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Occurrence and risk factors of bovine cryptosporidiosis in Brazil – Systematic Review

Ocorrência de criptosporidiose bovina no Brasil – Revisão sistemática

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ABSTRACT

Protozoa of the genus *Cryptosporidium* are parasites capable of causing animal rearing losses due to diarrhea outbreaks. This review aimed to determine the prevalence of and potential risk factors for bovine cryptosporidiosis in Brazil. The search was carried out in the databases Science Direct, Scopus, Web of Science, and SciELO. The search strategy used the descriptors "Cryptosporidium," "cryptosporidiosis," "cattle," "calves," and "Brazil." Nine studies conducted in different Brazilian states between 2008 and 2019 were selected. The age of the evaluated animals ranged from 0 to >36 months. The main risk factors were the low age of the animals, collective pens, presence of diarrhea, contact with other animals, and dairy breed. The average prevalence rate was 30.51%. The species identified were *C. ryanae*, *C. parvum*, and *C. bovis*. Optical microscopy was used as a diagnostic method for oocysts in fecal samples and to determine the species, and PCR was used in three studies. The average prevalence of *Cryptosporidium* spp. in cattle in Brazil was 30.51%, ranging from 10.2 to 62.5%. The main risk factors found were young animals, the presence of animals with diarrhea, contact with other animals, dairy breeds, and collective pens.
Keywords: *Cryptosporidium* sp.; diarrhea in cattle; parasitic diseases

RESUMO

Protozoários do gênero *Cryptosporidium* são parasitas capazes de causar perdas durante a criação animal devido à surtos de diarreia. Esta revisão foi realizada para determinar a prevalência e os potenciais fatores de risco para a ocorrência de criptosporidiose bovina no Brasil. A busca foi conduzida nas bases de dados Science Direct, Scopus, Web of Science e Scielo. A estratégia de busca usou os descritores "Cryptosporidium",

"cryptosporidiosis", "cattle", "calves" e "Brazil". Nove estudos conduzidos em diferentes estados do Brasil foram selecionados, com ocorrências entre 2008 e 2019. A idade dos animais avaliados variou de 0 acima de 36 meses. Os principais fatores de risco determinados foram animais jovens, baias coletivas, presença de animais com diarreia, contato com outros animais e raça leiteira. A taxa de prevalência média foi de 30,51%. As espécies identificadas foram *C. ryanae*, *C parvum* e *C. bovis*. Microscopia óptica foi usada como método de diagnóstico de oocistos nas amostras de fezes e, para determinação das espécies, a técnica PCR foi usada em três estudos. Concluiu-se que a prevalência média do protozoário *Cryptosporidium* spp. em bovinos no Brasil foi 30,51%, variando de 10,2 a 62,5%. Os principais fatores de risco foram animais jovens, presença de animais com diarreia, contato com outros animais, baias coletivas e raças leiteiras.
Palavras-chave: *Cryptosporidium* sp.; diarreia em bovinos; doenças parasitárias

INTRODUCTION

Protozoa of the genus *Cryptosporidium* are parasites of the gastrointestinal epithelium of vertebrates (Rieux et al., 2013), capable of causing considerable economic losses in animal rearing due to diarrhea outbreaks (Yap et al., 2016).

Cattle may be infected with *C. parvum*, *C. andersoni*, *C. ryanae*, and *C. bovis*; however, only *C. parvum* is associated with clinical diseases in neonatal calves, whereas *C. andersoni* is more frequently found in adult cattle, and the clinical signs include a reduction in weight gain and milk production (Thomson et al., 2017).

Parasitic diseases cause economic and productive losses (Schmidt et al., 2017) in addition to generating expenses for medicines, electrolyte replacement, fluid therapy, and hygiene measures (Graaf et al., 1999).

Several factors may interfere with the occurrence of diarrhea outbreaks caused by *Cryptosporidium* spp., such as the type and hygiene of the buildings, animal age, breed (Ayele et al., 2018), and season (Kashyap et al., 2021).

