ISSN: 2980-3063 (online) Review

Rats
2023; 1(2): 35-39
Doi: 10.5281/zenodo.10444415
https://ratsjournal.com

Gallbladder microbiota in laboratory animals: Insights and implications

İlayda Tendar[1]*, Hasan Erdoğan[1], Songül Erdoğan[1], Kerem Ural[1]

[1]Adnan Menderes University, Faculty of Veterinary Medicine, Department of Internal Medicine, Aydın, Türkiye
*Corresponding: ilayd.tend@gmail.com

Recevied: 14.09.2023 Accepted: 19.12.2023 Published: 30.12.2023

Abstract
The aim of this review is to give novel information about the gallbladder microbiome's clinical importance and alteration of the gallbladder microbiota of specific laboratory animals in health and disease. The significant involvement of the gastrointestinal microbiota (GM) in maintaining optimal health has been widely acknowledged. Their functions include a broad spectrum of mechanisms, such as facilitating digestion, enhancing the immune system and strengthening against bacterial pathogens and pathogenic commensal microorganisms. Until now, the majority of research on bacterial function and diversity has concentrated on the colon due to its easy accessibility for sample collection and the abundant presence of bacteria in that region. Despite the widespread interest in examining how the intestinal microbiota affects health and disease, it is unexpected that there has been a inadequate number of researchs focusing on investigating the microbial structure of bile. The gallbladder microbiome, although often neglected in human medicine, is a valuable source of discovery for veterinarians. Its applicability, especially in laboratory animals and hospitalized small animal patients, shows the prospective value of investigating the gallbladder microbiome as an encouraging avenue in the scope of veterinary medicine. Research in this field reveals important insights that can enrich veterinary clinical practice and offer new perspectives on animal health.

Keywords: *Gallbladder microbiome, mice, rabbit, microbiota*

1. Introduction

The human and animal digestive systems are home to a wide collection of microbiota. This complex microbiota acts as a protective shield against harmful agents from the outside and offers a variety of advantages to the host.[1,2] The significant involvement of the gastrointestinal microbiota (GM) in maintaining optimal health has been widely acknowledged. Their functions include a broad spectrum of mechanisms, such as facilitating digestion, advancing the immune system and strengthening against bacterial pathogens and pathogenic commensal microorganisms.[3]

GM has valuable systemic impacts on an animal's biological functions. Research with laboratory animals has played a central role in uncovering many broader effects of GM.[4] As we already know, the biological characteristics of an organism are strongly influenced by its environment, and it is reasonable to expect a similar effect on GM. Indeed, studies on laboratory animals have revealed significant differences in the microbiota composition of the same mouse strains housed in separate research units. This observation provides compelling evidence of the profound influence of the environment on an animal's microbiome.[5] Over the last few decades, numerous GM studies have focused on different diseases, but only a limited number of studies have examined bacterial community compositions in typical rodent strains. A large body of evidence suggests that there are significant differences in mouse phenotypes in disease models, which may be attributable to different microbiota compositions between animal facilities and commercial suppliers.[6,7]

Until now, the majority of research on bacterial function and diversity has concentrated on the colon due to its simple accessibility for sample collection and the abundant presence of bacteria in that region. Conversely, accessing other parts of the gastrointestinal tract (e.g., small intestine, the stomach, and gallbladder) has been challenging, resulting in limited knowledge about the microbiota characteristics in these areas.[8] Despite the widespread interest in examining how GM affects health and disease, it is unexpected that there has been a inadequate number of researchs focusing on investigating the microbial structure of bile.[9]

This review will focus on the gallbladder microbiome's clinical importance and alteration of the gallbladder microbiota of specific laboratory animals in health and disease.

