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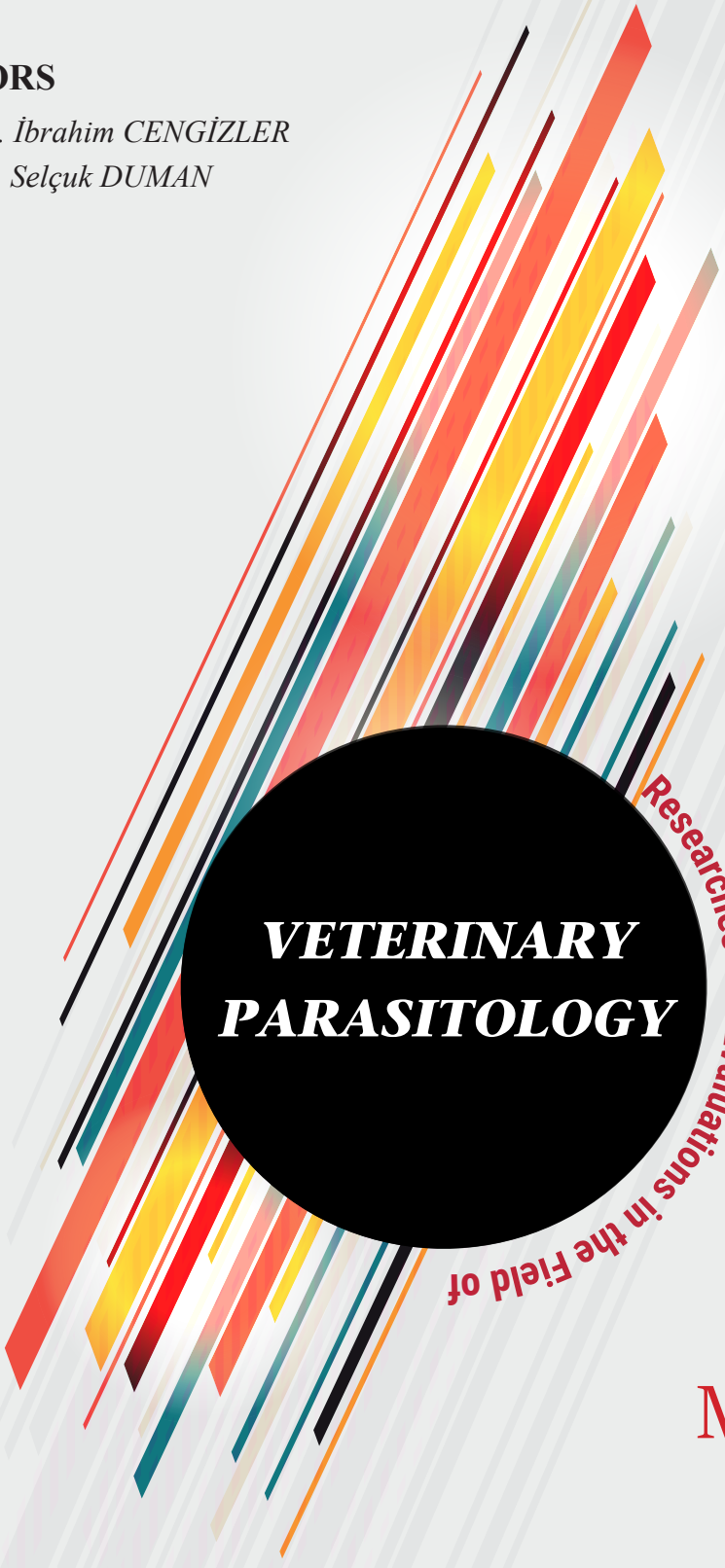

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Research And Evaluations In The Field Of Veterinary Parasitology

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Prof. Dr. İbrahim CENGİZLER
Doç. Dr. Selçuk DUMAN

İÇİNDEKİLER

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CHAPTER 1

THE POTENTIAL OF MONGOLIAN GERBILS AS A MODEL ORGANISM IN *TOXOPLASMA GONDII* RESEARCH: CURRENT FINDINGS AND FUTURE PERSPECTIVES

Asude Gülçe ORYAŞIN[1]

1 [1] Teaching Assistant Dr., Aydin Adnan Menderes University, Faculty of Veterinary Medicine, Department of Parasitology, 0000-0002-7219-2879.

