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Research article

INVESTIGATION OF *CORYNEBACTERIUM KUTSCHERI* INFECTION IN LABORATORY MICE IN TÜRKİYE: PREVALENCE, PATHOLOGY AND BIOCHEMICAL CHANGES

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The aim of this study was to investigate, for the first time in Türkiye, the prevalence of *Corynebacterium kutscheri* in 102 laboratory mice in the Aegean Region, together with associated histopathological lesions and biochemical alterations. *C. kutscheri* was isolated from five mice (4.9%), all of which exhibited abscess formation and neutrophilic infiltration in the liver, lungs, and heart. Infected mice also displayed significantly elevated AST, ALT, ALP, and LDH levels compared to controls ($P<0.001$). These findings demonstrate that latent *C. kutscheri* infections, although often subclinical, can cause systemic pathology and alter biochemical parameters, potentially compromising laboratory animal analysis safety and the reliability of biomedical research. As this study provides the first comprehensive dataset on *C. kutscheri* in laboratory mice in Türkiye, it establishes a critical baseline for future surveillance programs. The detection of this FELASA listed pathogen underscores the urgent need for robust national health monitoring systems aligned with international standards, thereby ensuring both animal welfare and the credibility of scientific outcomes.

Keywords: Biochemistry, *Corynebacterium kutscheri*, Histopathology, Laboratory mice, Latent infection.

INTRODUCTION

Corynebacterium kutscheri was first described by Kutscher in 1896 as a Gram-positive bacterium responsible for a pseudotuberculosis-like disease in mice. Subsequent investigations demonstrated that this pathogen can also infect rats, hamsters, and guinea pigs [1,2]. Infections are often subclinical, with the organism persisting latently

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in the oral cavity, cecum, oropharyngeal tissues, and cervical or maxillary lymph nodes of rodents [1,3-5]. Remarkably, *C. kutscheri* has been detected even in laboratory animal facilities operating under high hygienic standards, highlighting its ability for silent persistence and undetected dissemination [6].

Although human infections are extremely rare, the first documented case occurred in 2007 in a newborn who developed a soft tissue infection following a rat bite [7]. In rodents, however, disease development is typically associated with stressors such as overcrowded housing, poor nutrition, immunosuppressive treatments, radiation exposure, or invasive experimental procedures [8-11]. The primary route of transmission is fecal–oral, and experimentally infected animals may shed the bacterium in the feces for up to five months [3].

The Federation of European Laboratory Animal Science Associations (FELASA) lists *C. kutscheri* among the pathogens that must be routinely monitored in laboratory rodents [6]. This inclusion reflects its relevance not only to laboratory animal health but also to the reproducibility and validity of scientific research. Latent infections can alter physiological and biochemical parameters, thereby affecting experimental outcomes and potentially compromising animal welfare and the interpretation of research findings. Despite this, FELASA-aligned health-monitoring programs have not yet been fully implemented in Türkiye, creating a critical gap in both laboratory animal surveillance and research reliability.

To date, no comprehensive investigation has assessed the presence of *C. kutscheri* in laboratory mice in Türkiye. Therefore, the present study was designed to determine the prevalence of *C. kutscheri* in laboratory mice in the Aegean Region and to evaluate associated histopathological lesions and biochemical alterations. By integrating bacteriological, pathological, and clinical biochemical assessments, this research provides the first baseline dataset for Türkiye and represents a crucial step toward strengthening national laboratory animal health-monitoring practices and improving the reliability of biomedical research outcomes.

MATERIALS AND METHODS

Study design

A total of 102 adult Swiss-Albino laboratory mice, aged 12–24 weeks, were obtained from ten laboratory animal breeding units in the Aegean Region of Türkiye. The mean body weights were 32.3 ± 2.6 g for males and 28.5 ± 1.4 g for females. The mice were housed in groups in plexiglass cages measuring 30 × 20 × 13 cm under standardized environmental conditions, including a 12-h light/12-h dark cycle, ambient temperature of 22 ± 2°C, and relative humidity of 52 ± 10%. A maximum of 10 mice were housed per cage. The animals were provided with ad libitum access to water and a species-appropriate diet. Prior to necropsy, blood samples were collected via cardiac puncture

into serum tubes (MiniCollect® Tube, Austria) following deep CO_2 anesthesia (70% CO_2/30% O_2, 10% cage volume/min flow rate for 1 min) [8,11]. The depth of anesthesia was assessed by loss of the righting reflex and absence of response to external stimuli, including the pedal withdrawal/toe-pinch reflex. Blood collection was performed only after deep anesthesia was confirmed. While still under deep anesthesia, mice were euthanized by cervical dislocation, and liver, heart, and lung tissues were harvested aseptically for bacteriological and pathological examinations [10].