1.1. The gallbladder microbiome

Bile is a natural bodily fluid composed primarily of bile acids, proteins, phospholipids and cholesterol. It is synthesized in the liver and then accumulated and kept in the gallbladder. Its primary role is to assist in the digestion process by aiding in the absorption of fats in the small intestine.[10]

There are multiple potential pathways for bacterial translocation and establishment of a microbial community of the biliary system, including hematogenous entry into the liver and excretion into the biliary fluid or translocation through the duodenum. Based on recent studies, bacterial infections play a key role in the etiology of gallstone formation.[11,12] These findings remind that the impact of microbiota on gallbladder diseases cannot be underestimated.[13,14]

How to cite this article: Tendar I, Erdoğan H, Erdoğan S, Ural K. Gallbladder microbiota in laboratory animals: Insights and implications. *Rats*, 2023: 1(2); 35-39. Doi: 10.5281/zenodo.10444415

Although bile is an inhospitable environment for bacterial growth, many studies have shown that Listeria monocytogenes and Salmonella spp. can stay alive in the gallbladder for long periods of time and induce infections and gallstone formation.[15–17] One study showed that Prevotella, Streptococcus, Fusobacterium, Haemophilus and Veillonella, species were commonly found in bile duct of sclerosing cholangitis patients.[18] Furthermore, the connection between the GM and bilirubin is well-established. Once conjugated bilirubin enters the gastrointestinal tract, it undergoes a series of transformations, ultimately converting into urobilinoids, which is then eliminated through feces. This metabolic process in the intestines is carried out by four specific bacteria: C. perfringens, Clostridium ramosum, Bacteroides fragilis and C. difficile.[19]

