breeders and decision makers that can be responsible for the proper handling of cattle throughout the entire production chain.

3.2 HYPOTHESIS

- The number of animals slaughtered is directly related to the number of notifications performed.
- The socioeconomic index and the development of the local population is directly related to the higher the number of parasitic zoonosis identified in the carcasses during official inspection at the slaughterhouses.
- Regions with a higher rate of residents in the rural area have a higher prevalence of carcass injuries.

3.3 OBJECTIVES

3.3.1 General Objective

To determine the prevalence of bovine cysticercosis in Paraná state under the State Inspection Service through the quantitative survey of data obtained by the Paraná Agricultural Defense Agency (ADAPAR).

3.3.2 Specific Objectives

- To identify the municipality with the highest prevalence rate in slaughterhouses.
- To map the spatial distribution of bovine cysticercosis in the state of Paraná, southern Brazil.
- Correlate the observed prevalences with literature data from Brazil and around the world.

4. ARTICLE: PREVALENCE, GEOSPATIAL DISTRIBUTION AND RISK FACTORS ASSOCIATED TO BOVINE CYSTICERCOSIS IN SLAUGHTERHOUSES LOCATED IN PARANA STATE, SOUTHERN BRAZIL.

ABSTRACT

Increased beef production in Brazil reflects the globalization of markets and the improved purchasing power of consumers. The country has become the largest exporter of beef protein and also a remarkable consumer, that ranges around 42 kg per capta/year. Nevertheless, bovine cysticercosis (BCC) remains as the most frequently diagnosed disease through sanitary inspection during the post-mortem exam. Slaughterhouse's data are considered as a valuable source to describe the prevalence of BCC. Thus, the aim of this study was to determine the prevalence, geospatial distribution and risk factors associated to BCC in the state of Paraná, southern Brazil. Slaughterhouse's data from the State Inspection Service (SIE) and the Brazilian Institute of Geography and Statistics (IBGE) database were retrieved and analyzed during a two-years period. During this period, 2.896.142 cattle were slaughtered in the state. The SIE inspect around 35% of this total, which represents 1.019.339 animals. The geospatial distribution of 18.741 cattle (2%) was analyzed, this value represents the batches of overall affected animals. The number of BCC cases diagnosed in slaughterhouse's through overall inspected animals by the SIE was 1.461 (0.14%). Regarding the prevalence by municipality, in the Southern region of the state, Eneas Marques (0.66%), Planalto (0.50%) and Pinhão (0.50%) presented the highest prevalence's for BCC. Paiçandu, located in the Central North region of the state, had the highest prevalence for bovine cysticercosis (1.00%), while Ourizona and Floraí presented and (0.75%) in the same region. The main risk factors associated to BCC were the poor sanitary conditions of the municipalities, high human population density and the intensification in the extensive livestock sector. Despite this, the data obtained from cattle slaughtered remains as an extremely relevant tool to analyze the distribution of the BCC.

Keywords: Food Safety; Zoonoses; Beef; Cattle; Inspection; Cysticerci.

BACKGROUND

The global demand for animal protein has increased in recent years. The consumers expanded and diversified their consumption habits towards more expensive meat protein such as beef. Accordingly, the livestock sector has consistently exceeded the crop sector, resulting in the intensification for animal products in developing countries (FAO, 2018).

In Brazil, meat production had a growth due to the abundant supply of natural sources, feed and grassland. While the global annual meat consumption expects to increase from 25.5 to 37 kg per person in the forthcoming years (FAO, 2019), Brazil's last year consumption was approximately of 42.12kg per capta (ABIEC, 2019).

The growth is partially due to Brazil's advances in beef exportations and also to the domestic market in the country. According to the Quarterly Animal Slaughter Survey, released by the Brazilian Institute of Geography and Statistics (IBGE), the Paraná State had an increase in the number of slaughters in the last quarter of 2019, resulting in 380,000 thousand animals slaughtered (IBGE, 2020).

The livestock sector in the Southern region of Brazil represents an essential economic activity. The northwest region of Paraná State has the major beef cattle herds, with Umuarama municipality concentrating the largest and most expressive production. The comparative profile provided by the State Department of Agriculture and Supply (SDAS) established that Paraná's herds impact around 4.4% of the overall slaughtered animals in the country, producing 612.751 ton. of beef meat, of which 11% were exported (SEAB, 2019).

As a consequence, the increase in animal production and the accelerated production pace causes significant public health problems. To guarantee the ideal sanitary conditions during the entire production process and also avoid the streamed of foodborne diseases/zoonoses, the hygiene measures should be applied at all points in the food chain (FAO, 2019; OIE, 2018).

In slaughterhouses, the meat inspection should keep prioritized in order to diagnose, monitor and control pathological and zoonotic agents to animals and humans. The ante and post-mortem inspections in slaughterhouses, performed by trained professionals and also by veterinarians, contribute to reduce foodborne diseases and zoonoses risks to consumers. (ALVESEIKE, 2018).

