CLOSTRIDIUM PILIFORME: ANIMAL INFECTION AND ITS POTENTIAL ZOONOTIC RISK

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Recebido em: 15/05/2025 – Aprovado em: 15/06/2025 – Publicado em: 30/06/2025
DOI: 10.18677/EnciBio_2025B4

ABSTRACT

This study compiles information on *Clostridium piliforme*, the microorganism responsible for Tyzzer's disease, an obligate intracellular bacterium that affects various animal species, including rodents, domestic animals such as dogs and cats, and humans. This is concerning, as laboratory rodents are frequently used in research, and synanthropic rodents are a global problem. Dogs and cats can become infected through hunting and/or contact with synanthropic rodents, creating a zoonotic concern due to their proximity to humans. Tyzzer's disease presents nonspecific symptoms such as diarrhea, dehydration, weight loss, and lethargy, often progressing rapidly to death in young or immunocompromised individuals. The bacterium's ability to persist as spores and its fecal-oral transmission route are of great importance, promoting its spread. Periodic monitoring of animals is essential to detect asymptomatic carriers and prevent pathogen transmission, particularly when they are more exposed to the risk of contamination. Diagnosis involves identifying the pathogen through PCR, serological techniques, and/or histopathology. Veterinarians play a key role in controlling and preventing the disease by continuously monitoring animals and educating pet owners about the dangers of rodent contact and the importance of rodent control. Effective management and prevention in laboratory settings are vital to protect research animals and the professionals involved, ensuring scientific reliability. Professionals involved in human health must be able to identify suspected cases, adopt appropriate diagnostic methods, and ensure early treatment. Public awareness and preventive strategies can help reduce the spread of Tyzzer's disease, protecting both animal and human health.
KEYWORDS: Laboratory Animals, Public Health, Zoonosis.

RESUMO

Este estudo compila informações sobre *Clostridium piliforme*, microrganismo responsável pela doença de Tyzzer, bactéria intracelular obrigatória que afeta várias espécies animais, incluindo roedores, animais domésticos como cães e gatos, e humanos. Isso é preocupante, pois roedores de laboratório são frequentemente

usados em pesquisas, e roedores sinantrópicos representam um problema global. Cães e gatos podem se contaminar ao ter contato com roedores, criando uma preocupação zoonótica devido à proximidade com humanos. Doença de Tyzzer apresenta sintomas inespecíficos, como diarreia, desidratação, perda de peso e letargia, frequentemente progredindo rapidamente para a morte em indivíduos jovens ou imunocomprometidos. A capacidade da bactéria de persistir como esporos e sua rota de transmissão fecal-oral são de grande importância, favorecendo sua disseminação. Monitoramento periódico dos animais é essencial para detectar portadores assintomáticos e prevenir a disseminação do patógeno, principalmente quando eles estão mais expostos ao risco de contaminação. O diagnóstico envolve a identificação por PCR, técnicas sorológicas ou histopatologia. Os veterinários desempenham papel fundamental no controle e prevenção da doença, mediante monitoramento constante dos animais e educando os tutores sobre os perigos do contato com roedores e a importância do seu controle. O gerenciamento e a prevenção em ambientes laboratoriais são vitais para proteger os animais de pesquisa e os profissionais envolvidos, além de garantir a confiabilidade científica. Os profissionais envolvidos com saúde humana devem ser capazes de identificar casos suspeitos, adotar métodos de diagnóstico apropriados e tratamento precoce. A conscientização pública e as estratégias preventivas podem reduzir a disseminação da doença, protegendo a saúde pública.
PALAVRAS-CHAVE: Animais de Laboratório, Saúde Pública, Zoonose.

INTRODUCTION

Zoonotic infectious diseases constitute a priority area of research within public health, given their impact on human and animal populations. Among these diseases, Tyzzer's disease stands out, caused by *Clostridium piliforme*, a Gram-negative, obligate intracellular, spore-forming bacterium (FRANKLIN et al., 1994; MEREDITH; RAYMENT, 2000; CLIFFORD; PRITCHETT-CORNING, 2012). Originally described by Tyzzer in 1917 (CLIFFORD; PRITCHETT-CORNING, 2012; TYZZER, 1917), this condition affects a wide range of hosts, including animals and humans, and presents high lethality in immunocompromised and/or young individuals. The bacterium's ability to form spores grants it high environmental resistance, complicating control and eradication strategies (DUNCAN et al., 1993; FRANKLIN et al., 1994; NIEPCERON; LICOIS, 2010; STANNARD et al., 2017; ÜLKER et al., 2024).

Tyzzer's disease represents an emerging concern. The infection can silently establish itself in laboratory animal colonies, compromising the integrity and reproducibility of scientific experiments. Additionally, it poses a direct biological risk to professionals involved in research activities. In urban and peri-urban environments, the presence of synanthropic rodents - potential reservoirs of the pathogen - further increases the risk of infection spread. Domestic animals, such as dogs and cats, due to their close proximity to humans, may become infected through contact with these rodents, acting as potential vectors for zoonotic transmission (HANSEN; FRANKLIN, 2019; CARRIQUIRIBORDE et al., 2020; ECKE et al., 2022; CHARLES RIVER, 2024).

The lack of systematically organized data regarding the biology of the agent, its transmission pathways, clinical manifestations in different species, and effective therapeutic approaches complicates the early recognition of the disease and the implementation of appropriate containment measures. In this regard, the development of active surveillance strategies and sensitive, specific diagnostic

methods is necessary, particularly in biomedical research environments and areas with elevated zoonotic risk.

In light of this scenario, the present study aims to compile and systematize the main information available in the scientific literature regarding *C. piliforme* and Tyzzer's disease. Topics to be addressed include the agent's biology, host reservoirs, clinical manifestations, diagnostic methods, zoonotic potential, and therapeutic approaches. This compilation seeks to provide technical and scientific support for the early identification of suspected cases and the adoption of effective management, prevention, and control measures, fostering the integration of human and animal health disciplines.