According to the World Organisation for Animal Health (OIE) guidelines, the required sample size for a 5% expected prevalence, 5% margin of error, and 95% confidence level is at least 73 animals using the formula $n = Z^2 \times p \times (1 – p) / d^2$. To increase statistical power and ensure representativeness, 102 mice were included in this study [12].

Pathologic examination

Tissue samples from the liver, heart, and lungs of five mice showing gross lesions were fixed in 10% neutral buffered formalin for 24–48 h. Samples were routinely processed through graded alcohol and xylene, embedded in paraffin, and sectioned at 4–5 μm using a microtome (Thermo HM355S). Sections were stained with hematoxylin and eosin (H&E) and examined under a binocular light microscope (Olympus BX51).

Bacteriologic examination

During necropsy, liver, heart, and lung samples were inoculated onto 5% sheep blood agar and incubated aerobically and microaerophilically at 37°C for 24–48 h. After incubation, bacterial growth was assessed, and Gram staining was performed to evaluate microscopic morphology.

Biochemical identification was conducted using standard biochemical tests and the VITEK® 2 Compact (bioMérieux, France) CBC identification system. Isolates presumptively identified as *Corynebacterium kutscheri* were subjected to DNA extraction using a commercial kit (High Pure PCR Template Preparation Kit, Roche). Molecular confirmation was performed using species-specific primers [13]:

- Forward: 5'-CGT GAT GGC CAT CTT TGG TT-3'
- Reverse: 5'-AAT CGT ATT AGC AAA GGT ATG C-3'

PCR amplification was carried out in a TC-412 thermal cycler (Keison Products) under the following conditions: initial denaturation at 94°C for 3 min; 40 cycles of denaturation at 94°C for 15 sec, annealing at 55°C for 30 sec, and extension at 72°C for 15 sec; followed by a final extension at 72°C for 3 min. Xpert Fast Hotstart Mastermix (2×) (GRiSP) was used for amplification.

PCR products were separated on a 2% agarose gel stained with Xpert Green DNA Stain Direct (GRiSP) using GRS Universal Ladder (50 μg) as a molecular marker. Gels were visualized using a documentation system (Er Biyotek Fx51/FxT).

Biochemical analysis

For biochemical analysis, blood samples were allowed to clot for at least 20 min and then centrifuged at 1108 × g for 15 min (Sigma 3-16 KL, Germany). Serum samples from five mice with liver abscesses and from 30 randomly selected control mice were analyzed for aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total protein (TP), creatinine (Crea), and urea (UREA) using commercial diagnostic kits. Analyses were performed spectrophotometrically on a Mindray BS-240 autoanalyzer.

Statistical analysis

Data were analyzed using IBM SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, NY, USA). Differences in biochemical parameters between infected and control groups were evaluated using Student's t-test. Statistical significance was accepted at $p < 0.05$.

RESULTS

Bacteriological findings

Liver, heart, and lung samples from 102 mice were inoculated onto 5% sheep blood agar. Bacterial growth was detected in five samples. After 24–48 h of incubation, the colonies appeared small, circular, convex, smooth, grayish-white, and non-hemolytic on 5% sheep blood agar. The colonies were purified through subculturing. Gram staining demonstrated Gram-positive coccobacilli consistent with *Corynebacterium* spp. The isolates were identified as *C. kutscheri* using the VITEK® 2 Compact CBC identification system. Molecular confirmation using *C. kutscheri*-specific primers yielded a 540 bp PCR product in all five isolates (Figure 1).