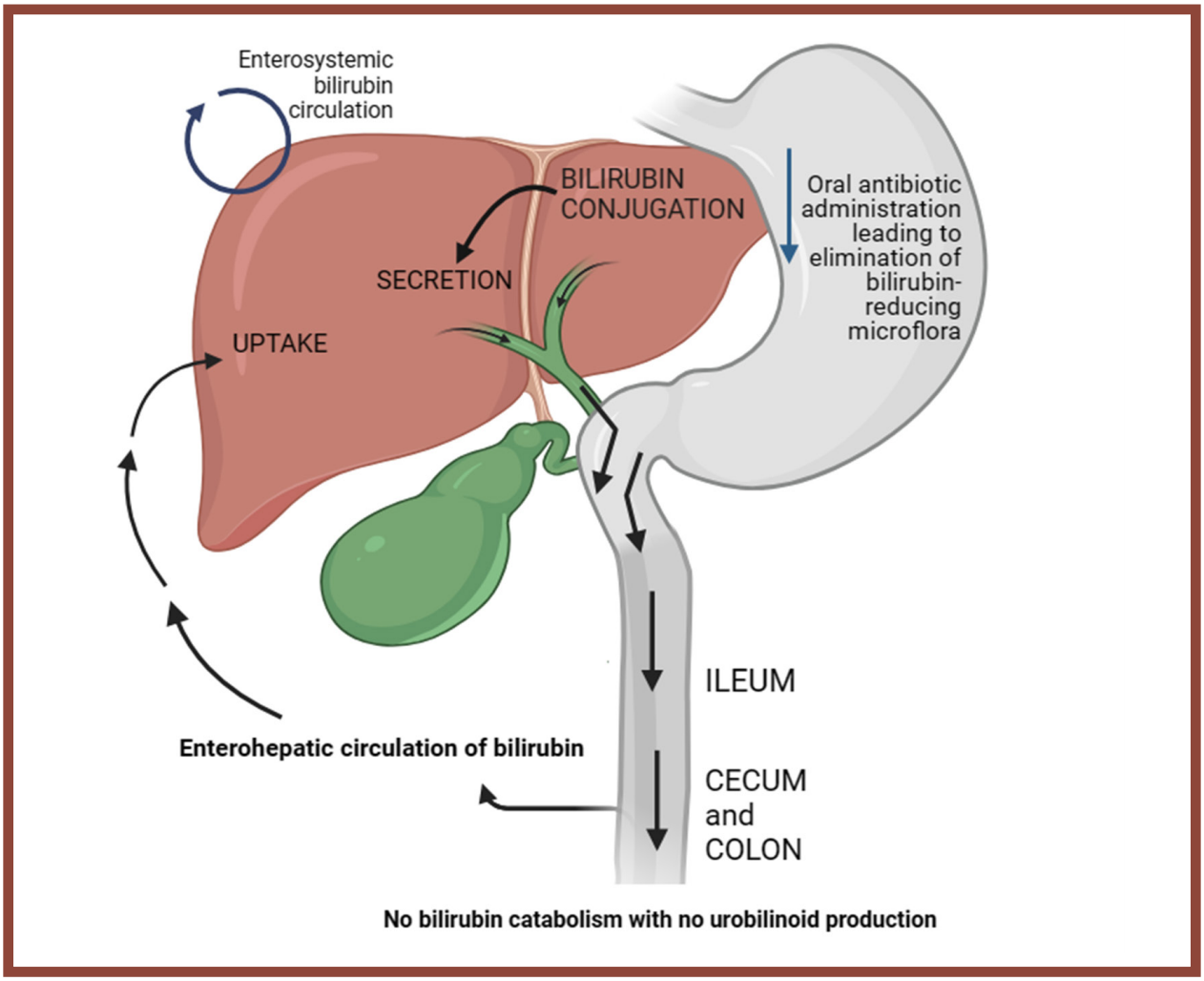

Figure 1. Intestinal metabolism of bilirubin in adults after eradication of bilirubin-reducing microflora. Adopted from Vítek et al.[20]

A study proven that serum bilirubin levels increased dramatically in mice whose faecal urobilinoids were eliminated by oral clindamycin/neomycin administration. Subsequently, it was observed that faecal urobilinoids reappeared with the intestinal colonization of C. perfringens. Current research reveals the important link between GM and the biliary system.[20]

Apart from diseased conditions, previously it was believed that a healthy biliary system was devoid of microorganisms, but it has now been discovered that the biliary tract and gallbladder contain diverse communities of microorganisms even in non-diseased conditions.[21–23]

The finding of Helicobacter pylori has caused a major reconsideration of the stomach, which was once assumed to be a sterile organ thanks to its acidic environment containing hydrochloric acid that was believed to prevent bacterial colonization. This has also led to a reassessment of the stomach's potential for bacterial colonization.[24] Likewise, the gallbladder does not have favorable environmental conditions for bacterial growth. However, many new studies have found many bacterial species that can colonize in these harsh conditions.[25,26] Nevertheless, the microbiota of the gallbladder is a relatively understudied and poorly understood subject due to the difficulty of sample collection and complications in diagnostic molecular biology methods. The most important limitations of human studies investigating gallbladder microbiota are the difficulty of collecting bile from healthy individuals and the use of non-aseptic methods during duodenal catheterization, which can result in contaminated bile. Consequently, DNA contamination can lead to inaccurate results. DNA contamination, especially in samples with low levels of biomass, is a factor to be considered in microbiota studies.[27]

According to a study of 102 people with biliary and

gallbladder-related diseases, patients with different gallbladder etiologies developed certain bacterial colonization. The results showed that *Enterococcus spp.*, *Pseudomonas spp.* and *Escherichia coli* were the most common bacterial species in patients with gallstones.[28] According to research involving 20 patients with gallstones, the analysis of bile samples taken during a laparoscopic cholecystectomy under sterile conditions indicated that the predominant bacterial species present were *Lactobacillus spp., Bacteroides spp., E. coli, and Bifidobacterium spp.*[29] In another study, healthy and gallstone diseased individuals' bile microbiota were compared and novelly, specific bacterial communities were found in healthy individuals. The most abundant bacterial colonies in the healthy control group were, *Bacteroidetes spp.*, *Actinobacteria spp., Firmicutes spp.* and *Proteobacteria spp.* while *Veillonellaceae, Porphyromonadaceae*, *Prevotellaceae,* and *Bacteroidaceae* were detected in patients with gallstones.[30]

On the other hand, investigating gallbladder microbiota in animals is much more applicable than in humans, as it can be performed in a hospital setting using laboratory and companion animals. Even so, gallbladder microbiome studies in animals are quite rare. In a new study comparing the bile microbiome of healthy dogs and dogs with gallbladder mucocele, researchers found differences in bacterial abundance and diversity similar to human studies. Then again, an important limitation of this research is the high prevalence of gene sequences from potential pathogens such as *Enterococcus spp.* and *E. coli-Shigella* that may occur as contaminants in extraction materials and reagents. The findings from this study do not provide evidence for a consistent core bile microbiome among the healthy control group. Instead, the data suggest that the microbiota in the bile is diverse and individual-specific, assuming there are no unaccounted contamination factors.[9]

