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Research Article

Presence of *Clostridium piliforme* in Rats from Laboratory Animal Facilities in the Aegean Region of Türkiye

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ABSTRACT

The health status of laboratory animals is essential for both research reliability and animal welfare. *Clostridium piliforme*, the causative agent of Tyzzer's disease, is an obligate intracellular and spore-forming bacterium. While it may cause high mortality in immunocompromised animals, the infection often remains subclinical and can persist in colonies for long periods. Such latent infections may compromise experimental outcomes, making regular health monitoring highly important. This study aimed to investigate the presence and prevalence of *C. piliforme* in rats from licensed laboratory animal facilities in the Aegean Region of Türkiye. A total of 80 faecal samples, 10 from each of eight facilities, were collected. DNA was extracted using a commercial kit and analysed by conventional PCR with species-specific primers. Of the 80 samples, four (5.0%) were positive, all originating from a single facility (40.0%, 4/10). The Wilson score method estimated a 95% confidence interval of 1.6–12.3, and Fisher's Exact Test indicated a statistically significant difference among facilities (P<0.001). These findings represent the first report of *C. piliforme* in rats from the Aegean Region and the second in Türkiye. The prevalence was lower compared to national and international reports, likely due to methodological differences and facility conditions. The study emphasises the potential risk of subclinical *C. piliforme* infections for research validity and highlights the necessity of including this pathogen in FELASA recommendations routine health monitoring programs.

Keywords: *Clostridium piliforme, conventional PCR, laboratory animals, rat, Tyzzer's disease.*

Türkiye'nin Ege Bölgesindeki Deney Hayvanı Tesislerinde Bulunan Ratlarda *Clostridium piliforme* Varlığı

ÖZET

Laboratuvar hayvanlarının sağlık durumu, araştırmaların güvenilirliği ve hayvan refahı açısından büyük önem taşır. Tyzzer hastalığının etkeni *Clostridium piliforme*, zorunlu hücre içi yaşayan ve spor oluşturan bir bakteridir. İmmün sistemi baskılanmış hayvanlarda yüksek mortaliteye yol açabilmesine rağmen, enfeksiyon genellikle subklinik seyredip kolonilerde uzun süre persiste olabilir. Bu tür latent enfeksiyonlar deneysel sonuçları olumsuz etkileyebileceğinden, düzenli sağlık izlemi büyük önem taşır. Bu çalışma, Türkiye'nin Ege Bölgesi'ndeki ruhsatlı laboratuvar hayvanı tesislerinde *C. piliforme*'nin varlığını ve yaygınlığını araştırmıştır. Sekiz tesisten her birinden 10'ar adet olmak üzere toplam 80 dışkı örneği toplanmış, DNA ticari bir kit kullanılarak ekstrakte edilmiş ve tür-spesifik primerlerle konvansiyonel PCR yöntemiyle analiz edilmiştir. Seksen örnekten dördü (%5,0) pozitif bulunmuş olup tamamı tek bir tesise aittir (%40,0; 4/10). Wilson skor yöntemiyle %95 güven aralığı 1,6–12,3 olarak hesaplanmış, Fisher'ın Kesin Testi ise tesisler arasında istatistiksel olarak anlamlı fark bulunduğunu göstermiştir (P<0,001). Bu bulgular, *C. piliforme*'nin Ege Bölgesi sıçanlarında ilk, Türkiye genelinde ise ikinci kez rapor edildiğini göstermekte; daha düşük prevalansın yöntemsel farklar ve tesis koşullarından kaynaklandığı düşünülmektedir. Çalışma, subklinik *C. piliforme* enfeksiyonlarının araştırma geçerliliği açısından oluşturduğu riski vurgulamakta ve bu patojenin FELASA tarafından önerilen rutin sağlık izleme programlarına dâhil edilmesinin gerekliliğini ortaya koymaktadır.

Anahtar kelimeler: *Clostridium piliforme, konvansiyonel PCR, laboratuvar hayvanları, rat, Tyzzer hastalığı.*

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Introduction

The health status of laboratory animals used in experimental studies is a crucial factor that directly influences both the reliability of research outcomes and the welfare of the animals. In addition to environmental and genetic factors, the presence of infectious agents can introduce variability in experimental results. Therefore, maintaining microbiological quality in breeding and research facilities has become essential. Particularly in scientific research, the use of pathogen-free animals is widely recognised as a prerequisite for achieving reproducible outcomes (Mähler et al., 2014; Matos-Rodrigues et al., 2020).