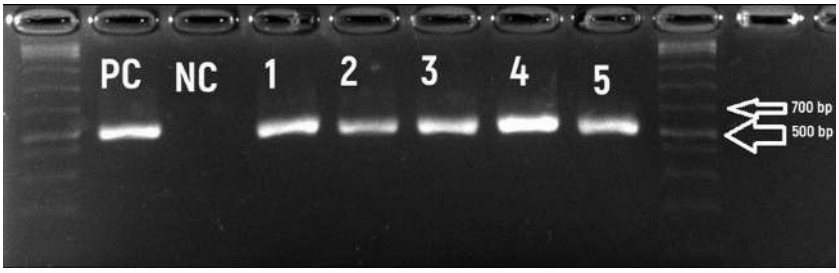

Figure 1: Agarose gel electrophoresis of PCR products specific for *C. kutscheri*. **PC**: *C. kutscheri* ATCC 43219; **NC**: *Escherichia coli* ATCC 25922; lanes 1–5: positive samples.

Pathological findings

Macroscopically, the liver was mildly enlarged and had a soft to friable consistency. On cut surface, the hepatic parenchyma appeared congested and contained multiple 2–5 mm grayish-white nodules with a multifocal distribution. These nodules were slightly raised and, on section, revealed purulent to caseous content consistent with microabscesses (Figure 2).

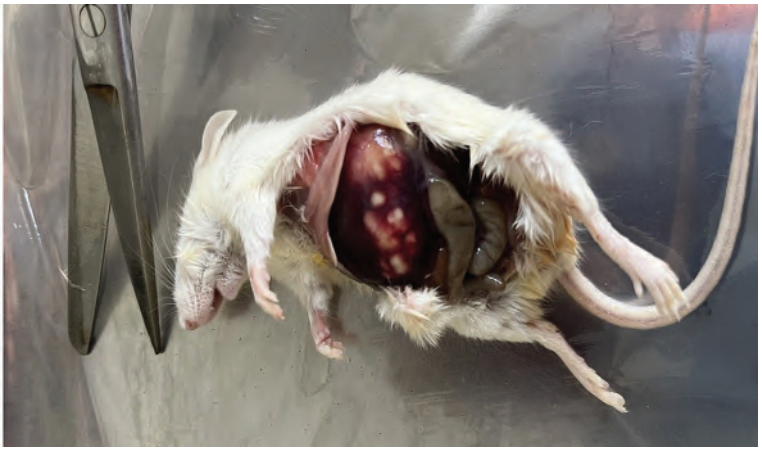

Figure 2: Gross hepatic lesions associated with *C. kutscheri* infection in a Swiss-Albino mouse. The liver shows multifocal, slightly raised, grayish-white nodules of varying sizes, consistent with microabscesses.

The lungs were dark red; on cut surface, they exuded moderate amounts of blood-tinged fluid without evident foamy or purulent exudate. The heart displayed pale, pinpoint discoloration at the apex. No intracardiac blood clots were observed, and on section, the myocardium appeared pale with focal discoloration but without purulent exudate. No remarkable macroscopic findings were noted in the other organs. Microscopically, the most severe lesions were detected in the heart and liver. In the heart, endocardial thickening and marked neutrophilic infiltration were evident, along with neutrophils located between cardiac muscle fibers (Figure 3A). The liver exhibited multifocal pyogranulomatous inflammation (Figure 3B). Some lesions were characterized by central accumulations of degenerated neutrophils surrounded by a fibrous capsule and mononuclear inflammatory cells, mainly macrophages and lymphocytes (Figure 3C). Other lesions displayed concentric lamellar structures with central calcifications and dense clusters of blue-purple stained bacteria, forming caseous necrotic foci bordered by macrophages and lymphocytes (Figure 3D). Mild mononuclear inflammatory cell infiltration, mainly composed of lymphocytes and macrophages, was also present in the portal areas. Lung tissues displayed mild hyperaemia and thickening of the interalveolar septa due to infiltration of inflammatory cells, predominantly macrophages and lymphocytes, with occasional neutrophils.