METHODOLOGY

This study is a narrative literature review focused on the biology, pathogenicity, diagnostic strategies, and zoonotic relevance of Clostridium piliforme. The bibliographic search was conducted between January and March 2025 using the PubMed, Scopus, Web of Science, and ScienceDirect databases. Controlled descriptors (MeSH terms) and free-text keywords were applied, including: “Clostridium piliforme”, “Tyzzer’s disease”, “zoonosis”, “laboratory animals”, “intracellular pathogen”, and “pathogenic bacteria”, combined using Boolean operators (AND, OR) to maximize retrieval sensitivity.

Original research articles, case reports, systematic and narrative reviews, and technical reports from reputable scientific agencies were considered. Although the search period covered publications from 1990 to 2024, preference was given to studies published between 2020 and 2025 due to recent advances in diagnostic methodologies. Following the criteria proposed by Silva *et al.* (2020) and Santos *et al.* (2023), earlier references were only included if published in high-impact journals or recognized as seminal contributions to the field.

Study selection occurred in two stages: initial screening based on titles and abstracts, followed by full-text reading of eligible studies. Relevant findings were grouped into thematic categories using the conceptual matrix method described by Santos *et al.* (2020) and de Jesus *et al.* (2021). The resulting classification comprised four key domains: (1) Agent biology; (2) Affected species and clinical manifestations; (3) Laboratory diagnosis; and (4) Zoonotic risk and biosafety.

All extracted data were systematized in Microsoft Excel® spreadsheets to ensure traceability, consistency, and cross-checking across sources. Reference management and citation insertion were performed using Mendeley® software (version 1.19.8), integrated with Microsoft Word®. Citations followed the ABNT referencing style. This methodological approach adheres to international recommendations for structured narrative reviews, as outlined by Ferrari (2015) and Baethge *et al*. (2019), ensuring analytical depth, transparency, and coherence in the synthesis of existing knowledge.

DEVELOPMENT – THEMATIC REVIEW

AGENT BIOLOGY

C. piliforme is an obligate intracellular, spore-forming, and Gram-negative bacillus (FRANKLIN *et al*., 1994; FRISK, 2012; HANSEN; NIELSEN, 2015; MEREDITH; RAYMENT, 2000; ÜLKER *et al.*, 2024), with a morphology that can vary significantly, ranging from long and thin bacilli to flattened and short forms (CLIFFORD; PRITCHETT-CORNING, 2012). It typically measures 0.5 x 8–10 µm in size, though its length can extend up to 40 µm (FRISK, 2012). Additionally, it is

considered a filamentous and flagellated microorganism (FRANKLIN *et al*., 1994; MEREDITH; RAYMENT, 2000; ÜLKER *et al.*, 2024).

The spores can survive for extended periods in the environment (MEREDITH; RAYMENT, 2000; AYALA *et al*., 2010; FRISK, 2012), and are inactivated when exposed to heat at 80°C for 30 minutes (AYALA *et al*., 2010). Regarding chemical agents, the spores are sensitive to formaldehyde, iodophors, peracetic acid, and sodium hypochlorite but resistant to ethanol, phenolic detergents, and quaternary ammonium compounds (AYALA *et al*., 2010; NOWLAND *et al*., 2015). Outside the host cell, the vegetative form of the agent is highly unstable and quickly loses its virulence (AYALA *et al*., 2010).

Because it is an obligate intracellular microorganism, *C. piliforme* does not grow on routinely used culture media. However, it grows well in embryonated chicken eggs inoculated in the yolk sac, as well as in primary cultures of hepatocytes from chicken or mouse embryos (AYALA *et al*., 2010; CLIFFORD; PRITCHETT-CORNING, 2012; TAO *et al*., 2024), in addition to intestinal cell lines (CLIFFORD; PRITCHETT-CORNING, 2012).

It is considered an opportunistic microorganism, multiplying within the host and causing disease when immunity is compromised, such as in situations of stress, poor management, or inadequate nutrition (AYALA *et al*., 2010; STANNARD *et al.*, 2017).

TYZZER'S DISEASE

The disease was first described in 1917 by Ernest Tyzzer and is characterized by nonspecific symptoms, primarily gastrointestinal, including diarrhea, anorexia, weight loss, abdominal tension, piloerection, and lordosis. These manifestations vary among species and even among individuals of the same species (TYZZER, 1917; AYALA *et al*., 2010; STANNARD *et al.*, 2017; ÜLKER *et al.*, 2024). The presence of the microorganism may occur without the appearance of symptoms in immunocompetent animals, facilitating its dissemination through fecal shedding (FRANKLIN *et al*., 1994).

Initially named *Bacillus piliformis* (TYZZER, 1917; DELONG, 2012), the causative agent of the disease was reclassified in 1993 as *Clostridium piliforme* due to its closer phylogenetic similarity to the *Clostridium* genus (DUNCAN *et al*., 1993; FRANKLIN *et al*., 1994; PRITT *et al*., 2010; DELONG, 2012; HANSEN; NIELSEN, 2015). Tyzzer's disease has been reported in numerous animal species, including rodents, and has also been observed in humans, where it is considered a zoonosis (TYZZER, 1917; DUNCAN *et al*., 1993; FRANKLIN *et al*., 1994; NIEPCERON; LICOIS, 2010; FRISK, 2012; STANNARD *et al*., 2017; ÜLKER *et al*., 2024).

Transmission occurs primarily through contact with the feces of carrier animals and ingestion of spores via the fecal-oral route (FRANKLIN *et al*., 1994; FRISK, 2012; STANNARD *et al*., 2017). Animal bedding contaminated with spores can remain infectious for up to twelve months (DUNCAN *et al*.,1993). Boot and Walvoort (1984) reported vertical transmission of the microorganism (BOOT; WALVOORT, 1984).

Upon ingestion, the microorganism penetrates the intestinal mucosa, which serves as the initial site of infection. It then enters the bloodstream, spreading to other organs such as the liver and heart, depending on the host's immune system (FRANKLIN *et al*., 1994; ÜLKER *et al.*, 2024). Frisk (2012) noted that in an experimental infection study with hamsters, the first site of microbial proliferation was the cecum (FRISK, 2012). Enterocolitis, hepatitis, and myocarditis may occur, with hepatic lesions being the most frequent in the majority of species (ÜLKER *et al.*,

2024). The pathogenicity of the microorganism has been linked to an exotoxin, which does not appear to be produced by all strains (CLIFFORD; PRITCHETT-CORNING, 2012).