Introduction

Toxoplasma gondii is a globally prevalent zoonotic protozoan parasite that can infect several warm-blooded animals, including humans. The significance of *T. gondii* in public health stems from its ability to cause congenital infections, induce severe complications in immunosuppressed individuals, and the absence of a safe and effective vaccine. Therefore, appropriate animal models are crucial for a better understanding of *T. gondii* pathogenesis and host immune responses to infection (Almeria & Dubey, 2021).

In the life cycle of *T. gondii*, feline species (*Felidae*) serve as definitive hosts, whereas humans and other warm-blooded animals act as intermediate hosts (Almeria & Dubey, 2021). Although *T. gondii* infections in intermediate hosts are often subclinical, they can lead to severe complications in immunocompromised individuals and during pregnancy. Congenital toxoplasmosis can cause birth defects and neurological disorders in fetuses, whereas immunodeficient individuals may develop severe brain infections, such as toxoplasmic encephalitis. In addition, the consumption of infected meat and exposure to contaminated water are among the primary routes of transmission (Almeria & Dubey, 2021).

Toxoplasma gondii exists in three main stages throughout its life cycle: the rapidly proliferating tachyzoite form, the bradyzoite form enclosed in tissue cysts, and the oocyst form responsible for environmental dissemination (Almeria & Dubey, 2021). Definitive hosts, such as cats, acquire the parasite by consuming infected intermediate hosts, allowing *T. gondii* to undergo sexual reproduction in the feline intestine and produce oocysts, which are then excreted into the environment. These oocysts can infect new hosts through contaminated water, food, or direct contact. In intermediate hosts, the parasite initially proliferates rapidly as a tachyzoite before transitioning into the chronic phase, during which it forms tissue cysts containing bradyzoite. These cysts can persist in tissues, particularly in the brain, muscles, and ocular structures, for extended periods in a latent state (Almeria & Dubey, 2021).

Mongolian gerbils (*Meriones unguiculatus*) have emerged as a valuable model organism for studying the pathogenesis and immune response mechanisms of various infectious diseases. Because of their susceptibility to *T. gondii* infection, gerbils serve as an effective animal model for both acute and chronic infection studies. This review explores the advantages of using Mongolian gerbils in *T. gondii* research and discuss their potential applications in future studies.

Toxoplasma gondii Immune Response

Toxoplasma gondii infection triggers a complex immune response in the host that involves both innate and adaptive immune components. During the early stages of infection, innate immune cells such as macrophages and dendritic cells interact with the parasite, initiating the release of proinflammatory cytokines. In this process, the interleukin-12 (IL-12)-mediated T helper 1 (TH1) response plays a critical role in parasite control by stimulating interferon-gamma (IFN-γ) production (Yarovinsky, 2014).

Mongolian gerbils exhibit higher susceptibility to both high- and low-virulence strains of *T. gondii* compared to murine models. This characteristic renders gerbils a valuable model for evaluating diagnostic and therapeutic strategies for toxoplasmosis, studying infection dynamics, and investigating host defense mechanisms. Experimental studies have demonstrated that as early as 1 day post-infection, *T. gondii* DNA can be detected in blood (66.7%), liver (73.3%), lungs (80.0%), spleen (80.0%), and peritoneal fluid (66.7%) samples of infected gerbils. These findings support the suitability of gerbils as a model for the early diagnosis and pathogenesis research in *T. gondii* infection (Dai et al., 2019).

The immune response to *T. gondii* infection differs in gerbils and other rodent models. While the early stages of infection are characterized by a strong pro-inflammatory cytokine profile associated with a TH1 response, whereas the chronic phase is shaped by the dominance of the TH2 response. Increased levels of cytokines such as IL-12, TNF-α, and IFN-γ have been observed, whereas immunomodulatory cytokines like IL-4 and IL-10 increase during later stages of infection (Bottari et al., 2014).