Only a subset of infections observed in rodent colonies manifests with obvious clinical signs. Nevertheless, even subclinical infections can alter immune responses, physiology, and behaviour, thereby potentially influencing experimental findings. This situation poses a greater risk for immunosuppressed animals compared to immunocompetent ones. For this reason, international organisations recommend that every institution establish a health monitoring program integrated into its quality assurance system and ensure the standardisation of such programs (Braken et al., 2017; Matos-Rodrigues et al., 2020).

The most comprehensive recommendations for health monitoring in laboratory animals have been issued by the Federation of European Laboratory Animal Science Associations (FELASA). According to these guidelines, *Clostridium piliforme* is included among the infectious agents that should be routinely screened in laboratory mice at three-month intervals (Nicklas et al., 2002; Mähler et al., 2014).

C.piliforme is an obligate intracellular, spore-forming, filamentous, and anaerobic bacterium that, although classified as Gram-positive, stains Gram-negative due to structural differences in its cell wall. It is notoriously difficult to culture in vitro; therefore, molecular diagnostic methods are usually preferred (Percy and Barthold, 2007; Clifford, 2008). As the causative agent of Tyzzer's disease in laboratory animals, *C. piliforme* can lead to high-mortality outbreaks in rats, characterised by lethargy, anorexia, diarrhoea, and sudden death (Besselsen, 2017). Nevertheless, many infections remain subclinical. Even in the absence of overt clinical signs, the pathogen can alter immune responses, metabolism, and behaviour, thereby compromising the reliability of experimental outcomes. In immunosuppressed rats, the disease tends to present more severely, often resulting in increased mortality (Jacoby and Lindsey, 1998; Mähler et al., 2014).

The aim of this study was to investigate the presence and determine the prevalence of *C. piliforme* in rats from laboratory animal facilities in the Aegean Region using molecular methods.

Materials and Methods

Faecal samples were collected from a total of 80 rats, randomly selected from individually housed cages across eight licensed laboratory animal facilities in the Aegean Region. The total number of rats present in these facilities was 903. From each facility, faecal material was obtained from 10 randomly chosen cages to ensure representative sampling at the colony level. All animals sampled were Wistar rats aged between 15 and 22 weeks. Discussions with facility managers confirmed that animals were singly housed, clinically healthy, and not immunosuppressed, ensuring that each faecal sample originated from an individual, immunocompetent rat. None of the eight facilities were classified as SPF units; instead, they operated as standard breeding and holding facilities with basic biosecurity practices rather than high-level barrier systems. This study was approved by the Bornova Veterinary Control Institute Directorate, Local Ethical Committee (Decision date and no.: 09.09.2025/6).

Since *C. piliforme* is an obligate intracellular bacterium that cannot be reliably cultured in vitro, bacterial culture was not performed, and PCR was used as the sole diagnostic method. The collected faecal samples were transported to the Bornova Veterinary Control Institute, Bacteriology Laboratory, under cold chain conditions. DNA extraction was performed using a commercial kit (Roche, Germany) with the manufacturer's instructions.

Specific primers for *C. piliforme*, Forward (5'-ACCATTGACAGCCTACGTAA-3') and Reverse (5'-GTCTCGCTTCACTTTGTTGTA-3'), were used to screen the extracted DNA samples. PCR amplification was carried out under the following conditions: an initial denaturation at 94°C for 3 minutes, followed by 40 cycles of 94°C for 15 seconds (denaturation), 55°C for 30 seconds (annealing), and 72°C for 15 seconds (extension), with a final extension at 72°C for 3 minutes. Reactions were performed in a Techne TC-412 thermal cycler (Keison Products, United Kingdom) using Xpert Fast Hotstart Mastermix (2×, GRiSP, Portugal) as the reaction mixture. PCR amplicons were analysed on a 2% agarose gel stained with Xpert Green DNA Stain Direct (GRiSP, Portugal) and visualised with a Vilber e-box gel documentation system (Vilber, France). Amplicons of 270 bp were considered positive for the *C. piliforme* gene (Aboellail et al., 2013).

Results

In this study, a total of 80 faecal samples obtained from rats in eight licensed laboratory animal facilities in the Aegean Region were examined by PCR for the presence of *C. piliforme*. Of the 80 samples analysed, 4 (5.0%) were positive, all originating from a single facility (40.0%, 4/10). It should be noted that although the total rat population across the eight facilities was 903, only 80 individuals were sampled. Therefore, the 5.0% value presented here represents the *sample prevalence*, not the true colony-level prevalence. True prevalence cannot be directly inferred without proportional or full-population testing. Statistical analysis revealed that the sample prevalence had a 95% confidence interval of 1.6–12.3 (Wilson score method; Newcombe, 1998; Brown et al., 2001). Fisher's Exact Test indicated a statistically significant difference in positivity among facilities ($P < 0.001$) (Fisher, 1922).