The prevalence of *Cryptosporidium* spp. in dairy calves organized in groups was higher (45%) than that in calves in individual pens (20%) (Åberg et al., 2019). In a study in Ethiopia, Ayele et al. (2018) noted a higher prevalence of this parasite in dairy calves under six months of age (28.4%) than in calves aged 6–12 months (15.8%). *Cryptosporidium* spp. may be found at all ages, and although calves under one month of age are the main carriers of *C. parvum*, adult cattle also play an essential role in cryptosporidiosis outbreaks, both in calves and humans (Díaz et al., 2021).

Cryptosporidium oocysts are robust and have a high survival capacity in soils and watercourses. From the moment oocysts are excreted in the feces, they may move to the ground and along the soil column to reach underground waters (Armon et al., 2016). According to Jenkins et al. (2002), oocysts may survive for months in agricultural soils, and consequently, have the potential to contaminate surface water.

The main source of oocysts is cattle grazing in the riverside areas. Oocysts that reach watercourses can be transported over long distances and get disseminated to animals and humans. Sunohara et al. (2012) observed an 88% reduction in oocyst load in the

watercourse when the animals were kept with restricted access to water and a 38% reduction when the animals had unrestricted access to water.
This systematic review aimed to determine the occurrence and risk factors of bovine cryptosporidiosis in Brazil.

METHODOLOGY

Search strategy

A search was performed in January 2022 to retrieve relevant studies from the Web of Sciences, SciELO, Scopus, and Science Direct databases, using the Descriptors in Health Science (DeCS/MeSH) AND and OR Boolean operators. To identify the search terms, we used the CoCoPop format (Munn et al., 2018), where:

Co – Condition – the presence of Cryptosporidium, cryptosporidiosis

Co – Context – occurrence in Brazil

Pop – Population – cattle, calves

The resulting strategy search was "(Cryptosporidium OR cryptosporidiosis) AND (cattle OR calves) AND Brazil".

Eligibility of the studies and inclusion and exclusion criteria

Two researchers evaluated titles and abstracts using the Rayyan platform (Ouzzani et al., 2016). The selected 3apers were evaluated based on the inclusion and exclusion criteria. The inclusion criteria were: (1) cross-sectional studies, (2) carried out in Brazil, (3) about the occurrence of Cryptosporidium in cattle, (4) peer-reviewed studies, (5) open access, and (6) published between 2011 and 2021. The exclusion criteria were: (1) thesis and dissertations, (2) case studies, (3) editorials, (4) duplicate studies, and (5) studies published before 2011. Discrepancies in the selection process were resolved by the research team.

Data extraction of the selected studies

Next, the papers were read by two researchers for a new evaluation based on the eligibility criteria, and the following data were extracted: authorship, publication year, state of the country, population (beef or dairy cattle), animal age, risk factors, size of the total samples and of positive samples, identified genus and species, and diagnostic method.

Assessment of the study quality

The risk of bias among the included studies was evaluated using a quality assessment checklist adapted from a study by Ding et al. (2017). The checklist comprised five items that were scored based on a scale system (2 = yes, 0 = no, or 1 = unsure). The studies were ranked as high, moderate, or low based on their analysis.

RESULTS

Nine studies met the inclusion criteria and were included in this systematic review (Table 1). The steps in the selection process are presented in a Preferred Reporting Items for Systematic Reviews and Meta-Analyses flowchart (Figure 1).

Characteristics of the studies

Nine studies were conducted in the following different states of Brazil: Minas Gerais (3/9), Paraná (5/9), Santa Catarina (3/9), Goiás (2/9), São Paulo (2/9), Ceará (2/9), Rio de Janeiro (3/9), Rio Grande do Sul (2/9), and Pernambuco (1/9). These cases were reported between 2008 and 2019. The age of the animals ranged from 0 to 36 months.