Histopathological and immunohistochemical studies have revealed the systemic effects of *T. gondii* infection in gerbils, with prominent lesions and inflammatory responses in the liver, lungs, and spleen. These findings highlight the potential of gerbils as a suitable model for investigating the tissue-level effects of *T. gondii* infection (Kahyaoğlu & Atmaca, 2022).

Furthermore, studies on the nonspecific effects (NSEs) of veterinary vaccines suggest that the immune response to *T. gondii* infection in gerbils can be modulated. Notably, the Bacillus Calmette-Guérin (BCG) vaccine has been reported to provide protective effects against parasitic infections in gerbils by enhancing immune function. These findings offer a new perspective for vaccine development studies targeting *T. gondii* in gerbils (Arega et al., 2022).

Investigations into oxidative stress parameters have shown that *T. gondii* infection in gerbils leads to an increase in erythrocyte malondialdehyde (MDA) levels and a decrease in superoxide dismutase (SOD) and

albumin levels. Additionally, infected gerbils exhibit an increase in neutrophil ratio and total leukocyte counts and decreased lymphocyte ratios. These hematological and biochemical alterations serve as significant indicators of the immune response to *T. gondii* infection in gerbils (Atmaca et al., 2015).

The excreted-secreted antigens (ESAs) of *T. gondii* play a crucial role in stimulating the host immune response. Studies using murine models have shown that these antigens elicit humoral immune responses and provide protection against infection. Notably, a 65-kDa protein band has been identified as a key factor in immune response activation. These findings hold importance for vaccine development efforts, and conducting similar studies on Mongolian gerbils could contribute to a better understanding of the immune response in this model organism (Daryani et al., 2023).

In conclusion, Mongolian gerbils emerge as a promising animal model for understanding the pathogenesis of *T. gondii* infection, developing novel diagnostic tools, and evaluating potential therapeutic strategies. Their susceptibility to *T. gondii* infection, ability to establish a systemic infection model, and distinct immunological features make them valuable tools for toxoplasmosis research. Future studies may focus on the use of the gerbil model for different *T. gondii* strains, the long-term infection dynamics, and the assessment of potential vaccine and drug candidates.

Advantages of Mongolian Gerbils as a Model

Mongolian gerbils have emerged as a valuable model organism for studying *T. gondii* infection, offering significant advantages. This species is considered to be a closer immunological model to humans than mice and rats. Gerbils have the potential to establish a more prolonged and stable infection model, which provides a crucial advantage for studying both acute and chronic infections (Mohanty et al., 2002; Suzuki & Tsunematsu, 1974).

The high susceptibility of gerbils to *T. gondii* infection allows for early detection of the pathogen. Experimental studies have demonstrated that *T. gondii* DNA can be detected in various tissues shortly after infection. This feature highlights the suitability of the gerbil model for developing and evaluating early diagnostic methods (Dai et al., 2019).

The high parasite burden observed during the acute phase of infection presents a significant advantage for pathogenesis studies. This allows for a detailed investigation of parasite dissemination and replication dynamics within host tissues. Additionally, the susceptibility of gerbils to both high-

and low-virulence strains of *T. gondii* enables comparative studies on the pathogenesis of different strains (Bottari et al., 2014).

Histopathological and immunohistochemical studies have demonstrated that the gerbil model provides an appropriate platform for understanding the tissue-level effects of *T. gondii* infection. Kahyaoğlu & Atmaca (2022) indicated that the systemic effects of infection and pathological changes in different organs could be thoroughly examined. This approach facilitates a comprehensive evaluation of infection progression and tissue damage caused by the parasite.

Another key advantage of the gerbil model is its potential to contribute to the assessment of novel vaccine and drug testing approaches. The species› susceptibility to *T. gondii* to infection and its infection dynamics provide a suitable framework for evaluating the efficacy of potential vaccine candidates and new therapeutic agents (Arega et al., 2022).