In Brazil, bovine cysticercosis (BCC) is the most frequently diagnosed parasitic zoonotic disease in slaughterhouses, causing significant economic losses due to carcass condemnations. (ROSSI et al., 2017). Even though the disease presents worldwide distribution,

the highest rates are reported in developing and underdeveloped countries with poor sanitary conditions, such as in regions of Africa, Asia and almost 21 of the 54 countries of the Americas (DERMAUW et al., 2018; EINCHENBERGER et al., 2020, BRAAE et al., 2018).

The BCC caused by *Taenia saginata* has been reported in several countries. This important zoonotic parasite causes taeniosis in humans (definitive host) and cysticercosis in cattle (intermediate host). Humans can be infected through the consumption of raw/undercooked beef or water containing cysticerci, while cattle acquire the infection accidentally, by ingesting sources of contaminated water or pasture (EICHENBERGER et al., 2020).

Slaughterhouse's data are valuable sources in the analysis of BCC prevalences. Most of these records include the age, gender and sanitary information. The regular analysis using slaughter data are willing to identify the most important diagnosed diseases, seasonality and spatio temporal trends. (HAREDASHT, 2018).

Therefore, the aims of the present study were to perform a retrospective analysis through data obtained from Paraná Agribusiness Defense Agency (ADAPAR) to determinate the (1) prevalence, (2) spatial-distribution and (3) risk factors associated to BCC in slaughterhouses under State Inspection Service throughout 2 years.

METHODS

Study area

Paraná state is located in the southern region of Brazil, with a total area of 199.305.236 km2, 399 municipalities, an estimated population of 11,433,957 inhabitants and a cattle herd of 9,275,271 heads (IBGE, 2018).

Data collection

The prevalence and geospatial analysis of BCC was performed through the database from official health inspection reports provided by ADAPAR, department responsible for slaughter sanitary conditions, meat inspection and the notifications of post-mortem findings, comprehending two years (2018 to 2020).

These data were provided by official inspection agents and veterinarians from the State Inspection Service in Slaughterhouses during the carcass examination. The access to the reports

was realized by online platforms containing information about sanitary conditions of the slaughtered animals. The monthly number of cattle sent to slaughterhouse was also provided by ADAPAR.

Analysis

To determine the prevalence and geographical distribution of BCC across Parana State data was analyzed by using R software, version 3.6.3 and the he packages readxl (data reading), tidyverse (general data manipulation), ggplot2, ggpubr (graphs) and the geobr (graphical coordinates for the maps).

In order to verify the integrity of this data, inconsistent information or case doubts were checked with ADAPAR. Incomplete or duplicated records were not included in this study.

The statistical analyses consisted in calculating summary measures, graphs and maps. The response variable and the prevalence of cysticercosis were calculated considering the ratio between the number of affected by the disease and the total number of animals.

All data obtained were also summarized by geographical unit (eg: municipality of origin of the animals) and were represented on maps with color scale according to the magnitude of BCC prevalence.

The risk factors associated to the BCC were determinate using online platforms provided by (IBGE) and also throught the Parana's Technology and Environmental Monitoring System (PTEM).

RESULTS

Through the period of two-years 2,896,142 cattle were slaughtered in Paraná, 1,019.339 under State Inspection Service. However, in this study were analyzed and included 18,741 animals. This value represents the batches of overall affected animals. Through overall animals inspected by SIE, 1.461 were diagnosed with BCC. This value represents a prevalence 0.14% in the state.

The geospatial distribution map of BCC prevalence in Paraná state was obtained through Global Positioning System (GPS) coordinates. The color scale is a gradient ranging from blue (0%) to red (100%). Municipalities with no cases diagnosed in slaughterhouses are shown in light gray (Fig.1)

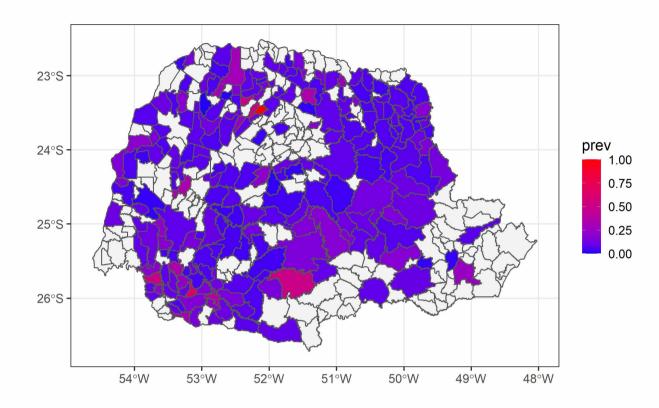


Fig.1 Geospatial distribution of BCC in Paraná from cattle slaughtered under State Inspection Service through two-years.