The determined risk factors for *Cryptosporidium* diarrheal disease (Table 1) were the young age of the animals (3/9), collective pens (3/9), presence of animals with diarrhea (2/9), contact with other animals (2/9), dairy breed (2/9), natural suckling and use of raw milk (1/9), hutches near the milking corral (1/9), bad hygiene in the hutches (1/9), ingestion of ration before 7 days of age (1/9), the permanence of the calves with mother for up to 12 hours after the birth (1/9), supply of colostrum 7 hours after the birth (1/9), hutches with pasture floor (1/9), presence of rotavirus in the flock (1/9), stress by transport (1/9), and consumption of contaminated water (1/9).

A total of 4,472 feces samples were evaluated, with 980 positive samples for *Cryptosporidium*. The average prevalence rate was 26.3%. Only three studies identified *Cryptosporidium* species in feces. *C. ryanae* (2/9), *C parvum* (3/9), and *C. bovis* (1/9), indicating a higher prevalence of *C. ryanae* and *C. parvum* infections. It is important to emphasize that these studies involved animals under 24 months of age, and *C. parvum* was identified in all animals. Optical microscopy was used to diagnose the presence of oocytes in feces in all studies. PCR was used to determine the species in three studies (Table 2).

Assessment quality of eligible studies

The results of the quality assessment of the eligible studies are presented in the Supplementary Table 1. All studies were of acceptable quality, except for the study by Volpato et al. (2017) because the authors neither presented the period of the study nor categorized the animals into subgroups.

Table 1. Characteristics of the included studies on bovine cryptosporidiosis in Brazil

Authorship	State[1]	Year	Animal age	Risk factors
Silva Jr. et al. (2011)	MG	2008-2009	≤ 12 months	Collective pens Natural lactation Calves housed near the milking corral Poor hygiene in the calves' pens Ingestion of water and rations before 7 days of age Ingestion of colostrum for 6 hours after the birth Separation of calves from their mother up to 12 hours after de birth
Fagundes et al. (2014)	RJ	2009-2011	≤ 100 days	A linear relationship between animal's age and infection
Toledo et al. (2017)	PR	-	0 - >24 months	European breed Presence of animals with diarrhea in the flock Animal's age
Volpato et al. (2017)	SC	-	1 - 60 days	Collective pens Contact with cats and dogs
Holsback et al (2018)	PR	2013-2014	<6 - >36 months	Young dairy animals
Cruvinel et al. (2020)	RS, PR, SC, RJ, SP, MG, GO, CE	2016-2017	1 - 135 days	Presence of animals with diarrhea or *Rotavirus* in the flock Collective pens
Agnol et al. (2021)	PR	2019	5 - 18 days	Stress due to transportation

Conceição et al. (2021)	PE	2018-2019	≤ 10 months	Contact with adult cattle, sheep, and goats Raising in an intensive system Poor hygiene in the pens
Oliveira et al. (2021)	CE, GO, MG, SP, RJ, PR, SC, RS	2016-2017	1 - 60 days	Young age (7-28 days) Presence of animals with diarrhea in the flock Collective pens Ingestion of water from natural sources