In a study in 2022, researchers examined the gallbladder microbiome of healthy dogs and dogs with liver and gallbladder disorders. The findings revealed that the canine gallbladder microbiome exhibited some bacterial diversity, particularly in Proteobacteria, Firmicutes and Actinobacteria, which partially resemble the microbiome found in humans. Interestingly, bacterial DNA was absent in the gallbladders of healthy animals, however in sick dogs, the bile microbiota showed differences according to the organs affected and the type of disease present. As a result, it was observed that there is no natural microbiome in the bile of dogs, but instead, they carry a specific group of bacteria that are linked to the type of disease they have.[31] Overall, it is clear that animal studies on the gallbladder microbiome have not reached a consensus and further studies are needed.

1.2. Microbiota of laboratory animals

In the late 19th century, the dangers of working with laboratory animals began to be recognized. The many zoonotic diseases they carried threatened researchers and animal health and endangered the reliability of studies.[32] In the mid-20th century, the application of the 3R (refinement, reduction, replacement) rule rendered laboratory animals free of specific pathogens through advances such as cesarean section and barrier applications.[33] Unfortunately, as a result, these laboratory animals, which are completely free of microorganisms, become entirely vulnerable to environmental opportunistic pathogens and are easily infected with bacterial and viral diseases, making research results misleading. Shaedler et al.[34], who recognized this challenge, identified commensal bacterial classes that are not related with disease and are naturally present in mice and was able to manipulate the microbiota of mice by introducing these selected commensal bacterial species to sterile mice or exposing them to their environment. In this way, only certain types of commensal bacteria are present in the microbiota of the mice and the influence of other bacteria is minimized. Shaedler flora, first described in mice, consists of eight aerotolerant and aerobic bacterial genus. These species were Lactobacillus acidophilus, Group N Streptococcus, Streptococcus faecalis, Lactobacillus salivarius, Escherichia coli, a Clostridium sp., Bacteroides distasonis, and a fusiform bacterial species.[34] The microbiota, later redesigned as ASF (Altered Shaedler Flora), is the primary microbiota of many rodent breeding colonies.[35]

Many studies involving laboratory animals show that mice sharing the same genetic traits and belonging to the same litter have similar microbiomes.[6,36] Especially in vaginally born mice, the first inoculum is the microbiome of their mothers. Subsequently, the microbiota is shaped by many factors such as different environmental conditions and gender and diversifies among different groups. As a result of a present study, the main phyla of healthy mice were Bacteroidetes and Firmicutes and the main genera were Ruminococcaceae, Bacteroides, Blautia, Lactobacillus, and Clostridioides. But, microbiome analysis in diseased mouse races showed differences compared to healthy rats in terms of abundance and predominant bacterial genus. While the Firmicutes genus in the diseased group decreased compared to the healthy group, Clostridioides, Helicobacter, Enterobacter, Blautia, Bacillus, Clostridiales and Paenibacillus species decreased, Saccharimonas, Rikenella and Odoribacter increased significantly.[37]

The 16s rRNA gene analysis in rats shows that the amount of bacterial genus in the rat intestines matches or exceeds those found in the human GM. Firmicutes, Actinobacteria, Proteobacteria, and Bacteroidetes were detected as the predominant bacterial groups in the rat intestinal microbiota, showing striking similarities to those found in humans.[38,39] Yet, the microbiota of rats have some uncommon features compared to humans. Contrary to humans, Lactobacillus spp. compose an important part of the rat microbiota.[38–40]