Several factors are associated with an increased predisposition to disease manifestation in carrier animals, particularly those involved in research activities (BACKER; PERKINS, 2015). These factors include corticosteroid treatments (BACKER; PERKINS, 2015; NOWLAND *et al*., 2015), high-protein diets, exposure to carbon tetrachloride, and intraperitoneal passage of ascitic tumors (BACKER; PERKINS, 2015). Inadequate husbandry conditions, such as overcrowding, poor hygiene, internal and external parasitism, and high temperature and humidity levels - factors commonly inducing stress in animals - can promote the transmission and development of the disease (BROWN; DONNELLY, 2012; NOWLAND *et al*., 2015; STANNARD *et al*., 2017).

Diagnosis is most often made post-mortem due to the acute and rapid progression of the disease. Necropsy findings typically involve the liver, which appears enlarged with necrotic foci of gray, whitish, or yellowish coloration, as well as intestinal inflammation accompanied by gas production, distension, and hemorrhage (AYALA *et al*., 2010; STANNARD *et al*., 2017; UZAL *et al*., 2018). Histopathological analysis of affected tissues may reveal the presence of the microorganism (CLIFFORD; PRITCHETT-CORNING, 2012; STANNARD *et al*., 2017).

ANIMAL INFECTION

Rodents

Clostridium piliforme and Tyzzer's disease hold significant importance, particularly in the context of research involving rodents, such as rats and mice. These animals are widely used as models in biomedical studies due to their genetic similarity to humans and their well-characterized physiology (DANESHIAN *et al*., 2015; TAYLOR; ALVAREZ, 2019). The presence of *C. piliforme* in research colonies can compromise experimental outcomes, as infected animals may display altered immune responses, metabolic changes, or variable disease progression, which can affect the reliability and reproducibility of scientific data (VAN ANDEL *et al*., 2000; FAHEY; OLEKSZAK, 2015; CHARLES RIVER, 2024).

Beyond its impact on research, the zoonotic potential of *C. piliforme* raises public health concerns. Although human infections are considered rare, given the scarcity of reports in the literature, the possibility of disease transmission from rodents to humans, especially in cases of immunocompromised individuals, cannot be overlooked (ÜLKER *et al*., 2024). The shedding of the bacterium through feces increases the risk of environmental contamination and exposure, emphasizing the need for stringent biosecurity measures in facilities that house research animals (STANNARD *et al*., 2017).

In addition, the presence of synanthropic rodents - those that live in close association with human habitats - poses a significant challenge in many countries. These animals can act as reservoirs and vectors for various zoonotic diseases, including Tyzzer's disease. Their proximity to human dwellings and food sources facilitates the spread of *C. piliforme*, increasing the risk of outbreaks. Effective rodent control and public health initiatives are essential to mitigate this risk and prevent the dissemination of this potentially serious pathogen (ECKE *et al*., 2022).

- Rat (*Rattus norvegicus*)

In rats, symptoms are typically more frequently observed in young animals and include diarrhea, which may contain mucus and/or blood, anorexia, emaciation, and abdominal distension, potentially progressing to death (BROWN; DONNELLY, 2012; MCCREADY; BARBOZA, 2024). At necropsy, hepatic and cardiac lesions are commonly observed (MCCREADY; BARBOZA, 2024). Adult carrier animals are usually asymptomatic, and the onset of symptoms may occur under stressful conditions (BROWN; DONNELLY, 2012).

Zenner and Regnault (2000) published a retrospective study in which they assessed the microbiological status of animal models used in research in France. The authors evaluated 581 rats from different strains and observed a positivity rate of 10.8% for the agent (ZENNER; REGNAULT, 2000). Carriquiriborde *et al.* (2020) also assessed the sanitary status of rodents used in research in Argentina through a retrospective study involving 1,198 animals tested by serology. They observed a 12.52% positivity rate for the agent, similar to the findings reported by Zenner and Regnault in their study (ZENNER; REGNAULT, 2000; CARRIQUIRIBORDE *et al.*, 2020). Schoondermark-Van De Ven *et al*. (2006), in their study, in which they assessed the prevalence of various microorganisms of importance for laboratory rodent models between 2000 and 2003 in Western Europe, observed a prevalence of 65% through an immunofluorescence test, a value significantly higher than that observed by the previously mentioned authors (ZENNER; REGNAULT, 2000; SCHOONDERMARK-VAN DE VEN *et al*., 2006; CARRIQUIRIBORDE *et al*., 2020).

Feng *et al.* (2015) published a study in which they assessed the prevalence of *C. piliforme* in rats and mice between 2010 and 2013 in China. The authors observed an increase in the prevalence of the agent in rats over this period, from 2.49% in 2010 to 7.58% in 2013 (FENG *et al.,* 2015). In a similar study, Pan *et al.* (2017) evaluated the prevalence of pathogens in laboratory rodent models between 2013 and 2015, including monitoring of the agent. The authors observed a 4.8% prevalence of the agent in rats (PAN *et al.*, 2017). Both studies showed lower percentages compared to those observed in the studies by Zenner and Ragnault (2000), Schoondermark-Van De Ven *et al*. (2006), and Carriquiriborde *et al*. (2020).
Ayala *et al.* (2010) published a study in which they experimentally infected 242 rats from 11 different strains via intravenous inoculation and evaluated their resistance. They observed that the alterations found showed no significant variation, and all strains were affected (AYALA *et al.*, 2010). Due to the fact that this microorganism has been observed in these animals, as described in the following studies, periodic monitoring of animal health status every three months is recommended (MÄHLER *et al*., 2014; HANSEN *et al*., 2019). Animals carrying the microorganism should not be used in scientific research (CHARLES RIVER, 2024).