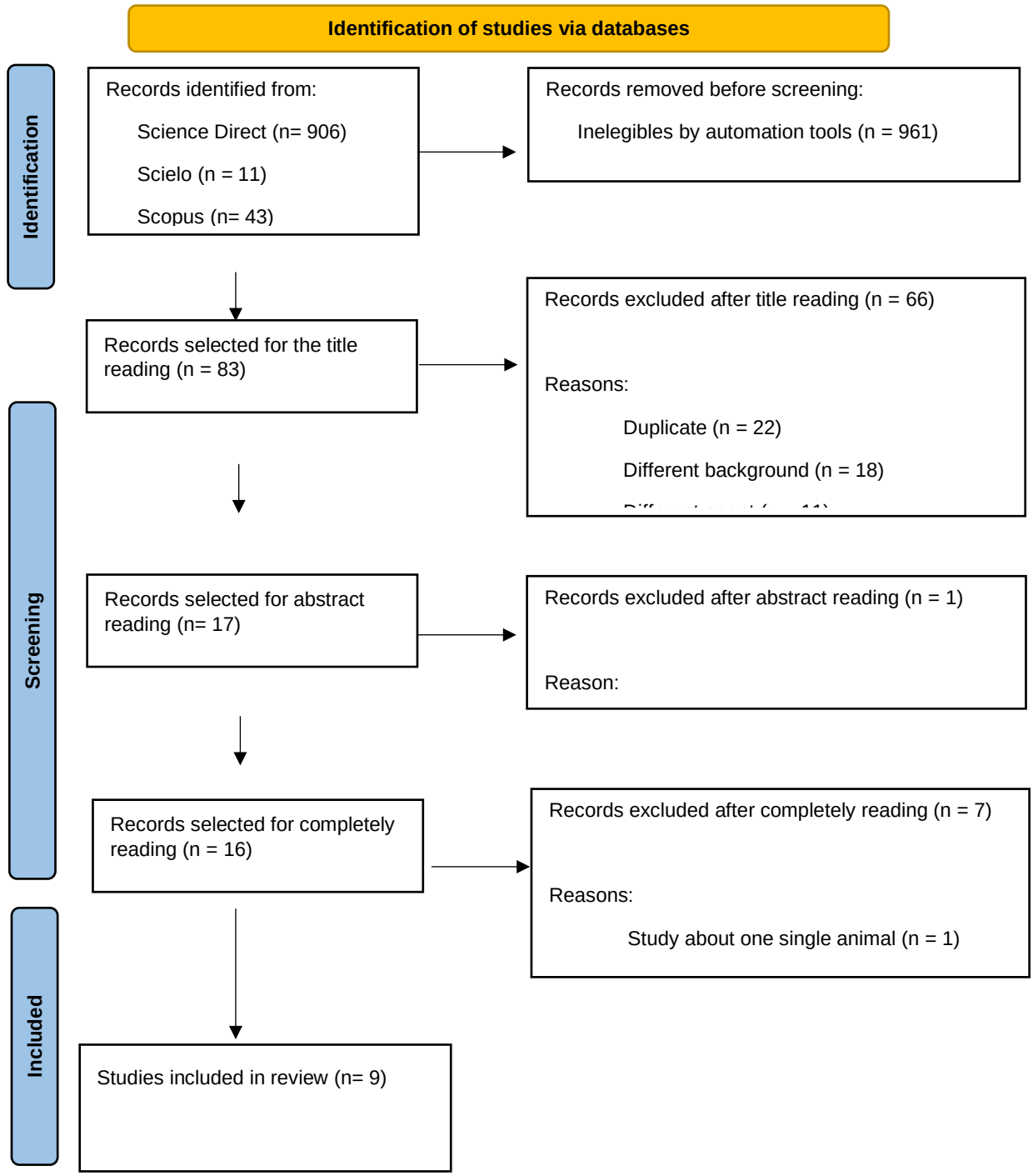

Figure 1. Flow diagram of the selection process of studies for the systematic review on bovine cryptosporidiosis in Brazil.

Table 2. Prevalence rate, identified species, and diagnostic methods of Cryptosporidium

Authorship	TNS	NPS	PR (%)	Identified species	Diagnostic methods
Silva Jr. et al. (2011)	356	77	21.6	-	OM
Fagundes et al. (2014)	1457	203	13.9	-	OM
Toledo et al. (2017)	937	96	10.2	*C. parvum* *C. ryanae* *C. bovis* *C. andersoni*	OM PCR
Volpato et al. (2017)	243	51	20.9	-	OM
Holsback et al (2018)	317	51	16.1	-	OM
Cruvinel et al. (2020)	361	182	50.4	-	OM
Agnol et al. (2021)	8	5	62.5	*C. parvum* *C. ryanae*	OM PCR
Conceição et al. (2021)	385	99	25.7	-	OM
Oliveira et al. (2021)	408	216	52.9	*C. parvum*	OM PCR

Discussion

Occurrence of bovine cryptosporidiosis

Among Brazilian regions, the southeast region led in milk production in 2019 with 34.35%, followed by the 8hey8 (34%), northeast (14%), midwest (11.6%), and north (6.1%). Bovine cryptosporidiosis occurs in most states in the southeastern and south regions, where higher milk production is concentrated (CNA, 2020).