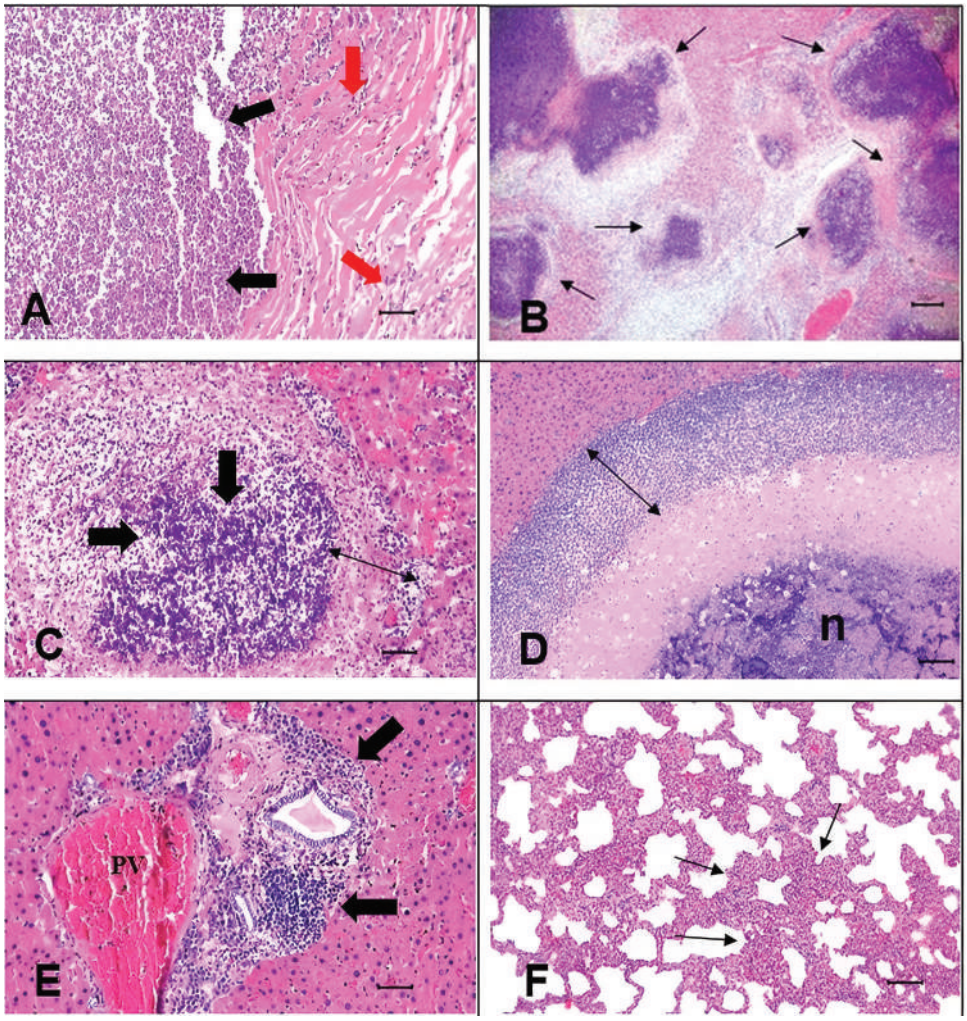

Figure 3 : Histopathological lesions associated with *Corynebacterium kutscheri* infection. **A)** Heart: Neutrophilic inflammatory cell infiltration in the endocardial and subendocardial regions (black arrows), extending between cardiac muscle fibers (red arrows). H&E, 50 µm. **B)** Liver: Multifocal pyogranulomatous lesions (black arrows). H&E, 200 µm. **C)** Liver: A focal pyogranuloma with degenerated neutrophils in the center (black arrows), surrounded by a fibrous capsule and inflammatory cells, mainly macrophages and lymphocytes (double-headed arrow). H&E, 50 µm. **D)** Liver: A concentric lamellar-structured pyogranuloma with central necrosis (n), calcifications, and bacterial clusters, surrounded by a sharply demarcated rim composed mainly of macrophages and lymphocytes (double-headed arrow). H&E, 100 µm. **E)** Liver: Mononuclear inflammatory cell infiltration, mainly lymphocytes and macrophages, in the portal area. H&E, 50 µm. PV: portal vein. **F)** Lung: Mild hyperaemia and inflammatory cell infiltration in the interalveolar septa, predominantly composed of macrophages and lymphocytes, with occasional neutrophils. H&E, 100 µm.

Biochemical findings

Serum biochemical analysis revealed significantly elevated AST, ALT, ALP, LDH, and urea levels in *C. kutscheri*–infected mice compared with the control group ($P < 0.001$). No statistically significant differences were detected in total protein or creatinine levels ($P > 0.05$) (Table 1).