In conclusion, Mongolian gerbils represent a promising animal model for understanding the pathogenesis of *T. gondii* infection, developing novel diagnostic tools, and assessing potential therapeutic approaches. The advantages of this species enable more comprehensive and detailed research on toxoplasmosis, contributing to significant advancements in both veterinary and human health.

Studies and Findings

The use of Mongolian gerbils as a model organism for studying *T. gondii* infection research has gained increasing interest in recent years. Various studies in the literature have examined the immune response of gerbils to *T. gondii* infection and the associated histopathological changes.

An experimental study conducted by Kahyaoğlu & Atmaca (2022) observed severe lesions in the liver, spleen, and lungs of gerbils infected with the *T. gondii* RH strain. The study revealed extensive parasite proliferation in liver tissue. Similarly, a study by Atmaca et al. (2015) reported a significant increase in liver enzyme activity (AST and ALT) in infected gerbils, indicating liver damage. These findings that oxidative stress and hematological changes contribute to *T. gondii* pathogenesis.

Bottari et al. (2014) demonstrated a high accumulation of tachyzoites and inflammatory cells in the peritoneal fluid of infected gerbils. A decrease in serum total protein and albumin levels, along with an increase in peritoneal fluid, was also noted. Histological exaexamination revealeddespread necrotic foci in the liver and cysts containing bradyzoites. These findings support the suitability of Mongolian gerbils as an important model organism for understanding the progression of *T. gondii* infection.

A recent study developed a quantitative real-time PCR (qPCR) method targeting the *T. gondii* B1 gene for early infection detection. This method demonstrated high sensitivity for detecting a single *T. gondii* tachyzoite, highlighting the potential of the gerbil model in advancing diagnostic techniques (Dai et al., 2019).

The susceptibility of gerbils to *T. gondii* infection has been recognized since the 1970s (Suzuki & Tsunematsu, 1974). However, knowledge regarding the gerbil immune system remains limited. The deficiency of T-independent antigens may explain their susceptibility to various parasites. Recent advances in the cloning and sequencing gerbil cytokine genes could enhance our understanding of *T. gondii* pathogenesis and protective immunity(Mohanty et al., 2002).

A study conducted in South Africa by Lukasova et al. (2018) investigated the presence of *T. gondii* in gerbil species. PCR analysis of 243 animals detected *T. gondii* in five cases (2%), with one positive sample belonging to a gerbil species. These findings emphasize the susceptibility of gerbils to *T. gondii* infection and the sensitivity of PCR for parasite detection.

Gerbils are also suitable model organism for parasites closely related to *T. gondii.* Dubey and Lindsay (2000) reported high susceptibility of gerbils to *Neospora caninum* oocysts. Similarly, Jie et al. (2013) demonstrated that gerbils provide an effective model for *Hammondia heydorni* infection. These findings suggest that gerbils offer comparable advantages in *T. gondii* research.

Detailed histopathological and immunohistochemical analyses by Kahyaoğlu and Atmaca (2022) revealed the systemic effects of *T. gondii* infection in gerbils. Observed alterations in the liver, lungs, and intestines further support the relevance of the gerbil model's relevance for studying *T. gondii* pathogenesis.

Studies by Sager et al. (2006) and Schares et al. (2005) reinforced the applicability of the gerbil model in *T. gondii* and related parasite research. These studies validated the use of serological monitoring and PCR-based parasite detection in gerbils.

Ukaji et al. (2011) highlighted the sensitivity of gerbils to various pathogens. Additionally, research on gerbil immunoglobulin production and characterization has opened new avenues for analyzing antibody responses to T. gondii and the development of gerbil-derived monoclonal antibodies specific to the parasite.

Overall, these studies demonstrate that Mongolian gerbils are a valuable model organism for *studying T. gondii.* The advantages they offer for

studying infection dynamics, immune response, and tissue-level changes support their broader application in future *T. gondii* studies.

Conclusions and Future Perspectives

Mongolian gerbils have emerged as a promising model organism for understanding the pathogenesis of *T. gondii* infection and the development of new therapeutic approaches. However, more comprehensive research is needed to investigate the immune response and disease progression. Expanding the use of the gerbil model and obtaining more detailed information about *Toxoplasma* infections in the future could significantly contribute to advancements in both veterinary and human medicine.