Nine studies reported the occurrence of oocyte excretion in dairy herds, and in one of them (Holsback et al., 2018), the authors reported the occurrence in beef herds, indicating the higher susceptibility of dairy breeds to *Cryptosporidium*.

Dairy cattle are subjected to subpar conditions of welfare compared to beef cattle. Due to the separation of the calves from their mothers, not having free access to the pastures, and the daily milking as an intervention in the animal's life (Silverlås et al., 2009; Mandel et al., 2022).

Animals allocated to pens with a considerable number of animals, where hygiene is precarious, are more susceptible to infection because the contamination of the animals is facilitated by quagmire, feces, and urine. In addition, this type of environment favors the presence and survival of oocytes, with possible contamination of drinkers and feeders, causing the animals to be infected when they ingest feed or water (Ayele et al., 2018; Thomson et al., 2019).

Age of the affected animals

We found that young animals are predominantly affected. Among the *Cryptosporidium*-infected animals, 62.6% were 7-28 days old(Oliveira et al., 2021), 10-15% were between 8-15 days of age (Cruvinel et al., 2020), 26% were 0-30 days of age, 37% were 30-60 days old, and 36% were >60 days old(Conceição et al., 2021), 35% were >46 days of age (Volpato et al., 2017), and 25.3% were 0-2 months old (Toledo et al., 2017), corroborating the findings of Garro et al. (2016, 2021) and Ayele et al. (2018).

The higher occurrence of cryptosporidiosis in younger animals is because they possess a less-developed immune system facilitating the infection (Ayele et al., 2018). In addition, newborns must adapt to a new environment with a diversity of pathogens, adjust the way of receiving nutrients from the umbilical cord to bottles or buckets, and then become ruminants. These changes generate a significant stress load in the animals (Osorio, 2020), reducing their immunity (Hulbert & Moisá, 2016). However, resistance to infection commonly develops with age owing to the maturation of the immune system (Kvac et al., 2006).

Risk factors associated with the occurrence of bovine cryptosporidiosis in Brazil

As mentioned before, young age has also been considered a risk factor for cryptosporidiosis (Toledo et al. (2017); Hoslback et al. (2018); Oliveira et al. (2021). In 33.33% of the studies, animal age was described as a risk factor for cryptosporidiosis. Younger animals are more likely to be infected by *Cryptosporidium* (Díaz et al., 2021).

Silva et al. (2011), Volpato et al. (2017), and Cruvinel et al. (2020) reported collective pens as a risk factor. In collective buildings, the contact among animals is higher, resulting in a greater probability of infection. Couto et al. (2015) reported that only 8.5% of calves reared in individual pens, with high feeders and drinkers, showed oocytes in their feces, compared to the 21.9% of calves reared in collective pens, with low feeders and drinkers, showing oocytes in their feces.

Animals with diarrhea contribute to the dissemination of *Cryptosporidium* in the environment, as mentioned by Toledo et al. (2017) and Cruvinel et al. (2020). This pathogen is strongly associated with diarrhea and economic losses. The incidence of infection is higher in animals with diarrhea than in those with normal feces (Garro et al., 2016, 2021; Ayele et al., 2018). This protozoan causes atrophy of the villi, which results in a smaller intestinal absorption surface and causes diarrhea due to the non-absorption of compounds such as glucose, water, and sodium (Radostits et al., 2007).