The *C. piliforme* specific PCR amplification products were

observed as 270 bp bands on agarose gel electrophoresis, as presented in Figure 1.

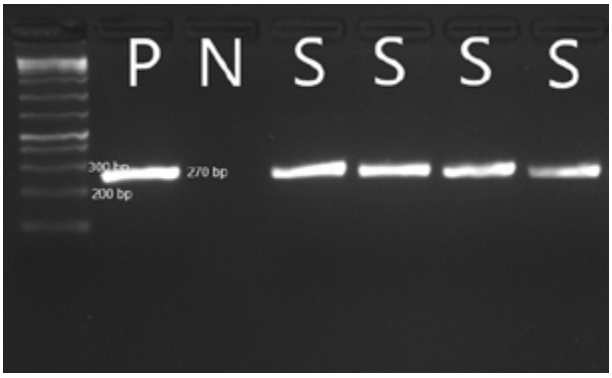

Figure 1: Agarose gel electrophoresis of PCR amplification products specific to *Clostridium piliforme*. P: Positive control; N: Negative control; S: Sample.

Discussion

Clostridium piliforme was first identified as the causative agent of Tyzzer's disease and is known as an obligate intracellular, spore-forming bacterium. It causes enteric, hepatic, and cardiac lesions, particularly in young animals, and can be fatal in species such as mice, rats, and hamsters. Stress factors including overcrowding, transportation, and poor sanitation play an important role in the occurrence of the disease. However, the infection often remains subclinical and spreads through asymptomatic carriers, which allows the pathogen to persist unnoticed within colonies. Furthermore, cases reported in immunocompromised humans have raised concerns about its potential zoonotic significance (Ganaway et al., 1971; Merck Veterinary Manual, 2023). The reliability of experimental studies conducted on laboratory animals depends largely on the health status and pathogen-free condition of the animals used. The presence of latent infections such as *C. piliforme* can directly compromise research outcomes, making a disease-free status in laboratory colonies essential. Therefore, regular health monitoring, certification of pathogen-free colonies, and the implementation of quality standards recommended by the FELASA are considered mandatory both for research reliability and animal welfare. In this context, FELASA recommends that *C. piliforme* should be included in the three-month health monitoring programs (Newcombe, 1998; Mähler et al., 2014).

In our study, 4 (5.0%) of the total 80 faecal samples obtained from rats in the Aegean Region were found positive for *C. piliforme*. Positivity was detected in only one of the eight facilities examined, where 4 out of 10 samples (40.0%) were found to be positive. According to the literature we reviewed, this study represents the first investigation conducted in the Aegean Region and the second study in Türkiye focusing on this disease in laboratory animals. It should be noted that the total rat population across the eight facilities was 903; however, only 80 individuals were sampled. Therefore, the 5.0% value presented here reflects *sample prevalence*. True colony-level prevalence cannot be estimated without proportional or full-population sampling. According to FELASA health monitoring recommendations, sample-based surveillance in non-SPF rodent colonies is an acceptable approach (Mahlör et al., 2014). Moreover, recent statistical design studies indicate that even low prevalence infections (~5 %) can be detected with relatively small sample sizes when designed appropriately (Sorzano et al., 2024).

In a LAMP-LFD based study conducted in China, *C. piliforme* was detected at rates of 5.08% in clean-grade animals and 9.96% in Specific Pathogen Free animals. The same study also reported that the pathogen was found at higher rates in laboratory animals used in universities and faculties compared to those maintained in companies and research institutes, which was attributed to differences in housing conditions and hygiene practices. In a study from Iran, using ELISA on 82 rats, the positivity rate for *C. piliforme* was reported to be below 10% (Fallahi and Mansouri, 2017). The positivity rates obtained in our study differ from reported in these findings. One of the most important reasons for this discrepancy may be the differences in diagnostic methods. While ELISA or LAMP-LFD detect antigen/antibody or genetic material with limited sensitivity, PCR-based approaches provide higher sensitivity and specificity, allowing the detection of even low-level infections. In addition, variations in colony management practices, animal age and immune status, housing density, and hygiene conditions are also likely to influence the prevalence rates observed in different studies.

Studies on infectious diseases in laboratory animals in Türkiye are quite limited. Therefore, the present findings contribute to addressing this gap in our country and allow comparisons with international data. In particular, the investigation of agents such as *C. piliforme*, which may follow a subclinical course and be overlooked during routine health monitoring, is of great importance for ensuring the pathogen-free status of laboratory animal colonies (İpek et al., 2023; İçil and Erbaş, 2024).