- Mice (*Mus musculus*)

In mice, the commonly observed symptoms include depression, diarrhea, dehydration, weight loss, piloerection, and a hunched posture (TYZZER, 1917; WHARY *et al*., 2015; STANNARD *et al*., 2017), but they may progress to death without showing symptoms (TYZZER, 1917; CLIFFORD; PRITCHETT-CORNING, 2012; STANNARD *et al*., 2017). It is described that susceptibility to the agent may vary according to the strain; C57BL/6 mice are less sensitive than DBA/2 mice (WHARY *et al*., 2015). Animals exposed to stressful conditions may develop the disease more easily (WHARY *et al*., 2015).

During necropsy, alterations are typically observed in the ileum, cecum, and colon, which appear slightly enlarged and reddened due to hemorrhage (CLIFFORD; PRITCHETT-CORNING, 2012; HANSEN; NIELSEN, 2015). Hepatic involvement may be noted, with necrotic foci (HANSEN; NIELSEN, 2015), which can also be present in the heart (TYZZER, 1917; CLIFFORD; PRITCHETT-CORNING, 2012). Tsuchitani *et al*. (1983) published a study in which they reported the occurrence of Tyzzer's disease in a colony of SPF mice, ddY strain, with high mortality and hepatic and cardiac lesions (TSUCHITANI *et al*., 1983).

Zenner and Regnault (2000) published a retrospective study in which they assessed the microbiological status of animal models used in research in France. The authors evaluated 3,663 mice from different strains and observed a positivity rate of 5.8% for the agent (ZENNER; REGNAULT, 2000). Schoondermark-Van De Ven *et al*. (2006) published a study in which they evaluated the prevalence of various microorganisms of importance for laboratory rodent models between 2000 and 2003 in Western Europe. To assess the agent, they conducted an immunofluorescence test, observing a prevalence of 4%, similar to the findings of Zenner and Regnault (ZENNER; REGNAULT, 2000; SCHOONDERMARK-VAN DE VEN *et al*., 2006). Carriquiriborde *et al.* (2020) also assessed the sanitary status of rodents used in research in Argentina through a retrospective study involving 1,774 animals tested by serology. They observed a 10.60% positivity rate for the agent, a higher percentage than that observed by Zenner and Regnault, and by Schoondermark-Van De Ven *et al*. in their studies (ZENNER; REGNAULT, 2000; SCHOONDERMARK-VAN DE VEN *et al*., 2006; CARRIQUIRIBORDE *et al.,* 2020).

Feng *et al.* (2015) published a study in which they assessed the prevalence of *C. piliforme* in rats and mice between 2010 and 2013 in China. The authors observed an increase in the prevalence of the agent in mice over this period, from 0.12% in 2010 to 2.10% in 2013 (FENG *et al.,* 2015). Similarly, Wei *et al.* (2021) published a study on the prevalence of the agent during the period between 2017 and 2019. The authors observed a prevalence of 7.1% during the analyzed period (WEI *et al.*, 2021), which was higher than the value observed by Feng *et al. (2015),* Zenner and Ragnault (2000), and Schoondermark-Van De Ven *et al*. (2006), but lower than that observed by Carriquiriborde *et al*. (2020).

Livingston *et al.* (1996) reported the occurrence of the disease in a colony of nude mice. The authors noted that the appearance of symptoms was rare, but when present, the animals showed rapid deterioration, culminating in death. Furthermore, only clinically affected animals exhibited lesions, which were identified during necropsy and histopathology, with hepatic and intestinal necrosis being observed (LIVINGSTON *et al.*, 1996). Ayala *et al.* (2010) published a study in which they experimentally infected 352 mice from 16 different strains via intravenous inoculation and evaluated their resistance. They observed that the alterations found showed no significant variation, and all strains were affected (AYALA *et al.,* 2010).

Damman *et al.* (2011) published a study in which they assessed animals purchased from pet stores, conducting immunofluorescence and ELISA tests to detect the presence of the agent. The authors found a prevalence rate of 10.7% in the animals analyzed (DAMMAN *et al.,* 2011). This finding is extremely important and reinforces the concern about the zoonotic potential of the agent, as mice are also kept as pets.

Due to the fact that this microorganism has been observed in these animals, as described in the following studies, annual periodic monitoring of animal health status is recommended (MÄHLER *et al*., 2014; HANSEN *et al*., 2019). Animals

carrying the microorganism should not be used in scientific research (CHARLES RIVER, 2024). As an example, the study conducted by Van Andel *et al*. (2000) demonstrated the interference in the expression of the pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-alpha) and interferon-gamma (IFN-gamma), which may compromise immunological studies (VAN ANDEL *et al*, 2000). The study conducted by Ayala *et al*. (2006) demonstrated that the microorganism also interferes in studies involving tumor response (AYALA *et al*., 2006).

- Hamster

Tyzzer's disease in hamsters is most commonly associated with weanling or immunosuppressed animals and can result in high mortality rates (FRISK, 2012; HANSEN; NIELSEN, 2015; MIEDEL; HANKENSON, 2015; DONNELLY, 2020). The symptoms observed, as in other species, are highly nonspecific, with the most frequent being ruffled fur, depression, dehydration, and diarrhea (FRISK, 2012; MIEDEL; HANKENSON, 2015; MIWA; MAYER, 2020). Some animals may die suddenly without showing any prior symptoms (MIWA; MAYER, 2020). Necropsy findings often reveal hepatic lesions (necrosis) along with enterocolitis, myocarditis, and lymphadenitis (MIEDEL; HANKENSON, 2015). Given that this microorganism has been observed in these animals, annual monitoring of the animals' health status is recommended (MÄHLER *et al*., 2014; HANSEN *et al*., 2019). Animals carrying microorganism should not be used in scientific research (CHARLES RIVER, 2024).

- Guinea pig (*Cavia porcellus*)

In this specie, the most commonly observed symptoms are diarrhea, lethargy, emaciation, and dehydration, which can progress to death (SHOMER *et al*., 2015; PIGNON; MAYER, 2020). The disease is more commonly associated with animals raised under improper management and stressful conditions, as well as young animals (O'ROUKE, 2004; DECUBELLIS; GRAHAM, 2013; MCCREADY; BARBOZA, 2024). The most frequently observed macroscopic findings include intestinal inflammation and hepatic necrotic foci, similar to those found in other affected species (O'ROUKE, 2004; DECUBELLIS; GRAHAM, 2013). Since this microorganism has been observed in these animals, annual periodic monitoring of animal health status is recommended (MÄHLER *et al*., 2014; HANSEN *et al*., 2019;). Animals carrying the microorganism should not be used in scientific research (CHARLES RIVER, 2024).