The contact of calves with other domestic animals is another risk factor, as demonstrated by Volpato et al. (2017) and Conceição et al. (2021). *Cryptosporidium* may be transmitted from an infected animal to a healthy one, and contact between animals, even from different species, may facilitate parasite dissemination owing to the low specificity of the protozoan (Clode et al., 2015; Ayele et al., 2018).

Dairy breeds are highly susceptible to cryptosporidiosis (Toledo et al., 2017; Holsback et al., 2018). The lower immune response in dairy breeds, mainly in high-yielding dairy cattle, can be either primary, associated with genetic selection for high milk production, or secondary to metabolic stress during the transition period (Bronzo et al., 2020; Vlasova and Saif, 2021).
Some researchers have observed that the milk supply, separation of calves from their mothers, consumption of water from rivers, ingestion of colostrum for short periods, co-infections, and poor hygiene in buildings may be risk factors for bovine cryptosporidiosis (Silverlås et al., 2009; Garro et al., 2016; Ayele et al., 2018; Brainard et al., 2020; Ebiyo & Haile, 2022).

The prevalence rate of bovine cryptosporidiosis in Brazil

The prevalence rate varied from 10.2% in the study conducted by Toledo et al. (2017) to 52.9% as observed by Oliveira et al. (2021), with an average prevalence rate of 26.3%. Agnol et al. (2021) used a small sample size (n = 8), which may have caused an overestimation of the prevalence rate. Several factors influence the prevalence rate, such as farming type (beef or dairy), animal age, country region, climate, season, management techniques, well-being, and diagnostic methods (Hatam-Nahavandi et al., 2019).
The highest prevalence rates were obtained by Cruvinel et al. (2020) and Oliveira et al. (2021), with animals aged 1-135 days (51.5%) and 1-60 days of age (52.9%), respectively, indicating a higher susceptibility of younger animals. Fagundes et al. (2014) verified a prevalence rate of 13.2% in animals up to 79 days of age; however, in this study, the animals were kept in individual pens, which may have contributed to the low *Cryptosporidium* prevalence.

Cryptosporidium species in bovine

C. parvum and *C. ryanae* were found in the feces of animals aged 0 to >24 months (Toledo et al., 2017), 5 to 18 days (Agnol et al., 2021), and to 1-60 days (Oliveira et al., 2021), whereas *C. bovis* was found in animals of 4-6 months and > 24 months (Toledo et al., 2017). According to Feng et al. (2014), 90% of *Cryptosporidium* infections in cattle are caused by *C. parvum* and *C. bovis*.
According to Santín et al. (2008), *C. parvum* was detected in 97% of pre-weaned calves examined at 2 weeks of age, while the initial detection of *C. bovis* occurred in calves at 4 weeks of age, with a peak occurrence at 16 weeks of age. *C. andersoni* was not found until the 19th month of age, representing 25% of infections in this age category. Recently, Díaz et al. (2021) observed the predominance of *C. parvum* in calves up to one month of age, *C. bovis* in animals between 1 and 24 months of age, and *C. ryanae* in calves aged between 2 and 12 months.

Diagnostic methods of cryptosporidiosis

Optical microscopy and PCR were used in nine of the included studies. *Cryptosporidium* can be diagnosed using several techniques, including optical microscopy of fresh material and smear staining. Immunological and DNA

detection methods are also available (Khurana & Chaudhary, 2018). Optical microscopy is the most commonly used method in veterinary medicine owing to its cost-effectiveness.
PCR technique has been used (Toledo et al., 2017; Agnol et al., 2021; Oliveira et al., 2021). This method indicates the presence of the parasite, but cannot be quantified (Thomson et al., 2019). The diagnostic method must be appropriately selected because a method with low sensitivity can lead to an underestimation of protozoan presence; however, PCR is useful for identifying *Cryptosporidium* species.

Conclusions

The average prevalence of cryptosporidiosis in Brazil obtained from the included studies was 30.51%, varying from 10.2 to 52.9%. The main risk factors were young age, the presence of an animal with diarrhea in the herd, contact with another type of animal, dairy breed, and collective pens.

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