Dai et al. (2019) emphasized that the gerbil model provides an effective platform for elucidating the pathogenesis of toxoplasmosis, improving diagnostic methods, and evaluating potential treatment strategies. The advantages of this model, particularly in the early detection of infections and the study of strains with different virulence levels, offer valuable opportunities for future research. Further studies using this model could contribute to the development of new strategies for controlling and treating *T. gondii* infections.

The research conducted by Kahyaoğlu & Atmaca (2022) demonstrated that gerbils serve as a valuable model for examining the histopathological and immunohistochemical characteristics of *T. gondii* infections. Future studies employing this model for different *T. gondii* strains and investigating long-term infection dynamics may allow for a more detailed understanding of toxoplasmosis pathogenesis.

Nasarre et al. (1998) demonstrated that Mongolian gerbils could serve as an important model for studying *T. gondii* and filarial parasites. Their study indicated that gerbil macrophages, when activated in vivo with BCG-PPD immunization, have the ability to kill *T. gondii*. However, gerbil macrophages differ from mouse macrophages in terms of in vitro activation and nitric oxide production. These differences may explain the unique susceptibility of gerbils to certain parasites. Macrophage activation in gerbils infected with *Brugia pahangi* was observed to change over time. These findings support the use of gerbils as a valuable model organism for studying *T. gondii* and filarial parasite research.

Ramamoorthy et al. (2005) demonstrated that Mongolian gerbils serve as a suitable model for *N. caninum* infection. Although this study was not directly related to *T. gondii*, it provided crucial insights into the use of gerbils as a model organism for parasitic infections. The researchers noted that gerbils were more susceptible to *N. caninum* infection than mice,

making them suitable for modeling acute infections. This model presents advantages for vaccine and drug developmbecause ofe to its ability to yield clear results in a short period and the ease of obtaining gerbils. Similar advantages also support the use of gerbils in *T. gondii* research.

Ribeiro et al. (2011) investigated the effects of probiotics and prebiotics on *T. gondii* infection. Although this study was not conducted on gerbils, a similar approach could be applied to the gerbil model. This study provided valuable insights into methods for examining the acute and chronic phases of *T. gondii* infection. For instance, in the acute phase, intestinal lesions and tachyzoite in organs were analyzed, while in the chronic phase (75 days post-infection), tissue cysts in the brain were investigated. Additionally, the use of PCR to detect *T. gondii* DNA is a technique that could also be applied to the gerbil model. The methodology of this study offers a framework for examining *T. gondii* infection in gerbils and exploring new research perspectives for testing potential treatment approaches (e.g., probiotics and prebiotics) in this model.

In conclusion, Mongolian gerbils represent an important model organism in *T. gondii* research. Future studies can provide more detailed insights into their immune response, disease progression, and potential treatment strategies. These investigations could lead to the development of new strategies for controlling and treating toxoplasmosis, leading to significant advancements in both veterinary and human health.

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CHAPTER 2

PREVENTION OF PARASITIC DISEASES IN LABORATORY ANIMALS

Asude Gülçe ORYAŞIN[1]

1 [1] Teaching Assistant Dr., Aydin Adnan Menderes University, Faculty of Veterinary Medicine, Department of Parasitology, 0000-0002-7219-2879.

Introduction

Laboratory animals constitute the cornerstone of biomedical research, and their health directly influences the reliability of experimental outcomes. Rodents, particularly rats (*Rattus norvegicus)* and mice (*Mus musculus*), serve as critical models in immunological, pharmacological, and genetic studies, playing an indispensable role in scientific investigations. However, these animals can harbor a variety of parasites naturally present in wild populations, and they may be introduced into laboratory settings through external sources or inadequate hygiene practices (Baker, 2007). Parasitic diseases can emerge unexpectedly, even under controlled laboratory conditions, posing threats to both animal welfare and research efficacy.