The only study conducted in Türkiye on *C. piliforme* was carried out by Ülker et al. (2024), who reported the presence of the pathogen in 40 (90.9%) of 44 faecal samples obtained from rats of different ages. Among the positive animals, 25 (96.1%) were female and 15 (83.3%) were male. An important aspect of their study is that all samples originated from a single facility, and the authors used the same species-specific PCR approach employed in the present study. In contrast, the substantially lower positivity rate observed in our study may be attributed to both the larger sample size examined and, more importantly, the inclusion of animals from eight different facilities with varying colony conditions. These differences suggest that the prevalence of *C. piliforme* can vary considerably depending on colony structure, biosecurity practices, population size, and sampling strategy.

The fact that all positive samples originated from a single facility suggests that colony-specific factors may have played a key role in pathogen circulation. Differences in hygiene routines, cage density, feed or water sources, ventilation conditions, and overall biosecurity practices can influence intra-colony transmission of *C. piliforme*.

It is also possible that historical exposure, animal stress levels, or previous introduction of infected animals contributed to the clustering of positivity in this facility. These factors may explain why no positivity was detected in the remaining seven facilities.

The lower positivity rate observed in the present study may be explained by several factors. First, faecal samples are known to have variable sensitivity for detecting *C. piliforme* by PCR, as spore shedding can be intermittent and may not be consistently present in all infected animals. Second, all sampled rats in this study were clinically healthy and immunocompetent, whereas *C. piliforme* infection is more frequently detected in immunosuppressed or stressed animals. This difference in host susceptibility may contribute to the lower detection rate. Third, the eight facilities included in this study were standard breeding and holding units with basic biosecurity practices, and differences in hygiene, husbandry and environmental conditions could reduce bacterial circulation and transmission. Taken together, these factors suggest that the low sample prevalence identified here likely reflects both the biological characteristics of the pathogen and the colony-specific conditions of the facilities studied.

Although all positive animals in the present study were clinically healthy, subclinical *C. piliforme* infection may still influence experimental outcomes. Previous reports indicate that this pathogen can alter immune responses, stress physiology, behaviour and metabolic parameters even in the absence of overt clinical signs (Ganaway, 1980; Livingston et al., 1997). Such hidden effects may confound the results of immunological, toxicological or behavioural studies, particularly in facilities where undetected infections persist. Therefore, routine molecular monitoring for *C. piliforme* is essential to minimise experimental variability and ensure the reliability of animal-based research (Baker, 1998).

Conclusion

This study provides the first data on the prevalence of *C. piliforme* in laboratory rats from the Aegean Region and represents the second investigation on this pathogen conducted in Türkiye. Our findings revealed a lower prevalence compared to previous national and international reports, which may be explained by differences in diagnostic methods, sample size, and the inclusion of animals from multiple facilities. These results emphasise that subclinical infections of *C. piliforme* can negatively affect both animal welfare and the validity of studies conducted using laboratory animals. Therefore, future research should focus on expanding surveillance programs and ensuring regular health monitoring in laboratory animal colonies, which is critical for maintaining the validity of studies performed with laboratory animals.

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Author contribution statement

ÇN, ÖFG, and Nİİ designed the research. ÇN performed the investigation, analysed the data, and prepared the original draft. All authors contributed to the methodology, validation, and review of the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

Aboellail, T.A., Naikare, H.K., & Mahapatra, D. (2013). A naturally occurring enterotyphlocolitis associated with dual infection by *Clostridium piliforme* and enteropathogenic attaching and effacing *Escherichia coli* in Syrian hamsters. *Journal of Veterinary Science and Medicine, 1*(1), 1–7. https://doi.org/10.13188/2325-4645.1000003

Baker, D.G. (1998). Natural pathogens of laboratory mice, rats, and rabbits and their effects on research. *Clinical Microbiology Reviews*, 11(2), 231–266. https://doi.org/10.1128/cmr.11.2.231

Besselsen, D.G. (2017). Infectious diseases of rats. In J.G. Fox, M.T. Davisson, F.W. Quimby, S.W. Barthold, C.E. Newcomer, & A.L. Smith (Eds.), Laboratory animal medicine (3rd ed., pp. 261–294). Academic Press.

Bracken, T.C., Cooper, C.A., Ali, Z., Truong, H., & Moore, J.M. (2017). Helicobacter infection significantly alters pregnancy success in laboratory mice. *Journal of the American Association for Laboratory Animal Science, 56*(3), 322–329.