- Gerbil (*Meriones unguiculatus*)

According to Miwa and Mayer (2020), Tyzzer's disease is the most fatal infectious disease in this species, which is highly susceptible to *C. piliforme* (HANSEN; NIELSEN, 2015; DONNELLY, 2020; MIWA; MAYER, 2020). Gerbils can succumb to the disease without displaying symptoms or with only mild signs, including depression, diarrhea, dehydration, ruffled fur, and a hunched posture (STANNARD *et al*., 2017; MCCREADY; BARBOZA, 2024). Among the most common necropsy findings are hepatic necrotic foci, similar to those observed in other species (MIWA; MAYER, 2020). There are no recommendations regarding the frequency of monitoring the agent for this species due to the scarcity of reports in the literature about its occurrence (MÄHLER *et al*., 2014). However, just like other rodents, animals carrying the microorganism should not be used in scientific research (CHARLES RIVER, 2024).

Other animals

- Rabbit (*Oryctolagus cuniculus*)

In rabbits, the microorganism can lead to acute cases of diarrhea, anorexia, lethargy, dehydration, and depression, potentially progressing to death, especially in young rabbits, which are highly sensitive (MEREDITH; RAYMENT, 2000; DELONG, 2012; DECUBELLIS; GRAHAM, 2013; NOWLAND *et al*., 2015; STANNARD *et al*., 2017). In outbreaks of diarrhea in rabbit colonies, particularly affecting weanling animals, Tyzzer's disease should be considered as the main suspect (PRITT *et al*., 2010). In these cases, animals may die within 72 hours (NOWLAND *et al*., 2015). The mortality rate in such cases can reach around 90% (DELONG, 2012; NOWLAND *et al*., 2015). At necropsy, foci of hepatic necrosis are observed, which can also be found in the cecum, ileum, colon, and myocardium (MEREDITH; RAYMENT, 2000; DELONG, 2012; DECUBELLIS; GRAHAM, 2013; NOWLAND *et al*., 2015). Edema may also be observed in the mucosal and serosal layers of the cecum (NIEPCERON; LICOIS, 2010).

Waner *et al.* (2005) published a study reporting an outbreak of the disease in Israel, affecting young animals aged 12 to 13 weeks. The primary clinical signs included diarrhea and anorexia, which progressed to death. Necropsy findings revealed hepatic and intestinal alterations, along with the involvement of mesenteric lymph nodes. Histopathological analysis identified the agent within hepatocytes (WANER *et al.,* 2005). Artuković *et al*. (2010) reported an outbreak of the disease in Croatia that resulted in the death of 148 young rabbits, which presented severe gastrointestinal symptoms, including diarrhea, abdominal distension, anorexia, weight loss, and apathy. Histopathology revealed intestinal and hepatic lesions in the vast majority of the animals and, in only one case, a cardiac lesion. The presence of the agent was confirmed through the PCR technique (ARTUKOVIĆ et al., 2010).

As with rodents, due to the observation of this microorganism in these animals, as described in the following studies, periodic monitoring of their health status every three months is recommended (MÄHLER *et al*., 2014; HANSEN *et al*., 2019). Animals found to carry this microorganism should not be used in scientific research (CHARLES RIVER, 2024).

- Dog (*Canis lupus familiaris*) and cat (*Felis catus*)

Regarding dogs and cats, it is important to consider the potential transmission of *Clostridium piliforme* to these animals due to their hunting behavior and frequent contact with synanthropic rodents. These pets, by preying on or interacting with infected rodents, could become exposed to the bacterium, potentially developing clinical symptoms or serving as asymptomatic carriers. This risk underscores the importance of monitoring and controlling rodent populations not only for human health but also to safeguard the well-being of companion animals and prevent the broader spread of Tyzzer's disease. The symptoms, as in the other animals mentioned earlier, are also nonspecific, as observed in the following studies.

Young *et al*. (1995) reported the case of a puppy, approximately 9 weeks old, that showed lethargy, diarrhea, and vomiting, progressing to death. At necropsy, intestinal and cardiac lesions were observed; however, the liver appeared normal. Histopathology revealed foci of necrosis in several tissues, including the liver, and the agent was also observed (YOUNG *et al*., 1995). Headley *et al.* (2009) reported the case of an animal, two months old, presenting with bloody diarrhea, vomiting, and weight loss, progressing to death. The necropsy examination revealed intestinal and hepatic changes, which showed areas of necrosis in histopathology, with the agent observed (HEADLEY *et al.,* 2009).

Jacobson *et al.* (2022) published a study in which they reported co-infection in two puppies, eight weeks old. The animals exhibited symptoms such as cachexia, lateral recumbency, jaundice, and seizures; one progressed to death and the other was euthanized. Histopathological analysis revealed foci of hepatic necrosis, with the presence of the agent. *C. piliforme* was confirmed by PCR (JACOBSON *et al.*, 2022).

Bennett *et al*. (1977) reported Tyzzer's disease in cats infected with the feline leukemia virus (BENNETT *et al*., 1977). Ikegami *et al.* (1999) reported co-infection of the agent in three one-month-old cats, which exhibited symptoms such as depression, diarrhea, and emaciation, with one of them progressing to death without apparent symptoms. Necropsy revealed foci of necrosis in the intestines, and histopathology confirmed the presence of the agent, which was further confirmed by PCR (IKEGAMI *et al.,* 1999). Neto *et al.* (2015) also reported co-infection of the agent in a one-month-old cat showing diarrhea, dehydration, dyspnea, and purulent nasal discharge, which progressed to death within two weeks. The animal belonged to a litter of three, and all of them died with similar symptoms (NETO *et al.,* 2015).