Gastrointestinal diseases are very common in rabbit breeding, especially after weaning, and are associated with high mortality rates despite the use of prophylactic antibiotics.[41] Especially in young rabbits, the cecal microbiome is a key point in the gastrointestinal system's health. Therefore, enrichment and stabilization of the cecal microbiome may reduce the risk of gastrointestinal diseases in weaning rabbits.[42] A new study investigating the gallbladder microbiome in rabbits based on age revealed that Firmicutes, Actinobacteria, Bacteroidetes, and Proteobacteria were the most shared phyla in the gallbladder and that these 4 main phyla differed

in young and mature rabbits. Young rabbits had more Firmicutes and Bacteroidetes compared to adult rabbits, while young rabbits had fewer Proteobacteria and Actinobacteria compared to adults. The same study compared the gallbladder and fecal microbiome and found that the dominant bacteria by genus were significantly different. The dominant species in the gallbladder were Bacteroides, Clostridiales and Acinetobacter while Ruminococcus Ruminococcaceae and Bacteroides were relatively abundant in the feces. The abundance of species was superior in fecal matter than in the gallbladder.[43]

2. Conclusion

As established through general microbiota studies and insights gained from the examination of both human and animal gallbladder microbiomes, the significance of these microbial communities in maintaining health and influencing disease states is undeniable. The gallbladder microbiota, often overlooked in both human and veterinary medicine, presents a realm of untapped knowledge so it's a topic that needs to be supported by further studies, and although it has complications such as the necessity of invasive methods and the fact that samples can be easily contaminated, It's believed that this could offer a fresh outlook in the realm of microbiomes, benefiting both human and animal healthcare.

Ethical approval

This study does not require approval from the Ethics Committee for Animal Experiments.

Conflict of interest

There are no conflicts of interest associated with this research publication, according to the authors.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgments

This situation does not exist.

Funding statement

This study has not received any financial support from any institution.

References

1. Collins SM, Surette M, Bercik P. The interplay between the intestinal microbiota and the brain. *Nat Rev Microbiol*. 2012;10(11):735-742. doi:10.1038/nrmicro2876
2. Xu J, Mahowald MA, Ley RE, et al. Evolution of Symbiotic Bacteria in the Distal Human Intestine. *PLOS Biol*. 2007;5(7):e156. doi:10.1371/journal.pbio.0050156
3. de Vos WM, de Vos EAJ. Role of the intestinal microbiome in health and disease: from correlation to causation. *Nutr Rev*. 2012;70 Suppl 1:S45-56. doi:10.1111/j.1753-4887.2012.00505.x
4. Ericsson AC, Gagliardi J, Bouhan D, Spollen WG, Givan SA, Franklin CL. The influence of caging, bedding, and diet on the composition of the microbiota in different regions of the mouse gut. *Sci Rep*. 2018;8:4065. doi:10.1038/s41598-018-21986-7
5. Ericsson AC, Davis JW, Spollen W, et al. Effects of vendor and genetic background on the composition of the fecal microbiota of inbred mice. *PloS One*. 2015;10(2):e0116704. doi:10.1371/journal.pone.0116704
6. Benson AK, Kelly SA, Legge R, et al. Individuality in gut microbiota composition is a complex polygenic trait shaped by multiple environmental and host genetic factors. *Proc Natl Acad Sci U S A*. 2010;107(44):18933-18938. doi:10.1073/pnas.1007028107
7. Tomkovich S, Stough JMA, Bishop L, Schloss PD. The Initial Gut Microbiota and Response to Antibiotic Perturbation Influence Clostridioides difficile Clearance in Mice. *mSphere*. 2020;5(5):e00869-20. doi:10.1128/mSphere.00869-20
8. Ley RE, Peterson DA, Gordon JI. Ecological and Evolutionary Forces Shaping Microbial Diversity in the Human Intestine. *Cell*. 2006;124(4):837-848. doi:10.1016/j.cell.2006.02.017
9. Gookin JL, Hartley AN, Aicher KM, et al. Gallbladder microbiota in healthy dogs and dogs with mucocele formation. *PLOS ONE*. 2023;18(2):e0281432. doi:10.1371/journal.pone.0281432
10. Begley M, Gahan CGM, Hill C. The interaction between bacteria and bile. *FEMS Microbiol Rev*. 2005;29(4):625-651. doi:10.1016/j.femsre.2004.09.003
11. Capoor MR, Rajni ND, Khanna G, et al. Microflora of bile aspirates in patients with acute cholecystitis with or without cholelithiasis: a tropical experience. *Braz J Infect Dis Off Publ Braz Soc Infect Dis*. 2008;12(3):222-225. doi:10.1590/s1413-86702008000300012
12. Swidsinski A, Lee SP. The role of bacteria in gallstone pathogenesis. *Front Biosci J Virtual Libr*. 2001;6:E93-103. doi:10.2741/swidsinski
13. Sayin SI, Wahlström A, Felin J, et al. Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. *Cell Metab*. 2013;17(2):225-235. doi:10.1016/j.cmet.2013.01.003
14. Sekirov I, Russell SL, Antunes LCM, Finlay BB. Gut microbiota in health and disease. *Physiol Rev*. 2010;90(3):859-904. doi:10.1152/physrev.00045.2009
15. Dowd GC, Joyce SA, Hill C, Gahan CGM. Investigation of the Mechanisms by Which Listeria monocytogenes Grows in Porcine Gallbladder Bile. *Infect Immun*. 2011;79(1):369-379. doi:10.1128/IAI.00330-10
16. Gonzalez-Escobedo G, Marshall JM, Gunn JS. Chronic and acute infection of the gall bladder by Salmonella Typhi: understanding the carrier state. *Nat Rev Microbiol*. 2011;9(1):9-14. doi:10.1038/nrmicro2490
17. Hardy J, Francis KP, DeBoer M, Chu P, Gibbs K, Contag CH. Extracellular replication of Listeria monocytogenes in the murine gall bladder. *Science*. 2004;303(5659):851-853. doi:10.1126/science.1092712
18. Pereira P, Aho V, Arola J, et al. Bile microbiota in primary sclerosing cholangitis: Impact on disease progression and development of biliary dysplasia. *PLOS ONE*. 2017;12(8):e0182924. doi:10.1371/journal.