Regarding the prevalence by municipality, in the Southern region of the state, Eneas Marques (0.66%), Planalto (0.50%) and Pinhão (0.50%) presented the highest prevalence's for BCC. Paiçandu, located in the Central North region of the state, had the highest prevalence for bovine cysticercosis (1.00%), while Ourizona and Floraí presented and (0.75%) in the same region.

Furthermore, as illustrated in Fig.1, a significant number of the municipalities did not diagnose cysticerci during slaughter. Few cases (0.01 to 0.025%) have been described in the west/north regions.

The distribution of the adequate treatment and disposal of human excreta and sewage, collection, transport, treatment disposal or reuse was representing in the maps (Fig.2). The color scale is a gradient ranging from blue (0%) to red (100%). Municipality with no data available are shown in light gray.

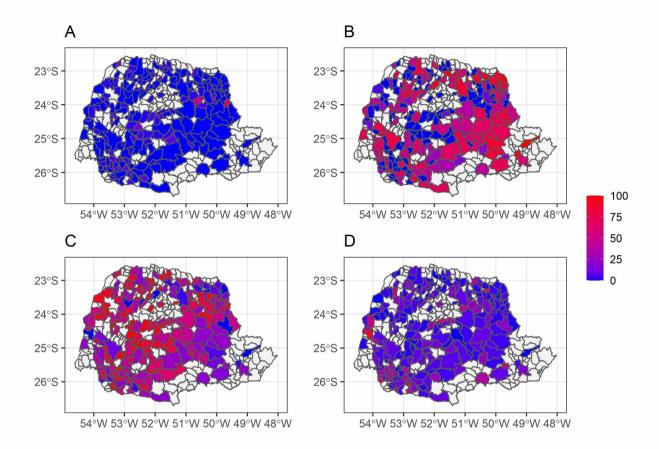


Fig.2 Sanitary conditions in Paraná state. **A.** Municipalities that performs individual solutions for collect and treatment. **B.** Municipalities with sewage collected and treated. **C.** Municipalities with sewage not collected and not treated. **D.** Municipalities with sewage collected and untreated.

DISCUSSION

BCC prevalence's as established in this study was performed based on meat inspection routine. The post-mortem examination of carcass in slaughterhouses remains as an obligatory method applied in slaughterhouses around the country and aims to detect infected animals through the visual inspection. During the exam, the carcass and/or viscera containing cysticerci may be conducted to the Final Inspection Department (DIF), where the Official Veterinary will realize the complementary inspection. (BRASIL, 2017).

The prevalence of bovine cysticercosis established in this study (0.14%) is lower than that reported in studies performed in the last years in the state. Nevertheless, higher results (2.23%) were observed in the analysis performed by Guimarães-peixoto et al; (2012) from 2004 to 2008, using data from slaughterhouses under Federal Inspection.

The prevalence established in this study can be underestimated due to the unworthy visual inspection performed by the professionals. The presence of cysticerci in uncommon muscles or mild infections with few cysticerci can hinder the visual inspection, bringing forth results that do not represent the real percentages of infected animals (GONZÁLEZ, et al., 2015). The post-mortem inspection during slaughter detect around 14.5 to 19.00% of cattle infected, while ELISA or serological exams bring forward more reliable results. (GUIMARÃES-PEIXOTO et al., 2020; BRAAE et al., 2018).

This different prevalence value can be related to the distinct criteria used by the authors, the number of animals slaughtered, the period of the analyze, the data obtained from different inspection service and also associated to the implementation of the Cysticercosis Control Program for the Paraná state (GUIMARÃES-PEIXOTO et al., 2012).

According to the IBGE, in the mentioned period 2.896.142 cattle were slaughtered in Paraná by the three instances of inspection. However, the Federal Inspection data registered 40% more animals slaughtered than State Inspection Service, used in this study. With that said, different criteria produce distinct results. (IBGE, 2020)

Paraná state has a high human density population, (52,40 hab/km²), the 5th among all states in Brazil, according to IBGE (2020). High human population density has been reported as a risk factor for BCC in the country. The southeast and south states represent the major scale of risk and contribute with the highest prevalence establish around the country (ROSSI et al.,2016).

Moreover, similar BCC prevalence's values (0.09%) was reported in Rio Grande do Sul, the 6th highest human population density (39,69 hab/km²) of Brazil, according to IBGE, (2020).

The study was conducted from 2013 to 2016 in the southern region of the state through data obtained from the official inspection service in slaughterhouses (ALBERTI et al., 2018), such as realized in this study.