Fingerhood *et al.* (2023) observed a 14.79% prevalence of Tyzzer's disease in orphaned kittens necropsied in 2021, aged 0-35 days. Following this, the authors conducted a retrospective study between 2000 and 2021, identifying 37 suspected cases in which the animals exhibited colitis, hepatitis, and/or myocarditis. In 19 of these cases, the agent was identified (FINGERHOOD *et al.,* 2023). Oliveira *et al*. (2023) reported an infection in a shelter cat that presented with intestinal, hepatic, cardiac, nervous, and cutaneous lesions. The authors were able to identify the agent in the cytoplasm of keratinocytes through PCR (OLIVEIRA *et al*., 2023).

Tyzzer's disease can also occur in other feline species, as observed in the study by Poonacha (1997), which reported an infection in a wild felid (*Felis capensis*) that was three weeks old and part of a litter of six animals. Three animals died at birth, and of the remaining ones, two died in the third week of life without showing symptoms. Necropsies were performed, revealing macroscopic changes limited to the gastrointestinal tract. Samples from different organs were collected for histopathological analysis, which showed foci of necrosis in the liver and intestines, with the agent present in the cytoplasm of hepatic, intestinal, and cardiac cells (POONACHA, 1997).

Veterinarians should be aware of the possibility of infection through potential contact with carrier rodents and be prepared to identify such suspicions, applying appropriate diagnostic methodologies and treatment. These professionals must also educate pet owners about the risks associated with rodent contact and promote the adoption of effective preventive measures.

- Horse (*Equus ferus caballus*)

In horses, the microorganism affects young animals, primarily during the first two months of life, who often do not show symptoms and rapidly progress to death (FOSGATE *et al*., 2002). When symptoms are observed, they are mostly nonspecific (FOSGATE *et al*., 2002; SWERCZEK, 2013; STANNARD *et al*., 2017). Stannard *et al*. (2017) mentioned that in foals, the microorganism can lead to cases of weakness, lethargy, anorexia, diarrhea, dehydration, jaundice, and tachycardia (STANNARD *et al*., 2017).

Borchers *et al.* (2006) published a study in which they evaluated clinical cases of seven foals under three months of age, with the diagnosis of six made through post-mortem examination and one, which did not progress to death, diagnosed through liver tissue biopsy. The observed symptoms included lethargy, recumbency,

fever, colic, and jaundice. The therapeutic protocol for the surviving animal involved the use of gentamicin, ampicillin, trimethoprim-sulfamethoxazole, in addition to other supportive medications (BORCHERS *et al.,* 2006).

Swerczek (2013) published a retrospective study in which he evaluated 148 cases of Tyzzer's disease in foals in the United States, demonstrating the rapid and often fatal progression of the disease in these animals. Among the observed symptoms were lethargy, fever, anorexia, and jaundice. As in other species, there is significant liver involvement (SWERCZEK, 2013). Garcia *et al.* (2022) also published a retrospective study in which they conducted a analysis of 25 cases of Tyzzer's disease in horses, with 24 of the cases occurring in animals under 45 days of age, between 1991 and 2015, in the United States. The authors observed that the animals presented symptoms such as diarrhea, fever, abdominal distension, depression, weakness, and in nine of the 25 animals, sudden death was observed without clinical manifestation. Most of the animals had hepatic lesions, but lesions were also observed in the heart and intestines, as mentioned in other species, and also in the lungs, spleen, lymph nodes, thymus, stomach, and kidneys (GARCIA *et al.,* 2022).

As with dogs and cats, and despite the lack of reports in the literature on transmission to humans through contact with carrier horses, the close interaction between these animals and humans makes frequent veterinary monitoring essential. All necessary precautions should be taken to prevent these animals from being exposed to the risk of contamination.

-Birds

The information available in the literature regarding reports involving birds is scarce, and the studies observed vary in relation to the affected animal species. Saunders *et al*. (1993) reported the first case of the disease in birds, a cockatiel (*Nymphicus hollandicus*) that was four days old and progressed to sudden death. Upon necropsy, hepatic changes were observed, which, in histopathology, revealed areas of necrosis, along with the observation of the microorganism. *C. piliforme* was confirmed by special staining and electron microscopy (SAUNDERS *et al*., 1993).

Raymond *et al.* (2001) reported the occurrence of the disease in a Rainbow Lorikeet (*Trichoglossus haematodus*), eight days old, found dead after experiencing difficulty in gaining weight since birth. Necropsy revealed changes in the liver, which, after histopathological analysis, showed numerous foci of necrosis, also observed in cardiac tissue. The microorganism was confirmed by PCR (RAYMOND *et al.,* 2001). Mete *et al.* (2011) reported the occurrence of Tyzzer's disease in a Taveta golden weaver (*Ploceus castaneiceps*) that was unable to fly or perch and exhibited neurological changes, leading to euthanasia. In histopathology, the most significant changes were observed in the brain, where the agent was also detected, as well as in the intestines. The microorganism was confirmed by PCR (METE *et al.,* 2011).

HUMAN INFECTION

Human infection is considered rare and is typically associated with individuals who have compromised immune systems (ÜLKER *et al.*, 2024). Smith *et al.* (1996) reported the case of an HIV-positive patient who developed painful verrucous papules on the chest, which appeared two weeks after being in an apartment infested with rats. A bacterial culture was performed, which returned negative, and through a biopsy of the lesions, intracellular microorganisms were observed, which, after PCR, were identified (SMITH *et al.,* 1996; PRITT *et al*., 2010; NOWLAND *et al*., 2015).

A study that evaluated the presence of antibodies in humans revealed that individuals who had closer contact with laboratory animal models, including professionals involved in the handling of laboratory animals, showed higher rates compared to those who had sporadic or no contact. This demonstrates the possibility of contamination by the agent originating from these animals and its zoonotic nature (TAO *et al*., 2024).

Based on this information, and given the nonspecific symptoms observed or even their absence associated with the presence of antibodies, it is evident that humans can be infected by *C. piliforme*. This raises the question of whether Tyzzer's disease in humans is truly rare or if it may be underdiagnosed. The lack of a thorough medical history, including the collection of information about possible contact with carrier animals or presence in areas with potential synanthropic rodent infestations, could aid in guiding diagnostic suspicion. The fact that animals spread the microorganism through feces and that it can persist in the environment as spores increases its potential for contamination, including through food and water. Healthcare professionals should be knowledgeable about the agent and the pathogenesis involved to ensure they have the necessary tools for a comprehensive approach to potential cases. This is especially important in high-risk areas due to the presence of possible reservoirs.