pone.0182924

19. Hamoud A rizaq, Weaver L, Stec DE, Hinds TD. Bilirubin in the Liver to Gut Signaling Axis. *Trends Endocrinol Metab TEM*. 2018;29(3):140-150. doi:10.1016/j.tem.2018.01.002

20. Vítek L, Zelenka J, Zadinová M, Malina J. The impact of intestinal microflora on serum bilirubin levels. *J Hepatol*. 2005;42(2):238-243. doi:10.1016/j.jhep.2004.10.012

21. Csendes A, Burdiles P, Maluenda F, Diaz JC, Csendes P, Mitru N. Simultaneous bacteriologic assessment of bile from gallbladder and common bile duct in control subjects and patients with gallstones and common duct stones. *Arch Surg Chic Ill 1960*. 1996;131(4):389-394. doi:10.1001/archsurg.1996.01430160047008

22. Helaly GF, El-Ghazzawi EF, Kazem AH, Dowidar NL, Anwar MM, Attia NM. Detection of Helicobacter pylori infection in Egyptian patients with chronic calcular cholecystitis. *Br J Biomed Sci*. 2014;71(1):13-18. doi:10.1080/09674845.2014.11669957

23. Zhang FM, Yu CH, Chen HT, et al. Helicobacter pylori infection is associated with gallstones: Epidemiological survey in China. *World J Gastroenterol WJG*. 2015;21(29):8912-8919. doi:10.3748/wjg.v21.i29.8912

24. Sheh A, Fox JG. The role of the gastrointestinal microbiome in Helicobacter pylori pathogenesis. *Gut Microbes*. 2013;4(6):505-531. doi:10.4161/gmic.26205

25. Hazrah P, Oahn KTH, Tewari M, et al. The frequency of live bacteria in gallstones. *HPB*. 2004;6(1):28-32. doi:10.1080/13651820310025192

26. Merritt ME, Donaldson JR. Effect of bile salts on the DNA and membrane integrity of enteric bacteria. *J Med Microbiol*. 2009;58(12):1533-1541. doi:10.1099/jmm.0.014092-0