A recent publication review conducted in Brazil, focused on bovine cysticercosis, established the prevalence for all administrative regions around the country (North, Northeast, Central-Western, Southeast and South). The North regional prevalence was (0.6%), Central-Western (0.9%), Northeast (1.5%), Southeast (2.7%) and South (3.4%). A possible correlation to the major prevalence in the southeast and the South region can be attributed to the population density (ROSSI et al., 2020).

According to Rossi et al., (2017), there were several situations that can be considered risk factors for BCC in Brazil, most were related to poor hygiene conditions, basic sanitary education, human population density, temporary works involving the production in the crop sector, recreation activities near to farms, extensive livestock production, animal purchases, flooded pastures, climate, river interferences, animal exposure to *T. saginata* eggs in the pasture, water or contaminated feed, among others. (ROSSI et al., 2017; LARANJO-GONZÁLEZ et al., 2018).

The deprived of basic sanitary conditions represents risks not only for the development of bovine cysticercosis, but also for several diseases that affect humans and animals. To analyze the situation of each municipality aspects related to potable water distribution, sewage collection, treatment, urban drainage and solid waste collection are evaluated (IBGE, 2017).

The geospatial distribution of BCC (Fig.1) Eneas Marques (0.66%), Floraí (0.75%), Ourizona (75%), Paiçandu (1.00%), Pinhão (0.50%) and Planalto (0.50%) strongly reflect the poor sanitary condition of the population. This prevalence also can be attributed to the livestock exclusively depending on the extensive pastures system and the developing urbanization nearby the areas of livestock breeding (DERMAUW V. et al., 2019; LARANJO-GONZÁLEZ et al., 2016).

According to the sanitary condition's maps (Fig.2), 75-100% of the Floraí and Ourizona municipalities does not perform sewage treatment. Eneas Marques and Planalto presents an even worse situation with almost 100% (Fig. 2) of the population without access to sewage treatment. Capanema, Paiçandu and Pinhão also presents high results, (50%) and (25-50%) does not perform sewage treatment, respectively. In Capanema almost 25% of the population realize individual solutions, that includes burn on the property, bury on the property, dumped on other lands or thrown into rivers (IBGE,2020).

Even if other municipalities have presented high values related to sanitary conditions, only those with significant flow of animals are mentioned in this study. This relation must be observed regarding the number of animals. While some municipalities gave rise to a significant number of the animals, other municipalities contributed only with one animal (ALVES et al., 2017; IBGE,2020).

While the high prevalence of BCC in Paiçandu, Eneas Marques and Planalto can be related to the deprived basic sanitation of this regions, the municipalities of Floraí, Ourizona and Pinhão reported considerable amounts of the disease due to the expressive number of farms, recreational activities near rivers, large herds of beef cattle and warm and humid climate (IBGE, 2020; ROSSI et al., 2020).

High intensification of agriculture involving cattle herds and increased irrigation has been known as a risk factor for bovine cysticercosis. The widespread occurrence in this municipalities also can be associated to the intense livestock production performed in the regions, which represents an important economic activity to the state (LARANJO-GONZÁLEZ et al., 2018).

Nevertheless, bovine cysticercosis was reported during meat inspection routines in other countries. In eastern Europe, for example, a prevalence of 1.7% was reported among 27 years of analysis by considering the low sensitivity of the post-mortem exam, false positives and underestimate results. This value is similar to those compared in the northeast region of Brazil, which had the lowest prevalence in the country. (TREVISAN et al., 2018; JANSEN et al., 2017).

During the period 1990 to 2017, BCC was reported in 19 countries of the Americas, such as Canada (4.02%), Ecuador (0.5%), Mexico (0,17%) and the USA (8.09%), what represents a widely distribution of the disease in these regions (BRAAE et al., 2018). In recent years, however, the USA and Canada have not diagnosed BCC, while in developing countries, like Brazil, the disease still remains as an economic and health problem.

On the other hand, the prevalence of BCC in eastern and southern Africa is ranging between 0.02 to 26.3%. The region has the cattle production as an important economic activity. The cattle herds are basically maintained by using the extensive beef production system. In this system, also used in the southeast and south regions of Brazil, the animals have free access to the pasture and sources of water, what is known to be a determining risk factor for bovine cysticercosis (DERMAUW et al., 2018; LAMPERT et al., 2020).

Ultimately, the high prevalence of BCC cases in the regions analyzed in this study are results of poor sanitary conditions, a commonly accepted risk factor for this disease. (GUIMARÃES-PEIXOTO et al., 2012).

CONCLUSION

Bovine cysticercosis is frequently observed in slaughterhouses in Paraná, Brazil. In addition, the State Inspection Service represents only 35% of the overall animals slaughtered in the state, which suggest that the real number of cases is even higher. The data obtained from cattle slaughtered remains as an extremely relevant tool to analyze the distribution of BCC. Finally, the main risk factor associated to the disease was the poor sanitary conditions, high human population density and the intensification in the extensive livestock sector.

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