Table 1. Serum biochemistry results in laboratory mice with and without *C. kutscheri* infection.

Measurand	SI Units	Laboratory Mice	
		C. kutscheri **Positive (n=5)**	***C. kutscheri*** **Negative (n=30)**
AST	**U/L**	364.34±261.66**	92.10±47.22
ALT	**U/L**	211.24±139.60**	27.03±14.30
ALP	**U/L**	229.69±48.67*	75.26±44.49
LDH	**U/L**	1125.59±781.17**	216.36±60.83
TP	**g/L**	61.54±6.10	64.08±13.04
Crea	**mg/dL**	0.25±0.07	0.29±0.13
UR	**mg/dL**	57.54±11.61*	31.74±15.33

*The difference between groups in the same line is significant ($P<0.05$).
**The difference between groups in the same line is significant ($P<0.001$).

DISCUSSION

The first recognition of *Corynebacterium kutscheri* as a causative agent of rodent infections dates back to 1952, when Lemaistre and Tompsett described its role in pseudotuberculosis like lesions in laboratory animals. Since then, the bacterium has been identified as an opportunistic pathogen capable of infecting various rodent species, with mice being particularly susceptible [14].

In Türkiye, the limited studies available have reported the detection of *C. kutscheri* mainly in rats, particularly under stress-induced conditions [15]. The detection of this pathogen in laboratory mice in the present study indicates that its distribution may be more widespread than previously assumed. This study represents the first comprehensive investigation of *C. kutscheri* infection in laboratory mice in Türkiye, providing a critical national dataset that may serve as a foundation for future surveillance and health-monitoring efforts. Considering that FELASA recommended health-monitoring programs have not yet been fully implemented in Türkiye, the likelihood of encountering such latent pathogens is expected to increase [6]. This points to an urgent need for more robust and standardized monitoring systems to safeguard research quality and laboratory animal welfare.

Stress is a well-known trigger for latent infections in rodents, and factors such as overcrowding, inadequate nutrition, and immunosuppression increase susceptibility [4,16]. Although latent *C. kutscheri* infections are often subclinical, our biochemical analyses indicate systemic effects, demonstrating that even inapparent infections may compromise animal physiology and research reliability.

The infection rate observed in our study (4.9%) is consistent with findings from other countries. Suzuki et al. (1986) reported antibodies in 0.5% of mice and abscesses in 4.1% of colonies in Japan. Similarly, we identified *C. kutscheri* in five out of 102 mice, indicating a prevalence comparable to international reports. These findings confirm that the agent can silently circulate within colonies, even at relatively low but epidemiologically significant rates.

Macroscopic findings in the present study, particularly multifocal grayish-white abscesses in the liver, are consistent with previously documented lesion patterns associated with *C. kutscheri* infections [17,18]. The histopathological findings pyogranulomatous inflammation, caseous necrosis, and concentric lamellar structures align with the typical lesions described in chronic infections. Multinucleated giant cells, reported in some studies, were not observed, which may reflect variation in disease progression, immune response, or infection chronicity. The clustering of caseous foci into larger coalescing lesions supports the chronic and progressive nature of the infection.

The localization of lesions in our study, particularly in the liver and heart with mild involvement of the lungs, is consistent with hematogenous dissemination of the bacterium. Previous studies have reported that lesion distribution varies by species; in rats, lesions commonly occur in the liver, kidneys, and lungs, whereas in mice, cardiac and pulmonary involvement is more frequent [4,17]. Our findings are consistent with this pattern, as cardiac and hepatic lesions predominated, while pulmonary changes were mild and characterized by active hyperaemia and interalveolar septal thickening associated with inflammatory cell infiltration.

Biochemical findings further supported the pathological results. Elevated AST, ALT, ALP, LDH, and urea levels in infected mice suggest hepatocellular injury and metabolic alterations, consistent with the observed hepatic and cardiac inflammatory lesions [4,17]. The absence of significant changes in total protein and creatinine suggests that the infection caused localized organ damage rather than generalized systemic dysfunction. Together, these findings demonstrate that even subclinical *C. kutscheri* infections can significantly affect biochemical parameters relevant to research outcomes.