27. Salter SJ, Cox MJ, Turek EM, et al. Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. *BMC Biol*. 2014;12(1):87. doi:10.1186/s12915-014-0087-z

28. Tajeddin E, Sherafat SJ, Majidi MRS, Alebouyeh M, Alizadeh AHM, Zali MR. Association of diverse bacterial communities in human bile samples with biliary tract disorders: a survey using culture and polymerase chain reaction-denaturing gradient gel electrophoresis methods. *Eur J Clin Microbiol Infect Dis*. 2016;35(8):1331-1339. doi:10.1007/s10096-016-2669-x

29. Simanenkov VI, Ильич CB, Suvorov AN, et al. Gallbladder microbiota in patients with gallstone disease. *Her North-West State Med Univ Named II Mechnikov*. 2020;12(1):37-44. doi:10.17816/mechnikov202012137-44

30. Molinero N, Ruiz L, Milani C, et al. The human gallbladder microbiome is related to the physiological state and the biliary metabolic profile. *Microbiome*. 2019;7(1):100. doi:10.1186/s40168-019-0712-8

31. Neo S, Takemura-Uchiyama I, Uchiyama J, et al. Screening of bacterial DNA in bile sampled from healthy dogs and dogs suffering from liver- or gallbladder-associated disease. *J Vet Med Sci*. 2022;84(7):1019-1022. doi:10.1292/jvms.22-0090

32. Hansen AK, Franklin C. Microbiota, laboratory animals, and research. *Lab Anim*. 2019;53(3):229-231. doi:10.1177/0023677219844538

33. The Principles of Humane Experimental Technique. *Med J Aust*. 1960;1(13):500-500. doi:10.5694/j.1326-5377.1960.tb73127.x

34. Schaedler RW, Dubos R, Costello R. The development of the bacterial flora in the gastrointestinal tract of mice. *J Exp Med*. 1965;122(1):59-66.

35. Dewhirst FE, Chien CC, Paster BJ, et al. Phylogeny of the defined murine microbiota: altered Schaedler flora. *Appl Environ Microbiol*. 1999;65(8):3287-3292. doi:10.1128/AEM.65.8.3287-3292.1999

36. Wen L, Ley RE, Volchkov PY, et al. Innate immunity and intestinal microbiota in the development of Type 1 diabetes. *Nature*. 2008;455(7216):1109-1113. doi:10.1038/nature07336

37. Guo J, Song C, Liu Y, et al. Characteristics of gut microbiota in representative mice strains: Implications for biological research. *Anim Models Exp Med*. 2022;5(4):337-349. doi:10.1002/ame2.12257

38. Brooks SPJ, McAllister M, Sandoz M, Kalmokoff ML. Culture-independent phylogenetic analysis of the faecal flora of the rat. *Can J Microbiol*. 2003;49(10):589-601. doi:10.1139/w03-075

39. Manichanh C, Reeder J, Gibert P, et al. Reshaping the gut microbiome with bacterial transplantation and antibiotic intake. *Genome Res*. 2010;20(10):1411-1419. doi:10.1101/gr.107987.110

40. Dalby AB, Frank DN, St Amand AL, Bendele AM, Pace NR. Culture-independent analysis of indomethacin-induced alterations in the rat gastrointestinal microbiota. *Appl Environ Microbiol*. 2006;72(10):6707-6715. doi:10.1128/AEM.00378-06

41. Cuniculture Magazine vol.33 pages 78-85. Accessed August 18, 2023. http://www.cuniculture.info/Docs/Magazine/Magazine2006/mag33-078.htm

42. Monteils V, Cauquil L, Combes S, Godon JJ, Gidenne T. Potential core species and satellite species in the bacterial community within the rabbit caecum. *FEMS Microbiol Ecol*. 2008;66(3):620-629. doi:10.1111/j.1574-6941.2008.00611.x

43. Xing Y, Liu J, Lu F, Wang L, Li Y, Ouyang C. Dynamic distribution of gallbladder microbiota in rabbit at different ages and health states. *PLoS One*. 2019;14(2):e0211828.