DIAGNOSIS

- Culture and isolation

In vitro diagnosis of the agent through culture and isolation using routine culture media is not possible due to the fact that the microorganism is intracellular and does not grow in commonly used media (NIEPCERON; LICOIS, 2010; DECUBELLIS; GRAHAM, 2013; NOWLAND *et al*., 2015).

- Histopathology

Histopathology is an important diagnostic tool in Tyzzer’s disease and can be used as a confirmation method (NOWLAND *et al*., 2015). Typically performed post-mortem, during necropsy, samples are collected from organs such as the ileum, cecum, colon, liver, and heart, which are potential targets of the agent after host infection. These samples are fixed in 10% formalin solution and embedded in paraffin blocks, followed by 4µm sectioning (PRITT *et al*., 2010). Stains such as Hematoxylin and Eosin, Warthin-Starry silver (MEREDITH; RAYMENT, 2000; PRITT *et al*., 2010; STANNARD *et al*., 2017), and Giemsa (AYALA *et al*., 2010) can be used for subsequent observation under a microscope (PRITT *et al*., 2010). Spores can be observed using Safranin stain (AYALA *et al*., 2010).

The microorganisms can be observed randomly distributed throughout the cytoplasm of host cells, most commonly found at the edges of necrotic areas (AYALA *et al*., 2010; POONACHA, 1997). It is also common to observe inflammatory cell infiltrates containing neutrophils and macrophages (POONACHA, 1997; AYALA *et al*., 2010). Hamsters and rabbits tend to present more severe enteric lesions, with the agent observed in the cells of the intestinal epithelium and mucosal muscle cells. In other species, hepatic necrosis is the most frequent finding (AYALA et al., 2010).

- Serology

Serological tests are routinely used in the monitoring of laboratory animal colonies (AYALA *et al*., 2010; NIEPCERON; LICOIS, 2010; PRITT *et al*., 2010; CLIFFORD; PRITCHETT-CORNING, 2012; NOWLAND *et al*., 2015; HANSEN *et al*., 2019), and other species (SWERCZEK, 2013), although they are considered less

sensitive and specific, with a risk of false positives or negatives (CLIFFORD; PRITCHETT-CORNING, 2012; HANSEN *et al*., 2019). A positive result, with high antibody titers, is a strong indication of a true positive (CLIFFORD; PRITCHETT-CORNING, 2012); however, confirmatory tests are recommended (CLIFFORD; PRITCHETT-CORNING, 2012; NOWLAND *et al*., 2015). Fries (1980) evaluated antibody production in spontaneously infected mice, rats, rabbits, dogs and humans. The author found no difference between these species, even though the antigen was isolated from different hosts (mouse, rat or rabbit) (FRIES, 1980).

a) Immunofluorescence

Indirect immunofluorescence can be used as a tool for the detection of antibodies, thereby allowing the monitoring of *C. piliforme* in laboratory animal model colonies (MEREDITH; RAYMENT, 2000; AYALA *et al*., 2010; PRITT *et al*., 2010; NOWLAND *et al*., 2015). Pritt *et al*. (2010) used indirect immunofluorescence to search for antibodies in rabbit samples (PRITT *et al*., 2010).

b) Enzyme-linked immunosorbent assay (ELISA)

The indirect ELISA for antibody detection has also been used as a tool for monitoring in laboratory animal model colonies (MEREDITH; RAYMENT, 2000). Franklin *et al.* (1994) mentioned that this methodology can be used to detect subclinical infections, which is important to prevent the spread of the agent (FRANKLIN *et al.,* 1994). It is important to note that positive results should not be considered definitive due to the possibility of cross-reactions with other microorganisms, and additional tests are needed for the definitive diagnosis of the agent (PRITT *et al*., 2010).

In the study conducted by Motzel *et al*. (1991), ELISA was used to detect antibodies in rats and mice experimentally infected with the agent. They were able to observe the prevalence of antibodies 11 weeks after inoculation of the microorganism (MOTZEL *et al*., 1991). Boivin *et al*. (1994) also used the technique to identify antibodies in rodents, rats and gerbils, also experimentally infected (BOIVIN *et al*., 1994). Similarly to the previously mentioned authors, Waggie *et al*. (1987) used the same methodology for antibody detection, but in rabbits (WAGGIE *et al*., 1987).

- Molecular biology

Polymerase chain reaction (PCR) can be used for the identification of the agent (BROOKS *et al*., 2006; PRITT *et al*., 2010; CLIFFORD; PRITCHETT-CORNING, 2012; SWERCZEK, 2013; NOWLAND *et al*., 2015; HANSEN *et al*., 2019) in fecal samples and tissues containing lesions (FELDMAN *et al*., 2006; FRISK, 2012; STANNARD *et al*., 2017). DeLong (2012) mentioned that one obstacle to identifying the agent by PCR is the nucleotide sequence diversity in the 16S rRNA genes found in samples from different hosts (DELONG, 2012). Animals aged 4-6 weeks are the most suitable for monitoring the agent in colonies, as older animals have greater immune competence, and younger animals are protected by maternal antibodies (CLIFFORD; PRITCHETT-CORNING, 2012).

Furukawa *et al* (2002) published a study in which they used polymerase chain reaction (PCR) to identify the agent in fecal samples. The authors followed the protocol proposed by Goto and Itoh (1994) but obtained low sensitivity in detecting the agent (GOTO; ITOH, 1994; FURUKAWA *et al.,* 2002). Considering that some components present in feces may reduce this sensitivity, Furukawa *et al* proposed adjustments in time and temperature, as shown in Table 1.