Taken together, our results highlight that *C. kutscheri* may remain latent in laboratory animal facilities and has the potential to compromise both animal welfare and the reliability of experimental results. Implementation of stringent, FELASA-aligned health-monitoring programs is crucial for early detection and control of such infections. Ensuring the microbiological health of laboratory animals is fundamental

for reproducibility, as unnoticed infections may lead to misinterpretation of data and invalid conclusions [19-21].

This study has certain limitations. The sampling was restricted to laboratory mice in the Aegean Region; therefore, the results may not fully represent the situation nationwide. In addition, cardiac-specific biomarkers such as cardiac troponin T (cTnT) and cardiac troponin I (cTnI) were not measured in the present study. Future studies including these biomarkers may provide a more specific evaluation of myocardial injury associated with *C. kutscheri* infection. Nevertheless, this investigation provides the first comprehensive evaluation of *C. kutscheri* in laboratory mice in Türkiye, integrating bacteriological, pathological, and biochemical findings. Larger-scale, multi-regional studies are needed to further clarify the epidemiological status of this pathogen and to support the establishment of national health-monitoring standards.

CONCLUSION

In conclusion, this study provides the first comprehensive assessment of *Corynebacterium kutscheri* infection in laboratory mice in Türkiye, combining bacteriological, pathological, and biochemical evaluations. The findings demonstrate that latent *C. kutscheri* infections, although often clinically silent, have the potential to alter physiological parameters and compromise both laboratory animal welfare and the reliability of biomedical research outcomes. The detection of this FELASA-listed pathogen highlights a critical gap in national health-monitoring practices and underscores the need for the development and implementation of robust, standardized surveillance programs in Türkiye. This study not only establishes essential baseline data but also represents an important step toward improving laboratory animal health-monitoring systems and ensuring the reproducibility and scientific integrity of future research conducted in the country.

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Authors' contributions

ÇN performed the conceptualization, data curation, formal analysis, and wrote the original draft of the manuscript. ÇO participated in the formal analysis and biochemical analyses and reviewed and edited the manuscript. EG and MK contributed to pathological examinations, laboratory diagnostics, and data validation. Nİİ contributed to data analysis, supervision, and validation, and reviewed and edited the manuscript. All authors have read and approved the final manuscript.

Declaration of conflicting interests

The authors declare that they have no conflict of interest.

Ethical Approval

All experimental procedures involving animals were approved by the Local Ethics Committee of the Bornova Veterinary Control Institute (Date: 12.02.2021; Approval No: 453425). All procedures were conducted in accordance with national and international guidelines for the care and use of laboratory animals.

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ISPITIVANJE INFEKCIJE SA *CORYNEBACTERIUM KUTSCHERI* KOD LABORATORIJSKIH MIŠEVA U TURSKOJ: RASPROSTRANJENOST, PATOLOGIJA I BIOHEMIJSKE PROMENE

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Cilj ove studije bio je da se, po prvi put u Turskoj, istraži prevalencija *Corynebacterium kutscheri* kod 102 laboratorijska miša u Egejskom regionu, zajedno sa povezanim histopatološkim lezijama i biohemijskim promenama. *C. kutscheri* je izolovan kod pet miševa (4,9%), od kojih su svi pokazali formiranje apscesa i neutrofilnu infiltraciju u jetri, plućima i srcu. Inficirani miševi su takođe pokazali značajno povišene nivoe AST, ALT, ALP i LDH u poređenju sa kontrolnom grupom (P<0,001). Ovi nalazi pokazuju da latentne infekcije *C. kutscheri*, iako često subkliničke, mogu izazvati sistemsku patologiju i promeniti biohemijske parametre, potencijalno ugrožavajući bezbednost analize laboratorijskih životinja i pouzdanost biomedicinskih istraživanja. Pošto ova studija pruža prvi sveobuhvatni skup podataka o *C. kutscheri* kod laboratorijskih miševa u Turskoj, ona uspostavlja kritičnu osnovu za buduće programe nadzora. Detekcija

ovog patogena sa liste FELASA naglašava hitnu potrebu za pouzdanim nacionalnim sistemima za praćenje zdravlja usklađenim sa međunarodnim standardima, čime se osigurava i dobrobit životinja i kredibilitet naučnih rezultata.