For instance, intestinal nematodes such as *Syphacia muris* or protozoa like *Eimeria* species can spread rapidly among colonies if biosecurity measures are insufficient. Ectoparasites, such as *Polyplax spinulosa* (rat louse) and *Ornithonyssus bacoti* (tropical rat mite) may cause cutaneous irritation and even zoonotic infections, potentially endangering laboratory personnel (Wang et al., 2020; Brito-Casillas et al., 2018). These parasites not only compromise the physical health of the animals but also alter the immunological and metabolic responses of experimental models, thereby jeopardizing the accuracy and reproducibility of scientific data (Pritt et al., 2012). In sensitive experiments, such as those involving the immune system, the presence of parasitic infections introduces confounding variables that complicate the interpretation of the results.

Moreover, natural parasites found in wild rat and mouse populations can trigger significant outbreaks in laboratory settings if not properly managed. Zoonotic parasites like *Hymenolepis nana* pose additional public health risks due to their potential for human transmission (Morand et al., 2015). Consequently, preventing parasitic diseases in laboratory animals is not only vital for maintaining research quality and for upholding ethical standards and occupational safety. This chapter explores preventive veterinary approaches to parasitic diseases in laboratory animals, discusses current strategies, and provides practical recommendations for controlling both endo- and ectoparasites.

Common Parasitic Diseases

Parasitic diseases in laboratory animals typically arise from housing conditions, animal transfers, or contamination of feed sources. The most prevalent parasites are nematodes, cestodes, protozoa, and ectoparasites. For example, *S. muris* and *S. obvelata*, commonly known as pinworms, colonize the intestines of mice and rats, affecting their overall health. These parasites are transmitted via the fecal-oral route and exhibit rapid disse-

mination in dense populations (Baker, 2007). A study in Nigeria reported a high prevalence of *Syphacia* sp. in laboratory animals, highlighting their zoonotic potential and adverse effects on colony health (Akanbi et al., 2022).

Another significant group of cestodes, such as *Hymenolepis nana*, possess zoonotic potential, affecting both laboratory animals and, occasionally, humans. Transmission typically occurs through contaminated feed or water, with severe complications observed in immunocompromised models (Morand et al., 2015). Among protozoa species, *Eimeria* species are notable, causing coccidiosis in rabbits and rodents, leading to diarrhea, weight loss, and potentially mortality (Pritt et al., 2012). Ectoparasites, such as *Polyplax spinulosa* (rat louse), induce pruritus and skin irritation in rats, compromising animal welfare (Wang et al., 2020), while *Ornithonyssus bacoti* (tropical rat mite) has been implicated in outbreaks within laboratory facilities (Brito-Casillas et al., 2018).

Naturally occurring parasites in wild rat and mouse populations can infiltrate laboratory environments via introduced animals. For instance, endoparasites like *Syphacia* species and *Hymenolepis nana* are commonly found in their intestines, whereas ectoparasites, including *Polyplax species*, *Ornithonyssus bacoti*, and occasionally *Xenopsylla cheopis* (rat flea), reside on their skin (Baker, 2007; Wang et al., 2020). These natural parasites pose a threat to laboratory colonies when quarantine protocols are neglected or hygiene standards are relaxed. Controlling these species is essential for ensuring animal welfare and experimental consistency.

Preventive Veterinary Approaches

Controlling parasitic diseases in laboratory animals requires a multifaceted approach. The first step involves establishing hygiene practices and housing management. Regular cage cleaning, prevention of fecal accumulation, and adequate ventilation reduce the spread of parasites like *Syphacia* (Baker, 2007). Environmental factors affecting the survival of *Eimeria* oocysts; for example, high humidity facilitates the survival of ectoparasites, whereas *Eimeria* oocysts, whereas proper temperature regulation can inhibit ectoparasite proliferation (Pritt et al., 2012). Preventing contamination from feed and water sources is equally vital. Almeria et al. (2021) emphasized that food- and waterborne parasites pose significant risks in veterinary medicine, advocating stringent measures to mitigate transmission. To prevent the introduction of natural parasites into wild populations, a minimum 14-day quarantine period for incoming animals, coupled with ectoparasite screening, is recommended (Wang et al., 2020). Personnel

training is indispensable in this process; adherence to standard operating procedures (SOPs) significantly minimizes contamination risks.