Table 1 - Primers and PCR programs used in protocols for the detection and identification of *C. piliforme.*

Target gene	Primer	Sequence (5´-3´)	PCR Program	Expect PCR product	Reference
16S rRNA	RJ-1	GTGCTAGGTGTTGGGAAG	25x 92ºC/2min, 50ºC/2min, and 70ºC/2min.	196bp	Furukawa *et al*., 2002; Ikegami *et al*., 1999.
	RJ-2C	TACTTTACGTAGCCTGTCAA			
16S rRNA	RJ-1	GTGCTAGGTGTTGGGAAG	35x 94ºC/1min, 56ºC/1.5min, and 72ºC/1min.	196bp	Furukawa *et al*., 2002.
	RJ-2C	TACTTTACGTAGCCTGTCAA			
16S rRNA	OP1	CCTAACACATGCAAGTC	First stage (OP1 and OP2 primers): 1x 94ºC/1min; 25x 94ºC/30sec, 50ºC/30sec, and 72ºC/1min. Second stage (PiliF and PiliR primers): same conditions, but hybridization temperature 55ºC and 30 cycles.	1140bp	Niepceron; Licois, 2010.
	OP2	GGCATGATGATTTGACG			
	PiliF	TGGGATAACATCGAGAAATC		850bp	
	PiliR	TACGTAGYCTGTCAATGGT			
16S rRNA	1st PCR	ACTAGAGTACAGGAGAGGTA	Fisrt stage (1st PCR primers): 25x 94ºC/1min, 55ºC/1min, and 72ºC/1min. Second stage (2nd PCR primers): same conditions.	No information	Goto *et al*., 2007.
		CCTAAACATAAGGGGCATGA			
	2nd PCR	GTGCTAGGTGTTGGG*AAG			
		*TACTTTACGTAGCCTGTCAA			
16S rRNA	F	ACCATTGACAGCCTACGTAA	94ºC/5min; 40x 98ºC/10sec, 55ºC/30sec, and 72ºC/1min; 72ºC/5min.	270bp	Aboellail *et al*., 2012.
	R	GTCTCGCTTCACTTTGTTGTA			
16S rRNA	F	ACCATTGACAGCCTACGTAA	94ºC/5min; 40x 98ºC/10sec, 55ºC/35sec, and 72ºC/1min; 72ºC/5min.	270bp	Ülker *et al.*, 2024.
	R	GTCTCGCTTCACTTTGTTGTA			
16S rRNA	F	AGCAAACGCAATAAGCACTCCA	95ºC/10min; 35x 95ºC/35sec, 53ºC/35sec, and 72ºC/35sec; 72ºC/5min.	162bp	Garcia *et al*., 2022.
	R	TACTTTACGTAGCCTGTCAATGGTATGT			
16S rRNA	16SF969	TACCATTGACAGRCTACGTAAAGT [R=A or G]	95ºC/14min and 15sec; 37x 95ºC/45sec, 55ºC/1min, and 72ºC/1min; 72ºC/5min	639bp	Feldman *et al*., 2006.
	16SR1608	TAACCRTTGTGTTTGTTATTCAATTTT			

*Bolds show antiexonuclease oligonucleotides

Goto *et al.* (2007) published a study in which they experimentally infected mice with the microorganism and evaluated its detection by PCR. The authors were able to detect the agent in tissue samples from the heart, cecum, and liver after the 8th day of inoculation. In this study, the authors did not identify the agent in fecal samples, and the protocol used is also shown in Table 1 (GOTO *et al.,* 2007). Ülker *et al.* (2024) published a study in which they used PCR to detect the agent in fecal samples from rats and mice. They obtained 90.9% positivity in rat samples and 83% in mouse samples (ÜLKER *et al.,* 2024). Table 1 presents the primers and PCR programs utilized in protocols described in the literature.

In addition to PCR, loop-mediated isothermal amplification (LAMP) is another methodology, also based on molecular biology, with high specificity, that can also be used for the confirmation of the agent. Tao *et al.* (2024) published a study in which they used this methodology for the detection of *C. piliforme* (TAO *et al*., 2024).

TREATMENT

The treatment of individuals affected by the agent is complicated by the intracellular presence of the microorganism, which reduces the efficacy of antimicrobial agents (DELONG, 2012). In rabbits, literature reports have highlighted the use of oxytetracycline and tetracycline with clinical improvement (MEREDITH; RAYMENT, 2000; DELONG, 2012); in gerbils, tetracycline and chloramphenicol (MCCREADY; BARBOZA, 2024); and in horses, gentamicin, ampicillin, and trimethoprim-sulfamethoxazole (BORCHERS *et al*., 2006).

FINAL CONSIDERATIONS

The discussions presented in this article underscore the significance of continuous monitoring and preventive strategies for controlling *Clostridium piliforme*, the causative agent of Tyzzer's disease. The complexity associated with the identification, treatment, and prevention of this pathogen arises from its obligate intracellular nature, the resilience of its spores in the environment, and the broad spectrum of species it affects. These factors highlight the necessity for stringent biosafety protocols, particularly in animal breeding facilities dedicated to scientific research, where the presence of this agent can compromise the quality of studies and pose risks to the health of professionals involved.

In addition to its impact on animal health, the zoonotic potential of *C. piliforme* also demands attention, as human infections have been reported, and antibodies against the agent have been detected in individuals exposed to potential reservoirs. This emphasizes the importance of proper hygiene practices and the protection of those handling these animals, whether in professional settings or pet care, thereby reducing the risk of interspecies transmission. Awareness and knowledge of these risks, particularly among healthcare professionals, are crucial for safeguarding public health.

Although diagnostic methods have advanced, they still present limitations in the early detection of infection. Techniques such as PCR, histopathology, and serological tests are valuable tools, but there is a need to develop faster, more sensitive, and economically feasible approaches for identifying the microrganism, especially in asymptomatic animals that serve as silent reservoirs of the agent.

Lastly, the importance of ongoing research into the biology of *C. piliforme* and its interactions with hosts is emphasized. This research aims to enhance control, diagnostic, and treatment strategies, as well as to ensure the continuous training of professionals involved in both human and animal health. Only through a thorough

understanding of the mechanisms of pathogenicity and transmission can effective preventive measures be implemented, safeguarding the health of both animals and humans.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

FUNDING

This work was internally funded.

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