Brown, L.D., Cai, T.T., & DasGupta, A. (2001). Interval estimation for a binomial proportion. *Statistical Science, 16*(2), 101–133. https://doi.org/10.1214/ss/1009213286

Clifford, C.B. (2008). Clostridium piliforme. In M.A. Suckow, S.H. Weisbroth, & C.L. Franklin (Eds.), The laboratory rat (pp. 411–424). Elsevier.

Fallahi, R., & Mansouri, M.A. (2017). Health monitoring of Razi Institute laboratory mice (NIH strain) to *Clostridium piliforme* in 1395. *Veterinary Research on Biological Products, 30*(4), 78–84.

Fisher, R.A. (1922). On the interpretation of χ^2 from contingency tables, and the calculation of P. *Journal of the Royal Statistical Society, 85*(1), 87–94. https://doi.org/10.2307/2340521

Ganaway, J.R., Allen, A.M., & Moore, T.D. (1971). Tyzzer's disease. *American Journal of Pathology, 64*(3), 717–730.

Ganaway, J.R. (1980). Tyzzer's disease: A review. *Journal of the American Veterinary Medical Association*, 177(9), 841–845.

İçil, N. İ., & Erbaş, G. (2024). The prevalence of *Helicobacter* spp. in licensed experimental animal facilities in Aegean Region, Turkey. *Laboratuvar Hayvan Bilimi Uygulamaları Dergisi, 4*(1), 33–43. https://doi.org/10.5281/zenodo.10894467

İpek, E., Epikmen, E.T., Nuhay, Ç., & Tunca, R. (2023). Intense exercise stress may trigger *Corynebacterium kutscheri* infection in Sprague-Dawley rats. *Veterinary Journal of Mehmet Akif Ersoy University, 8*(2), 136–144. https://doi.org/10.24880/maeuvfd.1249794

Jacoby, R.O., & Lindsey, J.R. (1998). Health care and control of disease. In G.J. Krinke (Ed.), The laboratory rat (pp. 385–400). Academic Press.

Livingston, R.S., Riley, L.K., Steffen, E.K., Besch-Williford, C., Hook, R.R., & Franklin, C.L. (1997).Cytokine responses in rats infected with Clostridium piliforme. *Laboratory Animal Science*, 47(1), 48–52.

Mähler, M., Berard, M., Feinstein, R., Gallagher, A., Illgen-Wilcke, B., Pritchett-Corning, K., & Raspa, M. (2014). FELASA recommendations for the health monitoring of mouse, rat, hamster, guinea pig and rabbit colonies in breeding and experimental units. *Laboratory Animals, 48*(3), 178–192. https://doi.org/10.1177/0023677213516312

Matos-Rodrigues, G.E., Masseron, C.C., Silva, F.J., Frajblat, M., Moreira, L.O., & Martins, R.A. (2020). PCR-based detection of *Helicobacter*

spp. in animal facilities of a university in Rio de Janeiro, Brazil. *Anais da Academia Brasileira de Ciências, 92*(4), e20200020. https://doi.org/10.1590/0001-3765202020200020

Merck Veterinary Manual. (2023). *Tyzzer disease in animals.* Merck & Co., Inc. https://www.merckvetmanual.com/digestive-system/tyzzer-disease/tyzzer-disease-in-animals

Newcombe, R.G. (1998). Two-sided confidence intervals for the single proportion: Comparison of seven methods. *Statistics in Medicine, 17*(8), 857–872.

Nicklas, W., Baneux, P., Boot, R., Decelle, T., Deeny, A.A., Fumanelli, M., & Illgen-Wilcke, B. (2002). Recommendations for the health monitoring of rodent and rabbit colonies in breeding and experimental units. *Laboratory Animals, 36*(1), 20–42. https://doi.org/10.1258/0023677021911740

Percy, D.H., & Barthold, S.W. (2007). *Pathology of laboratory rodents and rabbits*. Blackwell Publishing.

Sorzano, C.O. S., Sánchez, I., & Naranjo, A. (2024). Statistical design for health monitoring in laboratory animal facilities using sentinel animals. *Laboratory Animals*, 58(4), 345–353. https://doi.org/10.1177/00236772231219292

Ülker, U., Kızıl, S., Çeçen, E.M., & Aydın, E. (2024). Molecular diagnosis of *Clostridium piliforme*, the causative agent of Tyzzer's disease in mice and rats. *Laboratuvar Hayvan Bilimi Uygulamaları Dergisi, 4*(2), 78–82. https://doi.org/10.62425/jlasp.1426993