Second, routine parasite screening protocols are essential. Microscopic analysis of fecal samples is an effective method for the early detection of parasites such as *Syphacia* and *Hymenolepis* (Akanbi et al., 2022), while skin scrapings and microscopic examinations are employed for ectoparasites (Brito-Casillas et al., 2018).

The third critical step is the prophylactic use of antiparasitic drugs, with the type, administration method, and dosage varying according to the target parasite and animal species:

- *Syphacia* spp. (Pinworms): In mice and rats, *Syphacia* infections are commonly managed with ivermectin, administered orally (via drinking water) at a dose of 0.2 mg/kg (Baker, 2007).
- *Hymenolepis nana*: The eradication of this cathode involves a single oral dose of praziquantel (mixed with feed) at a dose of 25 mg/kg (Morand et al., 2015).
- *Eimeria* spp.: To prevent coccidiosis in rabbits and rodents, amprolium is added to drinking water at a concentration of 0.012% for 7 days (Pritt et al., 2012).
- *Polyplax spinulosa*: Control of this ectoparasite is achieved with topical ivermectin (applied to the skin) at 0.2 mg/kg (Wang et al., 2020).
- *Ornithonyssus bacoti*: The tropical rat mite can be killed by ivermectin (0.2 mg/kg, topical) or permethrin (0.05% solution, sprayed) (Brito-Casillas et al., 2018).

Challenges in Application

Implementing preventive veterinary measures against parasitic diseases in laboratory animals presents several challenges. First, contamination risks constitute a significant barrier. Even under sterile conditions, parasites can infiltrate colonies via feeding, water, or personnel. Zoonotic parasites like *Hymenolepis nana* pose additional risks to laboratory staff (Morand et al., 2015). Diagne et al. (2017) highlighted the difficulty of controlling the spread of natural parasites from wild populations, emphasizing the ecological and sanitary risks associated with biological invasions in rodent communities. Ectoparasites such as *Polyplax spinulosa* and *Ornithonyssus bacoti* can easily be introduced to external animals (Wang et al., 2020; Brito-Casillas et al., 2018).

Second, ethical and experimental limitations are significant. Although prophylactic drug administration effectively reduces parasite burden, such interventions may influence experimental outcomes. For instance, ivermectin use in immunological studies can alter immune responses (Baker, 2007). This creates a dilemma within the 3R framework (Replacement, Reduction, Refinement). The Refinement principle promotes parasite control to enhance animal welfare, whereas reduction encourages early detection to minimize the exclusion of infected animals from experiments. However, Replacement -eliminating animal use entirely- remains challenging in parasitology research. Animal welfare requirements may also restrict aggressive treatment or screening methods. Lastly, cost considerations impose a burden on large colonies, and prolonged use of antiparasitic drugs may elevate the risk of parasite resistance development (Pritt et al., 2012).

Conclusion and Recommendations

Preventive veterinary medicine against parasitic diseases in laboratory animals is essential for safeguarding animal health and research quality. Common parasites such as Syphacia, *Hymenolepis, Eimeria, Polyplax spinulosa*, and *Ornithonyssus bacoti*, which may be part of the natural flora of rats and mice, can be managed through early detection, appropriate drug administration, and biosecurity strategies. Molecular diagnostic techniques, such as polymerase chain reaction (PCR), offer rapid parasite identification (Pritt et al., 2012). Furthermore, the development of DNA vaccines for parasites like *Toxoplasma gondii* represents a groundbreaking advancement in preventive veterinary medicine (Yu et al., 2021). For ectoparasite control, routine skin examinations and environmental adjustments should be incorporated into standard protocols (Wang et al., 2020). To mitigate parasite resistance, drug rotation and alternative biocontrol methods should be investigated.

Collaboration among veterinary parasitologists, laboratory managers, and researchers will enhance the efficacy of these measures. Regular training programs and technological innovations will minimize parasitic diseases in laboratory settings, ensuring both animal welfare and scientific progress.

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